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The role of the Species Survival Plan in Maned wolf Chrysocyon brachyurus conservation

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The Association of Zoos and Aquariums Maned Wolf Species Survival Plan (MWSSP) was established almost 25 years ago. The goals of the MWSSP are to (1) maintain a viable self-sustaining captive population in North America, (2) enhance health and well-being of individuals living in North American zoos and (3) promote conservation of this species through education and field-conservation initiatives. Since its inception, the MWSSP and member institutions have supported studies on nutrition, medical management, behaviour and reproductive biology, and published a husbandry manual, which serves as a guide for captive management of Maned wolves Chrysocyon brachyurus in North and South American zoos. Furthermore, the MWSSP has provided funding for field studies aimed at identifying potential threats to wild populations in range countries, including Brazil, Bolivia and Argentina, as well as for the first Population and Habitat Viability Assessment Workshop for this species. Finally, the MWSSP has played an active role in promoting education and outreach efforts in both the United States and range countries. In this paper, we review and discuss the roles of the MWSSP in ex situ and in situ conservation of the Near Threatened Maned wolf.

Key-words: captive breeding; field research; husbandry; maned wolf; South America; SSP.

INTRODUCTION

The Maned wolf *Chrysocyon brachyurus* is a Neotropical canid that lives in habitats severely compromised by agricultural development. The species is native to the grasslands of South America (known as the Cerrado in Brazil) and found exclusively in Brazil, Bolivia, Argentina, Peru and Paraguay (Dietz, 1984; Rodden *et al.*, 2004). Although listed as Near Threatened on the *2008 IUCN Red List of Threatened Species* (IUCN, 2008), the

Maned wolf may be extinct in Uruguay (Dietz, 1985) with other populations increasingly at risk as a result of habitat loss to agriculture (Dietz, 1984; Rodden *et al.*, 2004). It was estimated during the first Population and Habitat Viability Assessment Workshop held in the Serra da Canastra National Park (PNSC), Brazil in October 2005, that about 20 000 individuals remain in Brazil, the country believed to have the most animals, with only a few thousand living in the other range countries.

Historical studbook records indicate that Maned wolves have been housed in zoological institutions for more that 40 years. European zoos, including Frankfurt, Krefeld and Berlin, all in Germany, and Antwerp in the Netherlands began importing Maned wolves from Brazil and Argentina in the 1960s (Dmoch, 2008). From 1965 to 1967, the first seven $[4.3 \ (3.9)]$ Maned wolves were imported from South America to several zoos in the United States (Dmoch, 2008). Partnerships among North American and European zoos facilitated exchanges between the two continents, and over the following 8 years, an additional six (3.3) and five (3.2) animals were imported to the United States from South American and European zoos, respectively. These animals, plus several more recent imports from South America and exchanges with European zoos, formed the core-breeding group represented in the current population of 80 (38.42) individuals

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descended from 32 founders. The Association of Zoos and Aquariums (AZA) added the Maned wolf to its fledgling Species Survival Plan (SSP) programme in 1985. The goals of the Maned Wolf SSP (MWSSP) are to (1) maintain a viable self-sustaining captive population in North America, (2) enhance the health and well-being of individuals living in North American zoos and (3) promote conservation of this species through education and field conservation initiatives. Since its inception, the MWSSP and its member institutions have participated in or provided financial or in-kind support to captive-management studies of nutrition, medical management, behaviour and reproductive biology. More recently, the MWSSP has provided funding for field studies conducted in range countries, including Brazil, Bolivia and Argentina.



Plate 1. Maned wolf Chrysocyon brachyurus at Smithsonian's National Zoological Park, Washington, DC. Jessie Cohen, National Zoological Park, Washington, DC.

CAPTIVE-BREEDING PROGRAMME

The ultimate goal of the captive-breeding programme is to maintain a healthy and viable population of Maned wolves in zoos (Plate 1). The MWSSP is a cooperative management programme involving all 28 North American zoos currently holding Maned wolves. While there are no formal ties between the MWSSP and similar programmes in Europe, such as the European Endangered Species Programme, and other regions of the world, exchanges of both animals and information within the global zoo community has proved invaluable in sustaining genetic diversity and increased our understanding of Maned wolf husbandry. The MWSSP is a committee-driven process composed of a Species Coordinator and a Management Committee, comprising a small group of elected or appointed representatives from the participating institutions. In addition, experts in the fields of reproductive biology, veterinary medicine and pathology, nutrition, education and behaviour act as scientific advisors to assist the Management Committee in formulating in situ and ex situ research goals and projects. The Management Committee produces an annual Population Management Plan that is distributed to all holding institutions and posted on the AZA website (http://www.aza.org). Genetic and demographic analyses of studbook data using PM2000 (Pollak et al., 2005) provide the basis for determining population growth goals, and making breeding and transfer recommendations for each individual. The primary objective of this activity is to maximize the retention of genetic diversity and minimize inbreeding.

Target population sizes, based on space surveys conducted at regular intervals, are determined for each species that falls within the purview of the AZA Canid & Hyaenid Taxon Advisory Group; the target population for Maned wolves is 100 animals. Between 2003 and 2007 the North American population remained close to 95 individuals through restricting breeding to a specific number of pairings. By pairing animals with the rarest

alleles (i.e. high Mean Kinship rank) and minimizing the inbreeding coefficients of offspring, the population has maintained gene diversity around 0.93 and the mean inbreeding coefficient (F) of c.0.015. Inexplicably poor reproduction for the past 2 years has reduced the population to 80 animals, although genetic parameters have not vet been adversely affected. The Management Committee has responded by increasing the number of pairings recommended to reproduce in 2009, in order to increase the population. Simultaneously, efforts are under way to examine more closely reproductive hormone levels and the role that stress and/or diet may be playing in recent reproductive failures.

Supporting research into all aspects of Maned wolf husbandry is another critical role played by the MWSSP. Since its inception, the MWSSP, working closely with several scientific advisors, has supported studies of reproductive behaviour and physiology, nutrition and medical management. Most of the research is conducted by scientists affiliated with zoos in the MWSSP. Proposals, including budgets, are submitted to the Management Committee for review and approval. The Committee prioritizes projects based on the needs of the programme and assists in funding through direct appeals to the MWSSP zoos. Working with the AZA, the MWSSP created a fund that is managed by the Dickerson Park Zoo (Springfield, MO, USA). Contributions from individual zoos and from external grants are deposited into the fund and utilized based on priorities determined by the Management Committee. While only a few zoos are able to offer direct funding to ex situ projects, virtually all zoos in the programme provide support; for example, by providing samples or collecting behavioural data or management information requested by the Principal Investigators of approved projects. The majority of funding for captive-management projects, totalling over US\$100 000, has been obtained through external grants awarded to MWSSP-affiliated scientists. Findings from these studies have been summarized and formulated into the Maned Wolf Husbandry Manual, serving as

a guideline for management of this species in captivity. The Manual was first published in 1987, updated in 1995 and completely revised in 2007 (MWSSP, 2007). It has been distributed to all holding institutions in North America and is posted on the AZA website. A Portuguese translation was sent to zoos and government agencies in Brazil, and a Spanish translation, once completed, will be made available to zoos and wildlife departments in Spanish-speaking range countries. The Manual will continue to be updated as new information becomes available.

Nutrition and medical management

In nature, the diet of Maned wolves as identified by scat analysis is typical for a generalist omnivore with c.50% being plant materials and the rest being small mammals [rodents, armadillos (Dasypodidae)], birds and insects (Dietz, 1984; Motta-Junior et al., 1996; Bestelmeyer, 2000). It has been shown that there are seasonal changes in the food habits of wild wolves. Small mammal remains are found more frequently in dryseason scats, while fruit and insect remains were more common in the wet season (Dietz, 1984; Motta-Junior et al., 1996). However, the seeds of the fruit 'lobeira' Solanum lycocarpum, the main plant item consumed by Maned wolves in Brazil, are found in wolf scat throughout the year.

Appropriate diet management is a key component for the health and well-being of wildlife maintained in zoos. Although the food habits of wild Maned wolves have been well characterized (Dietz, 1984; Motta-Junior et al., 1996; Bestelmeyer, 2000), formulation of optimal diets for captive Maned wolves has been a major challenge for North American zoos, possibly owing to the unique physiology of the species and susceptibility to cystinuria (Bush, 1980; Bovee et al., 1981). Maned wolves have a very sensitive gastrointestinal tract and often respond to minor management changes (e.g. diet manipulation) with diarrhoea and poor body condition (Bush, 1980). Diets fed to captive Maned wolves were originally based on formulations

for Domestic dogs *Canis lupus familiaris*. However, it has been shown that there is species specificity in digestibility (Childs-Sanford & Angel, 2006). Specifically, Maned wolves have lower digestibility of dry matter, energy and several minerals, including copper, magnesium, iron and sodium (Childs-Sanford & Angel, 2006), than Domestic dogs. Thus, diets formulated for the latter may not be completely appropriate for Maned wolves, emphasizing the need for additional research to develop a diet specifically for the Maned wolf.

The Maned wolf is predisposed to cystinuria, a metabolic disorder that results in the urinary excretion of the amino acid cystine (Bovee et al., 1981). It has been shown that cystinuria occurs in both wild and captive individuals (Bovee et al., 1981). Although the clinical significance of cystinuria for wild Maned wolves is unclear, this metabolic disorder has posed a considerable health threat to individuals in captivity. The clinical signs of cystinuria include urinary straining, frequent urination and obstruction of the urethra by calculi that can lead to obstruction and secondary rupture of the urinary bladder and death (Bovee et al., 1981). It has been suggested that the occurrence of cystinuria in captive Maned wolves is related to a diet high in animal protein, such as the carnivore diets originally fed to this species. High-protein diets tend to increase urine acidity that in turn increases the insolubility of cystine, resulting in the formation of calculi in the kidneys and bladder. In response to the increasing incidence of clinical illness related to cystinuria, efforts were made to formulate a Maned wolf diet that reduced and prevented the disease (Boniface, 1998). The resulting diet, having low cystine content and high fibre, was shown to reduce the concentration of urinary cystine and total nitrogen significantly. Subsequent modification of this formula increased the proportion of protein derived from plant materials and lowered the amount of sodium in the diet (Childs-Sanford, 2005). This modified formula has been shown to increase the urinary pH in Maned wolves. However, in 2002 concerns about a possible

linkage between long-term feeding of the new diet and poor reproduction prompted the MWSSP to err on the side of caution and discontinue feeding the new diet pending further investigation. US zoos have returned to feeding dog chow-based diets supplemented with a variety of fruits and vegetables as well as small amounts of whole prey items. Maned wolves fed these diets are generally in good health (15 year life span on average). However, identifying the optimal diet for captive Maned wolves remains a high priority for the MWSSP. A detailed survey of all diets being fed in US zoos was carried out late in 2006. The survey collected information about all components of the diet, body weights, current medical conditions and medications, urine pH and faecal consistency scores. No correlations have yet been found between diet, health and reproductive success, but analysis of the data is still under way. One finding was very clear: out of the 24 zoos responding to the survey, 15 different brands of chow were being offered and there was a great deal of variation in the amount and type of vegetable, fruit and whole-prey supplements fed (M. Rodden, unpubl. data).

Standardizing the nutrients we offer the Maned wolves in our care is a priority for the MWSSP. As a first step in that direction, the MWSSP has recently initiated a new project to characterize circulating nutritional profiles in captive animals. This pilot project aims to (1) determine baseline data on serum and plasma nutritional parameters, including amino acids, fatty acids, vitamins A and E, various minerals, cholesterol and triglycerides, and (2) correlate circulating nutritional profiles with demographic variables, such as age and gender, in addition to health status and diet composition. Once parameters in captive Maned wolves have been characterized, we hope to compare the results with data obtained from wild Maned wolves through collaborations with field researchers in range countries and use the information to formulate a standard diet that will better meet the Maned wolf's unique nutritional requirements. The MWSSP also hopes to initiate a dialog with colleagues in other regions of the world in

order to compare diets and health concerns throughout the global captive population.

Reproductive biology and behaviours

Because reproduction is the essence of species survival, the MWSSP has promoted significant efforts into studies aimed at characterizing reproductive physiology, determining the cause of poor reproductive success in captive individuals. In addition, these studies have laid the groundwork for the development of assisted-reproduction techniques (e.g. induction of oestrus, semen cryopreservation and artificial insemination), which will provide zoos with another tool for genetic management of zoo populations.

Behavioural field studies of primarily nocturnal, solitary and secretive animals that range over large areas can be extremely difficult, thus zoos provide an extraordinary opportunity to increase our understanding of reproductive behaviour. The Maned wolf is monoestrus with a well-known and distinctive 3–5 month breeding season in captivity, occurring from April to June in the southern hemisphere and October to February in North America and Europe (Maia & Gouveia, 2002). Pro-oestrus lasts for c. 2 weeks and is characterized by vaginal swelling and secretions (Velloso et al., 1998). The MWSSP sponsored a long-term study of Maned wolf social behaviour that involved standardized data collected by zoo staff and trained volunteers from 52 pairs housed at 17 zoos in the programme over seven breeding seasons. The published results describe breeding behaviours and present a technique for utilizing discriminant function analysis to help assess whether a pair has bred and, further, whether a ♀ is pregnant based on behavioural measurements (Rodden et al., 1996). The amount of time a pair spends in close proximity increases significantly during the oestrous period, which lasts from one to 10 days (Rodden et al., 1996). Courtship is characterized by frequent approaches, mutual anogenital investigation and playful interactions. Mounting may occur frequently during oestrus. Successful breeding includes a copulatory tie that may

last several minutes (Rodden et al., 1996, 2004).

There are no data on the precise onset of sexual maturity. However, analysis of the 2007 International Studbook for the Maned Wolf using PM2000 (Pollak et al., 2005; Dmoch, 2008) shows that both sexes can reproduce before 2 years of age, although rarely. Bitches can produce pups at 10–12 years of age but this is uncommon. However, for both sexes, the preponderance of breeding occurs from 3 to 8 years of age, an interval generally considered prime for reproduction.

Parturition in captivity peaks in June and December in South and North America, respectively (Maia & Gouveia, 2002). Litter size ranges from one to seven pups (mean SEM, $2 \cdot 6 \pm 0 \cdot 2$) following a c. 65 day gestation (Maia & Gouveia, 2002). Neonatal mortality rates in situ are not known but historical data from captive records indicates 53% of pups are lost in the first year, with almost 78% of the deaths occurring in the first 30 days (Maia & Gouveia, 2002). Pups remain in the den for the first 4 weeks. Nursing bouts begin to decline after the first month, when pups begin consuming solids regurgitated by the parents (Brady & Ditton, 1979; Rodden et al., 2004), and weaning is completed by around 15 weeks. PM2000 analysis (Pollak et al., 2005) indicates the maximum life span for captive Maned wolves is 16-17 years, although the mortality rate begins to increase significantly by 10 years of age.

Patterns of reproductive hormones in the Maned wolf, as measured by radioimmunoassays and enzyme immunoassay, reveal that profiles of gonadal hormones in ♀ Maned wolves are similar to those of Domestic dogs, especially during the preovulatory period (Velloso et al., 1998; Songsasen et al., 2006). Oestrogen concentration gradually increases beginning about 2 weeks before breeding and declines to baseline on the day of lordosis and copulation. Progestins steadily rise during late pro-oestrus and throughout oestrus and remain elevated throughout the pregnant or non-pregnant luteal phase (Velloso et al., 1998; Songsasen et al., 2006). When sexual behaviours are correlated with gonadal hormone profiles, copulatory ties are observed as early as 2 days before or 9 days after the oestrogen peak (Velloso *et al.*, 1998; Songsasen *et al.*, 2006). Non-pregnant \mathcal{P} excrete overall lower concentrations of progestins than their pregnant counterparts (Velloso *et al.*, 1998; Songsasen *et al.*, 2006). There is evidence suggesting that \mathcal{P} wolves may play a role in oestrus induction and/or ovulation in conspecific \mathcal{P} (Songsasen *et al.*, 2006).

Seasonality clearly affects semen production. Analysis of semen samples collected at 4–8 week intervals during two breeding seasons in North America (September through March) reveals a definitive temporal change in sperm concentration throughout the breeding season (N. Songsasen, unpubl. data). Semen samples contain low sperm-cell numbers in November, and a peak in cell concentration in December, followed by an immediate decline. Analysis of faecal testosterone metabolites shows a positive correlation between testosterone concentration and semen production (N. Songsasen, unpubl. data).

When data are analysed on the basis of reproductive history, 33 of proven fertility (n=6) have higher (P<0.05) proportions of structurally normal sperm than unproven counterparts (n=3). With one exception (seminal volume), there also is a strong tendency for other semen characteristics to be higher in proven wolves than in unproven individuals but the difference is not significant (P>0.05), probably owing to small sample size (D. Wildt, C. Platz & M. Bush, unpubl. data).

Reproductive success of Maned wolves housed in US zoos is relatively poor. During the past 5 years, the North American population has declined 20% (from 100 to 80 individuals), mainly as a result of the failure of paired individuals to reproduce and rear pups successfully. From 1996 through 2007, only 38% of 166 recommended breeding pairs produced pups, with 50% of pregnant \$\times\$\times\$ losing neonates within a few days of birth. Neonatal mortality has been a global issue for Maned wolves historically. In a review of the international studbook data, Maia & Gouveia (2002) reported that only 44% of 1532 pups

born over 1980–1998 survived to 1 year of age. They further stated that in 1982–1998 77.7% of pup deaths occurred in the first 30 days, of which 88 · 1% were classified as 'parental incompetence' (Maia & Gouveia, 2002). Thus far, the cause of neonatal mortality in this species has not been fully elucidated, although it has been hypothesized that stress associated with suboptimal management is a contributing factor to low reproductive success in Maned wolves. It has recently been demonstrated that dams experiencing neonatal deaths produced less progesterone during the second half of gestation compared with \$\text{9}\$ successfully rearing young (Songsasen et al., 2006). Retrospective evaluations also demonstrated that wolves losing pups produced higher faecal corticoid metabolite concentrations during the periovulatory interval than those that successfully raised their young (N. Songsasen, unpubl. data). The Maned Wolf Husbandry Manual recommends minimizing potential stress during gestation and pup rearing by providing multiple den sites to breeding pairs and installing video cameras so that pregnant a can be closely monitored (MWSSP, 2007).

Historically, zoos often removed a 3 before birth to avoid pup mortality associated with the aggression of a sire towards his young. However, it has been shown that the presence of the sire increases pup survival to 6 months of age (Bestelmeyer, 2000; see also Veado, 1997, 2005) partly owing to decreased aggression of dams to pups. Dams provide the majority of care while pups are in the den; however, the rate at which sires interact with pups significantly increases when the young emerge from the den (Bestelmeyer, 2000). Based on this study, the MWSSP currently recommends leaving the breeding pair housed together during gestation and pup rearing (MWSSP, 2007).

Zoo education

Educating zoo visitors about the plight of threatened species and habitats is a critically important role played by zoological institutions throughout the world. Several years ago, the

MWSSP developed an Education Bulletin for use in zoo education programmes for school children. The Bulletin summarizes Maned wolf natural history and describes the threats facing wild populations and habitats. It also includes an activity page for recording behavioural observations. The Bulletin has been translated into Portuguese and Spanish and several hundred copies have been distributed to zoos and wildlife parks in range countries.

IN SITU CONSERVATION

Although the original goal of the MWSSP was to maintain a viable and healthy population of Maned wolves in North American zoos, the SSP has become increasingly involved in field conservation initiatives in range countries. Recent projects include ecology and health studies conducted in the Noël Kempff Mercado National Park (PNNKM), Bolivia, and the PNSC, Brazil, an initial examination of genetic diversity in three range countries, as well as education and outreach efforts carried out in north-eastern Argentina. Furthermore, the MWSSP played an instrumental role in raising funds for the first Maned Wolf Population and Habitat Viability Assessment workshop, held in the PNSC, Brazil, in October 2005. The findings, recommendations and action plans resulting from the workshop have been published in Portuguese, English and Spanish (de Paula et al., 2008). Field collaborators submit research proposals to the Management Committee for review and approval, in much the same way as for ex situ projects. Over US\$20 000 has been contributed by c.25%of the zoos in the programme toward in situ conservation efforts since 2003, and close to half the zoos in the MWSSP donated c. US\$9000 to support the PHVA in 2005. The majority of support for in situ projects, c. US\$140 000, has been obtained through external grants awarded to zoo-affiliated scientists.

PNNKM, Bolivia

Bolivia's PNNKM hosts one of the largest protected Maned wolf populations, estimated

by Rumiz & Sainz (2002) at 118 breeding pairs fragmented among six isolated savannas within 3600 km² of suitable habitat in the Park (one-quarter of the area of the Park). However, data from radio-telemetry suggests that the number of breeding pairs is probably only about 30 (L. Emmons, pers. comm.). The Park is adjacent to the Brazilian states of Rondonia and Mato Grosso, and is diverse in habitat characteristics consisting of highground humid forests, flooded and riverside forest to dry forests, high plains and marshes. Because of its vast protected area and pristine state, PNNKM is a United Nations Educational, Scientific and Cultural Organization World Heritage Site. In comparison to other ecosystems in South America that are being destroyed at an accelerated rate, the majority of this Park remains untouched by human activities (http://www.discoveringbolivia.com).

The MWSSP has provided support to a long-term ecological study of mammal communities in PNNKM headed by Dr Louise Emmons, Smithsonian's National Museum of Natural History. The specific objectives of this multidisciplinary project are to (1) generate ecological information of wolves living in the PNNKM, (2) determine genetic diversity of individual living in this region and (3) evaluate the potential of diseases transmitted from local Domestic dogs to Maned wolves.

Ecological analysis showed that seasonality influenced the movement and habitat use of Maned wolves in this region (L. Emmons, pers. comm.). However, the most alarming result of this study was evidence of a significant decline in the Maned wolf prey base of small rodents within the study savanna (Emmons, 2009). The effects of fluctuations in prey availability on the area's Maned wolf population are currently being analysed.

To date, health analyses have been conducted on 11 Maned wolves. All wolves tested positive to adenovirus, with the majority exhibiting antibodies against *Dirofilaria immitis* (i.e. heartworm), *Toxoplasma gondii*, *Ehrlichia canis*, *Borrelia burgdorferi* and *Rickettsia rickettsii* (Deem *et al.*, 2008). In addition, several individuals had antibodies against rabies, canine distemper, canine parvovirus and

herpes virus (Deem et al., 2008). Serological analysis of Domestic dogs living around Park borders showed that the majority of dogs had antibodies against the same infectious diseases found in the Maned wolf population (Bronson et al., 2007), suggesting that these pathogens were directly or indirectly transmitted to wolves by dogs. Findings from this study demonstrate that despite the isolated and pristine location of the Park, disease exposure from domestic animals may be a significant cause of morbidity or mortality (Deem & Emmons, 2005; Bronson et al., 2007; Deem et al., 2008). Based on these findings, the investigators have recommended that Park managers develop vaccination campaigns against rabies and distemper virus in Domestic dogs (Deem et al., 2008). Furthermore, with support from the MWSSP, the researchers have produced and disseminated posters describing the importance of a pet vaccination programme throughout the region.

PNSC, Brazil

Within the Brazilian Cerrado, the PNSC is one of the habitats maintaining the highest density of Maned wolves in the country (IBAMA, 2004). The Park is located in the state of Minas Gerais in south-eastern Brazil, encompassing 2000 km²; 715 · 2 km² are regulated and managed by Chico Mendes Institute of Biodiversity Conservation (ICM-Bio), and the remainder is privately held. In contrast to the PNNKM where the majority of the habitat remains untouched by humans, the effects of prior human habitation and more recent eco-tourism are visible throughout PNSC. The area adjacent to the Park boundary comprises small ranches (1 km² or less), all of which have undergone extensive habitat alterations. Agricultural activities range from subsistence farming to large coffee and corn plantations. The Park comprises hills and ridges that rise abruptly from surrounding agriculture lands to elevations of nearly 1500 m and descend into valleys of c. 800 m. Approximately half of the Park is grassland with another 35% consisting of typical and diverse Cerrado vegetation interspersed

among transitional rocky fields, with the remaining being seasonal, tropical forest (Dietz, 1984).

A collaborative project including scientists from Brazilian universities, governmental [National Research Center for Conservation of Natural Predators (CENAP), ICM-Bio] and non-governmental (Institute for Conservation of Neotropical Carnivores) agencies, and the Smithsonian's National Zoological Park was initiated in 2004 with the main objective of determining the influence of human disturbance on wild Maned wolves living in the PNSC and the surrounding areas. The project involves longitudinal monitoring of the ecology, behaviour, health and reproduction of large numbers of wolves (43 individuals have been captured and radiocollared since 2004) living in low versus high human-activity areas. In addition, investigators are evaluating the impact of domesticated animals, especially Domestic dogs, on the health of the carnivore community, in particular Maned wolves. Finally, an education and outreach programme has been developed to target misperceptions of local communities about the species and to reduce human-wolf conflict (Songsasen & de Paula, 2007). Because the Cerrado habitat has been increasingly utilized by humans, findings generated from this study will not only improve our understanding of anthropogenic threats to Maned wolves living in the Serra da Canastra region but also can be applied to the development of wildlife management plans throughout the Cerrado ecosystem.

It is estimated that 80 wolves currently live inside the national Park (Songsasen & de Paula, 2007). Wolves living in this region have overlapping home ranges, especially those living inside the Park and around the Park border. This ecological characteristic is different from that described by Dietz (1984) and is probably the result of greater food availability and less competition for suitable habitat that exists in the Park today compared with the late 1970s. When Dietz conducted his study from 1978 to 1980, PNSC was only a 'paper park'; people and livestock were commonly found living inside the Park. In

1979 the Brazilian Environmental Agency, after strongly encouraging farmers to leave the protected area, began managing Canastra as a national park. Although development on the periphery of the Park has been increasing. a decline in human pressure inside the Park has increased its capacity to maintain wolves. Notably, there is significantly less homerange overlap for wolves living outside the Park boundaries than for those living within the Park. Landscape analysis using geographic information system has revealed that wolves living in cultivated areas avoid tended fields and appear to forage and use the remaining natural habitat fragments (Songsasen & de Paula, 2007).

Although ecological studies may suggest that Maned wolves in the Serra da Canastra area are able to exist in disturbed habitats, the health analysis suggested that direct or indirect disturbance by humans and domestic species may impact the well-being of Maned wolves. Haematology and blood biochemistries differed among animals living inside versus outside the Park boundaries (May et al., 2009). Specifically, erythrocyte count and serum cholinesterase were lower in wolves living inside the Park than in those living around Park borders and on surrounding farms. Furthermore, preliminary hormonal analysis of faecal samples revealed that wolves living on surrounding farmlands excreted higher corticoid concentrations in faeces compared with wolves living within the Park (K. Spercoski, unpubl. data). Based on these findings, it was suggested that interaction with humans and domestic species, and a lack of sufficient resources resulting from habitat alteration, may contribute to increased levels of stress experienced by wolves living in disturbed areas.

North-eastern Argentina

It is estimated that c.1000 Maned wolves inhabit the grasslands (Chaco) of northeastern Argentina, although the population is thought to be in decline (Soler & Carenton, 2008). As in other areas of the range of the species, conflicts with people (e.g. habitat

loss), road mortality, the potential for infectious diseases spread by Domestic dogs and persecution resulting from widely held superstitions and beliefs, are the primary threats facing Maned wolves in Argentina. Field studies have focused on determining population distribution and density, and habitat use in relation to the other carnivores in the region. Initial results indicate that Maned wolves show a preference for open grassland habitats and are also utilizing perturbed areas; for example, cattle ranches (Soler & Carenton, 2008). Direct competition with other carnivores has not been observed; however, scat analysis reveals that Maned wolves, Crab-eating fox Cerdocyon thous and Pampas fox Lycalopex gymnocercus (Pseudalopex gymnocercus) consume many of the same prey species (Soler & Carenton, 2008).

Educating the local people about Maned wolves and other endemic carnivores and involving them directly in the field project is a significant component of current conservation efforts in Argentina (Soler, 2008). Extensive surveys of farmers and ranchers have provided valuable information about the distribution of the species and also about people's attitudes towards Maned wolves and other carnivores. Education programmes aimed at changing negative perceptions of carnivores and increasing appreciation of the unique role carnivores play in maintaining biodiversity have been developed and presented to school children throughout the region, utilizing dramatizations, puppet shows, presentations and other educational tools (Soler, 2008). Support from the MWSSP enabled the project coordinators to print and distribute posters describing the fallacy of some of the negative local folklore surrounding the Maned wolf.

Genetic diversity

The MWSSP supplied partial funding for a collaborative pilot study aimed at providing insight into the evolutionary history of this species in South America in order to understand better the amount and patterns of gene flow and diversity between populations in

different areas of the geographic range. Mitochondrial DNA (mtDNA) analysis of both control region (D-loop) and cytochrome b sequences was used to characterize the genetic variability of 89 samples obtained in Bolivia and Argentina in addition to samples provided by captive animals of known Brazilian origin (Franco Berriel, 2005). The samples were primarily faeces but also included blood, hair and bone. Overall, gene diversity was quite low, as only seven different haplotypes (haplotypes A-G) were found in the rapidly-evolving D-loop region and two in the more slowly evolving cytochrome b region (Franco Berriel, 2005). In the control region, only one (haplotype B) was found in samples from all three geographic areas (Argentina, Bolivia and Brazil) (Franco Berriel, 2005). The most common haplotype (D) was found in Bolivian and Brazilian samples, but not in Argentina, and the remaining five were each unique to one of the three geographic areas, although all were found in low proportions. The mtDNA analysis combined with subsequent microsatellite analysis of a subset of samples indicates that the Argentinean population is significantly differentiated from the Bolivian animals and is more closely associated with the Brazilian population (Franco Berriel, 2005). The small sample size precludes any definitive conclusions but, generally, the results indicate that population size has fluctuated over history, possibly as a result of climate change affecting the grassland habitats and more recently from conversion of grasslands for agricultural use (Franco Berriel, 2005). Additional samples collected from more widely distributed areas of the species' range are needed to further elucidate genetic diversity and the degree of phylogenetic partitioning that exists in the remaining wild populations.

Population and habitat viability assessment workshop

The first Maned Wolf Population and Habitat Viability Assessment workshop was held in October 2005 at the PNSC, Brazil. The workshop was organized by CENAP/ICM-Bio and

funded by the MWSSP, Busch Garden Conservation Fund, US Fish and Wildlife Service, Wild Dog Foundation, ICM-Bio and the United National Development Program. There were 45 participants from Argentina (7), Brazil (30), Paraguay (3), Uruguay (1) and the United States (4), with six facilitators from the International Union for Conservation of Nature (IUCN) Species Survival Commission (SSC) Conservation Breeding Specialist Group. The goal of this workshop was to bring together wildlife biologists, educators, captive-breeding specialists, governmental agencies and wildlife nongovernmental organizations from range countries systematically to gather information regarding Maned wolf ecology, reproduction, social organization, health and possible threats.

During the 3 day workshop, issues including (1) threats and habitat management, (2) species status and distribution, (3) sociology and environmental education, and (4) *ex situ* conservation were discussed. Estimations of the Maned wolf population and an assessment of extinction risk for each range country were also conducted using vortex simulation models (Lacy, 2000). It should be noted that data were not available for the populations in Bolivia and Uruguay; thus simulation modelling was not performed for these regions.

It was estimated that there are ten populations of Maned wolves (600–800 individuals) remaining in Argentina. The main threat to Argentinean Maned wolf populations is persecution associated with harvesting of body parts and human conflict. Therefore, education campaigns, in collaboration with governmental agencies, aimed at reducing persecution rate, are considered the highest priority in this region. Among the ten populations, only one population in the Formosa region of north-east Argentina was identified as potentially viable with a low probability of extinction over the next 100 years; this population was designated as the highest conservation priority.

For Brazil, it was estimated that there are nine populations totalling $c.20\,000$ wolves in this country. The main threats to Maned

wolves in Brazil include habitat loss and mortality associated with road kill and human conflict. Because of the large number of Maned wolves in this country, there is low probability of extinction over the next 100 years, although the persistence in population threats may lead to local extinction in some regions. Therefore, conservation priorities in Brazil should include preservation and restoration of suitable habitat and reduction of human—Maned wolf conflict.

Eight hundred Maned wolves are estimated to exist in four suitable habitats in Paraguay. Very little is known about the status and distribution of Maned wolves in this country, and further research is required to estimate the population size accurately. The main threats to Maned wolves in Paraguay are mortality caused by road kill and persecution.

Regarding *ex situ* conservation, poor reproduction in captivity is the main concern in the United States, Argentina and Paraguay, while surplus animals and overcrowding resulting from large influxes of confiscated individuals are the most important issues in Brazil. Thus, conservation priorities for zoo populations include research addressing poor reproductive success in captivity and working with Brazilian agencies to (1) provide facilities to accommodate confiscated species and (2) control the number of wild wolves entering zoos by increasing efforts to reduce human—wolf conflict (see also Vanstreels & Pessutti, 2010).

SUMMARY

During the past two centuries, zoological parks have evolved from basic menageries into conservation organizations and resources for environmental educators (Kisling, 2000). Today, modern zoos play an increasingly important role in protecting and conserving global biodiversity through participation in cooperatively managed regional breeding programmes for threatened species, such as SSPs and EEPs, as well as supporting or conducting studies aimed at protecting wild populations.

The MWSSP is one of 113 SSP programmes established by the AZA since 1980. During the past two decades, the focus of the MWSSP has expanded from solely engaging in ex situ conservation (breeding and conducting scientific studies to improve health and well-being of captive individuals) to integrating in situ conservation and education projects into the Action Plan. Through the support of the MWSSP, scientists both in the United States and range countries have generated important information contributing to our understanding of the biology of Maned wolves (including ecology, diet, behaviour, reproductive physiology, susceptibility to diseases) and how this species responds to environmental changes related to human encroachment into the grassland habitats. However, several challenges remain, including (1) formulating a suitable diet for Maned wolves maintained in North American zoos, (2) improving the reproductive fitness of captive individuals, (3) evaluating the significance of disease threats to wild populations and (4) promoting and supporting conservation efforts in range countries to preserve habitat and reduce human-wolf conflict. The MWSSP is committed to the preservation of this unique canid and the critically threatened grasslands of South America, and will continue to collaborate with scientists and wildlife educators to ensure the existence of the species long into the future.

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