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# Factors That Affect Harem Stability in a Feral Horse (*Equus caballus*) Population on Shackleford Banks island, NC

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Factors That Affect Harem Stability in a Feral Horse (*Equus caballus*) Population on  
Shackleford Banks island, NC

A Dissertation

Submitted to the Graduate Faculty of the  
University of New Orleans  
in partial fulfillment of the  
requirements for the degree of

Doctor of Philosophy  
in  
Conservation Biology

by

Jessa Madosky  
B.S. Beloit College, 2005

August, 2011

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This dissertation is dedicated to my family, my fiancé,  
and all my friends that helped keep me sane through the process – both two and four legged.

I would like to acknowledge my advisor, Dr. Jerome Howard, for all of his help in this process, all of my committee members for their help and guidance, especially Dr. Dan Rubenstein, and Dr. Sue Stuska of the National Park Service for her endless help and support in and out of the field. I would like to thank the staff at Cape Lookout National Seashore for all of their assistance in the field. I would also like to acknowledge the Foundation for Shackleford Horses for their help and the Earthwatch Institute for their financial support.

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## Abstract

Mammal species often live in social groups, but the factors that promote group cohesion can be difficult to analyze due to the prevalence of strong group affiliations. Feral horses maintain stable harems of one or two males and several females, and harem stability is strongly related to individual fitness. Anecdotal evidence and an early study in the non-breeding season suggest that management of the Shackleford Banks island horses with immunocontraception reduces harem stability in the population, providing an opportunity to study the factors that influence harem stability. I investigated the effects of the immunocontraceptive PZP on harem stability during the breeding season and examined mare activity budgets and harassment rates to determine if these factors influence harem stability. I hypothesized that 1) immunocontraception would increase the rates at which mares changed harems during the breeding season 2) activity budgets of contracepted individuals would differ significantly from those of uncontracepted individuals, and 3) contracepted mares would experience greater levels of harassment associated with changing harems than uncontracepted mares. I found that the immunocontraceptive does increase harem changes during the breeding season. I also found that contracepted mares have different activity budgets than uncontracepted mares; as predicted, contracepted mares grazed less and moved more than uncontracepted controls. The factors that influence mare activity budgets included immunocontraception, harem stallion, number of individuals in the harem, number of mares in the harem and body condition of the mare, as well as some interactions between factors. I found that high harassment rates by both harem stallions and other mares in the harem are correlated with higher harem change rates and that contracepted mares are harassed more than uncontracepted mares. These results indicate that the immunocontraceptive does influence harem stability in this feral horse population, potentially through alterations in activity budgets and harassment rates.

Feral horse behavior, equine behavior, harem stability, social stability, immunocontraception, activity budgets, harassment, PZP, *Equus caballus*

## Introduction

Species form social groups in response to a variety of selective pressures, such as predation, food exploitation, resource localization, conspecific harassment, mating strategy and even the ability to assess habitat (Alexander, 1974; Armitage, 1981; Rubenstein, 1994; Gueron et al., 1996). Equid species exhibit two general types of social organization. Species such as the Grevy's zebra and the ass form unstable groups where a male defends resources needed by females (Rubenstein, 1986). Other species, such as the horse, plains zebra, and mountain zebra, form stable harem groups of adult females and their young defended by one or two males (Klingel, 1975; Rubenstein, 1986)

Social behavior in equines may have evolved to avoid predation (Rubenstein 1986). Despite the removal of predator pressure in many populations, feral horses still form social groups, even though herd behavior increases the internal parasite load (Rubenstein and Hohmann, 1989). The tendency to maintain stable groups may be related to social factors; Linklater et al. (1999) proposed that stable relationships between females and a harem male or group of males serve to reduce intraspecific aggression and the resultant reproductive costs. Rubenstein (1994) suggests that mares derive "substantial material benefits for themselves and offspring" from associating with males and that by defending harems males benefit from increased reproductive success. Females not harassed by males are free to spend time grazing while defended by the harem stallion who repels other males (Linklater et al., 2000). In equids, foraging success is limited by time spent grazing rather than by forage quality (Rubenstein, 1994) and equids are dependent on consuming large quantities of forage (Saltz, 2002). Any reduction in the amount of time an individual can graze may lead to a reduction in individual fitness and reproduction (Rubenstein, 1986). Rubenstein (1994) highlighted the benefit to a mare of associating with a stallion that repels other males, but does not alter her the time she spends in important activities such as grazing.

Feral horse harems are generally very stable (Klingel, 1975; Rubenstein, 1981; 1986; Linklater et al., 2000) and stable harems provide benefits to individual members of the harem. Harem stability is

positively correlated with both yearly (Rubenstein, 1986) and lifetime reproductive success in mares (Kaseda et al., 1995). Lower rates of harem change are associated with higher likelihood of reproduction and foal survivorship to independence (Rubenstein and Nunez, 2009). Mares that change harems less frequently may be able to spend more time grazing and thus be in better condition to support offspring (Rubenstein, 1986). Higher reproductive success by mares within the harem raises the reproductive success of the harem stallion as well, since 86.4% of foals in a harem are sired by the harem stallions in one population studied (Bowling and Touchberry, 1990). Harem stability is an important moderator of individual fitness for both males and females and thus the mean fitness of the entire population.

Despite the advantages of stable harem membership, multiple studies have noted harem changes by adult females. A study of a very small population on Santa Cruz Island found that only 2 mares changed harems for a total overall harem instability of 6.2% over 5 years, but their population only consist of two harems with a total of 7 mares (Blumenshine et al., 2002). A short study on the Rachel Carson Estuarine Sanctuary in North Carolina found that 30% of mares changed harems over two years (Stevens, 1990). In 1974 10.8% of mares changed harems in the study area of Shackleford Banks island (Rubenstein, 1981). After the implementation of immunocontraception in 2000, studies have indicated that contracepted mares in this population change harems more frequently than control mares in the nonbreeding season (Nunez et al., 2009).

The population on Shackleford Banks island, North Carolina, USA is managed by the National Park Service (NPS) via Porcine Zona Pellucida (PZP) immunocontraception in order to reduce population growth. PZP triggers an immune system response to the foreign zona pellucida protein injected (Barber and Fayrer-Hosken, 2000) and antibodies are then produced that bind to the sperm receptors on the zona pellucida of the mare's egg and block fertilization (Sacco, 1977). One of the major advantages of immunocontraception with PZP is that it is temporary, lasting approximately two years in horses (Powell and Monfort, 2001), although treatment for over seven years may not be reversible (Kirkpatrick and Turner, 2002).

Porcine zona pellucida immunocontraceptive has several advantages over other contraceptive methods. One advantage of this approach is that protein-based immunocontraceptives such as PZP would be deactivated by the digestive system if consumed, eliminating the risk of transferring the contraceptive to predators or humans (Warren et al., 1997). Another advantage that PZP has over other contraceptives is that PZP can be administered remotely through the use of darts rather than implanted under the skin and have been shown to be safe and effective in many animal species (Barber and Fayrer-Hosken, 2000; Martinez and Harris, 2000). Because of these advantages, PZP immunocontraception has been widely used in feral horses, wild deer, African elephants, feral water buffalo, feral burros, elk and more than 95 species of zoo animals (ZooMontana, 2000).

Contraceptives have been proposed as a useful method of reducing population without altering group size or composition (Garrott and Taylor, 1990) and proponents of PZP immunocontraception have claimed that “it has almost no effects on social behaviors” (ZooMontana, 2000). However, the results of studies that have assessed PZP impact on social behavior in feral horses are inconsistent. In a study of Assateague Island horses, no impact on “social behavior” was noted though the authors did not indicate which behaviors were studied (Kirkpatrick et al., 1995). Other studies have found no effect on aggression initiated or received, activity budgets of mares, sexual behavior, or proximity to stallions (Powell, 1999; Powell and Monfort, 2001; Ransom et al., 2010). However, several of these papers used “control” mares that had been previously treated with PZP (Powell, 1999, Powell and Monfort, 2001) rather than true untreated controls. Nuñez et al. (2009) documented an increased number of harem changes, an increase in the number of harems mares associated with, and elevated incidences of reproductive behaviors in treated mares compared to untreated controls on Shackleford Banks in a small study during the non-breeding season.

PZP immunocontraceptive is an important management tool for non-lethal population control, but since harem stability has such profound impacts of both the social structure and individual fitness of feral horses, it is critical to evaluate the impacts of the immunocontraceptive on harem stability during the

breeding season. If the immunocontraceptive program does decrease harem stability then its use in population management of equids and other mammals with highly structured social systems must be carefully evaluated for potential deleterious effects. However, if PZP alters social relationships between males and females, or alters the optimal activity patterns of contracepted females, then PZP can also be an important tool for exploring questions about the mechanisms that produce stability or instability of harems. These factors are normally hard to investigate due to the high stability of equine harems, thus this study system may afford a unique opportunity to investigate decisions made by mares in choosing a harem or changing harems.

In Chapter 1 I analyzed the effect of PZP immunocontraception on harem stability of the feral horse population on Shackleford Banks island in order to determine if PZP contraception increases harem changes among mares. I tested for differences in the number of harem changes and the number of harems visited between control mares and mares that were previously treated with immunocontraceptives. Given the previous work conducted by Nuñez et al. (2009), I hypothesized that contracepted mares would change harems significantly more often than control mares over both seasons and that contracepted mares would visit more harems than control mares. I also tested for patterns between historical contraception and current harem changes by examining the relationship between harem changes and the number of years contracepted, number of consecutive years contracepted, or number of years since past contraception. I predicted that harem changes would increase with number of years contracepted and the number of consecutive years contracepted. Conversely, I predicted that the number of harem changes would decrease as the number of years since contraception increased. Finally, I tested if actively contracepted mares changed harems more than mares that had been contracepted in the past but were not currently contracepted. I predicted that actively contracepted mares would change harems more than mares with lapsed contraception.

In chapters 2 and 3 I examined two reasons why harem changes may occur in this population. I first examined factors that influence activity budgets, since activity budget can provide important insight

into the impacts of management strategies (Powell, 1999) and social organization (Rubenstein 1986; 1994). In feral horses activity budgets, in particular grazing time, have a strong influence on individual fitness since foraging success is limited by grazing time (Rubenstein 1994). A reduction in grazing time may result in reduced body condition and provide motivation for mares to switch harems to a harem in which they are able to devote more time to grazing. Additionally, Neuhaus and Ruckstuhl (2002) argue that synchronicity of activity budgets is a requirement for stable group living. The reduction in pregnancy rates among mares caused by the immunocontraception may result in a shift in the “average” activity budget in a harem. This may result in a disruption of synchronous activity budgets and reduced harem stability as mares attempt to find a harem containing mares with similar activity budgets.

I first tested for differences in activity budgets between mares treated with contraceptives and untreated mares to determine if immunocontraceptive treatment altered activity budgets. I also tested for activity budget differences between mares with foals and mares without foals to determine whether reproductive status or contraceptive status provides a better explanation for differences in activity budgets. I predicted that mares with foals and control mares would spend more time grazing than mares without foals or treated mares. I also modeled the factors that influence activity budgets in an attempt to determine which sets of factors best explain variations in activity budget.

In Chapter 3 I then examined mare harassment rates by both harem stallions and other mares within the harem. Harassment is costly to females because it reduces the time mares are able to spend grazing and thus reduces their energy intake (Rubenstein 1986) and fitness. Linklater et al. (1999) suggested that harems evolved in equids to reduce these harassment costs. Mares that experience high harassment rates may change harems in an attempt to reduce the costs of harassment and increase their individual fitness. However, mares that are new to a harem suffer from a temporary increase in female harassment (Rutberg and Greenberg, 1990) and males often harass females when they first join a harem (personal observation). Thus changing harems may lead to an increase in harassment, at least

temporarily, rather than reducing harassment. I attempted to assess the relationship between harassment rates and harem changes.

I first tested for a correlation between either stallion or mare harassment rates and harem change rates to determine if high harassment rates were associated with high rates of harem change. I also tested for difference in harassment rates for mares that never changed over the season vs. mares that changed at least once in the season, harassment rates for contracepted mares vs. control mares, and harassment rates by the home stallion for mares that remained in a home harem over the season vs. mares that moved from their home harem. In order to determine if mares successfully reduced harassment by changing harems I tested the harassment rates by stallions for mares before and after harem changes.

The studies presented in this dissertation are unique in their ability to examine social behavior in feral horses with very fine grained temporal data. Due to the relatively small size of the island and the multiple observers involved in the following studies, I was able to collect data on all individuals much more often than other investigators in other areas. The social disruption due to the immunocontraception program also provides the unique opportunity to investigate the drivers of social stability that are so often hard to investigate due to the lack of harem changes or instability. I was also able to use the wide variety of data collected over numerous data points to investigate interactions between factors that have not been previously investigated. These studies represent uniquely in depth and thorough examinations of factors that influence social stability in feral horses and the impact of immunocontraception on social stability.



# Chapter 1: The effects of immunocontraception on harem fidelity in a feral horse (*Equus caballus*) population

Jessa M. Madosky, Daniel I. Rubenstein, Jerome J. Howard, and Sue Stuska

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## Abstract

Feral horses on Shackleford Banks Island, North Carolina, are managed by the National Park Service in order to reduce their impact on the fragile barrier island ecosystem. Management techniques include removal of young horses and immunocontraception of many of the mares using Porcine Zona Pellucida immunocontraceptive. This immunocontraceptive reduces the number of horses that need to be physically removed from the island, but there is concern that the contraception may be influencing the social behavior of the contracepted mares. We investigated the effect of immunocontraception on harem stability by tracking the number of harem changes of each adult mare through the breeding season over two seasons. In both seasons the mares that had been treated with the immunocontraceptive changed harems significantly more than mares never treated (2007,  $P = 0.037$  and 2008,  $P = 0.016$ ) and visited significantly more harems (2007  $P = 0.021$ , 2008  $P = 0.011$ ). The number of years treated did not have a significant effect on the number of harem changes (2007  $P = 0.145$ , 2008  $P = 0.848$ ), nor did the number of years a mare had been off contraceptive once the contraceptive was discontinued (2007  $P = 0.443$ , 2008  $P = 0.826$ ). Additionally, there was no significant difference in harem changes between mares that were actively contracepted and mares that had been treated in the past but were not currently actively contracepted (2007  $P = 0.336$ , 2008  $P = 0.533$ ). These results indicate that the PZP immunocontraceptive has a significant effect on harem stability and that once a mare has been contracepted the behavioral effect of the contraceptive treatment may not be readily reversed.

## Introduction

Feral horse populations are often considered to be a nuisance due to their competition with livestock and their effect on native habitats (Rubenstein, 2001). Although commonly associated with grassland habitats, feral horses also occur on barrier islands, where they have been shown to have significant effects on native flora and fauna (Turner, 1969). De Stoppelaire et al. (2004) found that feral horse grazing had a significant negative impact on dune formation and contributes to dune erosion. Overgrazing of marsh areas may result in a loss of important nursery habitat for many marine species (Levin et al., 2002). Due to the impact of feral horse populations on the environment, populations are often actively managed to reduce population size through a variety of strategies, including selective removals, adoption programs, and fertility control (Rubenstein, 2001).

Feral horses generally associate in harems or bands consisting of up to three adult males, a group of adult females, and their offspring (Linklater et al., 2000; Rubenstein, 1981; 1986). Although these social groups are generally stable (Klingel, 1975; Linklater et al., 2000) several studies have documented harem changes by adult females (Blumenshine et al., 2002; Rubenstein, 1981; Stevens, 1990). Harem stability has been found to positively correlate with yearly (Rubenstein, 1986) and lifetime reproductive success in females (Kaseda et al., 1995), possibly because mares that are more stable are able to devote more time to grazing and thus are in better condition to support a foal (Rubenstein, 1986). Females that change harems less frequently are more likely to reproduce and produce foals that survive to independence than mares that change harems more frequently (Rubenstein and Nuñez, 2009). Linklater et al. (1999) proposed that stable relationships between females and a harem male or group of males serve to reduce intraspecific aggression and the resultant reproductive costs. Altering harem stability may therefore alter overall reproductive rate, effective population size, and patterns of genetic diversity critical to maintaining long term stable populations. Thus, successful management of feral horse populations requires an understanding of how management strategies may alter social structure.

Population management through contraceptive use may minimize disturbance to social structure by preventing reproduction without altering group size or composition (Garrott and Taylor, 1990). Porcine Zona Pellucida (PZP) immunocontraception has been proposed as a contraceptive agent because it can be administered remotely through the use of darts, is relatively inexpensive, and has been shown to be safe and effective in many animal species (Barber and Fayrer-Hosken, 2000; Martinez and Harris, 2000). Immunocontraception with PZP is also temporary, lasting approximately two years in horses when conventional dosing schedules are used (Powell and Monfort, 2001), although treatment for over seven years may not be reversible (Kirkpatrick and Turner, 2002).

However, the results of studies that have assessed PZP impact on social behavior in feral horses are inconsistent. In a study of Assateague Island horses, no impact on social behavior was noted (Kirkpatrick et al., 1995). Other studies have found no effect on aggression initiated or received, activity budgets of mares, sexual behavior, or proximity to stallions (Powell, 1999; Powell and Monfort, 2001). In contrast, Nuñez et al. (2009) documented an increase in harem changes, number of harems mares associated with, and reproductive behaviors in treated mares. This study was conducted over a span of five weeks, included a limited number of mares, and occurred during the non-breeding season (Nuñez et al., 2009). Given the potential impact of increased rate of harem change on individual fitness, additional study is clearly necessary to determine if there is a significant effect of PZP immunocontraception on harem changes.

In this study we investigated the effect of PZP immunocontraception on harem stability of the feral horse population on Shackleford Banks, North Carolina, USA, during the course of two breeding seasons. We examined the number of harem changes each mare on the island made and the number of harems each mare visited during the course of the 2007 and 2008 breeding seasons. Every mare on the island was included in the analysis and data were collected over a period of several months each year. Given the previous work conducted by Nuñez et al. (2009), we hypothesized that contracepted mares would change harems significantly more often than control mares over both seasons and that contracepted

mares would visit more harems than control mares. We also analyzed the effect of foaling status on harem changes, hypothesizing that mares that foaled during the survey year would change less than non-foaling mares.

We also attempted to relate the historical record of immunocontraception with the current patterns of harem changes. We examined the relationship between number of years contracepted, number of consecutive years contracepted, or number of years off contraception and harem changes. We predicted that harem changes would increase as the number of years contracepted increased and the number of consecutive years contracepted increased. Conversely, we predicted that the number of harem changes would decrease as the number of years off contraception increased. Finally, we examined the number of harem changes made by females that were actively contracepted in comparison to those that had been contracepted in the past, but were not currently contracepted. We predicted that actively contracepted mares would change harems more than mares with lapsed contraception.

## Materials and Methods

### *Study area*

Shackleford Banks is a small barrier island located approximately three km off the coast of North Carolina, USA, in Cape Lookout National Seashore. The island is approximately 17 km long and one km wide with a variety of habitats including beach, swale, dunes, salt and freshwater marshes and maritime forest (Rubenstein, 1981). Individual horse bands are generally distributed along the entire island but individual harems occupy home ranges within a portion of the island. These home ranges can be grouped into far-east, mid-east, and western distributions. Each area contains at least one permanent water source, although digging may be necessary to access the water. Subjects in all areas of the island were observed for this study.

### *Study subjects*

There are currently approximately 120 horses on the island. Most horses on the island are found in harems including one to two males and a group of mature females and their immature offspring. Several harems have an alpha stallion and a beta stallion. Over the course of the study, harem sizes ranged from a single male and a single female to two males, multiple females, and multiple offspring. Both sexes disperse from their natal harems as found in other feral horse populations (Feh, 1990). Territoriality has been reported on the island in the past (Rubenstein, 1981), but does not appear to exist currently. This change may be the result of reduced water sources in the eastern end of the island where territories were found. Harems and solitary stallions tend to be found within the same general area of the island from season to season and year to year. There is some movement between areas, especially by dispersing juveniles, but most adult horses settle into one area of the island. There are exceptions – during a particularly dry summer several harems shifted areas or expanded their home range in order to access a better water source and there is more overlap between harems occupying the mid-east and far-east areas than between those in the mid-east and the west.

The minimum population of Shackleford horses is fixed by federal law at 100 horses (Prioli, 2007), and the National Park Service limits the population to approximately 130 individuals to reduce the potential for damage to the island ecosystem. The immunocontraception program was initiated in 2000 to reduce population size and control population growth (Nuñez et al., 2009). The contraceptive is administered by dart gun by the National Park Service between February and April every year. Each dose contains 100 µg of PZP mixed with 0.5 cc of either Freund's Complete Adjuvant, Modified, *Mycobacterium butyricum* (Calbiochem, Gibbstown, NJ, USA, #344289) for initial doses, or Freund's Incomplete Adjuvant (Sigma, St. Louis, MO, USA, #F5506) for subsequent doses (S. Stuska, personal communication; Nuñez et al., 2009). Booster doses are administered at least 14 days after the initial dose. At the initiation of the program eight mares were chosen as controls, chosen from eight distinct genetic lineages present on the island. These control have never been contracepted. One additional control was

added in 2007 in order to increase the age variation in controls and is included in the data analyzed for 2007, but died before the breeding season in 2008.

We observed harem affiliations for every mare on the island. Only mares that had dispersed from their natal harems were included in the analysis: nine controls and 55 contracepted mares in 2007 and eight control mares and 57 contracepted mares in 2008. The contracepted mares consisted of any mare that had ever been treated with the contraceptive, regardless of whether they had been treated in the previous year. We included all mares ever contracepted as there is some evidence that the contraceptive effect may last longer than one year (Powell and Monfort, 2001) and it is important to determine if contraception at any point changes the mare's subsequent behavior.

### *Behavioral Sampling*

The study was conducted by multiple observers supervised and trained by the primary investigator (J M). All observations were collected during the breeding season. Observations were collected between May 22<sup>nd</sup>, 2007 and July 29<sup>th</sup>, 2007 and between May 22<sup>nd</sup>, 2008 and August 12<sup>th</sup>, 2008, totaling 2965 total sightings in 2007 and 2985 total sightings in 2008. A sighting was defined as an independent positive identification of a mare and her harem association. Each observer recorded initial sightings upon identifying individuals. Unless a mare changed harems more than 20 min. after the initial sighting no additional sightings were recorded of the harem by that observer unless the harem was independently identified at a later point in the day. If two observers identified the same individuals within 20 min. only one identification was included in the sightings count. Harem changes were recorded regardless of their timing during the observation session. Individual horses were identified by freeze brand, distinctive markings on the face and body, color, and sex.

Harems were located an average of five times a week during 2007 and approximately four times a week during 2008. Each mare was sighted an average of 46.3 times in 2007 (S. E. 1.70) and 45.9 times in 2008 (S. E. 1.64), or approximately every 1.2 days (2007) and every 1.6 days (2008).

After examining the standardized Z score, no individuals were found to be outliers, and there was no correlation between the number of sightings and harem changes for either year (2007  $P=1.82$ , 2008  $P=0.502$ ). Each time a harem was located the harem members were all individually identified and the composition was recorded along with the GPS location of the harem. The number of harem changes for each mare on the island was quantified as a change from one harem to another over successive sightings. All sightings from all observers were compiled for each season and each move from one harem to another was counted. Absence from a harem without a sighting in a different harem was not counted as a harem change as in some rare cases mares were found without a harem stallion. The number of harems a mare visited was quantified as the number of harems a mare was affiliated with over a given season.

#### *Statistical analysis*

We analyzed the effect of contraception on the mean number of harem changes per individual per season using Mann-Whitney U tests. The Mann-Whitney U test was selected given the non-normality of the data and the large difference in sample size between the control (nine in 2007, eight in 2008) and contracepted (55 in both years) mares, which may cause problems when using t-tests (Zar 1999). One-tailed tests were used as we predicted that contracepted mares would change harems significantly more than control mares given the previous work by Nuñez et al. (2009). Effect size provides a measure of the strength of the relationship between the variables and was calculated using G-power (Faul et al., 2007) for a one-tailed t-test, which provides a reasonably equivalent estimate for the Mann-Whitney U analysis (Zar, 1999). Data were analyzed independently for each year and statistical outliers (one per year) were eliminated from the analysis. In this study both statistical outliers were contracepted mares that changed harems repeatedly over the season. Eliminating the outlier in each year made the analysis more conservative.

We examined the effect of age on harem changes using nonparametric correlation (Spearman's rho) between age on June 1<sup>st</sup> of the survey year and number of harem changes during that survey season. We ran Mann-Whitney U tests to determine if the average age differed between control and contracepted females. We also ran Kruskal-Wallis tests to determine if mares that foaled during a given survey year changed harems less than mares that did not foal based on their contraceptive status. All mares that foaled during the survey year (not just during the data collection season) were included as mares that foaled even if the foal did not survive. The mares eliminated as statistical outliers for earlier analyses were also eliminated from the age and foaling analyses.

To investigate the impact of historical contraception patterns on current behavior, we analyzed the effect of total number of years contracepted, number of years contracepted consecutively with the survey year, total number of years ever consecutively contracepted, and number of years off contraception prior to the survey year on harem changes using Kruskal-Wallis tests. Our assessment of contracepted status in a given year was based on National Park Service records, and included only contraception attempts deemed successful (solid hit with the dart and immunocontraceptive solution ejected from the dart) were included. We analyzed the effect of active versus lapsed contraception on harem changes using Mann-Whitney U tests. Mares were considered to be actively contracepted if contraception had been administered in the past two years (Powell and Monfort, 2001). Again, data were analyzed independently for each year and statistical outliers were eliminated from the analysis. All analyses were conducted in SPSS 15 (SPSS Inc., 2006).

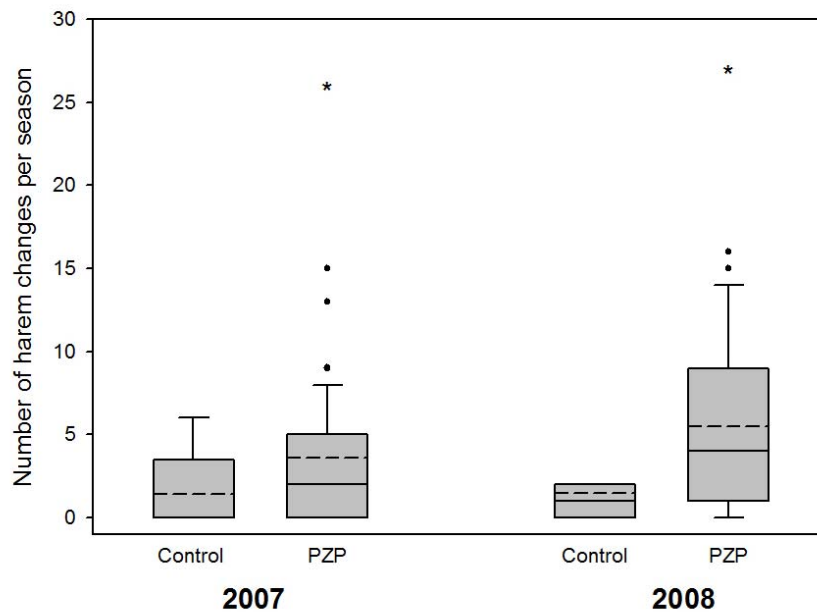
## Results

### *Immunocontraception and harem changes*

During 2007, there were nine controls and 55 contracepted mares; of the contracepted mares 25 received PZP in the previous year and 20 had not. Sixty-six percent of mares changed harems at least once over the season, with most changing harems multiple times. One individual mare changed harems



26 times over the course of the field season (the outlier in 2007 – a contracepted mare). Additionally, 70.9 percent of contracepted mares (39 of 55) changed harems at least once while 33.3 percent of control mares (three of nine) changed harems. Contracepted mares changed harems significantly more than control mares (Mann-Whitney U = 157.00, one-tailed P = 0.0415, effect size (d)= 0.61). The mean number of harem changes for contracepted mares was  $3.62 \pm 0.606$  S. E., while the mean number of harem changes for uncontracepted mares was only  $1.44 \pm 0.766$  S. E. (Fig. 1). Contracepted mares also visited more harems than control mares (Mann-Whitney U=145.5, one-tailed P=0.021, effect size (d)=0.80). The mean number of harems visited for contracepted mares was  $2.73 \pm 0.213$  S. E., while the mean number of harems visited for control mares was  $1.67 \pm 0.333$  S. E.



**Figure 1.** Box plot showing the number of harem changes by contraceptive status for both 2007 and 2008 with median(solid line), mean (dashed line), and statistical outlier (asterisk). The statistical outlier is presented, but not included in analysis.

During 2008, there were eight controls and 55 contracepted mares; 20 contracepted mares had received PZP in the previous year and 37 had not. Seventy-three percent of mares changed harem at least once, with a maximum of 27 harem changes (the outlier for 2008 – a different contracepted mare). In

2008, 77.2 percent of contracepted mares (44 of 57) changed harems at least once while only 50 percent of control mares (four of eight) changed harems. Again, contracepted mares changed harems significantly more frequently than control mares (Mann-Whitney  $u=124.5$ , one-tailed  $P=0.0205$ , effect size( $d$ )= $0.92$ ). In 2008, the mean number of harem changes for contracepted mares was  $5.47 \pm 0.758$  S. E., while the mean number of harem changes for control mares was only  $1.50 \pm 0.732$  S. E. (Fig. 1). Contracepted mares visited more harems than control mares (Mann-Whitney  $U=115$ , one-tailed  $P=0.011$ , effect size ( $d$ ) =  $1.10$ ). The mean number of harems visited for contracepted mares was  $3.05 \pm 0.222$  S. E., while the mean number of harems visited for control mares was  $1.63 \pm 0.263$  S. E.

#### *Age and harem changes*

Age of the mare was not found to correlate with harem changes in either year (2007  $\rho=-0.073$  (61),  $P=0.569$ , 2008  $\rho=-0.023$ (62),  $P=0.854$ ). Additionally, average age was not significantly different between contracepted mares and control mares (2007 Mann-Whitney  $U=200.5$ ,  $P=0.403$ , 2008 Mann-Whitney  $U=139$ ,  $P=0.084$ ).

#### *Foaling and harem changes*

In 2007, six control mares foaled and four non-control mares foaled; two non-controls had been off PZP for at least a year while 2 others had been contracepted the previous year. In 2008, seven control mares foaled and eight non-control mares foaled; again two mares contracepted the previous year foaled. In 2007, the 92% of treated mares did not foal while in 2008 90% of treated mares did not foal. There was a significant difference in the number of harem changes between mares that foaled and those that did not foal in 2008 (Mann-Whitney  $U=240.5$ ,  $P=0.042$ ) and a borderline significant difference in 2007 (Mann-Whitney  $U=165.0$ ,  $P=0.054$ ). In both cases mares with a foal changed harems less than mares without a foal. There was no significant difference in the number of harem changes between mares that were previously contracepted and foaled, mares that were controls and foaled, and mares that did not foal (Kruskal –Wallis 2007  $P=0.120$   $df=2$ , 2008  $P=0.112$   $df=2$ ), but in both years mares that had previously

been contracepted and foaled changed harems less than contracepted mares without foals and more than control mares with foals.

#### *Prevalence of harem changing*

The distribution of harem changes each season significantly differed from the Poisson distribution (2007: Chi-square= 269.11, df=10,  $P<0.001$ ; 2008: Chi-square=761.15, df=10,  $P<0.001$ ) suggesting that harem changes are not random. In general, there were more mares at the extremes and fewer mares in the center of the distribution than expected in a Poisson distribution. Prior to the immunocontraception program only 10.8% of mares changed harems in the same study area (Rubenstein 1981) as opposed to the 66% and 73% found over the two seasons of this study. This difference was significant for both years (2007: Chi-square=316.293, df=1,  $P<0.001$ ; 2008: Chi-square= 401.599, df=1,  $P<0.001$ ). In 2007, 94% of harems experienced at least one mare changing into or out of the harem and in 2008 97% of harems were affected by the harem changes.

#### *Historical PZP treatment and harem changes*

The number of years treated with PZP did not have a significant effect on the number of harem changes (Kruskal-Wallis 2007  $P=0.199$  df=6, 2008  $P=0.871$  df=7), nor did the number of years a mare had been off contraceptive after it had been discontinued (Kruskal-Wallis 2007  $P=0.310$  df=4, 2008  $P=0.823$  df=5). The number of years a mare had been treated consecutively with the survey year did not have a significant effect (Kruskal-Wallis 2007  $P=0.273$  df=7, 2008  $P=0.093$  df=4), nor did the maximum number of years a mare had ever been consecutively treated (Kruskal-Wallis 2007  $P=0.782$  df=6, 2008  $P=0.889$  df=6). There was no significant difference in harem changes between mares that were actively contracepted and mares that had been contracepted in the past but were not currently actively contracepted (2007 Mann-Whitney  $U=275.0$ ,  $P=0.196$ , 2008 Mann-Whitney  $U=365.0$ ,  $P=0.751$ ).

## Discussion

It is important in any management program to reduce the impacts of the management on the natural behavior of the population, though the importance of preserving behavior is often overlooked (Clemmons and Buchholz, 1997). In a highly social species such as feral horses it is critical to ensure that management strategies do not negatively impact social behavior. The results of this study indicate that the PZP immunocontraceptive used to control population numbers on Shackleford Banks Island has a significant negative effect on harem stability and that this behavioral effect is more persistent than the physiological contraceptive effect documented in previous studies (Powell and Monfort, 2001). This study demonstrates that the negative effect of the PZP immunocontraception on harem stability during the non-breeding season (Nuñez et al., 2009) was also present in the breeding season and that age was not related to number of harem changes in a given season. The significant increase in the historic rate of harem changes (from approximately 10 percent to approximately 66 and 73 percent) indicates that the overall rate of harem changes has increased since the immunocontraceptive program was initiated. Additionally, the high percentage of harems affected by harem changes indicates that the change in stable social structure after PZP immunocontraception affects the entire population, not just the mares contracepted.

Although the control sample size in this study was necessarily small due to management concerns, the replication of these results over two breeding seasons increases our confidence in the significance of differences in harem changes between contracepted and control mares. Given that mares stayed in the same general area, they frequently revisited the same harem several times during the season. The average number of harems visited for all mares was 2.58 in 2007 and 2.88 in 2008. Contracepted mares visited more harems than control mares in both years. It is important to note that some mares changed harems several times over the course of a week or even a day. High temporal resolution is critical to insure that these rapid harem changes between small numbers of harems are documented.

*Impacts of historical contraceptive treatment*

The fact that the number of harem changes did not differ among mares contracepted for different numbers of years, or different numbers of consecutive years, suggests that any exposure to PZP contraception may alter female behavior in fundamental ways. Additional years of contraception did not result in a higher number of harem changes, indicating that contracepting for multiple years does not decrease social stability further. However, increasing the number of years between contraception did not mitigate the behavioral effect of the contraception as expected. Surprisingly, mares that were not currently contracepted but had been in the past did not change harems less than mares that were actively contracepted. These analyses indicate that contraception schedule does not seem to mitigate the behavioral effects of the contraception and that the behavioral effects of the PZP contraception may persist long after the contraceptive should no longer be physiologically effective.

#### *Influence of foals on harem fidelity*

As seen in the non-breeding season (Nuñez et al., 2009), mares that had foals changed harems less than mares without a foal, regardless of their contraceptive status. The presence of a foal may increase mare fidelity. Unfortunately, it is difficult to separate the effect of the contraceptive itself from the result of the contraceptive – a lack of foals. If the presence of a foal is a driver of harem fidelity all contraceptive strategies may reduce social stability. Although not significantly different, the pattern of harem changes suggests that previously contracepted mares with foals may change more than controls with foals, but less than contracepted mares without foals suggesting that the contraceptive may have an effect beyond the presence of a foal. The failure to find a significant difference in this comparison may be due to low sample size. Further studies should be conducted with higher sample size to determine if contracepted mares with foals change harems at the same rate as control mares with foals.

It is possible that foaling returns mares to pre-contraceptive behavior – the sample size of this study was simply too low to conclusively answer this question. Relatively few mares that had been previously contracepted, but were not currently contracepted, foaled in either year – two out of 30 in

2007 and six out of 37 in 2008. Further studies should be conducted to determine if contracepted mares cease changing harems the season after they are reproductively successful or if they continue to change harems at a higher rate than mares that are never treated.

#### *Potential motivations for harem infidelity*

While it is clear that PZP immunocontraception causes a decrease in harem fidelity in this population regardless of season, it is unclear if this decrease is due to male or female choice. Males have been shown to discriminate between females based on female reproductive success in many taxa (Berven, 1981; Johnson and Hubbell, 1984; Verrell, 1985; Berglund et al., 1986; Jones et al., 2001; Szykman et al., 2001). This male choice may result in males defending reproductively successful mares (as evidenced by their foals) more than contracepted mares, allowing contracepted mares to change harems more often due to reduced male attentiveness. Previous studies found increased herding of mares with foals, but no difference in harem tending behaviors between currently contracepted mares and controls (Ransom et al., 2010). Alternatively, continuous cycling may lead to increased breeding attempts (Ransom et al., 2010) and male harassment of contracepted females. Contracepted females may elect to change harems more frequently to escape harassment or in an attempt to become pregnant. Future work aimed at distinguishing between male and female choice would be of particular interest.

#### *Fitness impacts of decreased harem stability*

Finally, the pervasive pattern of decreased harem stability may affect individual fitness of both males and females. In feral horses the breeding season corresponds to the foaling season (McCort, 1984) and thus a decrease in harem stability may lead to decreased grazing time while caloric demands on lactating mares are highest (Pagan, 1998; Pilliner, 1999) and decreased body condition even among mares that do not change harems. Ransom et al. (2010) found no difference in grazing time between treatment groups, but did not consider the overall effect of decreased harem stability on grazing. With decreased social stability, males may be required to spend more time guarding their harem to prevent

harem changes. This increase in time spend guarding harems may lead to reduced time grazing and result in lowered body condition for the harem stallions. Future work will test if body condition of males or females has decreased since the implementation of the immunocontraception program.

### *Management Implications*

The contraception of the majority of adult females on the island may have substantially changed the social structure of the population, and it is unclear if contracepted mares will ever return to pre-contraception social stability. Immunocontraception using PZP offers many potential benefits, but managers should also consider the potential impacts PZP may have on social structure and stability. One of the key benefits of PZP immunocontraception is the temporary nature of the contraceptive affect. However, even if mares can be physiologically restored to reproductive condition; their continuing propensity to move may reduce their subsequent fitness and complicate management. The persistent behavioral effects are not reduced by manipulating the number of years treated with PZP, so reducing these behavioral effects may not be possible.

Given that PZP immunocontraceptive is administered to a wide variety of species including deer, African elephants, feral water buffalo, feral burros, elk and more than 95 species of zoo animals (ZooMontana, 2000), it is critical to further examine the behavioral effects the contraception may have on other species. Although immunocontraception does not have some of the potential physiological effects of steroidal contraceptives and while behavioral effects may vary with different social systems present in different species, this study indicates that social behavior can be changed by PZP immunocontraception. If, as is known in horses, social behavior affects reproductive success, population management may be complicated by lingering changes in social dynamics. Managers of other species treated with PZP should be cautious in assuming that PZP immunocontraception ameliorates the behavioral effects of other types of contraception.

## Conclusions

This study, combined with previous work, demonstrates that there is a significant effect of PZP contraception on behavior in feral horses. Contracepted mares changed harems significantly more often than control mares. Further, a high percentage of mares change harems over the course of each season, indicating that this change is affecting the entire population. The duration of contraception does not have an effect on harem changes, nor does the duration of time off contraception reduce harem changes. Mare behavior is altered after contraception and this alteration appears to be long-lived. Given the pervasive behavioral effect of the PZP immunocontraception and the duration of this behavioral effect, it is especially important to investigate the potential costs of the reduction in harem stability to both sexes regardless of the direct effect of the contraceptive



## Chapter 2: Activity Budget Synchronicity and Factors that Affect Activity Budgets in a Feral Horse (*Equus caballus*) population

### Abstract

Activity budgets provide valuable insight into the ecological factors acting on individuals and can offer insight into the effects of management strategies on ecologically important behaviors such as foraging and resting time. In equines in particular, the amount of time spent foraging is a critical factor in fitness and reproductive success, and synchronicity of activity budgets is thought to increase harem stability and individual fitness. We examined the impact of management via immunocontraception on activity budgets in order to determine if activity budget asynchronicity may explain the harem instability observed in feral horses located on Shackleford Banks Island, NC. We tested for differences in activity budgets for mares with and without foals, and for mares that were contracepted or uncontracepted, as well as modeling the impact of multiple factors that may influence activity budgets. We found that the presence of a dependent foal increased time spent grazing and that mares with foals were recumbent and trotted or ran less than mares without foals. Uncontracepted mares spent less time moving (both walking and trotting or running) and recumbent than contracepted mares. We also found that immunocontraceptive status was an important model predictor of most activities (including time spent grazing, recumbent, and standing), but was not an important predictor for locomotion. Other factors found to be important include harem stallion, number of individuals in the harem, number of mares in the harem and body condition of the mare, as well as some interactions between factors. These results indicate that immunocontraceptive status plays an important role in predicting activity budgets in mares and that the asynchronicity in activity budgets between mares with different contraceptive status and reproductive status may play a role in increased harem instability.

## Introduction

Activity budgets can provide important information on the strategies adopted by individuals (Duncan, 1985) and offer insight into selection pressures (Boyd, 1988), impacts of management strategies (Powell, 1999), and social organization (Rubenstein 1986; 1994). Activity budgets are easily quantified by behavioral sampling and are a fundamental tool for understanding the evolution of social systems. In fact, Neuhaus and Ruckstuh (2002) argue that synchronicity of activity budgets is a requirement for stable group living.

In equids, foraging success is limited by time spent grazing rather than by forage quality (Rubenstein, 1994). Any reduction in grazing time can lead to a reduction in individual fitness and reproduction (Rubenstein, 1986). Previous studies in Przewalski horses have shown that mares lactating or pregnant spend more time feeding than barren mares (Boyd, 1988) and studies in horses indicate that mares increase grazing time during the peak of lactation (Duncan, 1985). Differences in female needs may be accentuated by environmental conditions and may lead to broken social bonds and harem instability (Rubenstein, 1994). A dramatic reduction in pregnancy rates among mares may result in a shift in the “average” activity budget in a harem as the proportion of barren mares increases and as the energetic demands of reproduction on mares are lessened. This may result in a disruption of synchronous activity budgets and reduced harem stability.

Mares who are allowed to determine their own activity budgets and are harassed less by males may have greater reproductive success due to an increase in grazing time (Rubenstein 1986; 1994). Females may use strategies such as associating with males of higher dominance rank (Rubenstein, 1986) or territorial males (Rubenstein, 1994) to maximize grazing time. Differences in the amount of time a harem stallion allows his females to graze may affect which stallions mares choose to associate with (Rubenstein, 1994). Differences in group size may also affect activity budgets, although these effects may be indirect, there is little competition for forage in feral horses and thus foraging time is not directly influenced by harem size (Rubenstein, 1994). Larger group size also has no influence on agonistic

encounter rates (Rubenstein, 1994). However, group movements are primarily initiated by either dominant females or lactating females depending on the season (Rubenstein, 1986) so an increase in group size may result in increased movement and decreased grazing time.

Immunocontraception with porcine zona pellucida (PZP) is currently used in several feral horse populations to reduce population growth (ZooMontana, 2000). Several studies have concluded that treatment with PZP does not affect the time budget of treated mares (Powell, 1999, Ransom et al., 2010). However, in one of these papers the control mares had been previously treated with PZP (Powell, 1999) and previous work has shown that previously treated mares, even when not currently contracepted, have altered behavior compared to mares that have never been treated (Madosky et al., 2010). Additionally, although Ransom et al. (2010) found there were few effects of treatment with PZP, they did find that band fidelity (whether or not a mare changed harems), foal presence, and body condition affected behavior. Given that PZP immunocontraception has been shown to increase harem changes in some studies (Nuñez et al. 2009; Madosky et al., 2010), the effect of band fidelity may be an indirect result of treatment with PZP.

We examined the factors that affect female activity budgets in feral horses using two methods. We first asked if there were differences in activity budgets between mares treated with the immunocontraceptive and untreated mares. Since some mares had been treated in the past but foaled during the study year we also tested the differences between both reproductively active mares (as measured by the production of a foal) and non-reproductively active mares. We predicted that mares with dependent foals and control mares would spend more time grazing than mares without foals or untreated, and that they would compensate by spending less time in other behaviors. To delve deeper into the factors that influence mare activity budgets we also modeled the impact of a variety of factors on activity budgets. Our objective was to determine which set of factors best explain variations in activity budget.

## Methods

### *Study Area*

Shackleford Banks is a barrier island approximately 17km long and 1km wide located off the coast of North Carolina, USA in Cape Lookout National Seashore (Rubenstein, 1981). Horses can be found along the length of the island, but individual bands tend to have home ranges within one of the three areas of the island – west, near-east, and east. Each of these areas contains a permanent source of water and a variety of habitats. Harems in all areas of the island were observed for this study.

### *Study Subjects*

Feral horses associate in generally stable groups of one or more males, one or more females, and their offspring (Klingel, 1975; Rubenstein, 1981; 1986; Linklater et al. , 2000). There were approximately 120 horses on the island during the course of this study and harem sizes ranged from one male and one female to two males with multiple females and their offspring. Both sexes disperse from their natal harem. Males that are unable to hold a harem are found either alone or in bachelor groups. Adult horses tend to stay in one area of the island, but there is some movement of juveniles between areas and occasionally harems move to a different area of the island, particularly when water is scarce.

The horses are managed jointly by the National Park Service (NPS) and the Foundation for Shackleford Horses, Inc. Population growth is restricted in order to prevent damage to the island ecosystem. Most adult mares have been treated at some point with an immunocontraceptive to prevent pregnancy. The immunocontraceptive program was initiated in 2000 (Nuñez et al., 2009) and is administered via dart gun by the NPS between February and April of each year. Each dose of immunocontraceptive consists of 100g of PZP mixed with 0.5 cm<sup>3</sup> of either Freud's Complete Adjuvant, Modified, *Mycobacterium butyricum* (Calbiochem, Gibbstown, NJ, USA #344289) for initial doses or Freud's Incomplete Adjuvant (Sigma, St. Louis, MO, USA, #F5506) for booster doses (S. Stuska, personal communication; Nuñez et al., 2009), with booster does administered at least two weeks after

initial dose. Before the immunocontraceptive program started 8 control mares were chosen from eight distinct genetic lineages to serve as controls. These controls were not treated with the immunocontraceptive.

We collected activity budget data on all females on the island and all males with a harem. Rarely, a female was found without a harem stallion. Data was collected on mares without a stallion but was not used in the model analysis. Only mares that dispersed from their natal harem were included in our analysis: eight control mares and 57 treated mares. All mares that had ever been treated with the immunocontraceptive were included in the treated group. We included mares that had been treated in the past in the treated group as there is evidence that the contraceptive effect of PZP last longer than one year (Powell and Monford, 2001) and that the behavioral effect of PZP contraceptive persists for numerous years (Madosky et al., 2010).

### *Behavioral Sampling*

Activity budget data was collected by the primary investigator (J. M.) and multiple observers trained and supervised by the primary investigator. Observations were collected during the breeding season of 2008 between May 22<sup>nd</sup> and August 12<sup>th</sup>. Observers conducted 39-min instantaneous scan samples of time budgets (Altmann, 1974) at 3 min intervals for all harem members. We collected 952 mare activity budget sessions totaling over 615 hours of mare activity over the course of the season. Observers also collected harem composition (including harem stallion(s), number of adult mares in the harem, and total number of harem members), presence or absence of a foal, and GPS location. Presence or absence of a control mare was determined by the primary investigator after all data was collected as most observers were not told which mares were controls in order to reduce any possibility of observational bias. Each individual was identified by freeze brand, markings, color, and sex. Data was not collected when individuals were not positively identified. Data points where individuals were out of sight or not positively identified are not included in the total observation time.

Observers were given a list of common behaviors in order to accurately record behavior. This list included graze, walk, drink, stand, fight, sternal (lying on breastbone), lateral (lying horizontally), vigilant, herd, elimination, and masturbate. Other behaviors were recorded in as much detail as possible and assessed by the primary investigator. Some of these behaviors included trot/run, scratch, flehmen, sniff, groom, and kick. Due to the infrequent nature of some behaviors some activities were grouped for analysis. For the mixed model analysis, walk and trot/run were combined into *locomotion* and sternal and lateral were combined into *recumbent*. Stand and recumbent were analyzed separately due to the fact that while both represent an inactive state, recumbence potentially indicates a higher level of comfort since it is more difficult for an individual to move quickly when recumbent. Some activities were too rare to analyze as part of an activity budget and were not considered further in this analysis. These included social (fight, herd, groom harem member), masturbate, scratch, flehmen, sniff, elimination, and kick. Thought potentially important, these behaviors are not frequent enough to provide enough data for time budget analysis. We plan to analyze these behaviors further in the future from ad lib data collected.

### *Statistical Analyses*

All observers were tested for inter-rater reliability and only data from observers that consistently had Kappa values of over 0.6, indicating “substantial” agreement, were included in either analysis (Landis and Koch, 1977). All analysis was conducted with SPSS 17.0 (SPSS Inc., 2008) and all percentages were arcsine square root transformed for analysis. Graphs show untransformed data.

#### T-tests

We performed t-tests on the average mean percentages of time spent grazing, walking, standing, recumbent, trotting or running, and vigilant for all mares with five or more time budget sessions. Grazing was analyzed as it is an important measure of energy intake. Walking and trotting or running were analyzed as important measures of energy outtake. Both standing and recumbent are resting behaviors, but were analyzed separately because mares that are recumbent are more vulnerable and because

recumbency indicates a more lasting resting session while standing may be a temporary state. Vigilance was analyzed as it may indicate the comfort level of harem members with higher rate of vigilance indicating less comfort.

The total number of recorded time points in an activity was divided by the total number of time points for that individual to determine the percentage of time spent in that activity state. All percentages were averaged to provide a mean percentage of time in the activity state over the breeding season. We tested the effect of contraceptive status (control or contracepted) and the effect of having a dependent foal. Only mares with foals for the entire breeding season were considered for the t-test analysis, yielding 50 mares without foals and seven with foals. There were 53 contracepted mares and 8 control mares considered for this analysis.

#### Model analysis

We used linear models to assess the effect of age, treatment status (control vs. contracepted), identity of the harem stallion, presence of a dependent foal, number of adult mares in the harem, total number of harem members, and body condition on variation in activity budgets. Age was provided from parturition records or estimation by an experienced veterinarian during previous captures. Treatment status was determined from NPS records of contraception. Body condition was determined by a single, experienced observer in order to eliminate variability in observer ratings. Rating was based on a 0.0-5.0 scale (Carroll and Huntington, 1988) and all scores were taken between April 1<sup>st</sup> and May 29<sup>th</sup> of 2008. Body condition may vary throughout the season, but we were only able to obtain one measure at the beginning of the season and used this measure as an approximation of body condition for that summer season.

Over the course of the season many mares changed harem stallions, harem size varied, and some individuals were born. Given these changes over the course of the season we used mixed models analysis to determine which factors predicted activity budgets. Mixed models analysis allowed us to use repeated

measures taken at different times. We used linear mixed modeling with the date data was collected as a repeated measure to account for correlations in the residuals of individuals measured repeatedly. Body condition was not available for all mares so only mares with body condition scores were considered for mixed model analysis. Only time budgets for mares with a stallion that had at least 15 observations were included to prevent bias in the data. This resulted in an analysis of 715 activity budget sessions (582 for contracepted mares and 133 for control mares) for 465 hours of activity budget data (377 hours for contracepted mares and 88 hours for control mares). Approximately 105 hours of activity budget observations were lost by only including mares with body condition scores, but this restriction was necessary to insure that the removal of mares without body condition scores from models with body score did not artificially reduce the corrected Akaike's Information Criteria ( $AIC_c$ ) value through a reduction in the number of levels for both the fixed effect and the repeated effect.

We first ran the models with variables that changed over the season (harem stallion, presence of a foal, total number of horses in the harem including the stallion, and total number of mares in the harem) with contraceptive status to determine which variables were most predictive and to determine if foal or contraceptive status was more predictive. For each activity (graze, locomotion, stand, and recumbent) we fitted all possible subsets of the variables with two-way interactions or without interactions. Foal and control were not entered into the model simultaneously as the contraceptive is intended to reduce foaling and there were only three mares who were treated in the past and foaled in 2008. Number of mares in the harem and number of individuals in the harem were also not entered into the model simultaneously as the number of individuals in the harem is highly influenced by the number of mares in the harem mathematically. Instead, for these variables, analysis was conducted with either of the two variables to determine which of the two provided more explanatory power. We then tested to determine if variables that did not change over the season (age and body condition at the beginning of the season) improved the predictive ability of the best models for each activity. We tested all models with a  $\Delta AIC_c$  of less than two from the best model with all possible combinations of age and body condition up to two-way interactions.



We also tested models with just age, just body condition, and in combination to determine if they predicted activity rates better than the previously tested models.

We fitted models to our data using linear mixed models analysis in SPSS Version 17.0 (SPSS, Inc., 2008). Models strength was assessed based on minimum AIC<sub>c</sub>. Models with the highest AIC<sub>c</sub> weight were considered the best model, but all models with a  $\Delta$ AIC<sub>c</sub> of less than two reported as possible best fit models (Burnham and Anderson, 2002). AIC weights were calculated for all models within  $\Delta$ AIC<sub>c</sub> of ten (Burnham and Anderson, 2002).

## Results

### *T-tests*

We found that presence of a dependent foal for the entire season made a significant difference for several activities. Mares with a foal grazed significantly more than mares without a foal ( $p=0.002$ , Table 1), averaging 12.6 percent more time spent grazing. Mares with a foal spent significantly less time recumbent ( $p=0.022$ , Table 1) with an average of 1.2% less time recumbent. Mares with a foal also spent significantly less time trotting or running ( $p<.001$ ) with no time spent running compared to 0.4% of time spent running for mares without a foal. Mares with a foal were vigilant significantly less than mares without a foal ( $p=0.003$ ) with an average of approximately 0.7% less time spent vigilant. There was no significant difference in time standing or walking though there was a trend of mares without a foal spending more time standing or walking (Table 1).

<b>Activity</b>	<b>t</b>	<b>df</b>	<b>p-value</b>	<b>Mean with foal <math>\pm</math>SE (%)</b>	<b>Mean without foal <math>\pm</math>SE (%)</b>
Graze	3.90*	12.91	0.002	75.8 $\pm$ 2.5	63.2 $\pm$ 1.9
Recumbent	2.50*	19.34	0.022	0.3 $\pm$ 0.2	2.1 $\pm$ 0.5
Stand	1.44	55	0.155	15.2 $\pm$ 2	21.4 $\pm$ 1.5
Trot/run	5.07*	49	<0.001	0	0.4 $\pm$ 0.1%
Vigilant	3.51*	15.06	0.003	0.1 $\pm$ 0.1	0.8 $\pm$ 0.2
Walk	1.09	55	0.280	7.3 $\pm$ 0.8	9.0 $\pm$ 0.5

**Table 1:** Activity budget t-test results comparing mares with foals and mares without foals (asterisks indicate that equal variance not assumed). Analysis was conducted with arcsine square root transformed data while percentages are for untransformed data.

We also found that contraceptive status made a significant difference for some of the same activities as the presence of a dependent foal and an additional activity. Control mares were recumbent for significantly less time than contracepted mares ( $p=0.005$ , Table 2) with an average of 1.8% less time spent recumbent. Control mares also spent significantly less time trotting or running ( $p<0.001$ , Table 1) with no time spent trotting or running compared to 0.4% of time trotting or running for contracepted mares. Control mares spent significantly less time walking than contracepted mares ( $p=0.016$ ) with an average of approximately 2.8% less time spent walking. There was no significant difference in time spent grazing for control mares but the trend paralleled the difference seen with mares with a foal with control mares spending approximately 6.4% more time grazing. There was no significant difference in time spent standing or vigilant.

<b>Activity</b>	<b>t</b>	<b>df</b>	<b>p-value</b>	<b>Mean for control <math>\pm</math> SE (%)</b>	<b>Mean for contracepted <math>\pm</math>SE (%)</b>
Graze	-1.33	59	0.190	70.5 $\pm$ 4.0	64.1 $\pm$ 1.8
Recumbent	3.08*	26.74	0.005	0.2 $\pm$ 0.2	2.0 $\pm$ 0.5
Stand	-1.12	59	0.903	21.1 $\pm$ 3.7	20.6 $\pm$ 1.4
Trot/Run	5.07*	52	<0.001	0	0.4 $\pm$ 0.1
Vigilant	1.16	59	0.252	0.4 $\pm$ 0.3	0.9 $\pm$ 0.2
Walk	2.64	19.51	0.016	6.8 $\pm$ 0.5	9.0 $\pm$ 0.5

**Table 2:** Activity budget t-test results comparing control mares and contracepted mares (asterisks indicate that equal variance not assumed). Analysis was conducted with arcsine square root transformed data while percentages are for untransformed data.

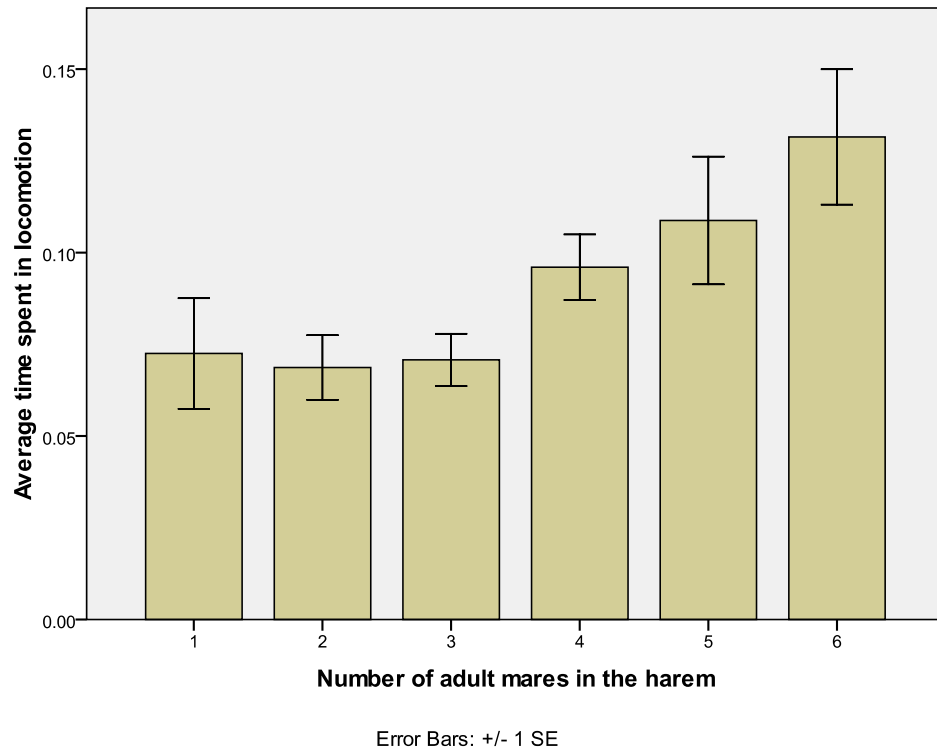
### *Models*

Locomotion was best predicted by the number of adult mares in the harem (Table 3), with higher numbers of mares resulting in more locomotion (Figure 2). We found significant effects of contraceptive status (Figure 3) and harem stallion on all other tested activities (Table 3). We also found that the total

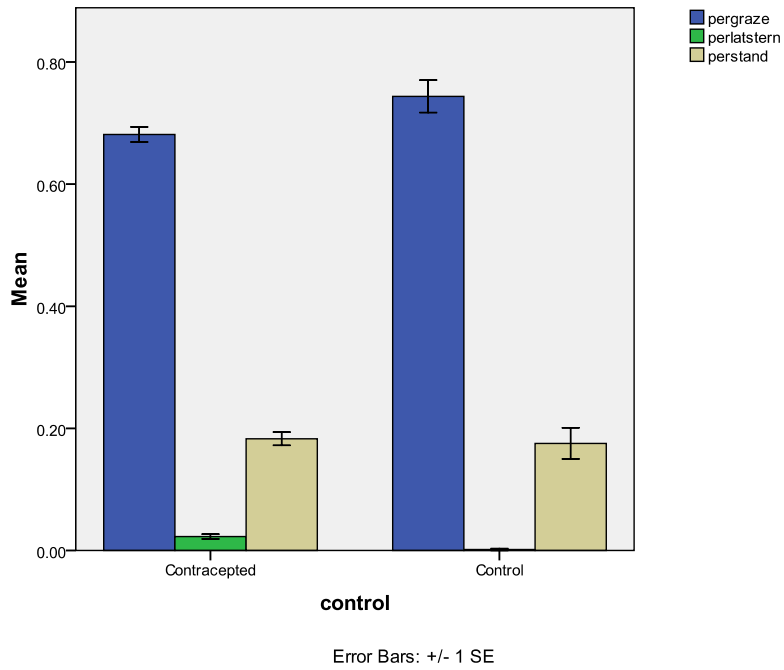
number of horses in the harem (Figure 4) and the interaction between total number of horses and the harem stallion were significant predictors for both time spent grazing and standing. For grazing and standing the best model also included either the interaction between contraceptive status and total number, or contraception and stallion. For recumbent the best fit model included stallion, contraceptive status, and the interaction between the two. Body condition and age in any combination did not add predictive ability to any of the best fit models and did not individually predict activity better than any of the best fit models, although body condition was nearly as predictive as number of adult mares on locomotion.

<b>Activity</b>	<b>Best model(s)</b>	<b>AICc</b>	<b>weights</b>
Graze	Stallion, Contracept, Total # in harem, Stallion*Total, Contracep*Total	710.14	.500
	Stallion, Contracept, Total # in harem, Stallion*Total, Contracep*Stallion	711.87	.211
Recumbent	Stallion, Contracept, Contracept*Stallion	-724.75	.738
Stand	Stallion, Contracept, Total # in harem, Stallion*Total, Contracep*Stallion	690.52	.444
	Stallion, Contracept, Total # in harem, Stallion*Total, Contracep*Total	692.39	.174
Locomotion	Number of Mares	-91.54	.616
	Body condition	-89.72	.248

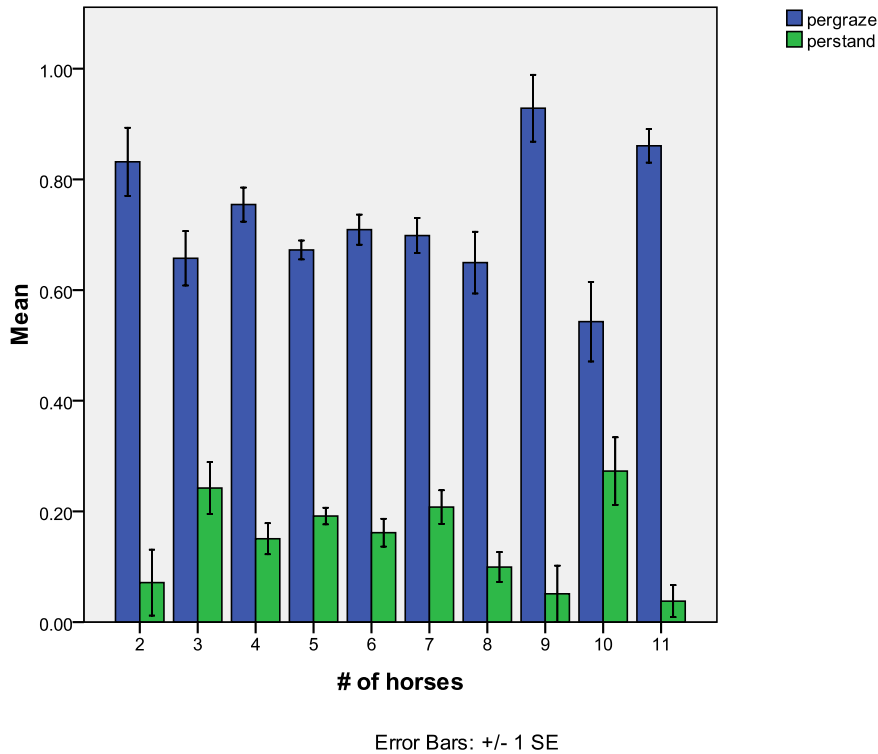
**Table 3:** Best fit models for each activity tested (all models with a  $\Delta AIC_c$  of less than 2 shown).



**Figure 2:** Graph showing the difference in locomotion between different harem sizes (as calculated by number of adult mares including the focal mare). Analysis was conducted on transformed data while the graph shows untransformed data. Graph shows data points used in the analysis without harem sizes with less than 5 observations.



**Figure 3:** Graph showing the effect of contraceptive status on percentage of time spent grazing, recumbent, and standing. Analysis was conducted on transformed data while the graph shows untransformed data.



**Figure 4:** Graph showing the effect of total number of horses in the harem (including the harem stallion and the focal mare) on percentage of time spent standing and grazing. Analysis was conducted on transformed data while the graph shows untransformed data.

## Discussion

### *T-tests*

T-tests indicate that for a variety of behaviors the presence of a foal had a significant impact on activity budget. The most dramatic difference in time spent between mares with a foal and those without was in time spent grazing. This is consistent with the increase in energy requirements in the last three months of gestation and first three months of lactation (Pagan, 1998) although another study in plains zebras found no difference in time spent grazing and an increase in bite rate for lactating female to compensate for increased energy demands (Neuhaus and Ruckstuhl, 2002). Our results parallel results

from Boyd (1988) in captive Przewalski horses that mares with foals spent significantly more time grazing, although we found no difference in time spent standing but did find a difference in time spent recumbent, with mares with foals recumbent less than those without foals (Table 1). Neuhaus and Ruckstuhl (2002) also found that plains zebra mares with foals spent less time recumbent than mares without foals, but found no other differences in activity budgets. These slightly different results may be due to differences between species including the need for zebras to monitor for predators (Neuhaus and Ruckstuhl 2002) or environmental factors such as supplemental feeding and captivity (Boyd 1988).

The results of the t-test also indicate that contraceptive status had a significant impact on activity budgets. Control mares spent significantly less time moving (both walking and trotting or running) and recumbent, and spent more time grazing although the difference for grazing (6.8%) was not significant. This parallels results from activity budget studies of PZP effect on white-tailed deer behavior (McShea et al., 1997) where contracepted deer spent more time active, but did not differ in feeding. On the other hand, it contradicts studies in free-ranging elk and feral horses indicating no differences in activity budgets due to contraception (Heilmann et al., 1998; Powell, 1999).

#### *Activity budget Models*

Our results indicate that the effect of treatment with immunocontraception and total number of individuals in the harem may be more important to activity budgets than previously thought (Ransom et al., 2010) and that harem stallion also plays an important role in activity budgets. Both immunocontraception and harem stallion were factors in the best fit models for graze, recumbent, and stand, while total number of individuals was a factor for both graze and stand. Interactions between these factors were also significant predictors of graze, recumbent, and stand. Ironically, the one activity we measured that Ransom et al. (2010) found to be best predicted by treatment effect was predicted best in our population by the number of adult females in the harem. However, Ransom et al (2010) did not consider the effect of the harem stallion or number of adult females (only total number of individuals),

nor did they include many interactions or multiple effects in their model analysis. Thus these results may not contradict their results, but offer more detailed insight into their suggestion that “body condition data exhibited considerable variation suggesting that other factors we did not measure may also influence time budget behaviors (Ransom et al., 2010).” We were unable to include more than one indicator of body condition due to limitations in collecting body condition data, but further work should consider the change in body condition over the breeding season and investigate if changes in body condition result in changes in activity budgets.

### *Immunocontraception*

The inclusion of contraceptive status as one of the predictor variables in all but one of the best fit models suggests that contraception significantly alters activity budgets. Control mares tended to graze more and stood less and were recumbent less than contracepted mares (Figure 3). This is reinforced by the significant differences in multiple activities comparing control and contracepted mares using t-tests. Additionally, contraceptive status was a better indicator in all best fit models than presence of a dependent foal, suggesting that the presence of a foal does not ameliorate the behavioral difference caused by the contraception. This suggests that the contraceptive (even when not active) has more of an impact than the natural reproductive state, reinforcing the finding that the behavioral effects of contraception last longer than the physiological contraceptive effect (Madosky et al., 2010). Contracepted mares may suffer more harassment from males to breed, reducing the time they are able to spend grazing. However, the sample size of mares with a foal that had been previously contracepted was very low, so future work is needed to determine the effect of foaling on previously contracepted mares.

The activity budget differences found in this study indicate that synchronicity of activity within social groups may be altered by the immunocontraceptive. Likewise, the inclusion of contraceptive status in the best fit model for all activities but locomotion indicate that the immunocontraception is an important predictor of activity budgets. Neuhaus and Ruckstuhl (2002) argue that synchronicity may be a



requirement for stable harem living; our results indicate that the contraceptive may be altering a key component that promotes harem stability. Conflicting mare needs within the harem may lead to mares separating from the harem in an attempt to find a harem with mares that have needs that mirror their own needs. For example, lactating mares may require more trips to watering holes than non-lactating mares, while non-lactating mares may prefer to spend their time in areas that offer good grazing. In untreated populations of feral horses between 57 and 81.4% of mares over the age of two were found to be pregnant ((Seal and Plotka, 1983; Wolfe et al., 1989), while during the study year in our population only 15 out of 60 adult mares (25%) foaled. Since activity budgets differ between mares with foals and mares without foals and between treated and untreated mares, these significant differences in activity budget may lead to the higher propensity of contracepted mares to change harems in this population (Nuñez et al 2009, Madosky et al. 2010).

While mares without foals may have always had different activity budgets, the fact that the proportion of mares that foal is dramatically reduced by the immunocontraceptive may accentuate these differences enough to elevate harem instability. Additionally, the contraceptive had more of an effect on activity budget than the presence of a foal so the contraceptive may result in larger inequities in activity budgets than natural differences between mares with and without foals. Alternatively another factor, such as harassment, that is also altered by the immunocontraceptive may cause both activity budget differences and elevated harem changes. More research needs to be conducted in order to determine the mechanism by which immunocontraception may reduce harem stability and to determine if these factors are location specific or vary in different ecological settings.

#### *Group size*

Total number of individuals in the harem (including the harem stallion) was a better predictor than number of mares in the harem for graze and stand, while number of mares in the harem was the single best predictor of all variables for locomotion. An increase in the number of mares in the harem led

to an increase in locomotion (Figure 2), while the results for the effect of the total number of individuals in a harem on time spent grazing and standing was less clear. Mares in harems with just themselves and the harem stallion and mares in harems with over 7 other individuals tended to graze more and stand less with the notable exception of mares in harems with 8 other individuals (Figure 4). The amount of time spent grazing did not diminish with an increase in total harem size as would be expected if there were increasing competition from conspecifics (Rubenstein, 1986) confirming that competition for forage is low within feral horse harems (Rubenstein, 1994). There were not enough harems of varying size to fully explore this result and the differences in graze and stand may be due to small sample size. Further work with addition harems of varying sizes is needed to illuminate the effect of group size on grazing and standing behavior.

#### *Harem stallion*

Harem stallion was an important factor for the percentage of time spent grazing, recumbent, and standing. This indicates that the individual stallion a mare associates with may have a strong impact on her ability to obtain enough energy given that energy intake in horses is limited mostly by time spent grazing (Rubenstein, 1994). Thus, females may be motivated to change harems to a different male to obtain more time grazing and thus more energy. Previous work indicates that control mares change harems less than contracepted mares (Nuñez et al., 2009; Madosky et al., 2010) and we found that control mares tended to graze more than contracepted mares and that mares with foals grazed significantly more than mares without foals ( Tables 1 and 2). Since most control mares foal every year and thus require more energy (Boyd, 1988) they may stay with stallions that allow them to graze for longer periods of time. Alternatively, control mares or mares with foals may change harems less because they are less willing to reduce time grazing by attempting to move to another harem. These factors were beyond the scope of this investigation, but we plan to analyze the role activity budgets play in harem changes further in the future.

The interaction between harem stallion and total number of harem members and between stallion and contraceptive status was a factor in the best fit models for graze, recumbent, and stand. These interactions are hard to interpret as mares in different harems had different activity patterns related to group size and contraceptive status. These differences may be due to harassment rates of different stallions, dominance of individual stallions, or different male strategies of harem management. The difficult to interpret effect of group size may in fact be more linked to individual stallion management style that leads to both the group size and the differences in activity budget. Rubenstein (1994) suggests that harassment rates by males play a significant role in limiting grazing time and that dominant stallions harass females less, thus resulting in an increase in grazing time for mares with dominant stallions. Rubenstein (1986) also suggests that when territoriality was present on the island (it is not present currently) mares in harems with territorial males suffered less harassment from intruding stallions than mares in non-territorial harems and thus were able to graze more. The harem management style of individual stallions may also influence harem changes by mares; some stallions tend to allow more freedom within their harems while others control mare movement more. Harassment rates were not analyzed in this study due to their rare occurrence, but another study we conducted on the same population indicates that harassment is an important factor in motivating harem changes (Chapter 3).

## Conclusions

Our study found that immunocontraception is an important factor in the activity budgets of feral horses, along with harem size and the harem stallion. Our results indicate that the best models for predicting activity budgets are in most cases more complicated than previously assumed. While the presence of a foal was important for several activities in simple t-tests, contraceptive status was a better predictor of activity budgets in the models. The importance of harem stallion suggests that mares may be motivated to change harems in order to change their activity budget. Further work is needed to evaluate the effect of the harem stallion on activity budgets, including the influence of harassment on harem changes. Overall, this study suggest that the factors involved in activity budgets in feral horses are more

complicated than previously shown and that immuncontraception has an important influence on activity budgets.

## Chapter 3: Harassment and Harem Stability in a Feral Horse (*Equus caballus*) population

### Abstract

Feral horse harems are generally stable and provide female members with reproductive benefits, but studies have still noted harem changes among feral horse herds. Treatment with PZP immunocontraception has been shown to increase harem changes, though the mechanism for this increase is not entirely clear. One potential driver of harem changes is harassment costs resulting from both male and female harassment to harem members. Mares may then attempt to change harems in order to minimize the harassment costs. In this study, we tested the relationship between harassment rates and harem change rates in order to determine if harassment drives harem changes. Higher stallion harassment rates were strongly correlated with higher harem change rates and higher harassment rates by other mares was also correlated with higher harem change rates. Mares that changed harems were harassed more than mares that stayed in harems. Treated mares were harassed more than control mares and mares that stayed in their home harem were harassed less than mares that moved from their home harem. There were no significant differences in harassment rates before and after harem changes. These results indicate that high harassment rates may cause mares to change harems, but that mares are generally unable to assess relative harassment rates in prospective harems potentially leading to multiple harem changes as mares attempt to minimize harassment.

## Introduction

Feral horses generally form stable social groups in which males defend harems of adult females and their offspring (Klingel, 1975; Rubenstein, 1981; 1986; Linklater, et al. 2000). However, multiple studies have noted harem changes by adult females despite the advantages of stable harem membership (Rubenstein, 1981; Stevens, 1990; Blumenshine et al., 2002; Nuñez, 2009; Madosky et al., 2010). Harem stability is positively correlated with both yearly (Rubenstein, 1986) and lifetime reproductive success in mares (Kaseda et al., 1995). Lower rates of harem change are associated with higher likelihood of reproduction and foal survivorship to independence (Rubenstein and Nuñez, 2009). Mares that have low harem change rates may be able to devote more time to grazing and thus are better able to support a foal (Rubenstein, 1986). Harem stability is positively related to fitness in feral horses and instability may significantly reduce mare fitness.

In general harem changes are relatively rare, but in the Shackleford Banks island, N.C. population, harem changes have increased from 1974, in which 10% of mares changed harems over the course of a season (Rubenstein, 1981) to 2007-8, when 66 and 73% of mares changed harems (Madosky et al. 2010). One potential explanation for the increase in harem changes is the implementation of an immunocontraception program as part of the management strategy by the National Park Service (NPS). The immunocontraceptive controls the population growth of the horses and protects the barrier island ecosystem, but contracepted mares in this population have been found to change harems more frequently than control mares in both the nonbreeding (Nuñez, 2009) and breeding season (Madosky et al., 2010). Though the harem changes in this population are well documented and are consistent with the idea that the immunocontraception can affect social stability in horse harems, it is unclear what behavioral mechanism drives this reduction in harem stability. Given the high harem change rate found in this population, the population affords a unique opportunity to investigate the mechanisms that drive harem changes.

One potential mechanism that may reduce harem stability is increased harassment. Harassment by males is costly to females because it reduces the time mares are able to spend grazing and thus reduces their energy intake (Rubenstein, 1986). Rubenstein (1994) and Linklater et al. (1999) suggested that the harem-defense mating strategy of feral horses may have evolved in order to reduce harassment costs. Linklater et al. (1999) also suggested that mares that change harems may have lowered body condition due to increased harassment, but did not track harassment of individual mares and their harem change rate. Evidence from other studies is equivocal; Linklater et al. (1999) suggested that mares suffer more harassment in multiple-stallion bands than in single stallion bands and thus incur higher harassment costs, but Stevens (1990) found that mares only left single stallion bands. If harassment is costly for females we would expect mares experiencing high harassment rates to change harems in an effort to reduce harassment costs. However, harem changes themselves could result in increased harassment. Females that are new to a harem suffer from a temporary increase in female harassment (Rutberg and Greenberg, 1990) and males often harass females when they first join a harem (J.M., personal observation). Mares may leave a harem in which they experience high rates of harassment, but may not always reduce harassment by doing so, and this may promote repeated harem changes by mares seeking a favorable social environment with reduce harassment.

We used individual level data on mares on Shackleford Banks island to determine if mares change harems in order to reduce harassment by both harem stallions and other mares. We first tested if there was a correlation between either stallion or mare harassment rates and harem change rates to determine if high harassment rates were associated with high rates of harem change. We then tested the differences in harassment rates of mares that never changed over the season vs. mares that changed at least once in the season, harassment rates of contracepted mares vs. control mares, and the rate of harassment in the home harem for mares that remained in the home harem over the season vs. mares that moved from their home harem over the season. Finally, we tested the harassment rates of mares before and after they changed harems to determine if mares were able to reduce harassment rates by changing harems.

## Methods

### *Study Area and Study Subjects*

A feral horse herd located on Shackleford Banks, N.C., USA in Cape Lookout National Seashore was observed for this study. The study area and subject for this study are described in detail in Madosky et al. (2010) and Rubenstein (1981). There were approximately 120 individuals on Shackleford Banks island over the two seasons of this study, organized into harem groups consisting of one or two stallions and at least one mare, single bachelor stallions, or bachelor groups of young stallions. Both sexes disperse from their natal harems, but young females join other harem groups after dispersing while young males tend to join bachelor groups (Klingel, 1974; Rubenstein, 1986). Population growth on the island is managed via PZP immunocontraceptive. The immunocontraceptive program was initiated in 2000 (Nuñez et al., 2009) and administered by the National Park Service. The immunocontraception procedure is described in detail in Madosky et al. (2010). For the purposes of this study contracepted mares are defined as mares that have been treated at any point with the contracepted mares while control mares are mares that have never been treated with immunocontraceptives.

### *Behavioral Sampling*

Harem membership data and harassment data were collected by the primary investigator (J.M.) and multiple observers trained and supervised by the primary investigator. Observations were collected during the breeding seasons of 2007 and 2008 between May 22<sup>nd</sup> and July 29<sup>th</sup> in 2007 and between May 22<sup>nd</sup> and August 12<sup>th</sup> in 2008 on all mares on the island. Limited observations on control mares, and contracepted mares associated with them, were collected in the breeding season of 2009 between May 26<sup>th</sup> and August 4<sup>th</sup> 2009. The 2009 data was only used for Wilcoxon Signed Ranks Tests of harassment rates before and after harem changes. Observers collected harem composition (including all members of the harem), GPS location of the harem, and ad lib data on harassment of mares by both the stallion(s) and other mares. Individuals were identified by freeze brand, markings, color, and sex. Harassment rate per



season was calculated by dividing the number of harassment events for that individual (the number of times the individual was harassed) by the number of hours of observation for that individual. A separate harassment rate was calculated for harassment by stallions and for harassment by other mares. Harem change rate was calculated by dividing the number of harem changes over the season for that individual by the number of times the individual was positively identified.

### *Statistical Analysis*

All analysis was conducted with SPSS 17.0. In order to standardize the data we used the harassment rate per season and the harem change rate per season in all analyses. All outliers (defined as having a Z score above 3.3) were eliminated for all analysis. One outlier was found in the 2007 data and two were found in the 2008 data. Results without outliers were similar to results with outliers so all analyses and graphs are presented with outliers eliminated in order to eliminate any potentially undue influence of the outliers.

#### Harassment rates and harem change rates

We tested the relationship between harassment and harem changes using correlation rather than regression because we cannot be sure if harassment rates result in higher harem change rates or if higher harem change rates result in higher harassment rates. We performed Pearson correlation analysis on the stallion harassment rates for each year and the harem change rate for the same year to determine if there was a relationship between stallion harassment and harem changes. We also performed Pearson correlations analysis to determine if there was a relationship between harassment from other mares in the harem and rate of harem changes. Wilcoxon Signed Ranks Tests were performed to determine if there was a difference between harassment rates by other mares for 2007 and 2008.

#### Contrasts of harassment rates

We used Mann-Whitney U tests to assess the significance of differences in harassment rates by stallions in different groups. We compared mares who stayed in the same harem for the entire season and mares who changed harems at least once throughout the season, control mares and mares that had been treated with contraceptive at any time in the past, and mares that stayed in their home harem either for the whole sampling season or after moving to that harem within the season and mares that moved in and out of their home harem. Home harems were defined as the harem a mare was associated with for the clear majority of the sightings (defined as 60% or more observations over the season). Only mares with at least 200 minutes of observation time with the stallion analyzed were used for analyses in order to insure that enough observation time was spent with the harem to observe potentially rare harassment events. We analyzed harassment rates for both 2007 and 2008.

#### Harassment rate before and after harem changes

We performed Wilcoxon Signed Ranks Tests to analyze differences in harassment rates before and after harem changes. For these analysis only mares that had at least 200 minutes of observation time with one stallion and at least 200 minutes of observation time with a different stallion immediately following the first stallion were considered. We compared harassment rates for 2007, 2008, and 2009 separately and with all years pooled. In 2007 and 2009 there was a mare that moved to three harems consecutively that fit the criteria for analysis (ie., A mare spent 200 or more minutes in three different harems in succession). In these cases analysis was performed both with and without these consecutive subsequent observations. The inclusion of these cases did not change the interpretation of the results and thus were included to maximize the power of the analysis.

## Results

#### *Stallion and mare harassment rates and harem change rate*

In both 2007 and 2008 there was a significant correlation between harassment rate of mares by stallions and the harem change rate (2007 Pearson Correlation=0.512, n=63, p<0.001.; 2008 Pearson

correlation=0.425, n=64, p<0.001). In both cases, higher harassment rates by stallions was associated with higher harem change rates by mares. In 2007 there was also a significant correlation between harassment rate of mares by other mares and harem change rate (Pearson Correlation=0.299, n=62, p=.018). Higher harassment rates by other mares were associated with higher harem change rates by the harassed mare. In 2008 there was no significant correlation between harassment rate by other mares and harem change rate (Pearson Correlation=0.043, n= 63, p=0.739). Mare-mare harassment rates were significantly lower in 2008 than in 2007 (Z=-2.475, p=0.013) which may account for the difference seen between the years.

#### *Harassment rate differences among groups*

Mares who stayed in a harem for the entire season (stayers) were harassed by the stallion significantly less than mares that changed harems over the course of the season (movers) in 2007 (Z=-3.76, p<0.001) (Table 4). In 2008 the difference was not significant (Z=-1.786, p=0.074), but the trend remained the same, with stayers harassed less than movers (Table 4). Control mares were harassed significantly less by the stallion than contracepted mares in 2007 (Z=-2.27, p=0.023) and the same trend was seen in 2008, though the difference was not significant (Table 4). Mares that stayed in their home harem were harassed significantly less by the stallion in their home harem than mares that moved from their home harem in 2007 (Z=-3.44, p=0.001). Once again this difference was not significant for 2008, although the trend remained the same (Table 4).

<b>Categories</b>	<b>n</b>	<b>Year</b>	<b>Z</b>	<b>p-value</b>	<b>mean harass/hr</b>
Stayers vs Movers	22, 42	2007	-3.76	<0.001	.42±.06, .83±.06
	17, 47	2008	-1.79	0.074	.93±.14, 1.2±.09
Control vs Contracepted	9, 57	2007	-2.27	0.023	.39±.10, .73±.06
	8, 56	2008	-1.64	0.100	.84±.21, 1.2±.08
Stayed in home harem vs.	24, 20	2007	-3.44	0.001	.42±.06, .98±.12
Moved from home harem	23, 10	2008	-1.65	0.100	.91±.10, 1.2±.18

**Table 4:** Results from Mann-Whitney U tests for differences in harassment rates for mares that stayed in their harem the entire season (stayers) and mares that moved at least once over the season (movers), mares that had never been contracepted (control) and mares that had been treated with contraceptives at some point (contracepted), and mares that stayed in their home harem for the season once they reached it (stayed in home harem) and mares that moved out of their home harem at least once over the season (moved from home harem) including mean harassment rate (harassment events per hour) ± SE.

*Harassment rate differences between harems*

Only 19 mares over the three years of observation met the criteria of associating with two harems consecutively for 200 minutes or more; six in 2007, seven in 2008, and six in 2009. There were no significant differences in harassment rates in the harem that mares left and the harem they subsequently joined for any of the three years or for the three years pooled (Table 5).

<b>Year</b>	<b>Z</b>	<b>P</b>	<b>mean harass/hr</b>
2007	-1.153	0.249	.46±.33, .97±.34
2008	-1.524	0.128	1.1±.27, .77±.11
2009	-0.314	0.753	1.0±.23, .89±.32
All years	-0.483	0.629	.89±.17, .87±.15

**Table 5:** Results from Wilcoxon Signed Ranks Tests for harassment rates before and after harem change for 2007, 2008, 2009 and all years pooled with mean harassment rate (harassment events per hour) ± SE before and after change.

## Discussion

The strong correlation between high stallion harassment rates and high harem change rates for both study years suggests that there is an important relationship between stallion harassment and the probability of harem change by mares. Harassment by other females in the harem was also significantly correlated with higher harem change rates for 2007. In 2008 no significant correlation was found, though this may be due to the significantly lower frequency of mare harassment in the 2008 season compared to 2007. Harassment from other females is a relatively rare event, so we simply may have been unable to observe enough instances of harassment in 2008 to confirm the 2007 results. It is unclear from these data if higher harassment rates drive higher harem change rates or if higher harem change rates result in higher harassment rate. Harassment may impose a large energy cost on mares as it does in other species (Boness et al., 1995; Schlupp et al., 2001), leading to harem changes in an effort to minimize harassment rates. Previous studies have indicated that mares entering a new harem experience higher harassment by mares already in the harem (Rubenstein, 1986; Rutberg and Greenberg, 1990) and mares entering a harem are often investigated more by the harem stallion (J. M., personal observation), potentially leading to higher harassment rates.

Unsurprisingly, given the strong correlation between harem change rate and stallion harassment rate, mares that stayed in a single harem over the course of the season were harassed by the stallion significantly less than mares that changed harems. Mares that were never contracepted were harassed significantly less by the stallion than mares that had been contracepted at any time. Previous work has shown that contracepted mares change harems more often than control mares (Madosky et al., 2010; Nuñez et al., 2009) but it is again unclear if the higher harem change rate drives the higher harassment or if higher harassment of contracepted mares drives those mares to change harems more often.

However, the significant difference in harassment rates experienced in home harems by mares that subsequently moved compared to mares that stayed provides direct evidence that harassment is related to decisions by mares to change harems. Mares that stayed in their home harem over the course of

the season experienced significantly less harassment than mares that changed harems in 2007, and a similar, though non-significant, trend was observed in 2008. This supports the idea that harassment rates may motivate harem changes by mares.

The lack of differences in harassment rate experienced by mares in the harems they moved to compared to those they moved from (Table 5) may indicate that mares are unable to accurately assess the harassment rate they will incur from a different harem. As a result, mares seeking to leave a harem may need to compare several different harems before finding one with lower harassment rates. This is consistent with a number of instances in which a mare changed from one harem to another and then returned to her original harem. Alternatively, mares may change harems for a variety of reasons apart from harassment rates, such as male quality or social interactions not directly related to harassment. Work on primates suggests that mature western lowland gorilla and Thomas langur females change groups in order to associate with a male better able to protect infants from male infanticide (Stokes et al., 2003; Sterck et al., 2005)

Earlier work has suggested that harem changes are influenced by contracepted status (Madosky et al., 2010; Nuñez et al., 2009) and asynchronicity of activity budgets that may result from the contraception program (Chapter 2). The results of this study indicate that harassment by the harem stallion may also play an important role in harem changes and that social interactions among harem members are one of the factors that influences harem fidelity. Further work aimed at quantifying the relative importance of these factors on individual decisions to change harems would be of significant value.

## Conclusions

Our study found that there is a strong correlation between stallion harassment rates and harem change rates, with higher rates of harassment associated with higher harem changes. We also found that harassment by other mares in the harem is associated with higher harem change rates. Mares that did not

change over the course of the season were harassed less by the stallion than mares that did change and control mares were harassed less than contracepted mares. Mares that stayed in their home harem were harassed less by stallions than those that left, indicating that harassment may drive harem changes. No significant differences were found between harems a mare left and the new harem she joined, indicating that mares may not be able to accurately assess harem rates in different harems and are testing new harems. Overall, this study indicates that social interactions between the stallion and his mares and between mares in the harem may have a strong influence on the likelihood harem changes.

## Discussion

Contraceptive use can help wildlife managers manage protected populations at sustainable levels, reduce the abundance of predator species, prevent the spread of diseases, and reduce human-wildlife space conflicts (Kirkpatrick and Turner Jr, 1985). In captive populations, contraceptives can be used to control breeding while allowing animals to remain in natural social groups instead of same-sex colonies (Powell, 1999). Population sizes can be controlled with trapping, relocating, poisoning or culling animals, but these solutions are often expensive, regarded as inhumane, potentially dangerous to non-target species, non-reversible, and generally opposed by the public (Kirkpatrick and Turner Jr, 1985). As a consequence, contraceptive agents are more widely accepted by the public than many other population control methods and various contraceptive agents have been tested for canids, birds, felids, rodents, ungulates, and equids (Kirkpatrick and Turner Jr, 1985).

Although there are clear advantages to immunocontraception, it is critical to also recognize the potential drawbacks of any management strategy. The preservation of behavior in a managed species is often particularly overlooked (Clemmons and Buchholz, 1997) but it is important to preserve natural behavior in managed species. The studies presented in this dissertation, along with work by another author (Nuñez, 2009), make it clear that PZP immunocontraception alters behaviors that are fundamental to the social structure of feral horses and has lasting effects that may reduce individual fitness across the population.

The studies in this dissertation show that PZP immunocontraception alters harem fidelity in the breeding season and that this effect is persistent beyond the physiological contraceptive effect. Mares that are contracepted seem to change behavior in persistent and not readily reversible ways. Unfortunately, this finding eliminates the possibility of removing or reducing the effect of PZP on behavior by manipulating the contraception schedule. At this point pregnancy does not seem to ameliorate the effect of PZP on harem changes, but more work should be conducted with a larger sample size of previously treated mares to confirm this result.



The mechanism by which PZP alters harem stability is not entirely clear, but the immunocontraceptive does appear to alter both activity budgets and harassment rates by the harem stallion. Treated mares graze less than control mares and are harassed more by harem stallions. A reduced need to graze and an increase in both resting behaviors and locomotion in treated mares results in time budget asynchronicity that may reduce social cohesion within the harem. The increased harassment rate from harem stallions may provide an incentive for mares to leave the harem in order to find a harem in which harassment is reduced. Given that mares seem to be generally unable to assess harassment in different harems and do not reduce harassment by changing harems, contracepted mares may continue to switch harems until they find a harem with lowered harassment. Both the dramatic increase in harem changes by Shackleford Banks mares since the implementation of the immunocontraceptive program and the high percentage of harems affected by harem changes indicate that the reduction in harem stability is affecting the entire population.

Since PZP immunocontraceptive has been widely adopted as a population control method in numerous species (ZooMontana, 2000), it is important to investigate whether the contraceptive has unforeseen deleterious effects in other species. Studies in white-tailed deer have also found differences in activity budget between treated and untreated does (McShea et al., 1997), though studies in elk (Heilmann et al., 1998) and another feral horse population (Ransom et al. 2010) did not find differences in activity budgets between treated and untreated individuals. Other species with differing social structures may not be affected in the same way as feral horses. Male harassment would be higher in species with long term associations of males and females; sexual segregation may provide less opportunity for males to harass females and making the effects of immunocontraception on harassment less important. However, in any species in which there is group living or male harassment of females this contraceptive may alter fundamental behaviors important to individual fitness. For example, though elephants have a different social structure in which females form groups without a harem male, time budget asynchronicity between pregnant or lactating untreated females and treated females with different energy demands may reduce

social cohesion and potentially lead to fragmentation of social groups. Further study is needed to determine if PZP can influence social cohesion in other social systems and if activity budget disruption is strong enough to reduce social cohesion.

One of the strengths of the studies presented in this dissertation is the fine temporal resolution of the data and the ability to track every individual in the population. Given that some mares changed harems more than once a day and that harem changes were often back and forth between nearby harems, this temporal resolution was critical to understanding the behavior of the population. Additionally, I was able to track every individual in the population over two field seasons. It may not be possible to observe large numbers of individuals daily over long time periods in all populations, but I feel that it is critical to assess the behavioral impacts of this contraceptive method. It is possible that the primary reason that effects of PZP have not been found in other populations is the lack of temporal resolution in the behavioral record.

The harem instability caused by the immunocontraceptive program provided a unique opportunity to investigate the motivation for harem changes and the factors that affect the formation of social groups. I examined both environmental factors and social factors in assessing harem changes across the population. In the activity budget model analysis I was able to model a variety of factors that may influence activity budgets (including age, contraceptive status, harem stallion, presence of a dependent foal, number of adult mares in the harem, total number of harem members, and body condition) and the interactions between these factors. Previous work did not consider some of these variables and did not consider many interactions (Ransom et al. 2010), so this analysis provides a more in-depth view of the factors that may influence time budgets. In the harassment analysis, I was able to consider individual level harassment data and the corresponding harem change rate in order to assess the influence of one important social interaction in harem changes. Previous work has not assessed this relationship at the individual level (Linklater et al., 1999), so the harassment analysis provides unique insight into the role of harassment in motivating harem changes.

Neuhaus and Ruckstuh (2002) argue that synchronicity of activity budgets is a requirement for the formation of stable social groups. My activity budget analysis supports this argument; I found significant differences in the activity budgets of treated vs. untreated mares in a population with higher than expected instability. The asynchronicity of time budgets in this population caused by immunocontraception and the resulting lack of foals may be one of the factors that influence harem changes, but it is important to note that there were other factors that were directly related to activity budgets and to harem changes in the population. The activity budget models indicated that the number of individuals in the harem was an important indicator of some activities (graze and stand). In addition, the number of mares in the harem was the best predictor for locomotion; as the number of mares increased so did the time spent in locomotion. Given the asynchronicity of activity budgets for mares in different reproductive states, it may be that with a higher number of mares in a harem there was a higher chance that some of the mares would have conflicting needs resulting in more movement. For example, lactating mares require more water than mares without dependent foals, and so may initiate movements to water holes in order to gain this resource. Then mares without foals may initiate movements back towards desirable resting or grazing areas.

The harem stallion was an important predictor for time spent grazing, recumbent, and standing. This indicates that the individual stallion influences a mare's foraging success, lending support to Rubenstein's (1994) theory that mares can benefit from associating with a particular stallion. The interaction between harem stallion and total number of harem members and between stallion and contraceptive status indicate that there is significant interaction between social factors such as harem stallion and non-social factors such as contraceptive status. The interactions were difficult to interpret from the activity budget models alone, but the harassment study indicates that differing levels of harassment may influence harem changes. Stallion harassment almost always results in movement by the mare and disturbs a mare from her chosen activity (personal observation). Harassment may thus reduce time spent grazing. Unfortunately, harassment is relatively rare, so it was not possible to investigate the

relationship between harassment and grazing, but further work to determine if mares in harems with higher rates of harassment spend less time grazing would illuminate the relationship between harassment and harem changes. Harassment could directly influence harem changes in so far that mares that are harassed change harems to avoid harassment. Harassment could also indirectly drive harem changes by reducing the grazing time of mares and thus causing them to change harems to increase grazing time.

Rubenstein (1994) and Linklater et al. (1999) suggested that harassment by stallions is one of the driving factors in harem formation as mares seek to avoid harassment by associating with a single stallion that will defend them from other stallions. Given that the harem stallion also harasses his females there is a benefit to belonging to a harem only if harassment of harem members is lower than harassment for mares not affiliated with a harem. Mares are rarely seen on their own, and usually change from one harem to another nearby harem (personal observation) suggesting that it is better to be with a harem stallion with a relatively high rate of harassment than to be without a harem stallion.

The inability of mares to reduce harassment rate when changing harems suggests that mares are unable to assess harassment in harems before changing and may be forced to test different harems in order to find a harem with low harassment. This sampling behavior may be similar to foraging sampling and risk-sensitive foraging behavior. Stephens and Krebs (1986) summarize risk-sensitive foraging experiments indicating that some species are “risk-averse when they have positive energy budgets but risk-prone when they have negative energy budgets.” In this case mares may be risk-averse (and thus stay in their harem despite high levels of harassment) when their energy budget is positive or their body condition is high, while they may be risk-prone (and thus leave their harem and sample other harems) when their energy budget is negative or their body condition is low. I was unable to obtain body condition scores for mares throughout the season, but it would be valuable to investigate the parallels between risk-sensitive foraging models and harem changes.

Since activity budget synchronicity may be another important factor for harem formation, the search for a suitable harem may be complicated by the need to find a harem with mares with similar activity budgets as well as a harem with a relatively low harassment rate. These two factors, along with other potential factors, may lead to the high rate of harem changes seen in this population as the mares, their behavior disturbed by the immunocontraception, test harems in an attempt to find a harem with low harassment and high synchronicity.

These studies, along with previous works, suggest that harem changes are affected by the immunocontraception. Immunocontraception alters the reproductive state of mares and thus their energy output since contracepted mares are released from the energetic demands of carrying and nursing a foal. Synchronicity of activity budgets and the ability to maximize energy intake may also affect harem changes, as do social factors such as harassment by other mares and the harem stallion. Harassment may in turn influence the ability of mares to maximize energy intake and may increase their energy output. Further work is needed to investigate the interactions between energy output, energy intake, and harassment rates in motivating harem changes in feral horses.

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## Appendix 1: Co-author permissions



Jessa Madosky <jessa.madosky@gmail.com>

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### IMPORTANT: Permission to use Harem Change Article in my Dissertation

3 messages

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Jessa Madosky <jessa.madosky@gmail.com> Tue, Jul 12, 2011 at 11:33 AM  
To: Sue Stuska <Sue\_Stuska@nps.gov>, Dan Rubenstein <dir@princeton.edu>

I just got an email back from the review department at our graduate school that told me I need to have proof of permission from all co-authors to use the published article (the harem change paper) in my dissertation. An email saying that you are fine with it is all they need, but I can't use it without permission.

If you could send me a quick email granting me permission I would really appreciate it!  
Thanks!  
Jessa

Jessa Madosky  
University of New Orleans  
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---

Dan Rubenstein <dir@princeton.edu> Tue, Jul 12, 2011 at 1:02 PM  
To: Jessa Madosky <jessa.madosky@gmail.com>

Hi Jessa,

Of course you have my permission to use the published harem change paper in your thesis.

best, dan

[Quoted text hidden]

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*Daniel I. Rubenstein*  
*Class of 1877 Professor of Zoology and Chair*  
*Department of Ecology and Evolutionary Biology*  
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Sue\_Stuska@nps.gov <Sue\_Stuska@nps.gov> Wed, Jul 13, 2011 at 1:57 PM  
To: Jessa Madosky <jessa.madosky@gmail.com>

You have my permission!

Sue

## Vita

The author was born in Albuquerque, New Mexico. She obtained her Bachelor's of Science degree in Ecology, Evolutionary, and Behavioral Biology and Philosophy from Beloit College in 2005. She started her work on Shackleford Banks island in the summer of 2005 with a team from Princeton University under the guidance of Dr. Daniel Rubenstein and collaborated heavily with Dr. Sue Stuksa and the National Park Service throughout her field work. She joined the University of New Orleans Conservation Biology graduate program to pursue a Ph.D. in conservation biology with a focus on animal behavior and became a member of Dr. Jerome Howard's lab in 2006.