

BULL-SWITCHING IN AFRICAN BOVID HERDS: ASSESSING BEST PRACTICES
FOR BREEDING MANAGEMENT IN WATERBUCK

A Thesis

by

RENEÉ CRYSTAL MICHELLE JONES

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Aug 2010

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

Chair of Committee,	Jane Packard
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ABSTRACT

Bull-switching in African Bovid Herds: Assessing Best Practices for Breeding
Management in Waterbuck.

(Aug 2010)

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Chair of Advisory Committee: Dr. Jane M. Packard

To implement sustainable ex-situ management of big ungulate herds such as African Waterbuck (*Kobus ellipsiprymnus*), one strategy is to place a vasectomized male with females during the sub-optimal season for breeding and subsequently replace him with an intact male during the optimal breeding season. However, information is needed on the effects of vasectomy and the long-term effects on social well-being of individuals used in this "bull-switching" treatment, which is designed to enhance well-being of the whole herd.

In this study, behavioral observations were conducted in three periods (pre-treatment, treatment, post-treatment) 2-months in length. Focal individual observations were used to systematically record (a) continuous samples on video (6-min duration; n = 595), (b) instantaneous samples of proximity (n = 951), and (c) field notes of all-occurrences of social interaction (courting and antagonistic).

The quantitative and qualitative analyses revealed a significant effect of treatment on three (courtship, escalation, and proximity) out of four measures (de-

escalation was not affected). Courtship and escalation behaviors increased significantly during the treatment ($G^2 = 46.35$; $df=1$, $P < 0.001$; $z=6.60$). The treatment was associated with a significant change in proximity for females ($G^2 = 17.21$; $df=1$; $P < 0.001$; $z=2.31$) and other males ($G^2 = 16.10$; $df=1$; $P < 0.001$; $z=-3.57$).

Overall, (1) there was no substantial change with social well-being of the vasectomized male before removal and after reintroduction; (2) the male proximity did not fluctuate significantly with the juvenile males, calves, and other species; and (3) the vasectomized male and the intact male exhibited similar social well-being characteristics. Considering current environmental changes, the treatment did not result in a decline of the social well-being of females and calves, but it did for non-breeding males. Courtship declined in post-treatment, but this was due to the increased percent of pregnant females. During post-treatment, a change in ratio of green grass and supplementary feed pellets possibly affected de-escalation in the context of interspecies interactions. The change of the proximity of the herd was associated with increased heat and use of shaded areas in the pasture. The treatment resulted in a change in relationship with the satellite male and juvenile males in the herd.

DEDICATION

This is dedicated to my Jones and McAdoo family. They are my rock and support system that I can count on each and every day. My dad Gary always said “the more you learn, the more you earn”. I treasure that quote as I studied along with encouraging words from my sister Le Teshia, my mother Linda, and Aunt Brenda. My work is for them; I want to show them what I am capable of and have them be proud of my accomplishments. And finally, I would like to also dedicate this to Toby, one of the African waterbuck at Fossil Rim Wildlife Center.

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1. INTRODUCTION

The majority of the captive exotic ungulate species have been non-seasonal breeders; issues arose when calves were born year-round in climates different from their origins. Most ungulates experience a decrease in body condition during harsh winters [Garroway and Broders, 2005; Weladji and Holand, 2003; Loison, et al., 1999]. One issue relates to lactating females that struggle to maintain their body condition throughout winter in order to meet their late summer and autumn newborn calves' needs. If lactating females declined in body condition due to winter conditions (i.e., decreased food resource, intense cold nights, decreased nutritional value of available grasses, etc.), both females and the calves would have been negatively impacted. If a female with poor body condition had a newborn calf, the calf would have been unlikely to survive. Furthermore, weaning calves were at a high risk during critical development stages when attempting to gain as much nutrition as possible; cold conditions were unforgiving and resulted in calf mortality.

Fossil Rim Wildlife Center is one of the first institutions to implement a non-domestic hoofstock male management plan to address the issues of the non-seasonal breeders in the ex-situ environment. The general goal of Fossil Rim is to establish and maintain reservoir populations of ungulates suitable for restocking wild populations during the next 100 years [Adam Eyres, personal communication, 2007]. The specific objectives of the management plan are to produce individuals

This thesis follows the style of *Zoo Biology*.

that meet the industry standards (benchmarks) for genetic viability, herd health, socially adept behavior, and ecosystem health. In the past, in intensive management systems, such as zoos, males can be separated from females to control breeding. Breeding of a species was managed with procedures such as contraceptives for common chimpanzees (*Pan troglodytes*) [Gould and Johnson-Ward, 2000], removal or separation of breeding pairs of Eastern barred bandicoot (*Perameles gunni*) [Krake and Halley, 1993], and artificial insemination of Asian elephants (*Elephas maximus*) [Brown et al., 2004]. “Bull-switching” practice is now under investigation for non-domestic bovids. “Bull-switching” has been employed with domestic cattle, in which a vasectomized bull is put in the pasture with females. Then he is removed and replaced by the intact male during the optimal breeding time.

Knowing that the African waterbuck (*Kobus ellipsiprymnus*), have an eight-month gestation period, [Spinage 1982], the veterinarians and managers at Fossil Rim projected the months of May and June as optimal time for breeding such that calf births would be synchronized in February and March. The early spring birthing period would help to solve the issues related to non-seasonal breeding. Many seasonal breeding ungulates have adapted to early spring as the birthing period because it is the time when protein levels peak in plants [Mysterud et al., 2002]. The non-domestic hoofstock male management helps to ensure the aseasonal waterbuck take advantage of the peak “green seasons” in a temperate environment.

The justification for implementing the “bull-switching” treatment for the waterbuck is based upon five factors. (1) One of the goals of Fossil Rim Wildlife Center and the Associations of Zoos and Aquariums (AZA) is maintaining a genetically diverse herd by avoiding inbreeding. For example, the territorial male had successfully bred and as the herd grew and individuals matured, he would be mating with his daughters. (2) Waterbuck originated in the tropical zones of East Africa and spread into the semi-arid regions of Southern Africa; due to their adaptability to a wide range of physical and social conditions [Spinage 1982], waterbuck are non-seasonal breeders in Texas terrains. There had been a decline in calf survival. Therefore, waterbuck served as an appropriate candidate for implementing a management plan to address non-seasonal breeding issues. (3) Old age of an ungulate is one reason for a low reproductive performance [Vanpé et al., 2009]. At Fossil Rim, the territorial male was over thirteen years old and in the wild would not be likely to reproduce past ten years. However, his experiences in the pasture with conspecifics and the other species would allow him to be a learning tool, and overall asset to developing calves while maintaining social interactions with the females. (4) The waterbuck have been the smallest herd in the main pasture at Fossil Rim. To be a viable resource for restocking wild populations, a larger herd would be necessary. Even though the conservation status of waterbuck is listed as least concern, poaching continues to be a problem [IUCN Red list, 2009]. (5) At Fossil Rim, the waterbuck are not attracted to feed from

visitors compared to the other species in the same semi-natural environment. This minimal effect of tourists on the waterbuck made observations more valid.

There is a lack of extensive well-being assessments of African waterbuck, especially for a non-domestic hoofstock male management in semi-natural environments. Furthermore, there is an information gap concerning how the “bull-switching” treatment could affect the social well-being of exotic ungulates in general, in a semi-natural environment. Developing tools and techniques to assess a modern male management treatment requires choosing to frame the research based on either welfare or well-being. Animal well-being is used to describe the current state of the animal [Swanson, 1995] (details on why well-being is used rather than welfare are stated in APPENDIX A). Therefore, the overall goal of the research project reported in this thesis will be analysis of the effects of a male management treatment on behavior and the well-being of individuals in a waterbuck herd in a semi-natural environment.

The research question is: “how to determine the effects of ‘bull-switching’ treatment on the ‘normal’ behaviors of the male?” What is considered “normal”? The expression of what is “normal” territorial behavior in male waterbucks in semi-natural environments remains undefined in modern scientific literature. That missing link is important because if the study were supposed to assess the behaviors of a territorial male waterbuck, one would need to know and understand the behaviors likely to occur in territorial males in general. In addition, because the definition of “normal” is based on information from studies of the waterbuck in their natural environments [Spinage, 1982; Walther 1984], the results from captive studies may be difficult to compare to the wild.

Establishing “normal” behaviors for diverse age and sex categories of waterbuck is essential for behavioral monitoring of well-being. Watters et al. [2009] suggest establishing a sound behavioral monitoring protocol as a key to understanding baseline behaviors for individuals. These “normal” (baseline) behaviors can be used as a baseline (control) upon which to measure changes in individual behavior due to treatment such as “bull-switching”. Information about territorial waterbuck males can play a substantial role “down the road” in defining best practices for managing species’ welfare in captivity. Furthermore, identifying behaviors and any changes associated with them can be utilized by management to adjust husbandry techniques and to implement veterinary care if necessary.

For the purpose of the present study “normal” behaviors are defined by a collection of expressive social behaviors described by past research. In African waterbuck, “normal” behaviors are not the same in all age/sex classes [Walther, 1984]. There are variations between individuals as well as changes in social and physical environments [Spinage, 1982; Wirtz and Oldekop, 1991]. In a pilot study, I outlined a list of behaviors and categorized them as courtship, escalation, and de-escalation [Jones et al., 2009]. For example, Walther [1984] has a category of “submissive” behaviors that are similar to my category of “de-escalation”. My reasoning for using the terms “escalate” and “de-escalate” was based on the logic of animal contests as described by Jennings et al. [2005]. By applying knowledge from the wild, assessing social well-being of waterbuck in semi-natural environments is the start.

The focus of this study is the “socially adept” objective of the Fossil Rim non-domestic hoofstock male management plan. Social problems can arise for an institution when implementing a breeding management plan within a semi-natural environment. Three issues are male aggression, inbreeding, and socialization of maturing males. These maturing males can be vulnerable to territorial males. Objectives were set to address and analyze these issues. The objectives of the study were to determine the effects of the bull-switching treatment on: (1) frequency and intensity of social behaviors based on courtship, de-escalating, and escalating behaviors; (2) the proximity of the territorial male to all age and sex categories within the herd and other ungulate species in the same pasture; and (3) well-being of the males and the herd. The study will provide the benchmarks for assessing social well-being of individuals that vary in age/sex/reproductive conditions. Continuing to practice the best technique for assessing well-being can lead to “formal data collection as a tool to monitor behavior” [Watters et al., 2009] that can become the norm at different institutions ranging from zoos to semi-natural environments.

2. METHODS AND MATERIALS

2.1 Subjects, Management, and Housing

Fossil Rim Wildlife Center in Glen Rose, Texas (32°10'03"N and 97°48'27"W) is known for its propagation and innovative management techniques for exotic species endangered around the world [Association of Zoos and Aquariums, 2008]. Fossil Rim's sustainable management plan is the foundation for implementing new strategies for the betterment of threatened and endangered species living at the wildlife center. Due to the experienced men and women with conservation on their minds, the breeding facilities have been an intricate part for preserving species. For years the institution has successfully bred such animals as cheetahs (*Acinonyx jubatus*), white rhinos (*Ceratotherium simum*), and Attwater's Prairie chickens (*Tympanuchus cupido attwateri*). To continue the trend of conservation by branching out to other endangered species, managers developed a non-domestic hoofstock male management plan for several species at Fossil Rim, including Addax (*Addax nasomaculatus*), African Waterbuck, and Sable (*Hippotragus niger*).

Fossil Rim Wildlife Center is approximately 1700 acres in the Central Texas Hill Country. Fossil Rim encompasses three Texas Ecoregions, i.e. Limestone Cut Plains, Western Cross Timber, and the Grand Prairie [Griffith and Omernik, 2009]. The 417-acre pasture (APPENDIX B) housing the waterbuck has six ponds and a stream as well as grass plain patches throughout the woodlands with various shelters to protect animals from inclement weather conditions. The main pasture includes waterbuck, addax, axis deer (*Axis axis*), white-tailed deer (*Odocoileus virginianus*), fallow deer (*Dama dama*),

gemsbok (*Oryx gazella*), and sable. All species have access to daily supplementary feed pellets from troughs and visitors. The pellets and troughs serve as an opportunity for managers to routinely monitor individuals' health status, reduce stress on the pasture, and maintain visibility for the visitors.

Two types of waterbuck mating systems have been observed and studied, low- and high-density populations. Their native habitat consists of high protein marshes along the eastern coast of Africa to south of the Sahara Desert [Estes, 1991; Spinage, 1982]. In low-density populations, waterbuck are polygamous with single territorial male mating with females as opportunities arise [Spinage, 1982]. Females are less likely associated in groups due to large range and access to widely distributed resources. In high-density populations [Wirtz and Oldekop, 1991], females wander in larger groups towards clumped food resources. Females move in and out of multiple male territories and each male has limited opportunity for defending female groups. In high-density populations or semi-natural environments, each territorial male may tolerate another male in his territory, referred to as the 'satellite male' [Wirtz, 1982]. The tolerance by the territorial male is a benefit for the satellite male because he has the opportunity to become the territorial male. He can acquire the territory when the owner dies or disperses [Wirtz, 1981; Wirtz 1982]. In dense semi-natural conditions, each male is more likely to defend food and water resources than females that pass through to access those resources [Mungall and Sheffield, 1994].

Literature reviews and a pilot case study conducted at Fossil Rim were the key components in developing definitions for normal social behaviors. The two categories of

normal social behaviors for a territorial male waterbuck include courtship and antagonistic with the latter having two subcategories: de-escalation and escalation (Table 1). Each behavior should occur within in normal range for the species derived from previous waterbuck studies. Based on the social and physical environment cues, the territorial male is predicted to respond with appropriate behaviors given the situation.

TABLE 1. Categories of social behaviors. Codes for behaviors in courtship and antagonistic social actions^a.

Category of behaviors	Code	Description of behaviors (events) in each category (state)
Courtship		
Low intensity	C1	Neck-stretch, foreleg-lift, nostril-lick, nose-lift (horn-toss), erect-posture, flehmen, follow-female, sniff-rear, sniff-inguinal (groin), nuzzle-female, nudge-female, horn-tap-female, head-rub-female
High intensity	C2	Chest-push (bump), partial-mount, penile-erection, mount, intromission, pelvic-thrust, ejaculatory-jump, dismount
Antagonistic		
De-escalating		
Low intensity	D1	Look-away, head-low (chin-out) posture, walk-away
High intensity	D2	Turn-tail, tooth-chomp (symbolic biting), escape
Escalating		
Low intensity	E1	Horns-high (display), erect-posture, horn-thrash, supplant
High intensity	E2	Lunge (rush threat), front-press, horn-contact, chase, bellow
All other behaviors	O	

^aBehavioral definitions are adapted from previous waterbuck studies [Estes, 1991; Spinage, 1982; Tomlinson, 1980; Walther, 1984].

Different management techniques and phases shaped the age/structure of the waterbuck herd observed at Fossil Rim Wildlife Center for this study (APPENDIX C). The founders of Fossil Rim provided one adult male to four adult females in 1984. From then until 1997, the waterbuck were allowed to breed freely. At puberty (2-3 years), all the males were removed from the herd. Several females were removed as well. The current vasectomized male (M530) was born towards the end of this phase in 1995. He was removed from the herd and placed in another pasture on the property. His half-brother, born in 1997, was hand-raised, castrated, and released back into the main pasture during the last year with his father, who died the same year at 13 years of age. By the end of this phase, there were approximately 6 males (juvenile non-breeding males) to 22 females. From 1997- 2004, there was not a breeding male and the herd remained stable without any new calves since 1997. In 2004, M530 was reintroduced to the females and showed interactions with his castrated half-brother similar to what has been described between territorial and satellite males in the wild. Due to the successful breeding from 2004-2007, M530 was vasectomized at age 13 to prevent inbreeding. Henceforth, M530 will be referred to as the vasectomized territorial male. The “bull-switching” treatment was initiated in January 2008 [Jones et al., 2009]. The new male for breeding arrived at Fossil Rim during the spring of 2008. He was 2-3 years old and not related to any individuals in the herd. Henceforth, the new male will be referred to as the intact territorial male.

2.2 Data Collection

The research design had three components, pre-treatment, treatment, and post-treatment. Focal-animal observation was conducted on the territorial male during each component. All-occurrence sampling was combined with focal-animal sampling [Lehner, 1996] to record behaviors relating to “normal” territorial male behavior. Qualitative approaches were used to assess “normal” behavior reported from field observations, both at Fossil Rim and past research. The research timeframe was a six-month period from March-August 2009. The pre-treatment was March through April with the vasectomized male in the pasture with the herd. The treatment period began with the switching of the vasectomized male and the intact male in May. May through June was the optimal breeding season with the intact male as the territorial male. The post-treatment was July and August with the vasectomized male back in the pasture. The vasectomized male was placed in a separate holding pen away from the herd and then reintroduced to the herd during the post-treatment. The intact male was in another pasture during the pre-, post-treatments, and remainder of the year.

Each week during the six-month research period, video recording observations were conducted from vehicles during daylight hours in a 24-hour period (APPENDIX D). The waterbuck were usually visible at sunrise until noon and then mid-afternoon until sunset, when waterbuck also were found most active in the wild [Wirtz and Oldekop, 1991; Estes, 1991]. Observers were close enough to record observations without disrupting the natural behaviors of the waterbuck. As suggested by Watters et al. [2009], both instantaneous and continuous recording procedures were followed. As

defined by Lehner, observers systematically searched a road survey transect until the territorial male was sighted [Jones et al., 2009]. Each video record began with the focal male in view and ended either after six-minutes or when he remained out of view for one-minute. All focals (including aborts) were used for qualitative analysis. After focal observation, a six-minute period of all-occurrence sampling of social behaviors was documented in field notes. A new six-minute video record began after a six-minute break. Between video records, opportunistic observations of social behaviors were recorded in qualitative field notes.

To determine the proximity of the male to the conspecifics or other species, an instantaneous sample was recorded every hour. Each individual in proximity to the male was categorized as: male (satellite); juvenile males; females; calves; and other species (APPENDIX E). Then the observer recorded yes/no if any individual was less than two meters from the territorial male (close proximity), between two to four meters (close proximity), or greater than four meters (not close).

2.3 Data Analysis

Social behavior bar graphs of the vasectomized male and the intact male were compared as well as the proximities of the vasectomized male for pre- and post-treatment. The log-likelihood ratio (G2) with a decision rule of $p=0.05$ [Lehner, 1996] was the statistical test for the hypothesis that given the treatment, there was a difference in the likelihood of close proximity for each age/sex/species category [Jones et al., 2009]. The matrices had two rows (close and not close) for proximity and three columns

(pre-treatment, treatment, and post-treatment). Furthermore, for each cell in each matrix, the binomial z-score [Lehner, 1996] was calculated (APPENDIX E).

The video clips were analyzed by using an instantaneous recording rule (APPENDIX F). Intervals between instances were 15 seconds. The behavior categories are described in Table 1. Criteria for inter-observer reliability was 85%, to ensure the accuracy of point sampling. Observer-reliability was measured using two indices: (1) index of concordance and (2) Kappa's coefficient [Martin and Bateson, 1993]. Three clips from each period were chosen containing social behaviors.

The log likelihood ratio (G^2) and the binomial z-scores were used to determine if there was a difference in the likelihood of social behaviors in all categories and intensity levels. To obtain information about the first objective, (frequency and intensity of social behavior displayed by the territorial males) three tests were conducted pertaining to the frequency of the social behaviors for each of the treatment periods. Each test evaluated the total number of one of the three observed social behavioral categories to what was expected based on the total number of social behaviors. Finally, these quantitative data were interpreted in the context of the qualitative data to determine whether the social well-being of the vasectomized male differed between pre- and post-treatment. In addition to quantitative analysis for assessing social behaviors, qualitative analysis will be included based on behavioral observations and literature reviews.

3. RESULTS

3.1 Frequency and Intensity of Social Behaviors

The treatment significantly affected courtship ($G^2=46.35;df=5; P=0.001$) and escalation ($G^2=22.33;df=5; P=0.001$), but not de-escalation ($G^2=5.06;df=5; P=0.079$). During the treatment, the intact male escalated more ($z=4.64$) and courted females more ($z=6.60$) than expected by chance (Appendix G). During the post-treatment, there were fewer courtship behaviors than expected by chance ($z=-1.99$). In the pre-treatment, the vasectomized male displayed fewer escalations ($z=-3.34$) and courtship than expected by chance ($z=-4.61$). Both territorial males showed courtship, de-escalation, and escalation (Fig.3). However, de-escalation was more frequent in the vasectomized male.

Courtship behaviors occurred 4% of the total number of observed behaviors. The majority of the courtship behavior was low intensity. High intensity behaviors only occurred in relation to erections, chasing, mounting, and dismounting. Although 45% of all low-intensity courtship was documented during the treatment, 55% of high-intensity courtship was documented during the post-treatment.

Escalating behaviors occurred 4% of the total number of observed behaviors. Fifty percent of all low-intensity escalation occurred during the treatment. The majority of escalation observed was low intensity. The intact male was observed horn thrashing at the ground around females and other species. Some of high intensity escalation behavior by the intact male was on days, or within one day, of high intensity courtship behavior. The field notes describe an example of a rare incident of high intensity escalation:

A juvenile male disappeared after he was chased by the intact male. The intact male was courting a female. Both low and high intensity courtship was observed. The particular female he was courting was the mother of the only calf in the pasture and both juvenile males were previously observed in close proximity to her for several months. As the female approached her calf, with the calf in close proximity to the juvenile males, this initiated a chase scene in the middle of the pasture. At this point in time, the intact male chased the juvenile in full sprint throughout the juniper loop for at least 15 minutes. The juvenile male displayed de-escalating behaviors the entire time. The chase continued as they both went out of view in the juniper trees. The juvenile male was never seen again.

Many one-sided encounters were observed as the intact male escalated from threat displays to high intensity escalation behaviors including front-pressing. Most escalation behaviors by the intact male were directed toward the satellite male. The satellite male began displaying de-escalating behaviors such as chomping, low-neck stretch and withdrawal; he moved to the edge of the pasture far away from the intact male. He moved throughout the main pasture as if he knew he was in the intact male's territory. The field notes describe another rare incident of high intensity escalation during the treatment period:

The satellite male was in the pasture by the cabins. The intact male saw him and chased the satellite male into one of the ponds. The intact

male charged him and struck him in the hind quarters. Every attempt by the satellite to leave the pond was interrupted by the intact male. He stood with an erect posture and horn display. This whole bout was about 15 minutes in length. The intact male walked around the perimeter of the pond to return right in view of the satellite male. The intact male left the satellite male and the satellite male remained in the water a while longer. A few days later the satellite male looked emaciated. Later the satellite male was found deceased. The necropsy was inconclusive.

De-escalating behaviors occurred 4% of the total number of recorded instants. High-intensity de-escalation occurred mostly during the post-treatment. All but two incidents were in the context of interspecific interactions at feed troughs. The two incidents of high-intensity de-escalation happened when the intact male was startled by distant stimuli during the first two weeks of treatment.

Collectively, all social behaviors (n=1064) were only 5% of total instants (n=19861) recorded. Courtship was the largest percent of all social behaviors at 80%. De-escalating behavior only occurred 3% of all social behaviors, mostly low-intensity. Escalating behavior occurred 17% of all social behaviors. Within this, high intensity occurred 8% of all escalating behavior.

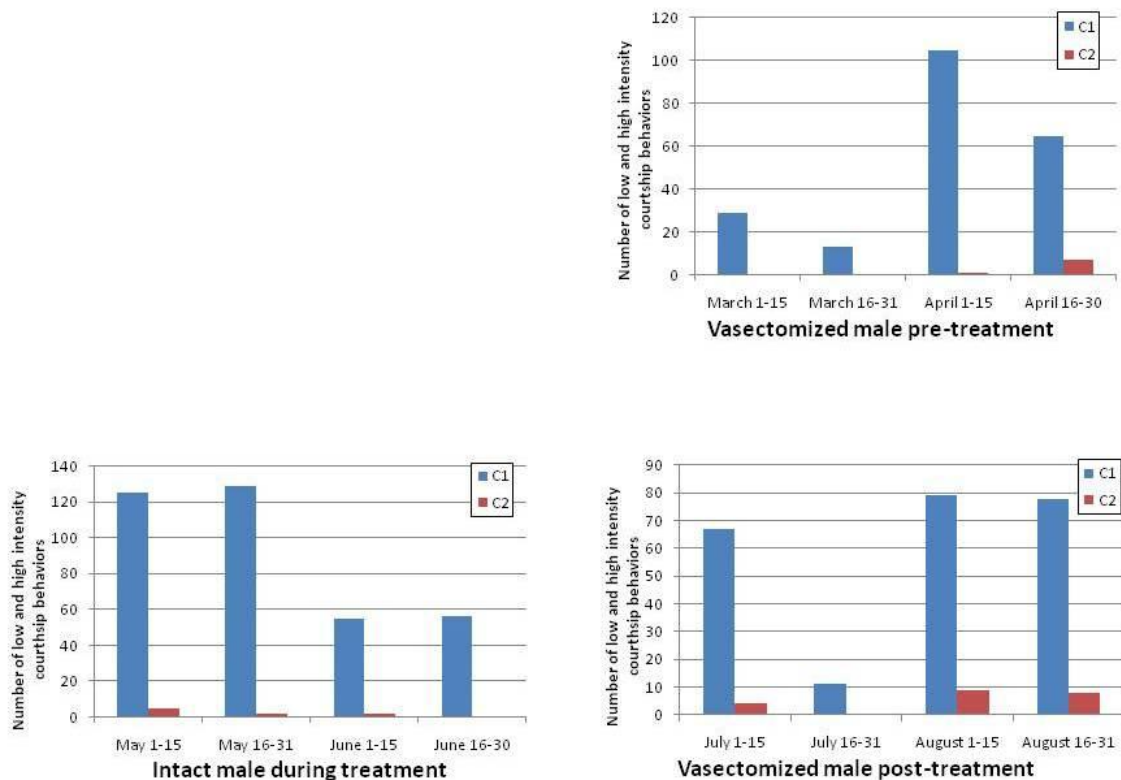


Fig. 1. Courtship behaviors of males. Intensity was coded as low (C1) or high (C2). Vasectomized male courtship behaviors were observed during pre-treatment and post-treatment. Intact male courtship behaviors were observed during the treatment period.

3.2 Evaluating Proximity

Proximity ($n=218$) of the territorial male changed significantly with respect to the satellite male ($G^2=16.10$; $df=2$; $P=0.001$), females ($G^2=17.21$; $df=2$; $P=0.001$), and other species ($G^2=8.16$; $df=2$; $P=0.05$). The proximity of the territorial males to the satellite male changed between the pre-treatment and treatment (Table 2). Throughout the pre-treatment, the satellite male was in close proximity ($z=3.57$) to the vasectomized territorial male in the context of lying down, chewing cud, grazing, and feeding at

troughs. During the treatment, the satellite male was not in close proximity ($z=-3.57$) to the intact territorial male. The females were in close proximity ($z=2.31$) to the intact territorial male and distant ($z=2.51$) from the vasectomized territorial male in the post-treatment. Other species were less than expected by chance in close proximity to the intact male (Table 2). Treatment had no significant effect on the proximity of juvenile males and calves (Table 2).

Proximity between males was related to their previous social relationships. The interactions observed between the vasectomized territorial male and the satellite male were consistent in the pre-treatment period. Neither engaged in high intensity escalation or de-escalation to one another. However, the previous relationship between the satellite male and the intact territorial male was reversed during the treatment period. During the first week of the treatment, the satellite male was in close proximity to the intact territorial male. It had been the same during the previous year [Jones et al., 2010], but conflict between those two males escalated in the second week. Subsequently, the satellite male was out of view and distant from the intact territorial male and food sources. When spotted, he was mostly near the fence as if he attempted to leave the territory.

TABLE 2. Proximity of territorial males to conspecifics and other species.

Social Behaviors Observed	G-squared	Pre-treatment	Treatment	Post-treatment	P-value
To females	17.21				0.001
Close (Within 4 meters)		0.55	2.31*	-3.05*	
Not Close (Beyond 4 meters)		-0.45	-1.90	2.51*	
To juvenile males	0.53				n.s.
Close (Within 4 meters)		0.64	-0.02	-0.58	
Not Close (Beyond 4 meters)		-0.16	0.00	0.15	
To calves	0.53				n.s.
Close (Within 4 meters)		0.31	-0.12	-0.17	
Not Close (Beyond 4 meters)		-0.05	0.02	0.03	
To other species	8.16				0.05
Close (Within 4 meters)		1.46	-2.38*	0.91	
Not Close (Beyond 4 meters)		-0.80	1.31	-0.50	
To satellite male ^a	16.10				0.001
Close (Within 4 meters)		3.57*	-3.57*	-	
Not Close (Beyond 4 meters)		-1.24	1.24	-	

^aSatellite male found dead before post-treatment

*Binomial test z-scores are different than expected by chance per contingency tests.

3.3 Assessment of Well-being

Escalation and courtship behaviors both gave insight on proximity measures. As the vasectomized male interacted with other species at feed troughs, it reduced the opportunity for close proximity to all conspecifics. The escalation behaviors observed by the intact male reduced opportunity for close proximity to males and other species. Both social behaviors and proximity need to be considered for the following assessment of well-being by comparison of 1) the vasectomized territorial male before and after treatment (Fig.2.) and 2) the vasectomized territorial male and the intact territorial male (Fig.3.).

The social behaviors were related to the proximity measures and vice versa. For example, the interactions of the satellite male and the vasectomized territorial male did not escalate and they remained in close proximity. In contrast, the intact territorial male escalated conflict and the satellite male kept his distance. When comparing the pre- and post-treatments, the vasectomized male's social behaviors increased significantly ($G^2=5.94$; $df=1$; $P=0.05$). Even though there were more social interactions during the post-treatment (Fig.2.), the vasectomized male's proximity to the females was less than expected (Table 2). The qualitative analysis on the video clips indicated social interactions increased in the context of the feed troughs. During the post-treatment period, the females fed on green grass in a different part of the pasture from where the vasectomized territorial male waited for feed pellets to be delivered to the troughs.

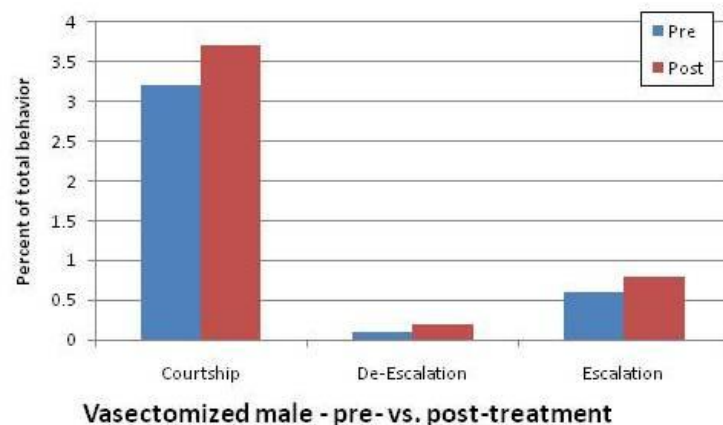


Fig. 2. All social behaviors of vasectomized male. Graph compares pre- and post-treatment.

There were similarities and differences when comparing the vasectomized male's and the intact male's social behaviors (Fig.3) and proximities (Table 2). Both males showed a similar probability of courtship behavior. Both males showed high intensity and low intensity courtship, although the frequency fluctuated (Fig.4). Courtship occurred post-treatment even though more females were likely to be pregnant.

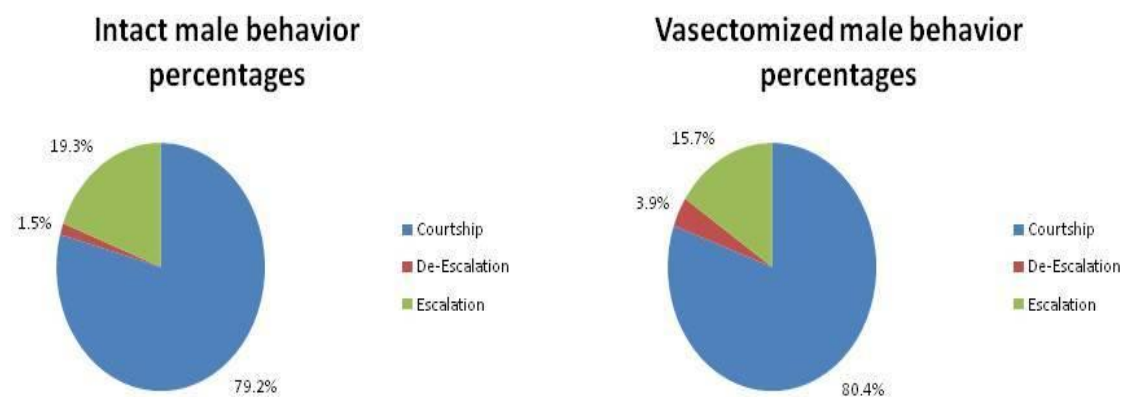


Fig. 3. Comparison of territorial males. Pie diagrams represent behavioral profile of territorial males during treatment (intact male) and before/after treatment (vasectomized male).

The biggest differences between the males were the number of instants of de-escalating behaviors. The older vasectomized territorial male was twice as likely to de-escalate compared to the younger intact territorial male (Fig.3). The younger intact territorial male was more likely to escalate. The qualitative data indicated high intensity escalation occurred on days when females were in standing estrus. "Present-threat" displays and erect postures were observed frequently with his stiff tail held away from the body and neck slightly arched.

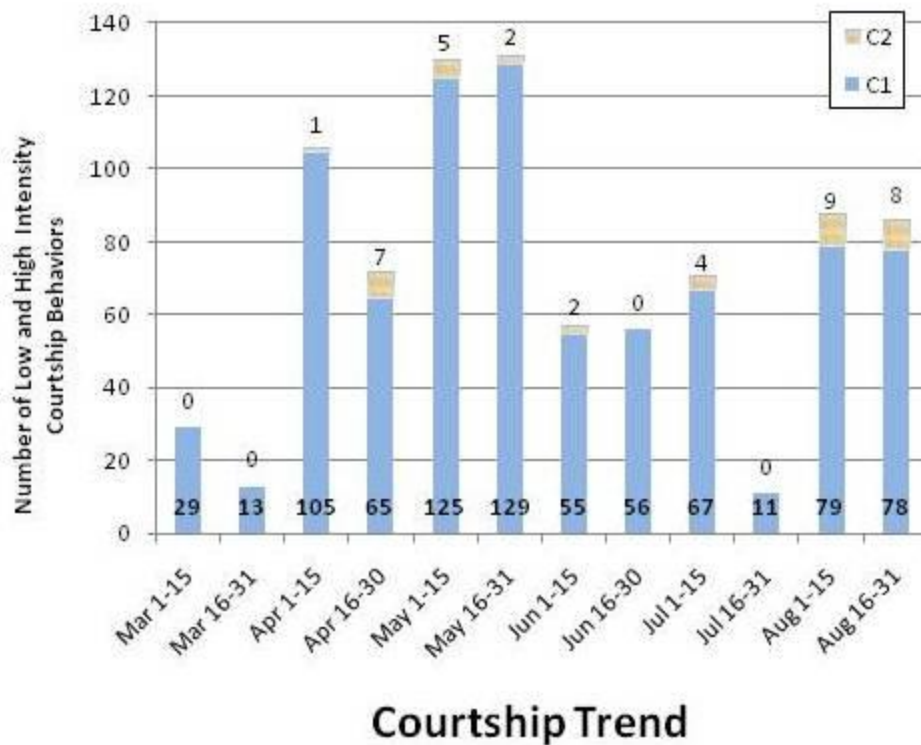


Fig.4. Detail of courtship trends throughout all periods. Codes indicate low intensity (C1) and high intensity (C2). The numbers at the bottom of the bars indicate low intensity and the numbers at the top indicate high intensity.

Overall, changes in the social and physical environments were associated with changes in social behaviors for both territorial males. These changes included the death of the satellite male, disappearance of one juvenile male, possibly more females pregnant in post-treatment and seasonal changes in distribution of food. Data available for interpreting death and disappearance are limited in the extensive pasture management system; this is a problem that needs to be addressed.

4. DISCUSSION

4.1 Social Behaviors

The three social behaviors analyzed occurred in range of “normal” behavior throughout the observation periods. Wirtz [1991] reported the “average waterbuck” at Lake Nakuru National Park, Kenya spent 2.6% of its day engaging in other activities including “sexual” and “antagonistic” behaviors. The majority of the daytime activities were feeding, standing, and lying. The 5% social behaviors recorded at Fossil Rim were similar.

The courtship behaviors by the vasectomized male, which occurred less than expected by chance, had two potential explanations: (1) there were fewer cycling females because of the successful copulations by the intact male, and (2) the hot weather decreased the number of hours for high behavioral activity, meaning more females were in the shade to keep cool.

There was a difference in the courtship in the pre-treatment compared to the treatment periods. For the intact male, the number of recorded courtship behaviors was higher. Possibly, the younger intact male had minimal experience with understanding how close a female is to standing estrus, which may have led to a higher number of courtship bouts. However, this does not mean there was a decline in well-being of the females and calves; only that there were individual differences of normal behaviors and different usage of time and resources. Possibly the older vasectomized male had more experience with the familiar females and courted less. However, surprisingly courtship

was lower before than after treatment. One would have expected less if more females were pregnant after the treatment period compared to before.

The increased escalating behaviors by the intact male positively correlated with the increased number of open (non-pregnant) females in the pasture. This behavior is “normal” and is evident in waterbuck in the wild and other exotic ungulates. Clutton-Brock [1979] reported more antagonistic behaviors between males with the presence of females. Especially during rut season of red deer (*Cervus elaphus*), escalating behaviors were positively correlated with courting behaviors [McElligott et al., 1998]. Spinage [1982] documented several encounters of threat and dominance displays by a territorial male with females in his proximity as another male entered his territory. However, displaying dominant and threatening behaviors does not always occur. The males in Uganda were flexible in their behaviors. They were not always likely to engage [Spinage, 1982] perhaps related to differences in age and experience of the males.

Both males behaved like “territorial bulls” even with individual character differences. Territorial bulls responded as if they knew when to take action with either escalation or de-escalation behaviors. There are three factors influencing de-escalation behaviors. First was the advanced age of the vasectomized male. In general, post-prime males would be more likely to de-escalate. Second, during feeding bouts at the troughs, more de-escalation occurred as if the vasectomized male evaluated the risk of escalating relative to the value of the resources. The vasectomized male reacted with horn presentations to other species when in close proximity before de-escalating. Third, the older vasectomized male may have been affected by the removal and reintroduction

process. The vasectomized male was in holding during the treatment, and his main source for nutrition was the supplementary feed pellets. Even after being reintroduced during the post-treatment, he exploited pellet resources rather than grazing on limited green grasses utilized by the females. Tomlinson [1980] described six types of social dynamics of normal expressive behavior of waterbuck and the normal sequence of events that occur depending on the individuals' status. This helped us to understand specific roles in the Fossil Rim herd. One of the social dynamics is the normal expressive behaviors between the territorial male and satellite male. The role of the satellite male is demonstrated by displaying de-escalating behaviors such as low-neck stretch and running off in the presence of a territorial male. This was normal behavior for the satellite male at Fossil Rim as if he viewed the intact male as the territorial male. However, the territorial intact male acted as if he viewed the satellite male as a neighboring territorial male or challenging bachelor. Tomlinson [1980] explained those roles' within social dynamics as two males engaging in escalating behaviors to defend (or even gain) resources. At Fossil Rim, the territorial intact male escalated repeatedly to the satellite male as if the signals from the satellite male were from one of two social dynamics (neighboring territorial males and territorial and challenging bachelor). The intact male's continued escalation to the satellite male may be related to: (1) the previous summer interactions, (2) lack of male socialization in the intact male's developing years, and (3) elevated testosterone levels during peak female estrus cycles. In general, both males behaved within "normal range" of expected behaviors.

4.2 Proximity

The treatment was associated with a significant change in proximity. The high number of courtship (as if the intact male was gaining information about females' peak estrus levels) could have explained close proximity of females to the intact male. For the post-treatment, females were more distant. The vasectomized male was located more around the feeding troughs. He was fed supplementary feed pellets for the two-month duration. He possibly became accustomed to pellets, and with his decreased body condition, gaining nutrition through pellets was best case scenario. In addition, the number of pregnant females increased and the hot temperatures led to females spending more time in the shade, distant from the feed troughs.

The territorial vasectomized male was more tolerant of the satellite male, which increased the measure of close proximity. The two males were born and raised at Fossil Rim. This tolerant behavior would be expected in an older territorial male [Estes, 1991; Spinage 1982].

The other species were not in close proximity to the intact male during the treatment. The main opportunity for other species to be in close proximity to the territorial male was at the feed troughs. The females did not feed much at the troughs and the intact male stayed in close proximity to the females that limited his interactions with and distance to other species.

Proximity measure results related to all three social behaviors. The qualitative and quantitative results of proximity and social behaviors illustrate that several factors need to be considered to truly understand "normal" behaviors. Previous experiences

between individuals can influence proximity and social behaviors. The relationship between the territorial intact male and the satellite male gives insight on this factor after observing the changes in both males behavior from one year to the next. This leads to social relationships in general in being a factor influencing proximity. For example, the intact male and females in the herd were observed in close proximity while engaging in courtship behaviors. Changes in the distribution of food resources also were tied to changes in proximity. Females and males utilized food resources differently. Females in their native habitat tend to travel to available green grasses, where males tend to remain in their territories longer [Wirtz and Oldekop, 1991].

4.3 Assessment of Well-being in the Herd

The waterbuck adjusted to the implementation of the male management breeding plan. The waterbuck behavior changed within the “normal” range expected for this species, given the associated environmental changes. Overall, (1) there was no substantial change with social well-being of the vasectomized male before removal and after reintroduction; (2) the male proximity did not fluctuate significantly with the females, calves, and other species; and (3) the vasectomized male and the intact male exhibited similar social well-being characteristics.

Even though there were no problems with any of the territorial males attacking calves, there was a decline in the well-being of the satellite and one juvenile male during the treatment. Both males displayed territorial behaviors in the presence of the satellite male. The erect posture and head-shaking escalating behaviors [Estes, 1991] caused de-escalating behavior reactions from juvenile males and calves. Even a low-intensity

escalating such as a head toss while lying down had calves respond with a quick withdrawal.

4.4 Complexity of Defining “Normal” Behavior

Throughout the study, male social behavior was “normal” based on literature [Estes 1991; Spinage, 1982; Walther, 1984; Wirtz, 1991] and previous observations where similarities were documented from time budgets of daytime activities to patterns in courtship behaviors. There was variation in individual behavior. The individual variation is linked to animals that have less experience within their environments and it can potentially reduce productivity [Seaman et al., 2002]. With slight differences due to individual personalities and socialization, both males’ social well-being did not decline. It is expected to see a difference in normal behaviors between the two males, especially with social and physical environment variations. However, the main point is even though there were differences in behaviors, all behaviors occurred within range of normal territorial male waterbuck.

Both males behaved as “normal” territorial males. One characteristic of “normal” territorial male behavior is displaying a group of specific behaviors. Observations showed the males displayed the five basic antagonistic patterns at the defense of this territory: (1) rush-threat, (2) weapon-threat, (3) present-threat, (4) broadside-present, (5) scar-threat (horn-thrashing on the ground) [Spinage, 1982]. There was a slight variation between the territorial males due to age and social interaction, but overall they behaved “normally”. The majority of the social behaviors were courtship. The large amount of courtship and escalation behavior during the treatment may explain why there was more

social behavior than expected by the territorial male. Season, age, and familiarity of the vasectomized male to the pasture and the inhabitants may be the reason for less social behavior observed during pre-treatment.

Taken as a whole, the “bull-switching” treatment solved the problem of intense aggression between the bulls. Estes [1991] mentioned how fighting can lead to death more common than not. If the bulls were in the pasture at the same time, critical to fatal injuries could have easily occurred. By controlling when each male was in the pasture with the herd, it reduced any encounters that may lead to antagonistic behavior. The treatment inevitably solved the problem of inbreeding. Inbreeding was likely to happen if the territorial male was not vasectomized. Unfortunately, the treatment did not solve the problem of young male socialization. Sub-dominant males in Uganda can learn social behaviors and status by reacting submissively to the territorial male to remain in a group or area [Spinage, 1982]. At Fossil Rim, the vasectomized older male did allow familiar younger males to stay in proximity, although the intact younger male did not. In general, bachelor groups form after breeding males escalate toward younger males [Mysterud et al., 2002]. Male/male socialization can occur between peers in bachelor male groups. Such factors should be considered in addressing the issue of male socialization within the semi-natural environment. In a semi-natural environment it is important to have socially adept males when it is time for managing the breeding season to maximize herd health and continued genetic diversity of this species.

5. CONCLUSION

The behavioral assays were effective at detecting changes in social behavior and proximity, associated with the “bull-switching” treatment. Even though there were behavioral changes, they were within what would be expected as normal, considering the environmental changes that also occurred during the study period. This study brings to the forefront a repeatable method that can describe “normal” behaviors for African waterbuck. In addition, the research method can be a benefit for assessing well-being of individuals before removal and after introduction.

This study illustrates that an objective list of criteria can be codified and measured for a specified well-being intervention, controlling for the variation across individuals, seasons, and species. This is a compilation of best practices for identifying changes in the behavior of individual animals as they adapt to the environment and treatments. The “bull-switching” treatment is an effective management strategy for the waterbuck and might be for other exotic ungulates in semi-natural environments. However, behavioral challenges that remain in need of investigation are how to manage the non-breeding males so they gain social experience with females and calves while they mature, without the risk of escalation from territorial males. Another ongoing challenge is the complexity of defining animal welfare (or in this study well-being) in a way scientists can agree upon [Appleby, 1999].

Managing captive ungulate populations will continue and the need to assess the effects of best practices will be pressing. Designing efficient and effective ways of

monitoring social well-being is essential in these times of economic shortage and fluctuation. Conducting research based on behavioral observation adds an element that can complement physiological or molecular measures.

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APPENDIX A

Defining welfare, well-being and “normal” behavior

The terms ‘welfare’ and ‘well-being’ are often used interchangeably. Gonyou's definitions suggest “animal ‘welfare’ be used as describing long-term implications for the animal’s well-being and that animal ‘well-being’ be used to describe the current state of the animal” Gonyou’s research [Swanson, 1995, p. 2745]. With the management plan and the “bull-switching” treatment focused on two individuals in relation to conspecifics and other species, assessing the welfare of the vasectomized and intact male and using a welfare assessment method as the foundation for all male waterbucks is not feasible. Therefore, the research project will be based on describing the well-being of two territorial male waterbucks in a semi-natural environment over a six month period. Over time with multiple well-being behavioral assessments and analyses, achieving a statement of what is the welfare of waterbuck can be obtained. Being able to assess well-being and understand behaviors in an efficient and practical matter is a considerable asset to animal caretakers and managers [Seaman, Davidson, & Waran, 2002].

The research question is: “how to determine the effects of ‘bull-switching’ treatment on the ‘normal’ behaviors of the male?” What is considered “normal”? The expression of what is “normal” territorial behavior in male waterbucks in semi-natural environments remains undefined by modern literature. That missing link is important because if the study were supposed to assess the behaviors of a territorial male waterbuck, one would need to know and understand the behaviors likely to occur in a territorial male. In addition, because the definition is based on information from the

origins of the waterbuck, the results can be compared to see if the territorial males in captivity or semi-natural environments are similar to the males in the “wild”. The next step is to establish a baseline to help guide the research. For the purpose of the study “normal” behavior is defined by a collaboration (or collection) of expressive social behaviors described by past research. The past research outlined a list of behaviors and categorized them based on every aspect of the social dynamics of the waterbuck. For example, Walther [1984] has a category of submissive behaviors and what actions express those types of behaviors. The literature-reviewed baseline compiled with the vast amount of preliminary observations conducted for the research can be utilized to begin creating a sound description of “normal” territorial male waterbuck social behaviors. This in turn is the start for establishing what social well-being is for the waterbuck.

APPENDIX B

Map and field data sheet

MAIN PASTURE



SPECIES:

DATE:

TIME:

OBSERVER:

ID'S:

BEHAVIORS:

APPENDIX C

Group history of waterbuck herd at Fossil Rim Wildlife Center

Time frame	Type of management	Total in herd	Male	Female	Comments
1984	No restriction breeding	5	1	4	First waterbuck herd at Fossil Rim
1985-1997	No restriction breeding	23	1	22	1 male sire. 36 males born, 31 dispersed and 5 dead. 44 females born, 17 dispersed and 5 dead.
1997-2004	No breeding	24	2	22	1 calf hand-raised and castrated. M530 born in 1997, kept in another pasture. Sire died in 1997.
2004-2007	Controlled breeding	33	2	31	6 males born, 5 sold, 1 euthanized due to injury. 12 females born, 3 dead. 7 calves born (not in total herd count).
2008-2009	Controlled breeding	32	4	29	Castrated and juvenile male found dead, intact male acquired, and juvenile male in another pasture. 2 females dead, 1 dispersed. 5 of 7 calves dead.
Present day as of 09/2009	Controlled breeding	32	3	29	Juvenile male vasectomized and reintroduced to herd with M530 as territorial male. 1 male calf and 1 calf with sex unknown, possible female.

APPENDIX D

1-hour behavior observation protocol

What to bring:

- Data sheets
- Pencil/pen
- Camcorder and extra battery
- Blank SD micro cards
- Watch
- Science In Action magnet for vehicle
- Binoculars

Process:

1. Begin by locating the vasectomized waterbuck. Start in Juniper Loop, which is the large pasture with the quarantine areas and it is the area just before arriving at Safari Camp. If no waterbuck are present, look around Safari Camp first and then back by the Lodge and creek. Please stay on designated roads for safety. (See map for survey route)
2. Once Lucifer is found, do the first proximity recording on data sheet. (see example data sheet) In addition, write the number one on the map, where his (and others) location is.
3. Begin recording 6-minute focal of territorial male no matter what his behavior is. Make sure he is the main focal individual. If others are in close proximity to him, they may be included in the view. Try to maintain a steady hand throughout focal.
4. After the 6 minutes are up. Record comments and focal time on the data sheet. In addition, if a mounting behavior is recorded for example, write down in the comment area the identity tag numbers of the female.
5. Wait another 6 minutes and then begin recording another 6-minute focal.
6. At 30 minutes into observation, record the second proximity on data sheet. Write the number 2 for second proximity location on map.
7. Continue 6-minute focal with a 6 minute break in between.
8. At the end of the hour record the third and final proximity. Write the number three on the map for third proximity location. At the end of one-hour observations, one should have approximately five 6-minute focal on a tape. Repeat one-hour observations from sunrise to sunset.

9. Return and follow instructions on Pre and Post Observation Protocol.

Frequently Asked Questions

1. What if at the end of a focal an interesting behavior is occurring? At the end of the 6-minute focal stop the focal and start another 6-minute focal immediately. Do not wait another 6 minutes.
2. What if I do not see the male? Continue to drive around until he is found. If he is absolutely nowhere to be found. Do a focal on the calves. Then look again for him. Make a note on data sheet if a calf focal is recorded.
3. What if the male goes out of sight during a focal? End the focal after one minute and write abort on data sheet and reasoning. Try to locate him.
4. What happens if the tape runs out? Change the tape if there is less than six minutes left on the tape.

APPENDIX E

Proximity, field data, and analysis sheets

FOSSIL RIM WATERBUCK STUDY- DATA SHEET FOR V-BULL MS30 "LUCIFER"

1-HOUR PROXIMITY Date: Observers:

Time	MS30 visible?	Juv. Males			Females			Calves			Other species							
		<2m	2-4m	>4m	<2m	2-4m	>4m	<2m	2-4m	>4m	<2m	2-4m	>4m					
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N

FOCAL VIDEO TAKEN DURING THIS 1-HOUR PROXIMITY SCAN (6 minutes) TAPE #:

Time Start	Time Stop	Location on map	Comments (behaviors seen during video-taping)

GENERAL COMMENTS (includes comments on context and behaviors not captured on video)

APPENDIX E

Proximity, field data, and analysis sheets continued

This Spreadsheet is set up to calculate CONTINGENCY TESTS for a 2X3 matrix.

Date: 11-11-2009 Observer: Renee Jones Sampling rule: [focal indiv] [focal group] [group scan]
 Site: Fossil Rim Wildlife Center Animal ID's: M530 & Y42 Recording rule: [instant] [interval] [all occurrence] [sequence]
 Video: 2009 Thesis SD videos see Lehner (1996:411-412) Sample duration/interval:

Describe the Category codes:
 Y: YES, N: NO, L: M530, D: Y42, ESCALATING BEHAVIORS
 PRE: PRE-TREATMENT, DUR: DURING TREATMENT, POST: POST TREATMENT

INSTRUCTIONS: (1) In the Data Entry Box, type in the categories for the variables
 (2) Type in your values for each cell in the data entry matrix
 (3) decide on which test, and look for results in the Test Results Box

DATA ENTRY BOX

Categories for T1 (observed values), eg. receiver for T0 (before)

	PRE	DUR	POST
Y	40	91	53
N	6935	6884	6922
Column Totals	6975	6975	6975

Chi-square Goodness of fit test
 Expected values
 eg. $E = MT / ((O-E) * (O-E) / E)$

Row Totals	Expected values
RT1= 184	10462.5 10097.7359
RT2= 20741	10462.5 10097.7359
Matrix Total	column= Chi-square=
MT= 20925	3 20195.472
rows= 2	

TEST RESULTS BOX

Choose a contingency test: decide which of these null hypothesis you are testing; look for results in the corresponding box

- 1) Chi-square goodness of fit (GF-X2): Values of cells in an array (nx1) do not differ from what is expected by chance.
- 2) Chi-square contingency test (X2): Values of cells in a matrix (nxc) do not differ from what is expected by chance.
- 3) Log-likelihood ratio test (G2): The probability of co-occurrence of events tallied in a matrix do not differ from chance.
- 4) Freeman-Tukey deviate (FTD): A particular cell does not contribute to the significance of chi-square.
- 5) Binomial score (z): Probability of co-occurrence of the events tallied in a cell does not differ from chance.

The decision rules for rejecting the null hypothesis for each of these tests are as follows. Table A2 from Conover(1980) p.432

df in descending order used for match function	1) GF-X2 Degrees of freedom: $df=(r-1)*k=1$ critical values are shown in the table below If the GF-X2 is less than the critical value at 0.05, then do NOT reject the null hypothesis Reject with a confidence level of 0.001	2) X2 (at bottom of column on ne: 23.11) Degrees of freedom: $df=(r-1)*(c-1)=k=2$ critical values are shown in the table below If the X2 is less than the critical value at 0.05, then do NOT reject the null hypothesis. Reject the null with a confidence level of 0.001
25	(1-p) = 0.05 0.01 0.001	3) G2 (at bottom of column on ne: 22.33) Degrees of freedom: $df=(r-1)*(c-1)=k=2$ critical values are shown in the table below If the G2 is less than the critical value at 0.05, then do NOT reject the null hypothesis. Reject the null with a confidence level of 0.001
20	df = 1	4) FTD (from next page) for cell (1,1) is -2.967 Do NOT reject the null hypothesis if $ z < 0.95$
16	2	27.88 Use the binomial distribution (Table A3 in Conover 1980). Reject the null hypothesis
15	3	5) z score (from next page) for cell (1,1) is -3.34 Do NOT reject the null hypothesis if $ z < 1.96$ See Bakeman & Gottman 1986 page 134. Reject the null hypothesis
12	4	
10	5	
9	6	
8	8	
8	9	
6	10	
5	12	
4	15	
3	16	
2	20	
1	25	

CALCULATION BOX

This section of the spreadsheet is to calculate the expected values, G, chi-square and z scores for each cell.
 Note that every cell in the matrix is listed below in order in the array in the first column.
 columns E&G are used to avoid problems caused by data cells with value 0, undefined calculations are set to 0

Cell address	Observed O= cell value	Expected E= CT*RT/MT	Log likelihood ratio (scratchpad) O*Ln(O/E)		Chi-square (scratchpad) (O-E)*(O-E)/E		Freeman-Tukey Deviate z= sqrt(O)+sqrt(O+1)-sqrt(z)	Binomial test z= (f(r,c)-f(r)*p(c))/sqrt(f(r)p(c)(1-p(c)))
PREY	40	61.33	-17.10	-17.10	7.4203	7.42	-2.967	Reject
PREN	6935	6913.67	21.37	21.37	0.0658	0.07	0.259	don't reject
DURY	91	61.33	35.90	35.90	14.3496	14.35	3.436	Reject
DURN	6884	6913.67	-29.60	-29.60	0.1273	0.13	-0.354	don't reject
POSTY	53	61.33	-7.74	-7.74	1.1322	1.13	-1.066	Reject
POSTN	6922	6913.67	8.34	8.34	0.0100	0.01	0.103	don't reject
			G-square= 2*SUM		Chi-square=SUM			
			22.33		23.11			

REFERENCES:
 for log-linear analysis, binomial test and Freeman-Tukey deviates:
 Bishop, Y.M.M., S.E. Fienberg, & P.W. Holland. 1975. Discrete Multivariate Analysis. The MIT Press: Cambridge. (pp136-139)
 Gottman, J.M. & A.K. Roy. 1990. Sequential Analysis: a guide for behavioral researchers. Cambridge University Press: Cambridge.

for chi-square and chi-square goodness of fit:
 Conover, W.J. 1980. Practical Nonparametric Statistics. J.Wiley and Sons: New York.

Work space for determination of rejection of null hypothesis for parts 1-3

1) GF-X2	2) X2	3) G2
Index into df table	Index into df table	Index into df table
0.001 critical value	1.00	2
0.01 critical value	10.83	0.001 critical value
0.05 critical value	6.635	0.01 critical value
	3.841	0.05 critical value
		5.991
		5.991

APPENDIX F

Instantaneous point sampling data sheet

INSTANTANEOUS POINT SAMPLE

DATE:	OBSERVER:	TIME START	TIME STOP	ANIMAL ID	INTERVAL	TAPE #
				Lucifer	15 sec.	

Interval #	Focal Start Times									
	Clip Time	Clip Time	Clip Time	Clip Time	Clip Time	Clip Time	Clip Time	Clip Time	Clip Time	Clip Time
:00										
:15										
:30										
:45										
1:00										
1:15										
1:30										
1:45										
2:00										
2:15										
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4:45										
5:00										
5:15										
5:30										
5:45										
6:00										

clip:	clip:		clip:		clip:		clip:		clip:		clip:		clip:		Total	
	1's	2's	1's	2's	1's	2's	1's	2's	1's	2's	1's	2's	1's	2's	1's	2's
C			C		C		C		C		C		C		C	
D			D		D		D		D		D		D		D	
E			E		E		E		E		E		E		E	

CODES		
Courtship	De-escalating	Escalating
C1: Neck stretch, Foreleg kick, Nose lift, Erect posture, Follow female, Sniff rear or inguinal (groin), Nuzzle, nudges, taps or rubs horns at rear of female, Flehmen	D1: Looking away and head-low/chin-out posture.	E1: Display of horns, erect posture, horn thrashing, and supplanting.
C2: Partial mount, Penile erection, Chest push or bump, Mount, Intromission, Pelvic thrusting, Ejaculatory jump, Dismount	D2: Turning tail and symbolic biting.	E2: Front-pressing, chasing and rush-threat display.
0 = None or out of view 1 = Low Intensity 2 = High Intensity	0 = None or out of view 1 = Low Intensity 2 = High Intensity	0 = None or out of view 1 = Low Intensity 2 = High Intensity

APPENDIX G

Supplementary tables

TABLE 3. Observed social behaviors by category throughout treatment.

Social Behaviors Observed	G-squared	Pre-treatment	Treatment	Post-treatment	P-value
Courtship	46.35	-4.61*	6.60*	-1.99*	0.001
De-escalation	5.06	-1.16	-1.16	2.32*	0.079
Escalation	22.33	-3.34*	4.64*	-1.30	0.001

*Binomial test z-scores were different than expected by chance per contingency tests.

TABLE 4. Observed social behaviors combined throughout treatment.

Social Behaviors Observed	G-squared	Pre-treatment	Treatment	Post-treatment	P-value
All social behaviors	64.78	-5.70*	7.63*	-1.93	0.001
All other behaviors	64.78	1.32	-1.77	0.45	0.001

*Binomial test z-scores were different than expected by chance per contingency tests.

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