SOCIALITY OF SABLE ISLAND HORSES: POPULATION, GROUP, AND INDIVIDUAL INTERACTIONS

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ABSTRACT

The social structure of feral horses (*Equus ferus caballus*) is strongly influenced by their female-defense polygynous mating system, and in turn, social structure is known to impact individual fitness. What regulates structure of social groups is not as well understood. I examine how population density and adult sex ratio (ASR) influence sociality in the feral horse population of Sable Island, (Nova Scotia, Canada), and characterize the duration of associations and the differences in social characteristics between reproductive and non-reproductive adult members of the population. I also explore how concentrations of testosterone and cortisol recovered from tail hair relate to physiological and sociological correlates in this free-living population.

Density and ASR influence a number of social characteristics in this population. High local density is associated with larger harems and increased probability of adult females switching between social groups. Male ability to compete for sexual opportunities is reached 2–3 years after females leading to a typically female biased ASR. However, as ASR becomes more neutral or male-biased group size decreases and adult females become less likely to change bands. Foal production decreased with increasingly male-biased ASR while the same conditions improved foal survival. Female reproductive success increased when they maintained long-term associations with specific males and minimized their overall associations. While several studies suggest that female–female associations in the harem are important, I present evidence to suggest that could be a side-effect of females attempting to remain in association with the same male and to avoid antagonistic interactions associated with establishing their position in the social hierarchy of a new group. Investigation of hair cortisol concentrations (HCC) also revealed that the endocrine response to particular stressors may be different between males and females. Top models describing male HCC included only physiological factors of age and body condition and also year. While the best model describing female HCC included body condition, age, and presence of a foal, they also included social variables of harem size and abundance of males in vicinity. Males in reproductive roles as dominant harem stallions had higher hair testosterone concentrations than non-reproductive males. This research provides a better understanding of the interactions between population level processes and indirect actions on individual fitness, opportunities for sexual selection, and endocrinology as they relate to the social and mating structure of an island-bound population of feral horses.

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ABBREVIATIONS

AIC_c Akaike Information Criteria (corrected for small sample size)

ΔAIC_c change in Akaike Information Criteria (corrected for small sample size)

ASR   Adult Sex Ratio

HCC   Hair Cortisol Concentration

HPA   Hypothalamic-Pituitary-Adrenal

HPG   Hypothalamic-Pituitary-Gonadal

HTC   Hair Testosterone Concentration

LL   Log-Likelihood

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Overview and Research Objectives

Recent work in population ecology has brought more attention to the interactions between population and group-level dynamics (Dobson and Oli 2001; Packer et al. 2005; Bateman et al. 2011; Vander Wal et al. 2012; Armitage 2012; Perrin et al 2012). Among social species, individual success in reproduction and survival can be strongly linked with the number, type, and duration of associations an individual maintains (Linklater et al. 1999; Dunbar and Shultz 2010; Silk et al. 2010; Armitage 2012). The evidence suggests that population dynamics may not only influence reproduction and survival through resource competition and reproductive opportunities but also may have indirect influence on fitness through pressures on social structure and resulting behaviour of individuals within the population (Bonenfant et al. 2004; Székely et al. 2014).

Density-dependent impacts on individual fitness and population growth are well studied (Clutton-Brock et al. 1997; Gaillard et al. 2000; Kokko and Rankin 2006; Coulson et al. 2008). More recently, it is recognized that sex ratios also impact fitness and population trends (Le Galliard et al. 2005; Cheney et al. 2012, Marjamäki et al. 2013; Gerber and White 2014; Székely et al. 2014). Biased sex ratios have been shown to increase aggression, destabilize social structures, delay sexual maturation, lower recruitment, and even increase mortality in a variety of taxa (*Microtus canicaudus*, Wolff et al. 2002; *Lacerta vivipara*, Le Galliard et al. 2005; *Papio hamadryas ursinus*, Cheney et al. 2012; *Parus major* , Nicolaus et al. 2012). These impacts will inherently be different among species given the diversity of mating systems (Emlen and Oring 1977; Clutton-Brock 1989). In extreme cases, biased sex ratios can create evolutionary traps, such as when a high number of males negatively affects survival of females perpetuating the bias and threatening population persistence (Le Galliard et al. 2005; Lee et al. 2011; Rankin et al.

2011). Understanding how sex ratios interact with social or mating systems and individual fitness is important as we learn more about how local sex ratios can be influenced by external factors such as climate (Manning et al. 2015), and that many management practices are often biased towards one sex (Rankin and Kokko 2007).

The overarching question of my research examines how population structure, specifically density and sex ratio, might influence group structure and stability and how this may in turn influence individual reproductive success and physiology (Figure 1.1). Using the feral horse (*Equus ferus caballus*) population on Sable Island, Nova Scotia I first look at the relationship between population density and sex ratio to determine whether it influences the social structure (Chapter 2). Using location and association data obtained from annual surveys of the entire population (2008–2015) I compare the density and adult sex ratios measured at different spatial scales to examine whether associations become more or less stable, effects on individual fitness, and influences on group size. Initial work on the Sable Island population have already suggested density-dependent impacts on social dispersal and survival (Marjamäki et al. 2013; Richard et al. 2014; Laforge et al. 2015) and I anticipate the longer dataset used in this research will support the observed positive relationships with the probability of an adult individual changing bands, a negative relationship with individual survival, and a positive relationship with band or harem size when focal individuals are in high density regions. Previous research on the population has established spatial variation in sex ratios are attributable to weather and distribution of resources (Manning et al. 2015). A higher adult sex ratio is likely to occur when non-reproductive (bachelor) males are abundant. I anticipated that a higher number of bachelors (higher ASR) would have a disruptive influence on band stability leading to increased band changes by females and males, smaller harem or group sizes, and may also impact adult female fitness.

Stability and group size in feral horse social structure is known to impact female fitness (Berger 1986; Kaseda et al. 1995; Goodloe et al. 2000; Cameron et al. 2009; Debeffe et al. 2015). In Chapter 3 I take a closer look at sociality in the population by examining population-level trends (lagged association rates and similarity of annual social networks), as well as how individual social characteristics influence reproduction and survival of offspring (among females), or to the attainment of dominant breeding position (males). Using the 5-year production and survival of offspring for females as a response variable it is anticipated that having fewer associates, fewer

stallion (dominant harem defending males) associations, and remaining in the same band would all have a positive influence on their 5-year reproduction because of the social stability these factors suggests. Having both long-term stable female associates as well as male associates should improve reproductive success based on the importance identified for these associations in horses (Cameron et al. 2009; Heitor and Vicente 2010*b*; Stanley et al. 2017) and in other harem-defense polygyny mating systems (Silk et al. 2003; Frère et al. 2010; Silk et al. 2010; Cheney et al. 2012). For males, it is anticipated that the social interactions of bachelors (males which do not defend harems), as measured by degree (number of associations) or strength (relative weight of associations), would be related to their future success in becoming a stallion as these variables characterize potential social experience and knowledge of potential competitors (Berger 1986).
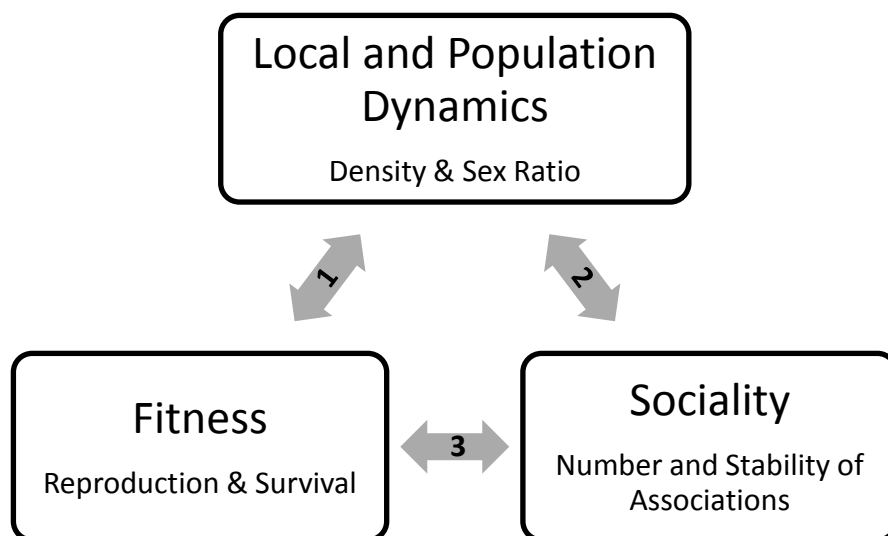
Figure 1.1. The interactions between population dynamics, sociality and fitness. Link 1, between population dynamics and fitness, has been well documented (for density at least, less so for sex ratio). Less research is available on how population dynamics can influence group structure and individual associations while at a more local scale (e.g., home range), group structure will also influence local measures of sex ratio and density (link 2). Link 3 suggests an indirect link between population dynamics and their influence on individual fitness through the more proximate effects of sociality.

The remainder of my thesis focuses on individual physiology, namely concentrations of cortisol and testosterone recovered from tail hair of horses. Androgen hormones such as testosterone along with glucocorticoid hormones (e.g., cortisol or corticosterone) have been shown to play important roles in how individuals interact with available resources and conspecifics (Hirschenhauser and Oliveria 2006; Rubenstein 2007; Bryan et al. 2013[a]; Lymburner et al. 2016). Social structures and mating systems also can influence the endocrine responses in animals (Wingfield et al. 1990; Creel et al. 1993, 2013; Hennessy et al. 2009; Gleason et al., 2009). Evaluating hormone concentrations in wildlife can inform on how an individual is responding to physiological events such as reproduction, predation, inadequate resources, illness, or injury (Hoffsis and Murdick 1970; Merl et al. 2000, Rietmann et al. 2004; Bechshøft et al. 2013; Bryan et al. 2013*a*, 2013*b*).

The majority of research on hormones and their production come from controlled studies on captive or laboratory animals which have provided important baselines for transitioning to exploring how animals are responding to complex, real-world circumstances. In Chapter 4 I investigate hair cortisol concentrations in the free-living feral horses on Sable Island in relation to physiological and sociological variables, including social measures obtained in previous chapters, and determine the most influential variables for both males and females. Hair cortisol concentration is likely to be increased in individuals with high metabolic demands such as females rearing offspring, particularly females nursing foals (Sapolsky 2004; Bonier et al. 2009). Individuals in poor body condition would also likely have high hair cortisol concentrations according to the traditional cort-fitness hypothesis, (Bonier et al. 2009). I also investigate if social structure and stability at the group level and at different spatial scales may influence hair cortisol levels.

Testosterone was similarly investigated among males using hair testosterone concentrations; along with the relationship between the two hormones (testosterone and cortisol) and the role their combined actions can play in shaping male reproductive success (Wingfield and Sapolsky 2003; Mehta et al. 2008; Mehta and Josephs 2010). Hair testosterone concentrations should be higher amongst stallions defending harems then those males that did not (bachelors). Hair testosterone and hair cortisol may also have a negative relationship with each other because of

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known inhibitory effects of one hormone pathway upon the activity of the other (Sapolsky 1987; Viau 2002; Hardy et al. 2005; Rubinow et al. 2005).

1.2   Literature Review

1.2.1   The Social Organization of Feral Horses

Feral horses exhibit female-defense polygyny where the reproductively active group, referred to as a 'band', consists of a dominant adult male (stallion) and a harem of females and their offspring (Hoffmann 1982; Berger 1986). Bands which include additional unrelated adult males subordinate to the dominant stallion (called "tags" or "tag males") also occur in most feral horse populations with varying prevalence (Miller 1981; Berger 1986; Asa 1999; Linklater 2000; Boyd and Keiper 2005). Male horses which are not band stallions often congregate into 'bachelor' groups which are known to change their size and composition of individuals frequently. Dominant band stallions generally father most of the offspring in their harem; however, some research suggests that subordinate band males, followed by other stallions, or bachelors, may obtain some reproductive opportunities (Miller 1981; Asa 1999).

Bands can be as small as two individuals (one stallion and female pair) or up to 35 as seen in the feral horses of the Llanos of Venezuela (Pacheco and Herrera 1997). The mean size of bands recorded for feral horse populations varies from 3.4 to 18.1 (Boyd and Keiper 2005). Average band sizes on Sable Island range from 5.21 (Welsh 1975) and 6.1-6.6 (Contasti 2011). Group size has been found to influence reproductive success of feral horses (Welsh 1975; Kaseda and Khalil 1996; Curry et al. 2007). Kaseda and Khalil (1996) observed that a stallion's reproductive success was greatest when 5 mares were present in the band; over 5 mares and the number of foals produced dropped to an average of 2. Welsh (1975) observed 100% foaling rates when Sable Island bands contained 3 adult females, however, increased foal survival was observed in bands with 4 adult females. Total band size (i.e. harem and any non-dispersed juveniles) was suggested as an important factor for Sable Island horses for overwinter survival through protection and minimizing heat loss during severe storms (Welsh 1975).

Within bands, members establish dominance hierarchies. Mare rank is most strongly correlated to their arrival into the band with original mares having higher rank than newly obtained mares

(Van Dierendonck et al. 1995; Monard and Duncan 1996). Perhaps related to this finding, older mares tend to hold higher rank than younger females (Keiper and Sambraus 1986; Rutberg and Greenberg 1990; Van Dierendonck et al. 1995; Kimura 1998; review in Boyd and Keiper 2005; Vervaecke et al. 2007). The literature both supports and refutes the position of stallions at the top of the intraband hierarchy. This lack of consensus could be the result of what behaviours or activities the researcher used to establish dominance (Keiper and Sambraus 1986; Boyd and Keiper 2005) or an artifact of ecological or population level interactions (Keiper and Sambraus 1986). There is a clear hierarchy established between the adult males in multi-male bands (Miller 1981; Salter and Hudson 1982; Linklater and Cameron 2000). Dominance hierarchies also exist amongst bachelor males; however, the clarity of bachelor hierarchies are complicated by frequent testing of dominance as well as by higher frequencies of individuals joining and leaving all-male groups (Tilson et al. 1988; Vervaecke et al. 2007; Bourjade et al. 2009; Heitor and Vicente 2010*a*). Once established, dominance hierarchies allow for reduced rates of aggression which reveals the importance of the establishment and identification of social rank among individuals (Rutberg and Greenberg 1990; Van Dierendonck et al. 1995; Granquist et al. 2012).

Bands and bachelors occupy home ranges which overlap and may vary seasonally with resource availability (Berger 1986; Linklater 2000, Manning et al. 2015). Sable island horses occupy small home ranges (0.9-6.6 km^2; Welsh 1975) compared to other feral horse populations (review in Boyd and Keiper 2005). Stallions frequently defend their band's position at a resource patch (e.g. water) from a challenging band or bachelors, or he may submit to their approach by moving his harem away (Stevens 1988). Only in one population has territoriality been observed (Rubenstein 1981) and this finding has been disputed by other authors (Linklater 2000).

The strongest argument made for female-defense polygamy in feral horses is that social interactions, and, in particular, female avoidance of harassment by males, plays a pivotal role in driving the observed social structure (Linklater 2000; Sundaresan et al. 2007; Cameron et al. 2009; Rankin et al. 2011; Gray et al. 2012). Given the driving factors behind this social structure, it suggests that it could be vulnerable to changes in adult sex ratio and that an increase in males would result in increased pressure on established bands. Minimal research has investigated the influence of population dynamics on group structure in feral horses and looked for feedback between group structure and population dynamics. In his comprehensive book on feral horses of

the Great Basin in Nevada, Berger (1986) briefly discusses social influences on population and concludes that social behaviours likely influence the rate of population change. Berger (1986) and Goodloe et al. (2000) both noted that band sizes are generally smaller when the sex ratio is skewed towards males. Two studies have also acknowledged that a female biased sex ratio was likely related to the delayed dispersal of juvenile females as well as earlier attainment of band stallion status for young males (Monard et al. 1996; Khalil and Murakami 1999).

Evidence from the first three years of the feral horse survey on Sable Island have indicated that group size is linked to local density along an East–West resource gradient (Contasti et al. 2012; Marjamäki et al. 2013). Dispersal, i.e. emigration from bands, is also suggested as being positively density dependent in Sable Island horses and influenced by band size (Marjamäki et al. 2013). Literature suggests strong links between feral horse group size, structure, and social relationships and an individual's likelihood to successfully reproduce (Kaseda et al. 1995; Linklater et al. 1999; Cameron et al. 2009). As Sable Island is naturally a closed population with no predators, inter-specific competitors, or unnatural manipulations (which are common in other feral horse populations) we are using an ideal population to test a strongly social ungulate for hypotheses on density- sociality, and sex ratio- sociality interactions.

1.2.2   Social Associations in Feral Horses

Bands undergo several immigration or emigration events throughout their existence (review in Boyd and Keiper 2005). Band members are lost most commonly through juvenile dispersal but adult females may be stolen away by a bachelor, another stallion, or otherwise leave the band. In other instances, complete or partial harem takeovers occur when a successful challenge is made by a competitively superior male (mature bachelor or subordinate stallion). Feral horse harems generally have low levels of female secondary dispersal with populations experiencing between 2-29.6% in adult female transfers per year (review in Boyd and Keiper 2005).

Juveniles disperse from natal bands at approximately 2 years of age (range 1-5; Berger 1986; Boyd and Keiper 2005). Sub-adult males generally leave their natal harem and enter bachelor groups (Salter and Hudson 1982; Berger 1986; Khalil and Kaseda 1998). Among juvenile females, natal dispersal is thought to primarily function as inbreeding avoidance since stallions

may retain their breeding role in the band for longer than it takes for females to mature (Berger and Cunningham 1987; Linklater and Cameron 2009). Juvenile females frequently disperse when they reach sexual maturity or shortly after (Berger 1986; Monard et al. 1996). Young dispersing females may consort with bachelors and this can lead to the formation of a new band; otherwise, dispersing females are quickly (if not directly) assimilated into an already established band (Berger 1986; Monard et al. 1996).

Feral horses are not well-known for showing kin preferences among adult individuals since both male and female juveniles disperse (Linklater 2000, Heitor and Vicente 2010*b*). The strength of associations between female feral horses have been described as weaker than in true female philopatric species (e.g. baboons, *Papio cynocephalus*, Silk 2007) in part likely due to un- or distant-relatedness between the band members (Clutton-Brock and Lukas 2012). Despite a lack of strong or numerous kin relationships, the importance of long-term associations in feral horses is well documented (Berger 1986; Kaseda et al. 1995; Goodloe et al. 2000; Cameron et al. 2009). When a new female is acquired by a stallion she is often the recipient of agonistic actions and even harem exclusion by original resident females while at the same time being frequently herded by the stallion to encourage unification with the band (Berger 1986; Rutberg and Greenburg 1990; Monard and Duncan 1996). Over time, social integration eventually reduces this aggression from the males and other band members (Linklater et al. 1999; Cameron et al. 2009).

There are many other known advantages to maintaining established social associations. Reproductive success was improved, regardless of age, band type, or position in the dominance hierarchy, when the female is found to be socially integrated into the band (Berger 1986; Kaseda et al. 1995; Goodloe et al. 2000; Cameron et al. 2009). Inter-birth intervals are also reduced when females maintain stable associations (Kaseda et al. 1995; Debeffe et al. 2015). Females that frequently perform secondary dispersals by changing bands, or that avoid joining harems altogether (a rare occurrence), tend to have low reproductive success (Welsh 1975; Rutberg and Greenberg 1990; Kaseda et al. 1995; Linklater et al. 1999). In addition, sociality generally entails mutual grooming activities and other proximity benefits which may have positive physiological consequences in horses (Welsh 1975; Feh and de Mazières 1993) as it has been observed to do in non-human primates (Sapolsky 2005; Foerster et al. 2011).

1.2.3 Ecology of Sable Island horses

The horses on Sable Island are descendants of those brought over in the mid-1700s for transport and agricultural purposes as well as those brought to assist in the recovery of human life and goods from the shipwrecks which historically occurred in the shallow sand bars surrounding the island (Armstrong 1981). The present day Sable Island horse has close phylogenetic relationships to sport and light draft horses with more distant ancestral relations to Newfoundland, Icelandic, and Fjord pony breeds (Plante et al. 2007; Prystupa et al. 2012). There have been several different breeds introduced to Sable Island resulting in what is now a unique genotype (Welsh 1975; Plante et al. 2007; Prystupa et al. 2011). Some of the past introductions were made with the intention to improve herd quality to the benefit of those who once collected and sold the horses (Christie 1995). The gatherings and removals were viewed inhumane by the public and in response to the outcry; Prime Minister Diefenbaker protected Sable Island, and its feral horses, by means of the 1960 amendments to the Canada Shipping Act (2001). Further protection is now attained by the inclusion of Sable Island into the Canada National Parks Act (2000) in 2013, and the creation of Sable Island National Park Reserve.

Similar populations of feral horses occur on the Atlantic barrier islands of the North American coastline (Rubenstein 1981; Keiper and Sambraus 1986; Goodloe et al. 2000). However, there are some noteworthy differences which enhance the Sable Island Horse's value as a population ideal for long-term study. First, the isolation of the island prohibits loss or gain of individuals to causes other than death and birth. In addition, its remoteness prevents the introduction of predators and inter-specific competitors, of which there are currently neither on Sable Island. The size of the population, currently near 500, improves statistical power of analyses. The vegetation community is relatively simple with no trees or high shrubs to conceal individuals and make observations and surveys difficult. And lastly, due to federal protection, there have been no management activities on Sable Island horses in the last fifty-plus years to disrupt natural processes in the population's social system or population dynamics.

The feral horses are the only non-human terrestrial mammal inhabiting Sable Island. Grey seals (*Halichoerus grypus)*, as well as harbour seals (*Phoca vitulina*) frequent the island, with the grey seals using the island for moulting and reproductive activities. Several marine birds use the island for nesting and occasionally, as is likely the case with bats (*Chiroptera*), there are

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individuals which may have been forced by weather to seek refuge on the island. Apart from possible benefits through nutrient cycling (McLoughlin et al. 2016), these species do not influence the horse population through competition or predation.

1.2.3.1 Resource Use

As with most species, feral horse behaviour is strongly dependent on the distribution of resources across the landscape. The allocation of time spent grazing, resting, and travelling varies among global feral horse populations (review in Boyd and Keiper 2005) as well as seasonally (Welsh 1975; Berger 1986). Horses are grazing animals with hind gut fermentation. Sable Island horses use Marram (*Ammophila breviligulata*) grassland habitat preferentially as this grass species also composes the majority of their diet (Welsh 1975; Freedman et al. 2012, van Beest et al. 2014). Other important forage species for Sable Island horses are beach pea (*Lathyrus japonicus*) and sandwort (*Honckenya peploides*).

On Sable Island there is an obvious difference in the availability across the island of one essential resource. In the west, fresh water ponds are accessible throughout the year; however, in the east, the water for most of the summer and fall is only accessible by digging to the freshwater lens which is near the surface in low lying areas (Rozen-Rechels et al. 2015).

1.2.3.2 Sexual Maturity and Reproduction

Gestation period in Sable Island horses is on average 337 days (range: 323–354 days) which makes it similar to other feral horse populations (Welsh 1975). Females give birth to one foal and may go into post-partum estrus in the 6–10 days following parturition (Welsh 1975). Peak parturition occurs in May and most mares become pregnant during either their post-partum estrus or second estrus (Welsh 1975; Lucas et al. 1991). Female horses can potentially become pregnant after their first year of life and give birth to their first foal at 2 years of age; however, successful recruitment of offspring at this age is rare (Berger 1986; Lucas et al. 1991). It is much more common to see primiparity at 3 years of age or later (Berger 1986; Lucas et al. 1991; Welsh 1975). Females on Sable Island may give birth each year but it is more common that offspring are produced in 2 out of every 3 years (Welsh 1975). Male horses have spermatozoa in the epididymis tubules by their third year but generally lack the body size and competitive ability to obtain reproductive opportunities until 5 or 6 years of age (Welsh 1975).

1.2.3.3 Mortality

Mortality among Sable Island horses is ultimately through starvation; however, this may be exacerbated by stochastic events, predominantly bad weather, or injury and illness (Welsh 1975). The sandy substrate has been attributed to high rates of dental problems as well as providing inadequate friction to maintain ideal hoof condition (Welsh 1975). Horses on Sable Island also contend with various internal parasites which contribute to illness and mortality (Welsh 1975, Debeffe et al. 2016).

1.2.4   Hormones (cortisol and testosterone) and their contribution to wildlife ecology

1.2.4.1   Cortisol and the Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-Pituitary-Adrenal (HPA) axis responds to stress (physical or psychological) by activating the adrenal cortex to release glucocorticoid hormones (GCs) initiating a response in virtually all body systems (Habib et al. 2001; Sapolsky 2004). Levels of one of these hormones, cortisol, is frequently used as an indication that an individual is under some form of stress and has been investigated in many species, tissues, and situations. Under non-stressful situations, baseline levels of cortisol fluctuate in a circadian rhythm related to general periods of activity (Chrousos 1998). In stressful situations an increase in cortisol promotes the metabolism of stored energy to support elevated performance of cardiac, pulmonary, and muscular systems (Habib et al. 2001, Sapolsky 2004). Prolonged high levels of cortisol, and other hormones related to activation of the HPA axis, have been linked to undesirable physiological responses such as immunosuppression, anti-growth, and anti-reproductive responses (Chrousos and Gold 1992; Habib et al. 2001, Sapolsky 2004).

1.2.4.2   Testosterone and the Hypothalamic-Pituitary-Gonadal Axis

Testosterone is produced via activation of the Hypothalamic-Pituitary-Gonadal axis. The hypothalamus produces gonadotropin-releasing hormone which stimulates the anterior pituitary to produce and release luteinizing hormone to act upon the interstitial cells of the testes to produce testosterone. Testosterone is known to produce many of the secondary sexual characteristics that are important for mate competition and reproductive success (Folstad and Karter 1992, Mcglothlin et al. 2008; Mills et al. 2009). Like glucocorticoids, testosterone levels

are modified by a number of physical and psychological processes (Mazur and Booth 1998; Goymann et al. 2007; Earley and Hsu 2008). Testosterone increases in preparation for contests and generally winners of these contests proceed to generate even greater amounts of testosterone (Wingfield et al. 1990, 2001; Mazur and Booth 1998; Hsu and Wolf 1999; Oyegbile and Marler 2005; Mcglothlin et al. 2008; Gleason et al. 2009; Lennartsson et al. 2012; Maruska 2015). Circulating testosterone levels also rise with an increase in sexual behaviour, particularly in mammals (Kirkpatrick et al. 1977; Borg et al. 1992; Lynch et al. 2002; Hirschenhauser and Oliveira 2006; Gleason et al. 2009; Khalil et al., 2009) including those behaviours related to mate acquisition (Borg et al., 1992; Mills et al. 2009) and in anticipation of the breeding season (Goymann et al. 2007).

1.2.4.3   *Measuring Hormones Concentrations in Wildlife*

Neuroendocrine responses in wildlife has been a recently emerging area of research in ecology and conservation (Reeder and Kramer 2005; Walker et al. 2005; Dantzer et al. 2014; Kersey and Dehnhard 2014) but the important role of hormones in relation to individual behaviour, reproduction and survival have been long established. For example, relationships are often observed between testosterone levels and aggressive behaviours, social position, and mating success (Wingfield et al. 2001; Gleason et al. 2009; Mehta et al. 2009). Additionally, numerous reviews have recently been published outlining the advances in understanding the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the complex role of glucocorticoids in species ecology and population dynamics (Bonier et al. 2009; Creel et al. 2013; Boonstra 2013*a*; Dantzer et al. 2014).

Obtaining information on hormone levels from free living wildlife without biasing the results through capture, or by choosing tissue or excreta with limited temporal inference has been one challenge facing wildlife endocrine researchers. Because of its systemic release, cortisol and testosterone can be detected in several different body tissues and their accumulations represent snapshots of different periods of time (review in Sheriff et al. 2011). Measurements from blood plasma provides an estimate of extremely recent hormone levels occurring from the systemic stress response. In fact, wildlife studies which use plasma cortisol concentrations risk being confounded by a stress response initiated by the capture, handling, and plasma sampling. Cortisol concentrations recovered from saliva and urine represent a slightly longer period of time for

cortisol release and in the case of urine, there is a considerable delay between a serum cortisol peak and its echo peak in urine. Faeces are simple to collect in a non-invasive manner and therefore reduce the likelihood of researchers influencing upon cortisol release. The cortisol and testosterone metabolites can be measured in faeces to reflects a period related to how long food takes to pass through the digestive tract (Möstl and Palme 2002; Schmidt et al. 2010).

Cortisol and testosterone are also incorporated into the developing hair tissue (Yang et al. 1998; Liu et al., 1988; Antignac et al. 2001; Koren et al. 2002). Because hair is slow growing, the hormone concentration determined from a section of hair will reflect the quantity of free hormone circulating over a significantly longer period of time than any of the above-mentioned tissues or eliminations (Sheriff et al. 2011). Cortisol and testosterone levels from hair would represent near baseline average but would dampen the observations of peak release from a few or infrequent episodes, including any caused by researcher sampling. Hence hair should be very useful in identifying individuals which are chronically stressed or experiencing frequent and intensive stress exposure over the period of time concurrent with hair growth (Davenport et al. 2006; Accorsi et al. 2008; Koren et al. 2008; Sheriff et al. 2011).

1.2.4.4   *Hair as a Recording Matrix of Systemic Hormone Levels*

Two common approaches have been used when validating the concept of systemic hormone incorporation into hair in a representative fashion. The first is to manipulate the circulating levels of the hormone in question followed by testing the hair. Studies using cortisol or corticosterone as an indicator of HPA axis function have used adrenocorticotropic hormone (ACTH) challenges to validate their work. Multiple ACTH injections produced a detectable increase in hair cortisol concentration in both lynx (Terwissen et al. 2013) and eastern chipmunk (Mastromonaco et al. 2014). In cattle, three injections one week apart produced a detectable effect in hair collected seven days after last injection, but this peak was diluted when looking at longer hair segments sampled 30 days after the last injection (González-de-la-Vara et al. 2011). A single injection of ACTH in caribou and reindeer did not increase hair cortisol levels in most hair samples (Ashley et al. 2011).

Strong support for systemic sources being the predominant modifier of hair hormone levels is found in animals biologically incapable of producing the normal systemic (i.e. adrenal or

gonadal) endocrine response. Testosterone was undetectable in hair from male horses which had undergone gonadectomy (Anielski et al. 2005; Boyer et al. 2007) or had known androgen deficiencies (Thomson et al., 2009), and immature male and female rats treated with estradiol valerate or testosterone (respectively) produced hair containing the treatment hormone and not what would normally be synthesized by the mature functioning organs (Snoj et al. 2012). Adrenalectomies in rats resulted in significant reductions in hair corticosterone collected 15 and 30 days post-operatively (Scorrano et al. 2015). Cushing's patients, with high adrenal cortisol output, also have high hair cortisol concentrations (Thomson et al. 2010; Manenschijn et al. 2011) as do dogs with hyperandrenocorticism (Ouschan et al. 2013). Addison's patients (high ACTH but no adrenal cortisol response) have low hair cortisol concentrations (Manenschijn et al. 2011) while individuals receiving oral treatment for adrenal insufficiencies show a positive correlation between oral hydrocortisone dosage and hair cortisol levels (Gow et al. 2011). The drastic reduction or absence of measurable hormone in hair from an adrenalectomized or gonadectomized animal, along with the observed relationships to induced or natural increases or decreases in circulating hormones strongly suggests a systemic origin of the hormones recovered in hair but does not clearly identify the pathway of incorporation (e.g., blood, sweat, sebum, interstitial fluids).

The second approach to validating hair hormone concentration representing systemic levels of hormones compares naturally occurring levels in hair with concurrent samples of other matrices. For the most part, these have resulted in positive correlations (Yang et al. 1998; Davenport et al. 2006; Koren et al. 2006; Accorsi et al. 2008; Moya et al. 2013; Mastromonaco et al. 2014). However, three of the studies were unable to correlate hair cortisol concentrations with faecal metabolites (Bryan et al. 2013*b*; Moya et al. 2013; Yamanashi et al. 2013) or blood serum concentrations (Feng et al. 2011; Bryan et al. 2013*b*). In some cases, the reason for the lack of correlation could come from inappropriate sampling. Feng et al. (2011) used a single sample of blood that was taken within 8 minutes of capture which may also have been preceded by chase which could have had considerable effect on circulating cortisol levels (Walker et al. 2005; Sheriff et al. 2011). Chen et al. (2014) also recognized the likelihood that the period of growth of the collected hair sample did not correspond to the overnight urine collections due to the delay in the eruption of hair above the surface of the skin.

Some researchers have argued that locally synthesized cortisol (metabolized near the developing hair) can influence the observed hair concentrations (Sharpley et al. 2009; Keckeis et al. 2012). In adrenalectomized rats (Scorrano et al. 2015) the corticosterone was still measurable in newly grown hair 30 days post-surgery (levels measured in blood were not reported). While in other studies, the regression of hair cortisol measurements compared to cortisol levels from other matrices produced a line passing above the origin of the graph (Davenport et al. 2006, Bennett and Hayssen 2010) which could also suggest that hair hormone levels are increased slightly by non-systemic sources.

1.2.5   Hormone Correlates in Wildlife

1.2.5.1   Sociality

A strong portion of the following research investigates what can drive changes in individual associations and the relative importance (as measured by reproductive success) of maintaining associations. Animals with complex social organization and interactions are likely to experience psychological stress associated with disruptions to established relationships and their position within hierarchies, as well as to changes in the number of conspecifics within their social group (Alberts et al. 1992; Creel 2001; Engh et al. 2006*a*; Kikusui et al. 2006; Hennessy et al. 2009; Dunbar and Schultz 2010). There are noted neurobiochemical benefits to social animals not only to being grouped with conspecifics, but also maintaining relationships with specific individuals (Hennessy et al. 2009; Kikusui et al. 2006; Engh et al. 2006*b*).

Group size alone may or may not influence cortisol concentrations. In spotted hyenas, males of large groups had significantly higher faecal corticosteroid levels than males in smaller groups (Goymann et al. 2003). The same relationship was found in elephants (Foley et al. 2001), and gerbils (*Rhombomys opimus*, Rogovin et al. 2003). In ring-tailed lemurs (*Lemur catta*) cortisol concentrations were minimized in intermediate sized groups and increased in both abnormally small and abnormally large groups (Pride 2005*a*). This last study implied that cortisol could be useful to investigate optimal group size. However, in degus (*Octodon degus*), faecal cortisol concentration was not at all related to social group size but was related with reproductive success (Ebensperger et al. 2011). In species where fitness is positively linked to social group size, or to

the abundant resources supporting a large group size, an accompanying response in cortisol could be misinterpreted (Ebensperger et al. 2011).

Where social hierarchies exist, males with the highest testosterone levels tend to maintain more dominant positions while subordinates have lower levels of this hormone, particularly among mammals (Pelletier et al. 2003; Hirschenhauser and Oliveira 2006; Gesquiere et al. 2011). The role of glucocorticoids in relation to social position is often less clear with high levels potentially found in both dominant and subordinate individuals depending on the type and stability of the social structure (Creel 2001; Goymann and Wingfield 2004; Gesquiere et al. 2011; Mendonca-Furtado et al. 2014). Only a handful of studies compare naturally occurring variation in hormone concentrations to social correlates among free-living wildlife (Koren et al. 2002; Pelletier et al. 2003; Bortolotti et al. 2009; Mendonca-Furtado et al. 2014)

1.2.5.2 Population Dynamics

Direct links of cortisol to population dynamics such as density and sex ratio are likely to be greatly confounded by the more direct effect of an individual's access to resources, competition, and social interactions. For example, population density was positively correlated with glucocorticoid levels in gerbils (Rogovin et al. 2003), house mice (*Mus musculus*; Chapman et al. 1998), rhesus macaques (*Macaca mulatta*; Dettmer et al. 2014), red deer (*Cervus elaphus* L; Forristal et al. 2012), and testosterone in grizzly bears (*Ursus arctos*; Bryan et al 2013*a*); however, in all cases the authors observed more proximate causes such as increased social interactions, social instability, aggression, or physiological factors such as disease transmission that occurs at higher densities. Some studies have also failed to find a correlation between hair cortisol levels and population densities even when some of these correlations between cortisol and density have been seen in other tissues (Martin & Réale 2008; Mastromonaco et al. 2014).

1.2.5.3 Reproduction

Physiological states relating to pregnancy and lactation have been associated with an increase in circulating cortisol (baboons, Engh et al. 2006*a*; bats *Tadarida brasiliensis*, Allen et al. 2010; hamsters *Mesocricetus auratus*, Chelini et al. 2011; polar bear *Ursus maritimus*, Macbeth et al. 2012). It has also been observed that the stress response can influence offspring survival and even function to produce a male or female biased birth sex-ratio depending on the timing of the

major stress event during gestation (Linklater 2007). Stress events early in gestation will frequently result in loss of female embryos; events in mid- to late-gestation tended toward male biased embryo death (Linklater 2007).

High cortisol has been linked to decreased fitness (Cort-Fitness Hypothesis, C-F), have no effect on fitness, or even an increase in fitness (Cort-Adaptation Hypothesis, C-A) (review in Bonier et al. 2009). The C-F hypothesis was the earliest prediction of a cortisol relationship to fitness and followed the logic that high cortisol meant that an individual was in poor condition and likely to have decreased reproductive success (Bonier et al. 2009). However, the C-F hypothesis does not fit conclusively amongst all taxa. The theory behind the C-A hypothesis is that an individual in good condition may proceed to invest more into reproduction; however, the increased physiological demands of a high reproductive effort (e.g. producing larger or more offspring) is accompanied by an increase in the release of cortisol to facilitate the reallocation of energy to sustain successful reproduction. Therefore, this situation presents as high cortisol along with high fitness; conversely, where individuals are not responding to the environmental or physiological stressors with an increase in cortisol, or responding to a lower degree, they will contend with lowered reproductive success or survival (gerbils, Rogovin et al. 2003; cliff swallows (*Petrochelidon pyrrhonota*), Bonier et al. 2009).

Testosterone is highly related to reproductive success in males. High circulating testosterone in males is linked to embellishment of secondary sexual characteristics (e.g., ornamentation, plumage) and behaviours (e.g., calls or competitive ability) that lead to higher reproductive success (Folstad and Karter 1992, Ketterson and Nolan 1999, McGlothlin et al. 2008). Additionally, behaviours related to mate acquisition (Borg et al. 1992; Mills et al. 2009), in anticipation of the breeding season (Goymann et al. 2007), and completing reproductive opportunities can also increase circulating testosterone for males (Kirkpatrick et al. 1977; Borg et al. 1992; Lynch et al. 2002; Hirschenhauser and Oliveira 2006; Gleason et al. 2009; Khalil et al. 2009).

1.2.5.4 Other Physiological Variables

Physiological stress can be the result of numerous environmental and ecological variables such as food availability (Forristal et al. 2012; Bryan et al. 2013*a*), climate (Bechshøft et al. 2013) ,

predation (Bryan et al. 2015), or human disturbances (Brearley et al. 2012; Bourbonnais et al. 2013; Fourie et al. 2015*a*). Parasites also have the potential to negatively affect the physiological well-being of individuals and are reflected by higher cortisol levels (Foley et al. 2001; Raouf et al. 2006). Bull elephants with injuries causing lameness had increased faecal GC metabolites (along with a decrease in body condition) during the period of recovery (Ganswindt et al. 2010). And finally, mortality was found to be more likely among individuals who had higher levels of cortisol (Rogovin et al. 2003; Pride 2005*b*).

1.2.6   Cortisol and Testosterone in Horses

Very few studies were found which investigated hormone levels in feral, or free-living, populations of horses. Nuñez et al. (2014) detected an increased level of faecal cortisol metabolites among females that were undergoing social dispersal from one band into a new social group. Another paper also uses faecal testosterone metabolites in a feral population of horses (Khalil et al. 2009) where they observed an increase in a stallion's testosterone with an increase in harem size. Male testosterone levels were also tested regularly in a minimally managed group of intact males where a 'stallion-like' position was frequently contested which provided the most dominant male with across-the-fence privileges to consort with females (McDonnell and Murray 1995). The successful male which obtained this stallion-like position rapidly developed higher serum testosterone levels upon gaining the role and showed a decline in testosterone when he was eventually removed or lost to challenging males (McDonnell and Murray 1995).

Other research has looked at cortisol concentrations in serum from feral horses which had undergone a significant disturbance to their natural behaviour in the form of chase and capture (Kirkpatrick et al. 1977, 1979; Seal et al. 1985; Plotka et al. 1988). The above papers report serum cortisol measurement taken from corralled and restrained horses (Kirkpatrick et al. 1977, 1979; Plotka et al. 1988), or chemically immobilized horses aerially darted after a chase (Seal et al. 1985). The collection methods quite likely influenced the observed levels of plasma cortisol (Sheriff et al. 2011). The designs of these experiments are not suited, and indeed were not intended, to improve our understanding of cortisol responses in unmanaged feral horses living in self-regulated social structures.

Research on cortisol and the domestic horse is more prevalent. From these studies information on a horses HPA response to reproduction, social instability, and injury or illness have provided relevant information to this proposed research. Reproductive state of mares (lactating or not lactating) was not found to be correlated to observed faecal cortisol concentrations (Sinderbrand 2011); however, a peak release in cortisol was observed in serum and saliva in prepartum females in a separate study (Nagel et al. 2012). Manipulated social structures suggested that new group individuals, and subordinate newcomers (as opposed to more dominant newcomers) had higher free cortisol than the established residents (Alexander and Irvine 1998). Pain or acute illness is also linked to high corticosteroids, including cortisol and cortisol metabolites (Hoffsis and Murdick 1970; Merl et al. 2000; Rietmann et al. 2004).

CHAPTER 2

INFLUENCE OF SEX RATIO AND DENSITY ON INDIVIDUAL FITNESS AND THE STRUCTURE AND STABILITY OF FERAL HORSE SOCIAL GROUPS ON SABLE ISLAND

2.1 Abstract

Population parameters, density, and sex ratio, can have profound effects on population growth. Through what mechanisms (e.g., competition for resources, mate finding, immigration, or emigration) these parameters act on individuals to influence reproduction and survival is varied and often dependent on mating strategy. Feral horses often have large home ranges and asynchronous estrus among females which promotes harem-defense polygyny and leads to a species which derives numerous benefits from maintaining stable associations and group structure. I use eight years data of the Sable Island feral horse population to investigate how local measures of density and sex ratio could affect group stability and structure. Predictive models of harem size are mediated by local measures of adult sex ratio (ASR), density, number of bachelors, and availability of preferred forage. When the local ASR became more male biased there was a reduced probability of adult females to change bands as well as limited impact on adult survival. This supports the theory that the harem-defense polygyny mating system does indeed act as a buffer for adult females to avoid additional male harassment. This would also suggest that the competition between males for access to females is intensified as the local sex ratio becomes more male biased and promotes stronger sexual selection.

2.2   Introduction

At the level of the population, groups are most often described by their density in a given area or habitat and by the ratio of males to females (sex ratio). Density is well known to influence population growth rates through impacts on survival, reproduction, and migration via competition for resources or through opportunity to connect with potential mates (Clutton-Brock et al. 1997; Coulson et al. 1997; Kokko and Rankin 2006). The consequences of biased adult sex ratios have had less recognition, but recent studies have found several reasons why sex ratio is an important measure for understanding population trends (Kvarnemo and Ahnesjö 1996; Rankin and Kokko 2007; Székely et al. 2014). Biased sex ratios can both increase or decrease the strength of sexual selection (Kvarnemo and Ahnesjö 1996; Pettersson et al. 2004; Fitze and Le Galliard 2008, Székely et al. 2014); however, this is likely dependent on mating strategy. When modelling demographic stochasticity of hypothetical populations, mating system and sex ratio contribute the most to high demographic variance, and small populations with polygynous mating systems were susceptible to earlier extinction (Lee et al. 2011).

Polygynous mating systems evolved from a number of pathways to support a variety of ecological, physiological or behavioural tendencies of animals (Emlen and Oring 1977; Clutton-Brock 1989). Harem-, or female-defense polygyny often results when distribution of resources, the movements of females, and a long protracted reproductive season with asynchronous estrus makes defending a group of females more effective then defending a territory or home range (Rubenstein 1986; Clutton-Brock 1989; Moehlman 1998). Additional benefits of grouping often include a reduced risk of predation, nutritional (e.g., resource access) or thermal advantages (Welsh 1975). The combination of ecological factors and mating system can lead to the establishment of permanent social groups which become an integral component in the species persistence. In fact, when social species are deprived of their optimal social structure there are often increased costs in the form of reduced reproductive success (decreased production or survival of offspring) or individual survival (Linklater et al. 1999; Pride 2005*a*; VanderWaal et al. 2009; Armitage 2012).

One of the most notable advantages of harem-defense polygyny is the buffering of females from harassment from multiple males (Linklater 2000; Cameron et al. 2009; Sundaresan et al. 2007;

Rankin et al. 2011). This harassment is a form of sexual conflict, where the behaviour or physiology which increases fitness in one sex can have negative effects on fitness in the opposite sex (Hiruki et al. 1993; Wolff et al. 2002; Le Galliard et al. 2005; Fitze and Le Galliard 2008; Rankin et al. 2011; Székely et al. 2014). In experimental approaches, biased sex ratios have sometimes disproportionate effects on female survival and reproduction than the effects of resource competition via density (Le Galliard et al. 2005; Fitze & Le Galliard 2008). Harem-defense polygyny would appear to be more resilient to the potential effects of a male biased sex ratio in that females are defended by a dominant male, and that males defending these groups would have outcompeted local competition. In mating systems where females are not buffered through mate guarding, male biased sex ratios lead to increased harassment and mating events leading to higher energetic costs, injury, or higher rates of mortality (Le Galliard et al. 2005; Dreiss et al. 2010; Rankin et al. 2011).

Stability of the social structure of feral horses is known to be vital for maximizing female fitness (Berger 1986; Kaseda et al. 1995; Goodloe et al. 2000; Cameron et al. 2009). What is not well studied is how the structure and stability of these social groups respond to changes in density and sex ratio at local spatial scales. A marked effect of sex ratio on social structure has previously been observed in a population of feral horses of Shackleford banks (Rubenstein 1986). For several years a female biased adult sex ratio supported larger bands and higher birthrates. When mild winters increased juvenile male survival this resulted in higher pressure on the aging band stallions leading to a larger number of smaller sized bands as well as a decrease in foal production. Similar correlations between biased sex ratios and harem size were observed in Misaki feral horses (Japan) although this was a managed population with an artificially maintained female bias (Kaseda and Khalil 1996). Among temporary harem-defense polygynists, red deer, mean harem size was negatively associated with an increasingly male-biased population (but not with density) and the number of harems also increased (Bonenfant et al. 2004).

The research included in this chapter focuses on how local density and sex ratio influence the group structure and social affinities of adult feral horses. The decision to focus on only adult individuals is due to the anticipated natal dispersal from social groups of juvenile females at one to three years old. Results from earlier studies in this population suggest that high density will

lead to increased departure from bands as well as larger band sizes (Marjamäki et al. 2013) but I build on that study with an additional five years of data. I also investigate the direct relationships between local parameters of density and adult sex ratio and their impacts on fitness (survival and offspring production). Male biased sex ratios can have a negative influence on female survival in mating systems which involve male harassment (Le Galliard et al. 2005; Lee et al. 2011; Rankin et al. 2011), while negative density-dependent effects on survival are well noted (Gaillard et al. 2000; Laforge et al. 2015). What has not received attention is how variation in local sex ratio influences social structure and maintenance of associations. I anticipate that male biased sex ratios would be disruptive to social stability and lead to increased rates of adult individuals changing band associations. Second, with increasingly male biased sex ratios I anticipate a diluting effect leading to a higher number of smaller bands, rather than few large bands which would be expected at female biased sex ratios. To complete this portion of my research I build a model using the most probable significant variables of local population parameters, habitat characteristics, and social characteristics to infer the strongest factors driving the observed harem and band sizes.

2.3 Methods

2.3.1 Study Area

Sable Island National Park Reserve is located 175 km from mainland Nova Scotia Canada, isolated in the Atlantic Ocean (43°55'N, 60°00W). The 42 km long, but narrow (1.6 km max width) island is composed entirely of sand and the vegetation which helps hold it together. The island has supported a feral horse population since they were first introduced in the mid-1700s. During the operation of life-saving stations established to support maritime travel and trade, there were some introductions and removals of the horses, however round-ups ceased after 1960 saw protection granted to the horses though the Canada Shipping Act (2001). From that time there has been no or very minimal disturbance to the horses and they have been maintaining a natural social structure. Currently, the entire island is protected through the development of

Sable Island National Park Reserve in 2013, and the horses are protected as wildlife under the Canada National Parks Act (2000).

2.3.2   Survey Design and Field Work

Survey of the horse population has been conducted annually from 2008 to present, however this research will use the information obtained up to 2015. Census of the population predominantly occurred over the summer months of July and August, although in 2009 some data from May and June are also available. A summary of the survey dates and sampling effort can be found in Table 2.1. Survey consisted of travel on foot across the island to locate horses. Individuals were classified in the field as either being solitary bachelor, part of a bachelor group (no females present), or member of a band (mixed sex group with at least one mature male). For bands, total band size (a count of all individuals including foals) as well as harem size (number of females 2 years old and older) was calculated.

The geography of the island, being quite narrow with low vegetation, ensured a very high probability that all horses would be observed (>99 % accuracy; Marjamäki et al. 2013). The horse's high tolerance of human presence made it easy to collect information on group type and size, location, and characteristics of the individuals, along with photographs to later use for identification. Individual identification was done using natural markings and colouration, shape of chestnuts, position of hair whorls, whiskers, and nose crease patterns. Individuals were distinct enough in these characteristics to not require invasive or disruptive forms of marking or tagging. Overall, 582 observations were made of 174 unique bands over the 8 years of this study. Survey and observations were taken following the guidelines provided by the permitting agency, the Canadian Coast Guard (2008–2012), or Parks Canada (2012–2015 – Research License 14668), and in accordance with our institutional animal care permit (University of Saskatchewan UCACS #20090032).

Table 2.1. Timing, number of days of survey, and mean number of sightings for individual feral horses each summer of the University of Saskatchewan census on Sable Island, NS.

Year	First Day	Last Day	Days	Mean # of sightings per horse ($\pm$SE)
2008	08/12/2008	9/9/2008	29	2.6 $\pm$ 0.05
2009	05/23/2009	7/30/2009	48	5.5 $\pm$ 0.14
2010	07/2/2010	09/01/2010	37	5.4 $\pm$ 0.07
2011	07/21/2011	08/23/2011	24	3.1 $\pm$ 0.46
2012	07/20/2012	08/17/2012	27	7.3 $\pm$ 0.11
2013	7/15/2013	9/15/2013	50	7.6 $\pm$ 0.07
2014	7/21/2014	9/9/2014	51	7.7 $\pm$ 0.06
2015	7/24/2015	9/1/2015	38	7.7 $\pm$ 0.08

2.3.3 Data Analysis

2.3.3.1 Scale of Measure for Density and Sex Ratio

Individual median locations were calculated from all observed locations in a given year. Using ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, CA) buffer radii were calculated at 2000 m, 4000 m, and 8000 m which correspond to the 80th, 95th, and 99th percentile of annual movements of all horses (Laforge et al. 2015). Population characteristics density and sex ratio were calculated at each of these scales. Several social characteristics were also calculated such as number of bands or bachelors within a given buffer.

Density (horses/km^2) was calculated based on the amount of vegetated area available within the buffer. Although the non-vegetated areas are used by the horses for travel and rest, and does have a role in spatial distribution of the horses, it is the resources of the vegetated portions of the island that are critical to sustaining the population. The base map used to quantify available habitat types within the buffers was created in 2009 using aerial photography, Light Detection and Ranging (LIDAR), and ground-truthing (produced by Applied Geomatics Research Group at Nova Scotia Community College, Middleton, Nova Scotia).

Adult sex ratio was calculated based on the reproductively active or potentially reproductively active individuals of the population. Male horses can produce semen as young as two years old however, males less than 5 years old are rarely seen consorting with females in the wild as they lack both the size and competitive skill to acquire and defend females (Berger 1986). Females in the population also do not successfully produce offspring until they are at least 3 years old; becoming pregnant in their second year of life (Lucas 1991; Welsh 1975; this study). For this reason, Adult Sex Ratio (ASR) is calculated using males aged four and up and females aged 2 and higher using the following equation:

$$ASR = \frac{Males_{4+}}{Males_{4+} + Females_{2+}}$$

Equation 2.1

2.3.3.2 *Statistical Analysis*

To analyze how changes in density and sex ratio can influence social structure, I used generalized linear mixed effects models to account for the binary structure of the response variable (dispersal y/n; survival y/n) or binomial response (harem or band size) while the inclusion of random effects accounted for temporal variation not specifically investigated here (Year), and repeated measures of individuals among years (Horse ID). Models were run using R Version 3.2.1 (R Core Team 2013). Models were developed separately for males and females to highlight difference in their response to changes in population parameters. Analyses were also run at the three different spatial scales (2000 m, 4000 m, and 8000 m) to detect at what ecological or spatial scale these population effects are acting most strongly on. Models were fit by maximum likelihood (Laplace approximation; Bolker et al. 2009). Analysis on dispersal and survival were limited to the adult (4 years old and older) males and females of the population. Analysis was run on 673 observations from 198 females and 874 observations from 245 males over the 8-year period.

For analyses in which social group characteristics were response variables we limited the observation to one point per band by using the dominant band stallion as the focal individual. Year was also considered a random effect not particularly of interest in this question and Horse

ID was also considered a random factor to account for repeated sampling of individuals between years. Analysis was on 582 annual observations of bands led by 174 different stallions across the 8 years. Generalized linear mixed models were run with package glmmADMB using a truncated Poisson distribution to account for non-zero count data as the response variable (i.e., you cannot have a harem or band size of zero). Model selection was based on AIC_c (Burnham and Anderson 2002) and model fit was evaluated by visually assessing the residuals.

2.4   Results

2.4.1   Basic Population Trends

The horse population on Sable Island has seen a population increase between 2008 and 2015 with two notable overwinter mortality events in 2010–2011 and 2014–2015 respectively (Figure 2.1). The adult sex ratio has become increasingly male biased despite the fluctuation in population size. There has been a continual increase in the number of adult males over the past 8 years while the number of adult females has shown greater variability (Table 2.2). However, despite observing a 111.6% increase in the number of adult males over the 8-year period, the increase in the number of bands experienced a more modest 55.8% increase. This is reflected by the increase observed in the percentage of the male population existing as bachelor males from 28.2% in 2008 to 59.9% in 2015.

The number of multi-male bands did not appear to increase with either the increase of adult males or the increased number of bands as the population grew and the sex ratio became more strongly male-biased. However, since there was an increased number of bands this did mean that the number of males per band was slightly lower as the study progressed. Also of note in this table in the first three years of the study, where the sex ratio was more strongly biased towards females, we see both higher numbers of females per bands but also higher number of males per bands.
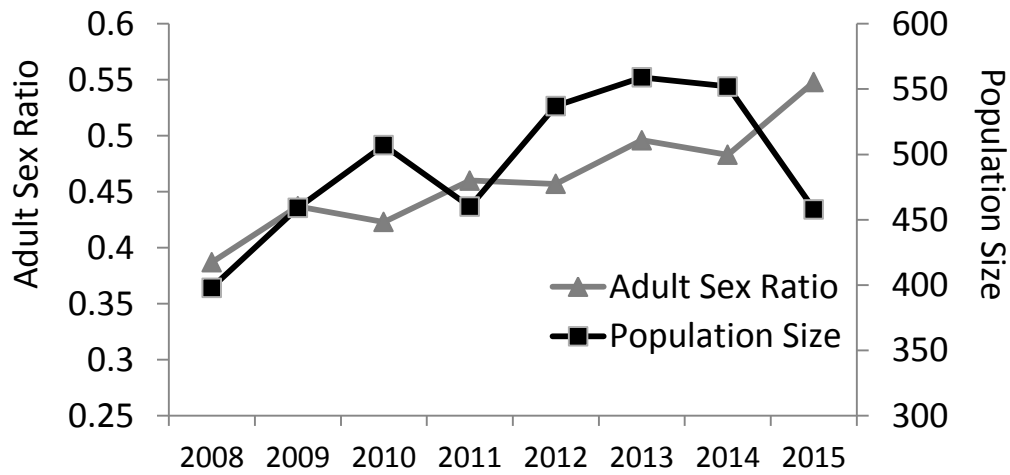
Figure 2.1. Annual population size and whole-island adult sex ratio for the population of feral horses living on Sable Island, NS (2008-2015).

Table 2.2. Social distribution of the adult feral horses of Sable Island, NS.

YEAR	# of Males 4+	% as Bachelors	% in Bands	# of Bands	# of Multi-male bands	Mean Males per Band	# of Females 2+	Mean Females Per Band	ASR
2008	86	28.2	71.8	52	12	1.23	136	2.62	0.387
2009	114	35.9	64.1	58	12	1.25	147	2.53	0.437
2010	122	27.0	73.0	72	12	1.24	165	2.29	0.425
2011	123	30.9	69.1	72	8	1.18	147	2.04	0.456
2012	145	37.9	62.1	78	11	1.15	175	2.24	0.453
2013	177	41.8	58.2	86	13	1.19	178	2.07	0.499
2014	180	58.3	41.7	88	10	1.11	193	2.19	0.483
2015	182	59.9	40.1	81	13	1.16	150	1.85	0.548

2.4.2   Effects of Density and ASR on Changing Associations (i.e., band changes)

Increased density increased the probability that a female horse would experience either band collapse or disperse to a different band when measured at the 2000 m buffer (Table 2.3). Males also saw an increased likelihood to experience significant social disruptions (band collapse, band formation, or entire loss of original females but maintaining status as a band stallion with new harem) but the change in probability was not significant (Figure 2.2). Interestingly, when the local adult sex ratio is more male biased we saw a reduced probability of females to change bands when measured at 4000 m buffer; males, on the other hand, were trending towards experiencing a change in sociality or social position but the differences in probability were not significant.

Table 2.3. The probability of an adult female or adult male to socially disperse (change bands) depending on local measures of adult sex ratio (ASR) or density of horses (horses/veg km2) measured at three spatial scales based on the individual's centroid location (radii in m). Model fit to a generalized linear mixed effect model with binomial distribution (random terms are year and horse ID). Models in bold indicate a $\Delta AIC_c \geq 2$ from the null model.

Model	Scale (m)	Estimate	*P*-value	AIC$_c$	LL
FEMALES					
ASR + (1|Year) + (1|HorseID)	2000	−1.340	0.196	885.4	−438.7
	4000	**−3.280**	**0.028**	**882.1**	**−437.1**
	8000	−3.606	0.117	884.4	−438.2
Density + (1|Year) + (1|HorseID)	**2000**	**0.010**	**0.003**	**878.1**	**−435.0**
	4000	0.009	0.052	883.4	−437.7
	8000	0.013	0.049	883.3	−437.7
Null	all			885.1	−439.6
MALES					
ASR + (1|Year) + (1|Horse ID)	2000	1.102	<0.001	991.4	−491.7
	4000	1.001	<0.001	992.9	−492.5
	8000	0.242	<0.001	993.4	−492.7
Density + (1|Year) + (1|Horse ID)	2000	0.004	0.002	992.2	−492.1
	4000	0.003	0.039	993.2	−492.6
	8000	0.004	0.004	993.2	−492.6
Null	all			991.5	−492.7
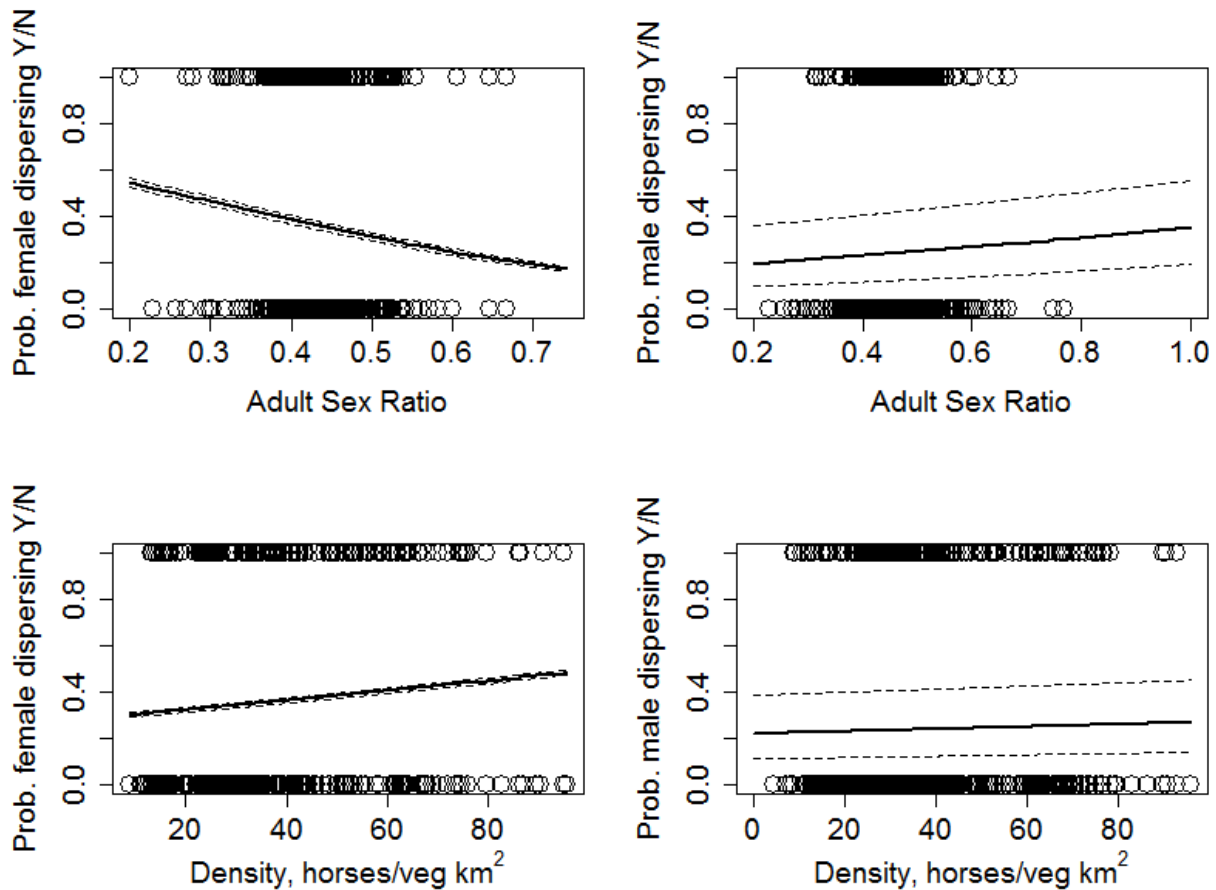
Figure 2.2. Probability of social dispersal (change bands) for adult females (left) and males (right) depending on adult sex ratio (top) or the local density of horses (horses/ veg km2; bottom) as measured in a 4000 m radius from individual's median location. Probability $= 0 -$ individuals did not disperse, Probability $= 1 -$ individual did disperse. Dashed lines indicate the 95% confidence interval.

2.4.3   Effects of Density and ASR on Fitness

Adult sex ratio and density did not have a strong direct correlation to survival for females nor on males at any buffer size (Table 2.4). However, there was a slight trend present that females experience lower survival probabilities when in an area with more male-biased sex ratios (Figure 2.3). The probability of adult females to produce a foal was reduced in regions with more male-biased ASRs but this was found to only be significant when measured at our largest scale of an

8000 m buffer (Table 2.5). Also significant at only the 8000 m buffer, foal production was increased in areas with higher density, but foal recruitment was found to be lower at higher densities (Figure 2.4). Recruitment of offspring was not strongly correlated with the adult sex ratio during the year of their birth.

Table 2.4. Overwinter survival of adult females and adult males to depending on local measures of adult sex ratio (ASR) or density of horses (horses/ veg km2) measured at three spatial scales from the individual's centroid location (radii in m). Model fit to a generalized linear mixed effect model with binomial distribution (random terms are year and horse ID). No models were found to have a $\Delta AIC_c \geq 2$ from the null model.

Model	Scale (m)	Estimate	*P*-value	AIC_c	LL
FEMALES					
ASR + (1|Year) + (1|Horse ID)	2000	−2.240	0.096	675.1	−333.6
	4000	−2.509	0.194	676.1	−334.1
	8000	−4.121	0.215	676.3	−334.2
Density + (1|Year) + (1|Horse ID)	2000	0.007	0.176	675.9	−334.0
	4000	0.004	0.509	677.4	−334.7
	8000	−0.006	0.517	677.4	−334.7
Null	all			675.8	−334.9
MALES					
ASR + (1|Year) + (1|Horse ID)	2000	−0.864	0.377	643.5	−317.7
	4000	−1.543	0.357	643.4	−317.7
	8000	−4.860	0.101	641.7	−316.8
Density + (1|Year) + (1|Horse ID)	2000	0.007	0.153	642.1	−317.0
	4000	0.005	0.478	643.7	−317.8
	8000	0.004	0.695	644.0	−318.0
Null	all			642.2	−318.1
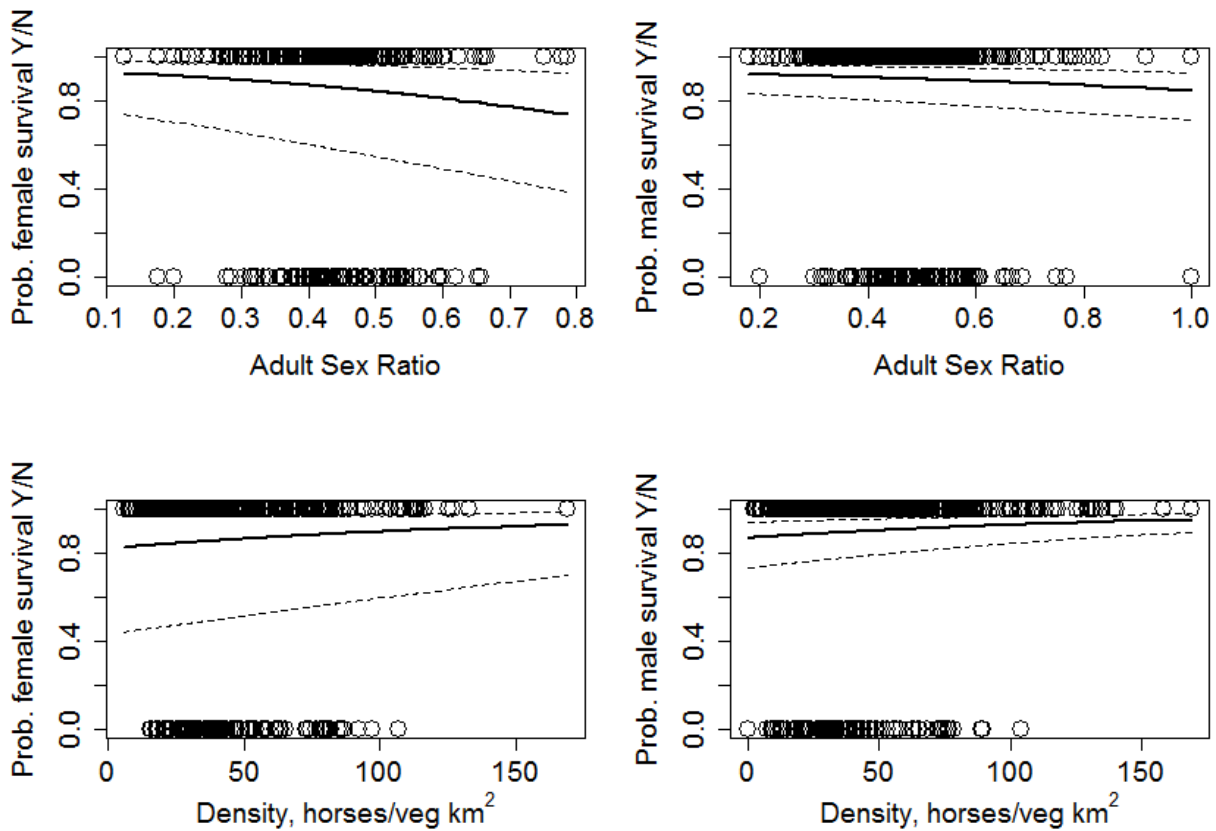
Figure 2.3. Overwinter survival of adult female (left) and adult male (right) feral horses based on the adult sex ratio (top) and density (horses/ veg km2; bottom) experienced in the previous summer. Density and ASR measured using the 2000 m radius from individual's median location. Probability = 0 – individuals did not survive overwinter, Probability = 1 – individual did survive overwinter. Dashed lines represent the 95% confidence interval.

Table 2.5. Production of foals and foal survival to 1 year of age of adult females depending on local measures of adult sex ratio (ASR) or density of horses (horses/ veg km2) measured at three spatial scales from the individuals centroid location (radii in m). Model fit to a generalized linear mixed effect model with binomial distribution (random terms are year and horse ID). Models in bold type were found to have a $\Delta AIC_c \geq 2$ from the null model.

Model	Scale (m)	Estimate	P-value	AIC_c	LL
Foal Produced (Y/N)					
ASR + (1|Year) + (1|Horse ID)	2000	−0.718	0.427	1212.3	−602.2
	4000	−1.633	0.181	1211.2	−601.6
	8000	**−4.930**	**<0.001**	**1203.3**	**−597.7**
Density + (1|Year) + (1|Horse ID)	2000	0.003	0.271	1211.7	−601.9
	4000	0.008	0.065	1209.4	−600.7
	8000	**0.018**	**0.006**	**1204.8**	**−598.4**
Null	all			1210.9	−602.5
Foals Survival to 1 y.o.					
ASR + (1|Year) + (1|Horse ID)	2000	−0.336	0.850	462.0	−227.0
	4000	−0.416	0.869	462.0	−227.0
	8000	3.445	0.479	461.5	−226.8
Density + (1|Year) + (1|Horse ID)	2000	−0.008	0.175	460.3	−226.1
	4000	−0.012	0.124	459.8	−225.9
	8000	**−0.024**	**0.021**	**457.0**	**−224.5**
Null	all			460.0	−227.0
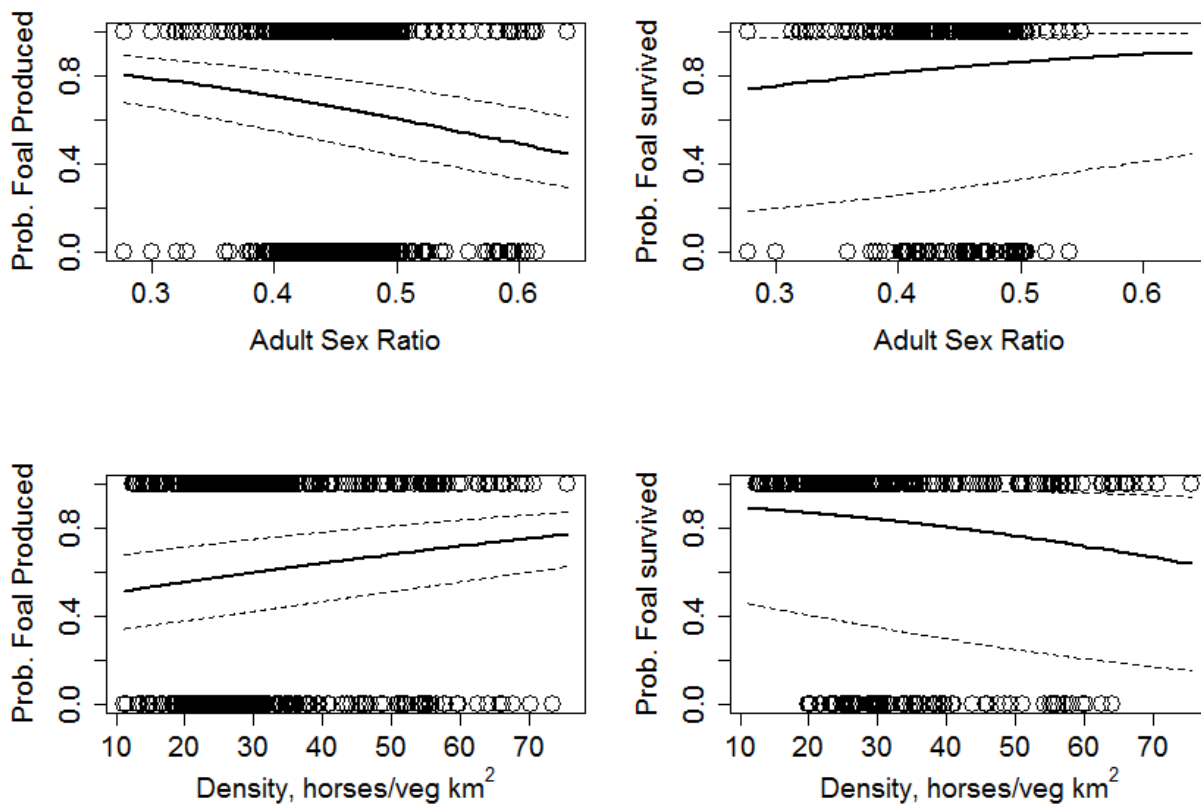
Figure 2.4. The probability of a female to be accompanied by offspring (left) and survival of offspring to 1 year of age (right) based on the adult sex ratio (top) and density (horses/ veg km2; bottom) experienced in the previous summer. Density and ASR measured using the 8000 m radius from individual's median location. Probability = 0 – individuals did not produce a foal, Probability = 1 – individual did produce a foal. Dashed lines represent the 95% confidence interval.

2.4.4   Effects of Density and ASR on Group Size.

Both density and adult sex ratio returned significant models describing group size (harem and band) at all three scales of analysis (Table 2.6). As adult sex ratios became more male biased we saw that group sizes decreased (Figure 2.5). Conversely as density increased we tended to see

band sizes increase, although only significantly for band size when measured at the finest scale of 2000 m. Observations consisted of 582 bands over the 8 years and 174 unique bands.

Table 2.6. Influence of adult sex ratio (ASR) and density (horses/ veg km^2) measured at three spatial scales from the individual's centroid location (radii in m) on the observed harem and band size of feral horses. Models fitted with the variables year and horse ID as random effects using a truncated Poisson distribution accounting for non-zero response variable. Observations from 582 bands, 8 years and 174 individual bands.

Model	Scale (m)	b	P-value	AIC$_c$	LL
HAREM SIZE					
ASR + (1|Year) + (1|Horse ID)	**2000**	**–3.279**	**<0.001**	**1680.0**	**–836.0**
	4000	**–3.234**	**<0.001**	**1701.5**	**–846.7**
	8000	**–3.268**	**<0.001**	**1710.8**	**–851.4**
Density + (1|Year) + (1|Horse ID)	**2000**	**0.005**	**0.0015**	**1725.0**	**–858.8**
	4000	**0.005**	**0.031**	**1730.6**	**–861.3**
	8000	**0.005**	**0.130**	**1730.6**	**–861.3**
Null	all			1733.2	–863.6
BAND SIZE					
ASR + (1|Year) + (1|Horse ID)	**2000**	**-1.613**	**<0.001**	**2542.2**	**-1267.1**
	4000	**-1.640**	**<0.001**	**2559.6**	**-1275.8**
	8000	**-1.848**	**<0.001**	**2561.2**	**-1276.6**
Density + (1|Year) + (1|Horse ID)	**2000**	**0.003**	**0.002**	**2575.7**	**-1283.8**
	4000	0.002	0.12	2582.6	-1287.3
	8000	0.002	0.24	2579.6	-1285.8
Null	all			2583	-1288.5
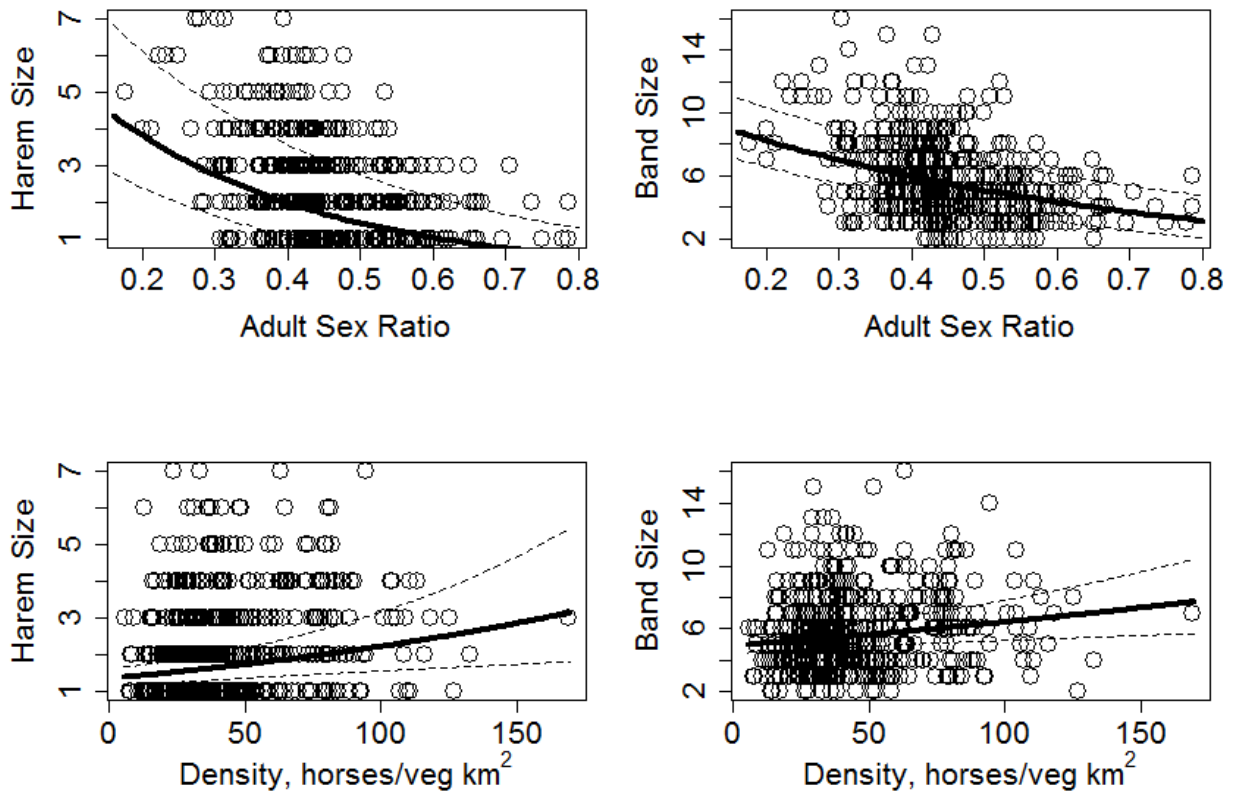
Figure 2.5. Harem size (left) and band size (right) tended to be smaller when adult sex ratios (top) in the population became more male biased. Density (horses/ veg km^2; bottom) also had a positive correlation to harem and band size when measured at the 2000 m radii from the bands median location. Figures represent models from the 2000 m buffer with zero truncated model with year and horse ID as random variables. Dashed lines represent the 95% confidence interval.

2.4.5 Models Describing Group Size

The scale at which each variable included in the model was chosen based on how they performed in single variable correlations with either harem size or band size (Table 2.7). These variables were then evaluated for their collinearity with each other and with Year, which was included as a random factor (Figure 2.6). There is some evidence of collinearity between Bachelor and year (Pearson correlation coefficient = 0.70) and between Bachelor and Stallion counts (Pearson

correlation coefficient = 0.64) but investigation of all variance inflation factors for the covariates indicated all VIF < 10 (Zuur et al. 2010).

The strongest model describing harem size included the population parameters density and adult sex ratio as well as the habitat variable grass and the number of bachelors (Table 2.8). An almost equally strong model included the above variables but dropped grass availability. Overall band size was best predicted by just density and adult sex ratio alone (Table 2.9).

Table 2.7. Univariate evaluation of covariates calculated at different spatial scales (2000 m, 4000 m, and 8000 m buffer from band centroid). The strongest variable (in bold) was selected to use in developing the full model to describe harem and band size.

Variable	Scale	Harem Size			Band Size		
	m	T	P-value	R^2	T	P-value	R^2
Dense Grass	2000	-1.612	0.108	-0.067	-2.143	0.033	-0.089
	4000	**-2.974**	**0.003**	**-0.123**	**-3.032**	**0.003**	**-0.125**
	8000	-2.574	0.010	-0.106	-1.784	0.075	-0.074
Surface Water	**2000**	**2.420**	**0.016**	**0.100**	**2.508**	**0.012**	**0.104**
	4000	1.896	0.058	0.078	1.939	0.053	0.080
	8000	1.876	0.061	0.078	2.227	0.026	0.092
Stallions	2000	-0.419	0.676	-0.017	-0.327	0.744	-0.014
	4000	-2.291	0.022	-0.095	-2.641	0.009	-0.109
	8000	**-2.996**	**0.003**	**-0.123**	**-2.864**	**0.004**	**-0.118**
Bachelors	2000	-1.482	0.139	-0.061	-3.090	0.002	-0.127
	4000	**-2.614**	**0.009**	**-0.108**	**-4.163**	**<0/001**	**-0.170**
	8000	-2.532	0.012	-0.105	-3.70	<0.001	-0.155
Horse Density (veg)	**2000**	**4.292**	**<0.001**	**0.175**	**3.566**	**<0.001**	**0.146**
	4000	2.665	0.008	0.110	1.471	0.142	0.061
	8000	1.705	0.089	0.071	0.775	0.439	0.032
Adult Sex Ratio	**2000**	**-9.172**	**<0.001**	**-0.356**	**-9.202**	**<0.001**	**-0.357**
	4000	-7.179	<0.001	-0.286	-7.584	<0.001	-0.300
	8000	-5.408	<0.001	-0.219	-6.597	<0.001	-0.264
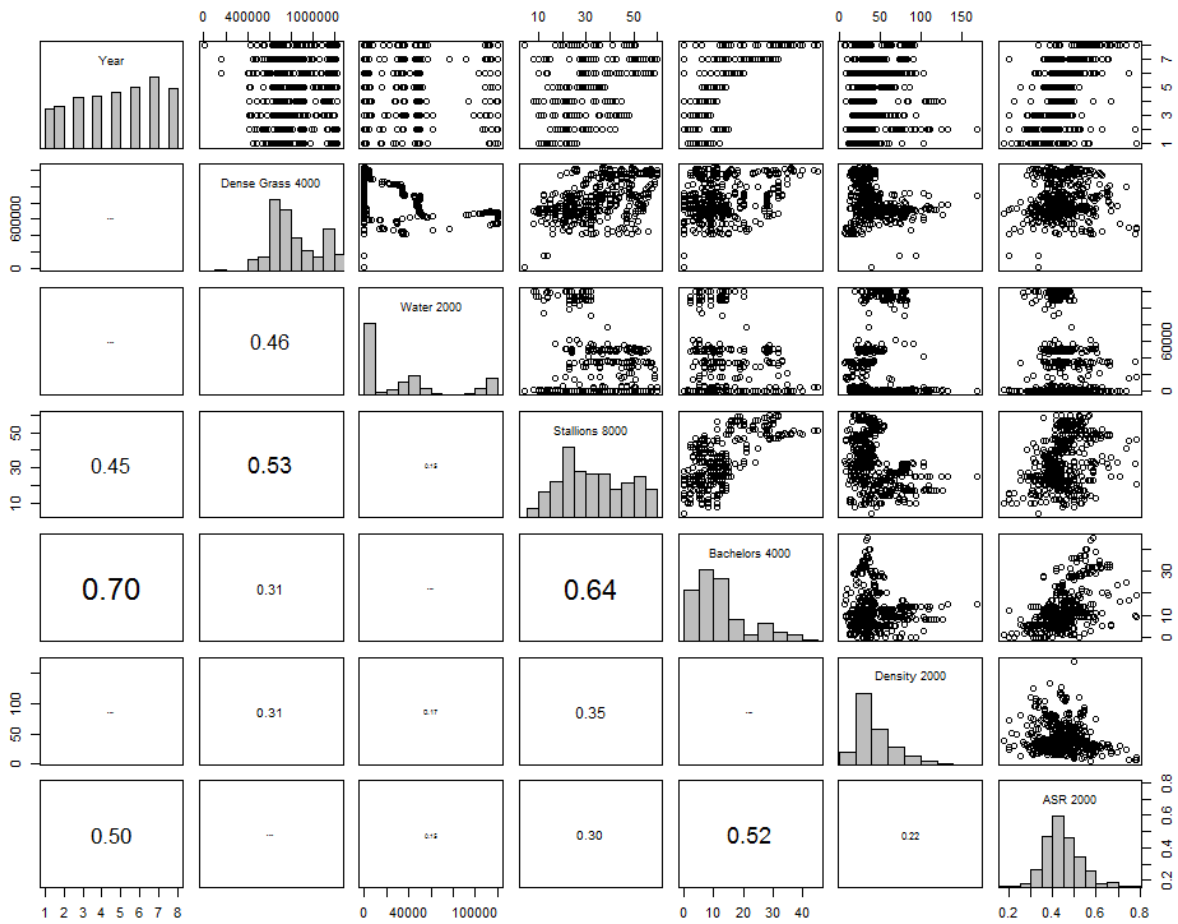
Figure 2.6. Scatterplot (upper right panes) and Pearson correlation coefficients (lower left panes) of covariates used to develop models for harem and band size. Variable names appear in the diagonal (Adult Sex Ratio–ASR).

Table 2.8. Best fit models for harem size with relation to population parameters, habitat, and social environment. Models fitted with the variables year and horse ID as random effects using a truncated Poisson distribution accounting for a non-zero response variable.

Intercept	ASR 2000m	Density 2000m	Grass 4000m	Water 2000m	Stallions 8000m	Bachelors 4000m	AIC_c	ΔAIC_c	LL
2.17	**–3.76**	**0.00285**	**–2.93e-07**			**0.0122**	**1673.9**	**0**	**–830.0**
1.89	–3.71	0.00356				0.0098	1674.0	0.1	–831.0
2.09	–3.75	0.00293	–2.33e-07	6.39e-07		0.0118	1675.5	1.6	–829.7
1.79	–3.20	0.00381					1675.8	1.9	–832.9
2.09	–3.75	0.00295	–2.39e-07	6.37e-07	2.99e-04	0.0115	1677.5	3.6	–829.7
1.995	–3.28						1680.0	4.1	–836.0
0.517							1733.2	59.3	–863.6

Table 2.9. Best fit models for band size with relation to population parameters, habitat, and social environment. Models fitted with the variables year and horse ID as random effects using a truncated Poisson distribution accounting for a non-zero response variable.

Intercept	ASR_2 000m	$Density_{20}$ 00m	Grass 4000m	Water 2000m	Stallions 8000m	Bachelors 4000m	AIC_c	ΔAIC_c	LL
2.34	**–1.55**	**0.00152**					**2540.9**	**0**	**–1265.4**
2.46	–1.65	0.00137	–1.94e-07		0.00262		2541.2	0.3	–1263.6
2.45	–1.55	0.00124	–1.20e-07				2541.3	0.4	–1264.7
2.43	–1.61						2542.2	1.3	–1267.1
2.44	–1.64	0.00137	–1.77e-07	1.80e-07	0.00255		2543.0	2.1	–1263.5
2.44	–1.65	0.00137	–1.77e-07	1.78e-07	0.00253	5.26e-05	2545.0	4.1	–1263.5
1.71							2583.0	42.1	–1288.5

2.5   Discussion

Local demographic properties such as adult sex ratio and density were observed to influence group structure in feral horses on Sable Island. In fact, population parameters appear to more strongly affect the social structure of the population than their direct comparison to survival or social dispersal. Harem and band size were both affected by male biased sex ratios at all spatial scales from local (2000 m buffer) to maximum home range (8000 m buffer). Increasingly male-biased sex ratios led to smaller harem and band sizes as predicted; however, it did not appear to occur through disrupting associations of adult females. As the sex ratio became more male-biased the probability of an adult female to change bands was observed to decrease. Conversely, when density increased we saw an increase in the probability of adult females to change bands. At the local scale density continues to influence harem and band size with larger groups found in regions of higher density. Among males, increased local characteristics of ASR and density also appeared to increase the probability that their social status would change indicating that there is more likelihood of either gaining, losing, or switching harems.

Perhaps the most critical influence density and sex ratio have is on their effect on social group size. There is a strong negative relationship between the adult sex ratio and harem or band size among harem defense polygynous mammals (Berger 1986; Rubenstein 1986; Goodloe et al. 2000; Bonenfant et al. 2004). Although the correlations were less strong, there was an observed increase in harem or band size with increased density. The high-use areas tend to have higher quality vegetation which attracts more individuals, but that those individuals group into larger bands rather than clusters of smaller bands is notable. Feral horses are not known for being territorial with one exception: when the geography made defense of high quality habitat feasible, and the population was strongly female biased, the stallions of Shackleford Banks were observed to exclude bachelors from high quality habitat and had larger harems (Rubenstein 1986). The horses on Sable do not exhibit territorial behaviour, however, where females are drawn into better quality habitat it is likely easier for a single male to monopolize access to more females. This could be why we also see more frequent changes in band membership for females in high density areas (this study; Marjamäki et al. 2013). Males may also be able to take advantage of high quality resources to expend more effort in harem growth and defense. The highest density areas on Sable tend to also coincide with the lowest ASR, which one would expect of an area

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dominated by larger bands, but it suggests that bachelor males are also less likely exploit resources in these areas (Manning et al. 2015).

It was hypothesized that high numbers of males would put excessive strain on band stallions and that we would see more social movement through band collapses or successful attempts to steal adult females from existing bands. Instead what was observed was that male-biased sex ratios resulted in fewer adult female movements between bands compared to when adult sex ratios are neutral or female biased. These observations speak to one of the known benefits of harem-defense polygyny among equids which is to buffer females from excess harassment by bachelor males (Linklater 2000; Sundaresan et al. 2007; Cameron et al. 2009; Madosky 2011; Gray et al. 2012). It could also speak to the higher selective pressure through male-male competition resulting in higher quality males fulfilling reproductive roles (Clutton-Brock et al. 1982; Berger 1986; Feh 1990; Kvarnemo and Ahnesjö 1996; Székely et al. 2014). Adult males in this study were also observed to experience a higher probability of either obtaining or losing a harem when ASR was male-biased with an overall trend of a lower proportion of adult males becoming successful harem holders. The mating system of horses may in fact have or maintain a stronger selection on quality males by having proportionally fewer mating opportunities for the 'excess' males and results in competitively superior males to secure harems (Clutton-Brock et al. 1982, 1997; Kvarnemo and Ahnesjö 1996). However, this conclusion should be made cautiously, in some systems male biased sex ratios have resulted in lower intensity in intrasexual selection; as more males become successful at acquiring mates as females attempt to avoid harassment by being less choosey (Kokko and Rankin 2006; Fitze and Le Galliard 2008; Dreiss et al. 2010).

Generally, the sex which matures, or enters their reproductive phase in life earlier, is the one which dominates in the calculated sex ratio (Lovich and Gibbons 1990; Székely et al. 2014). For horses, females enter into reproductive phase at 2 years of age while males usually don't begin successfully defending harems until five years old. A female biased sex ratio was common during the start of the research on Sable Island but in the later years mature males compose a greater proportion of the population (Table 2.2). Adult female survival was lower than juvenile survival during a large portion of this study (Richard et al. 2014). The exact cause of this is not explicitly examined here but is likely linked to a difference in mortality among females who bear the heavier costs of reproduction and possibly experience higher overwinter mortality (Richard et

al. 2014). Several studies have shown a link between male biased sex ratios and increased mortality of females (Le Galliard et al. 2005; Lee et al. 2011; Rankin et al. 2011). The results of this study showed a trend towards increased mortality of females with an increase in males within their home ranges however it failed to be significant factor in the models. It may be that density and adult sex ratio have less impact on individual survival but their action on population growth could act through reproductive success then survival (Richard et al. 2014).

Not evaluated in this study but another possible source of the decreasing adult female component of the population are negative effects of biased sex ratios on the recruitment or survival of young females (Gaillard et al. 2000; Dreiss et al. 2010). Adult females appeared less likely to change bands with increased sex ratios however we do observe that band sizes decreased while at the same time observe an increased number of bands in years with male biased ASRs (Table 2.2). It could be that instead of dispersing into existing bands juvenile females are more likely to form associations with bachelors. Studies have also suggested that a female biased sex ratio were likely linked to a delayed dispersal of juvenile females implying that dispersal happened earlier in male-biased populations (Monard et al. 1996). We also observed that adult females were more likely to change bands when the population was more female biased. In social primates (*Papio hamadryas ursinus*) female biased sex ratios led to higher rates of female–female aggression and associations became less stable (Cheney et al. 2012).

As expected based on earlier research on this population (Marjamäki et al. 2013), we see that females tend to change bands more frequently when density increased. However, Marjamäki et al. (2013) did not detect a relationship between density and band size, while the larger dataset provides strong evidence of a relationship between density and both band or harem size with the inclusion of additional years of data. It is likely then that areas of high density are becoming so by the formation of larger bands and not by a high numbers of smaller bands. There is opportunity for strong selective forces acting on males as they compete for the opportunity to defend these larger harems occupying in high density regions.

When considering both ecological and social variables that are likely to refine harem size we find that the best model includes abundance of preferred vegetation along with the local density. However, the best model also includes ASR as well as just simply the number of bachelors within the 4000m buffer. For band size, the variables in the best model remain solely density and

adult sex ratio. The relationship between social structure and ecological factors has also been linked in other equids (Rubenstein 1986; Moehlman 1998; Sundaresan et al. 2007).

2.6 Conclusions

Group structure and social stability among Sable Island horses is mediated by the local measures of adult sex ratio, density, abundance of bachelors, and availability of preferred forage (marram grass; *Ammophila breviligulata*). The reduced probability of adult females to change bands and the limited impact on adult survival when the ASR is male biased suggests that the mating system of harem-defense polygyny does act as a buffer for adult females to avoid additional male harassment. This would also suggest that the competition between males for access to females is intensified when the local sex ratio becomes more male biased, and promotes stronger sexual selection to be occurring among that sex.

CHAPTER 3

PERSISTENCE OF SOCIAL ASSOCIATIONS OVER TIME AND THEIR LINK TO REPRODUCTIVE SUCCESS OF MALE AND FEMALE FERAL HORSES ON SABLE ISLAND

3.1 Abstract

Characteristics of associations in species considered social in their nature have been frequently linked to individual fitness. The frequency of social changes can also have important consequences on the genetic diversity of the population. Feral horses maintain a year-round social structure that reflects their harem-defense polygynous mating system. I used Lagged Association Rates (LAR) and compared annual social network similarity for both the reproductive portion of the Sable Island horse population ('bands') as well as the non-reproductive male 'bachelors' to determine the persistence of associations. LARs suggested that the association between females and band stallions is the most persistent, followed by female–female associations. Associations between bachelors were least likely to be maintained over time. Additionally, the annual network representing the associations between individuals living in bands goes through a restructuring where all previously existing associations are broken in approximately five years. To evaluate how associations may impact individual reproductive success I used female 5-year foal production and foal survival to one year old. Females with a higher number of associates over this time and those who belonged to more bands, had lower fitness. Maintaining longer associations with a specific stallion increased female fitness while maintaining longer associations with specific females had no effect. Social network measures of degree, strength, and betweenness were compared to the future social position of males to see if

there was any predictive capacity to identify bachelors destined for reproductive success; no such correlation was observed. However, for bachelors 5 years old and older there was a decrease in degree and strength measures suggesting that males in that age group associate less strongly with specific individuals or groups. Last, evidence is found of a potential conflict between the sexes with regards to band or harem size. Having more females provides a better likelihood for the dominant males of fathering at least one offspring but it comes at a cost of a lower proportion of females producing a foal.

3.2   Introduction

Ranging across taxa, group living may be a short-lived temporary artifact of predation pressure (e.g., shoaling in fish, Hoare et al. 2004) or associated with mating strategies (red deer, *Cervus elaphus*, Carranza and Valencia 1999), while at the extreme end, group living is permanent and comprised of complex social and mating systems (Krause and Ruxton 2002; e.g. primates, Silk 2011; spotted hyenas (*Crocuta crocuta*) Holekamp et al. 2012). In social species, it is well documented that the type and quality of associations have a noticeable impact on individual fitness (Silk 2007; Cameron et al. 2009; Dunbar and Shultz 2010). Stronger or more preferred associations are often seen between kin or near-kin, which provides an individual indirect fitness benefits (Silk 2007; Holekamp et al. 2012; Seyfarth and Cheney 2012) but maintaining non-kin associations have also been shown to be important in some species (horses: Cameron et al. 2009, Heitor and Vicente 2010*b*; Stanley et al. 2017, dolphins (*Tursiops aduncus*): Wiszniewski et al. 2012; reviews: Clutton-Brock and Lukas 2012; Seyfarth and Cheney 2012).

Feral horses maintain year-round social groups between one or more males and a harem of generally unrelated females (Berger 1986; Linklater 2000). Commonly, both males and female offspring natally disperse: males between 1–4 years of age and females at around 2 years of age, usually with first estrous, therefore females in a harem are seldom related to one another (Berger 1986). Despite being non-kin, long-term associations between female horses have been suggested to both increase reproductive success and decrease levels of harassment from males (Berger 1986; Kaseda et al. 1995; Cameron et al. 2009). In rare instances where females do not

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associate with a band they receive higher rates of male harassment and often have poor body condition and low reproductive success (Linklater et al. 1999).

Strong variation in band size is reported for feral horses, they can be as small as two individuals (a stallion and mare pair) or up to 35 as seen in the feral horses of the Llanos of Venezuela (Pacheco and Herrera 1997). The mean size of bands recorded for feral horse populations varies from 3.4 to 18.1 (review in Boyd and Keiper 2005). Based on the results of Chapter 2, variation in ecological factors and population demographics come together to influence the observed differences in group size and stability. A male's reproductive success will inherently be linked with their ability to obtain a position as a dominant stallion, the number of females within his harem, and his ability to maintain this role over time. However, there is evidence that more may not always be better when it comes to harem size (Kaseda and Khalil 1996; Silk 2007, Welsh 1975). Larger bands often have lower foal production then smaller bands, and there is a greater risk of rival stallions or bachelors mating with females from larger groups (Kaseda and Khalil 1996). Optimal group sizes are often intermediary with groups that are too small or too large enduring costs that reduce reproductive performance of males and females (Silk 2007; Pride 2005*a*).

Among male horses, there is evidence suggesting that the experiences and associations between natal dispersal and band formation can influence future reproductive success. Males in bachelor groups are thought to benefit from interacting with conspecifics gaining social experience and developing role-specific behaviours (Hoffmann 1982; Berger 1986; Levréro et al. 2006). Bachelors will establish dominance hierarchies amongst their peers, resulting in rank positions which have been linked to future reproductive success (Tilson et al. 1988; Bourjade et al. 2009; Heitor and Vicente 2010*a*). Formation of long-term bonds between bachelor males have also been thought to lead to cooperative behaviours in future attempts of harem formation leading potentially to multi-male bands (Feh 1999; Fischhoff et al. 2009).

The assortment of individuals in a social species has strong implications in population genetics, disease or information transmission, resource access, and avoiding predation (Wey et al. 2008; Croft et al. 2011). Social network analysis has provided ecologists with a new set of tools for evaluating and quantifying associations in animal societies (Krause et al. 2007; Whitehead 2008; Farine and Whitehead 2015). The basis of the analysis is the network created by determining

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which individuals ('nodes') interact creating the link ('edge') between associated nodes (Whitehead 2008). Additional behaviour data about the nature of the association such as persistence or direction of action (e.g., aggressor and receiver) can allow for development of weighted or directed networks and more detailed analysis (Wey et al. 2008; Whitehead 2008). For example, measure of neighbor proximity was used as a representation for association strength in feral horses of the Carneddau Mountain Range, N. Wales (Stanley et al. 2017). The Sable Island feral horse survey data is comprised of the most basic of association information being that of an individual's presence or absence in a group. Known as "Gambit of the Group" data, this supports undirected networks only with association strength calculated based on the proportion of times an individual is seen associated with a specific individual based on all observations of both individuals (Whitehead 2008; Franks et al. 2010). Although 'gambit of the group' data, the Sable Island dataset is one of the temporally longest as well as containing one of the largest number of individuals analyzed for an equid population (Sundaresan et al. 2007; Fischhoff et al. 2009; Nuñez et al 2015; Stanley et al. 2017).

This closed-population dataset allows us to accurately evaluate the longevity of associations. The Lagged Association Rate (LAR) calculates the probability of associations persisting through time (Whitehead 2008) and provides insight on the stability and non-randomness of groups within populations (Best et al. 2013; Carter et al. 2013; Francesiaz et al. 2017). Measures of centrality such as degree (the number of associates an individual is seen with), strength (an index measure based on the proportion of times two individuals are seen associating), and betweenness (the number of shortest paths that connect other individuals which pass through the focal individual) are also used to help distinguish social characteristics of individuals (Whitehead 2008). Additionally, matrices used to define the network can be statistically compared allowing for interannual comparisons (Whitehead 2008; Croft et al. 2011; Rimbach et al. 2015).

Feral horses provide a very interesting problem when using social network analysis in that they contain two very different patterns of associations (Figure 3.1). The first consists of the fairly rigid band structures which show very little fission-fusion activity within years (although do have intra-annual movements of interest). Among bands, measures such as degree and strength are very much a function of band size and using social network measures provides very little additional information. The more itinerant bachelors consist of individuals which typically

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interact with several different bachelor groups throughout the year. Therefor the number of associates and the relative strength of these associations are more diverse and difficult to identify in large datasets without the tools social network analysis provides. The differences between band and bachelor group suggests using different approaches in analyzing social characteristics of females versus males against reproductive success.
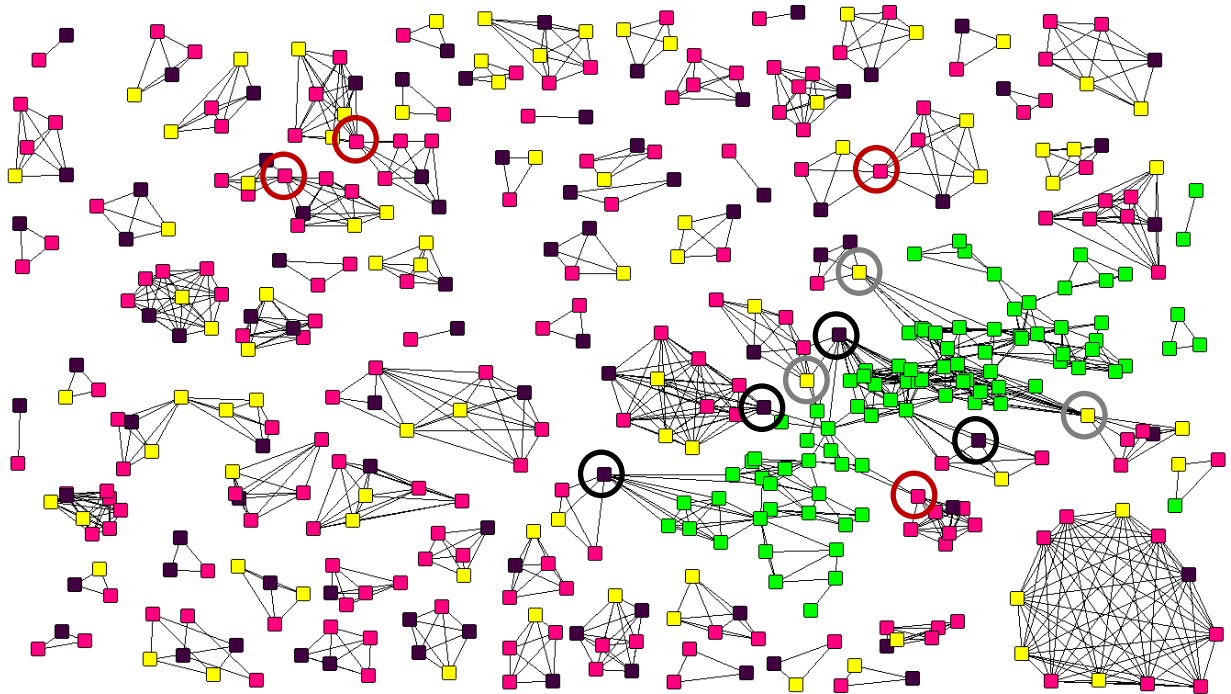
Figure 3.1. Social network of feral horses for the observed associations in 2012 showing the very distinct bands (Black – Band Stallion, Pink – Female, Yellow – Juvenile) and the highly connected group of bachelors (Green). Stallions do occasionally leave their harem temporarily to engage with bachelors, or bachelors engage with stallions (black circles) while some juveniles will also foray into bachelor groups (grey circles). You can see a few instances where females are observed to consort with more than one band during at least one observation during the survey period (red circles).

Here I investigate trends in feral horse associations and how characteristics of these associations can influence individual reproductive success. I use informative approaches from the field of social network analysis (LARS, network comparisons, and centrality) and some of the metrics

produced in these processes are then further evaluated using more traditional modelling approaches. Female foal production and recruitment over a 5-year period is used as a measure of reproductive success in females and compared to the number of associates over that period of time, as well as the number of band stallions, and times that a female changed bands. Female reproductive success is also compared to the duration of their longest female or stallion partner. Measures of sociality (degree, strength, or betweenness) of bachelor males was evaluated for its usefulness as a predictor of male success in obtaining status as band stallion and associated reproductive opportunities. If experiences in playing or fighting with other bachelors aids in their development and future success we would likely see individuals with higher degree, strength, or betweenness be more likely to succeed in obtaining a harem.

3.3   Methods

3.3.1   Study Area

Sable Island National Park Reserve is located 175 km from mainland Nova Scotia Canada, far offshore in the Atlantic Ocean (43°55'N, 60°00W). The 42 km long, but narrow (max 1.6 km width) island is composed entirely of sand and the vegetation which holds it together. The island has been supporting a feral horse population since they were introduced in mid-1700s. During the operation of life-saving stations (established to support maritime travel and trade) there were some introductions and removals of the horses however round-ups ceased after 1960 saw protection granted to the horses though the Canada Shipping Act (2001). From that time there has been no or very minimal disturbance to the horses and they have been maintaining a natural social structure of their own choosing.

3.3.2   Survey Design

Surveys of the horse population have been repeated annually from 2008 to present; however, this research will use the information obtained up to 2015. Census of the population predominantly occurred over the summer months of July and August however in 2009 some data from May and

June is also available. Survey consisted of travel on foot across the island to locate horses. Individuals were classified in the field as either being a solitary bachelor, part of a bachelor group (no females present), or member of a band (mixed sex group with at least one mature male). For bands, total band size (a count of all individuals including foals) as well as harem size (number of females 2 years old and older) was calculated. The dominant male or 'stallion' was the adult male of a band which maintained closer proximity to the females, was observed to conduct most of the matings with the group females, and/or was dominant to all other adult males in the group. Other adult males that were associated with a band but were not the dominant male were considered 'tag' males.

The geography of the island, being quite narrow with low vegetation, ensured a very high probability that all horses would be observed. Their high tolerance of human presence made it easy to collect the information on group type and size, location, and characteristics of the individuals along with photographs to later use for identification. Individual identification was done using natural markings and colouration, shape of chestnuts, position of hair whorls, whiskers, and nose crease patterns, etc. Individuals were distinct enough in these characteristics to not require invasive forms of marking or tagging. Survey and observations were taken following the guidelines provided by the permitting agency, the Canadian Coast Guard (2008–2012), or Parks Canada (2012–2015, Research License 14668), and in accordance with our institutional animal care permit (University of Saskatchewan UCACS #20090032).

3.3.3   Characteristics of Male and Female Social Structure

3.3.3.1   5-year Summary of Associations and Reproductive Success of Females

Female reproductive success was measured by the number of foals produced, as well as the number of those foals surviving up to 1 year of age, for all adult females that survived for at least five additional years. Observations were limited to females three years old or older in their first of five consecutive years. In addition to reproductive success, information on the cumulative number of associates, number of dominant and tag stallions, number of bands the female consorted with, and length of their longest association (both male and female) were determined for the concurrent five years.

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The data, being predominantly count data, has a Poisson distribution and therefore generalized linear models were used to analyze the data. Data were evaluated for significant random effects of both the first year of entry into the analysis as well as for the age of entry into the analysis. Based on these results generalized linear mixed effect models were used to evaluate the influence of social factors on foal production and recruitment over 5 years. All analyses were performed using R v. 3.0.2 (R Core Team 2013).

3.3.3.2 Social Network Analysis

3.3.3.2.1 Measures of Associations and Centrality

Annual social network values were calculated based on all observations of bachelors during each summer survey. To test that the networks constructed from observations are significantly different than random, 1000 permutations of the observations were done by swapping individuals between groups (Croft et al. 2011; Farine 2013*a*). Due to some of the limitations in our data, namely the absence of behavioural measures, three descriptive measures were chosen for further analysis (Whitehead 2008). Degree is an integer value indicating the number of unique individuals that the focal individual was observed associating with in a given year. Strength is a measure reflecting how frequently an individual is seen with another specific individual (i.e., if always seen together the edge weight is one). Strength is a cumulative value of all edge weights of an individual. Betweenness reflects how central (or well connected) an individual is within the network by calculating how many 'shortest paths' the individual occurs on between any other dyad. The higher the betweenness value the more 'central', or more strongly connected, the individual is said to be. The above variables were calculated using R with the packages sna (Butts 2014), asnipe (Farine 2013*b*), and igraph (Csardi and Nepusz 2006).

Generalized linear mixed effects models were used to see if there was a relationship between degree and the age of the individual. Horse ID was added as a random effect as we have repeated measures of individuals. Linear mixed effect models were used to investigate association strength against age using package *lme4* (Bates et al. 2014). Generalized linear mixed effect models were also used to investigate if degree, strength, or betweenness of an individual was a significant predictor of their obtaining position of dominant band stallion or for any changes in

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social position the following year. For these analyses both Age and Horse ID were considered random factors.

3.3.3.2.2 Lagged Association Rates

Lagged association rates measure the probability that a dyad will be observed together over a period of time (Whitehead 2008). For this study, it was of interest to find out how the persistence of associations between females and the stallion, or females and other band females, as well as look at the persistence of associations among bachelor males. I restricted LAR analysis of individuals in bands to only adult animals since foals have higher mortality and younger animals tend to move for natal dispersal. I looked at both the persistence of relationships between adult females, as well as between females and adult males (i.e., the dominant or tag band stallions). This study is more interested in what is occurring during prime reproductive phases of an individual's lifetime. For bachelors however, I have included all bachelors from 1 year old to adult as the bonds which occur early in bachelorhood have potential to persist for a much longer period of time (Berger 1986; Levréro et al. 2006). To calculate the standard error I used a jackknife technique, removing one observation and calculating the lagged association rate of the remaining data (Whitehead 2007; Whitehead 2008; Farine 2013*a*). Lagged Association Rates were calculated and drawn using R with the packages igraph (Csardi and Nepusz 2006), sna (Butts 2014), and asnipe (Farine 2013*b*).

3.3.3.2.3 Mantel Tests for Matrix Similarity

Mantel tests return a correlation statistic representing the similarity between two matrices as well as probability that the observed correlation between two matrices (networks) is higher than the correlation statistic returned from one network undergoing permutations (Whitehead 2008; Croft et al. 2011; Farine and Whitehead 2015). Social network matrices were created for all horses (excluding foals) for each year representing who associated with whom during at least one observation during the annual survey. Mantel Tests were then used to compare the similarity of the matrices using R statistical software and the packages asnipe (Farine 2013*b*) and vegan (Oksanen et al. 2015). Rimbach et al. (2015) similarly used Mantel tests to see if differences between annual networks were significant.

3.4 Results

3.4.1 Social Networks Analysis

During the annual surveys performed from 2008 to 2015 there were 304 days of observations (Table 3.1). A total of 5388 observation of unique groups or individuals: 1541 bachelors or bachelor groups and 3847 bands. Over the seven years 216 adults were classified as bachelors and 432 adults classified as band members and were included in the following analyses.

Table 3.1. Summary of the feral horse survey data for years 2008–2015 (Sable Island, NS).

Year	First Day	Last Day	Days	Mean # of sightings per horse ($\pm$SE)
2008	08/12/2008	9/9/2008	29	2.6 $\pm$ 0.05
2009	05/23/2009	7/30/2009	48	5.5 $\pm$ 0.14
2010	07/2/2010	09/01/2010	37	5.4 $\pm$ 0.07
2011	07/21/2011	08/23/2011	24	3.1 $\pm$ 0.46
2012	07/20/2012	08/17/2012	27	7.3 $\pm$ 0.11
2013	7/15/2013	9/15/2013	50	7.6 $\pm$ 0.07
2014	7/21/2014	9/9/2014	51	7.7 $\pm$ 0.06
2015	7/24/2015	9/1/2015	38	7.7 $\pm$ 0.08

3.4.2 Lagged Association Rates

The lagged association rates give us an indication of how long the associations last between individuals. As the overall network shows some very clear isolation between individuals in bands and bachelors (Figure 3.1) I look at the two as separate networks. Amongst individuals living in in bands we see slightly higher probability of an associations between females and the adult males of the bands (this test does not distinguish between dominant and subordinate males) being maintained across the years than we do between females of the same harem (Figure 3.2). We also

see that the longest male–female association lasted 6 years while the longest female–female association was only 5 years. With bachelors, there is a much lower probability of a dyad being seen together in the following year or subsequent years (Figure 3.3) than there was for individuals living in bands.
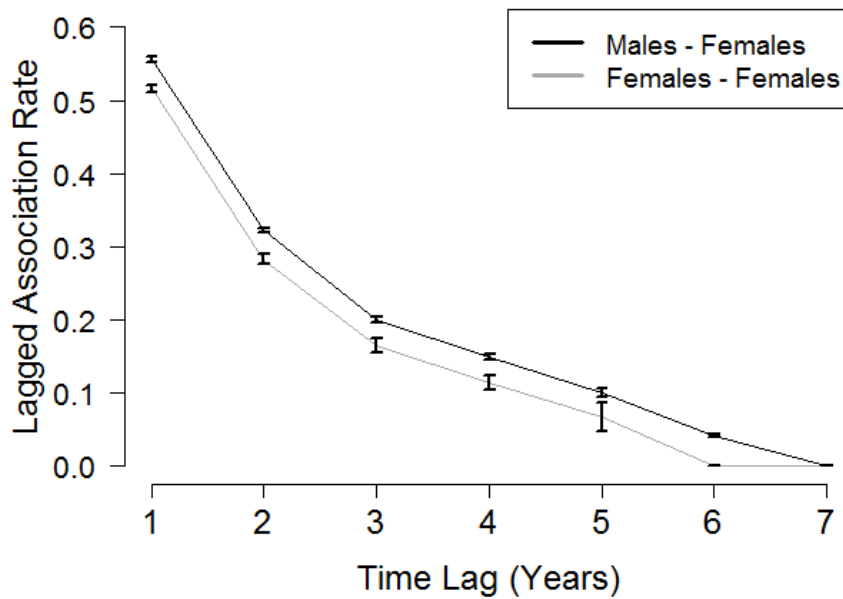
Figure 3.2. Lagged association rate (probability of a dyad being together after a period of time) of adult horses (4+ years of age) in bands (excludes bachelors). Line colour indicates mean lagged association between females and band stallions (black) or between harem females (grey). SE bars generated by jackknife production of 1000 random matrices.
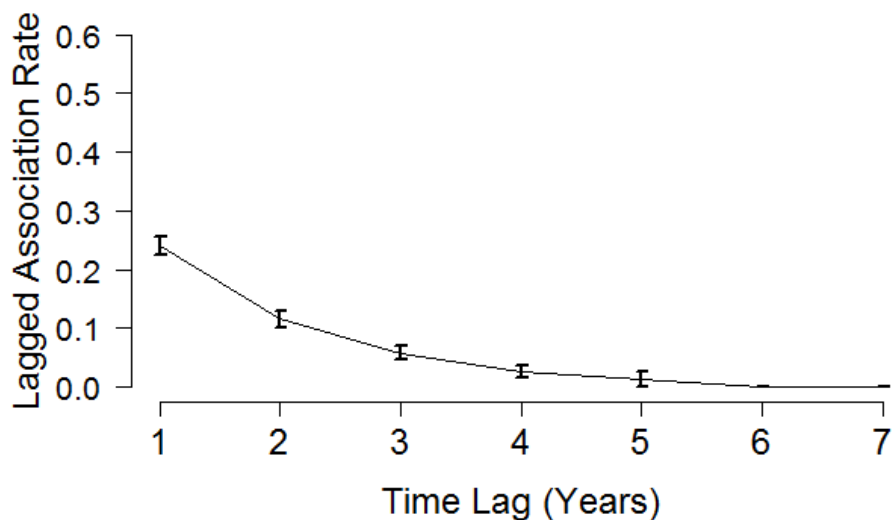
Figure 3.3. Lagged association rate (probability of a dyad being together after a period of time) of bachelor males (1+ years of age). SE bars generated by jackknife production of 1000 random matrices.

3.4.3   Similarity of Annual Networks

Again, due to the vastly different social behaviours between individuals in a band and those which are bachelors I performed Mantel tests on independent networks. Mantel tests of the annual matrix for adult horses in bands suggest that it takes up to 6 years for the associations between individuals to break down enough for the lack of correlation to be considered significant (Table 3.2). There is also variation in the correlation statistic between consecutive networks depending on the year. For example, the social network in 2009 was the most similar to the network in 2008 (0.642) while the transition from 2010 to 2011 had a low measure of network correlation (0.346). In comparison, it is clear from the results of the Mantel tests on the annual bachelor networks that the similarity between years is much less and does not persist for much more than 2 to 4 years (Table 3.3).

Table 3.2. Mantel test of the annual networks of horses (4+ years old) that were members of a band between 2008 and 2015. Bold indicates that the probability that the network correlation statistic is higher than the correlation statistic derived from 999 randomized permutations of one matrix.

YEAR	2008	2009	2010	2011	2012	2013	2014
2009	**0.642**						
2010	**0.334**	**0.472**					
2011	**0.151**	**0.180**	**0.346**				
2012	**0.091**	**0.119**	**0.224**	**0.586**			
2013	**0.033**	**0.064**	**0.102**	**0.273**	**0.497**		
2014	0.008	**0.036**	**0.068**	**0.130**	**0.246**	**0.438**	
2015	–0.002	0.008	**0.025**	**0.048**	**0.090**	**0.150**	**0.418**

Table 3.3. Mantel test correlation statistic of annual networks for bachelors (1+ year old) between 2008 and 2015. Bold indicates that the probability that the network correlation statistic is higher than the correlation statistic derived from 999 randomized permutations of one matrix.

YEAR	2008	2009	2010	2011	2012	2013	2014
2009	**0.220**						
2010	**0.067**	**0.217**					
2011	–0.001	**0.066**	**0.181**				
2012	–0.003	–0.004	**0.061**	**0.308**			
2013	–0.003	–0.005	**0.017**	**0.112**	**0.185**		
2014	–0.003	–0.005	–0.004	**0.032**	**0.047**	**0.206**	
2015	–0.003	–0.005	–0.004	**0.024**	**0.013**	**0.077**	**0.279**

3.4.4 5-year Summary of Associations and Reproductive Success of Females

We had a full five years of adult female reproductive history for 106 horses. Fifty-two of the females were 3 years old in the first of the five years, the remaining 54 females were 4 or older. The majority of the females had their five year period of study start in 2008 ($n = 65$), the other years were lower (2009: $n = 16$; 2010: $n = 14$; 2011: $n = 11$). There was no significant differences between the numbers of associates an individual has and when the start of the 5 consecutive years were ($F_{1,104} = 0.890$, P-value $= 0.348$) . However, the years included in the analysis did have a significant influence on both the number of foals produced (Figure 3.4; $F_{1,104} = 7.812$, P-value $= 0.006$) as well as the number of foals that survived ($F_{1,104} = 7.698$, P-value $= 0.007$). Additionally, if the individual was 3 years old in the first of the five consecutive years then there was also a likelihood that they would have lower 5-year foal production (Figure 3.5; $F_{1,104} = 8.518$, P-value $= 0.004$) and foal survival ($F_{1,104} = 9.638$, P-value $= 0.002$) These results suggest that the start year for the 5 consecutive years as well as the age of the individual at the start requires consideration when trying to understand the influence of social structures on female reproductive performance.
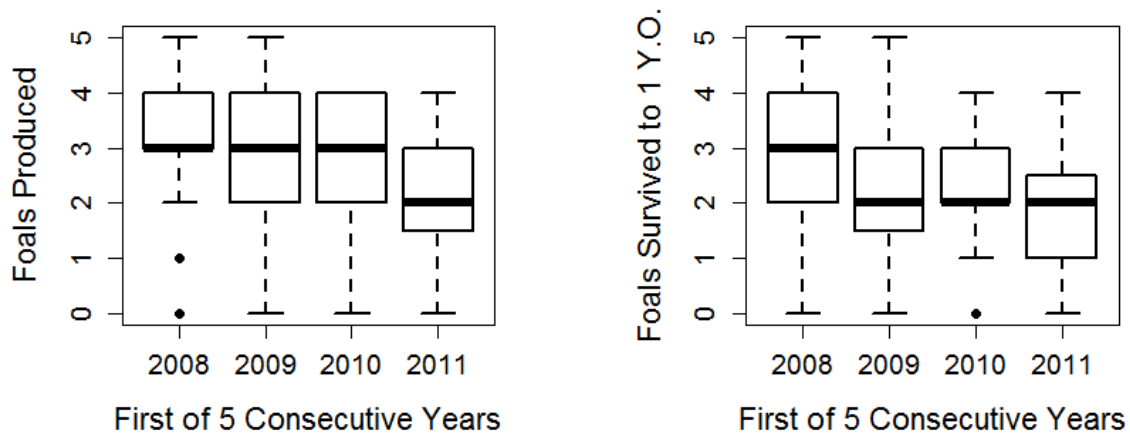
Figure 3.4. Five-year foal production by females (left) and 5-year foal survival (right) did have a tendency to decrease the later the first of the 5 consecutive years of analysis started. Foals Produced: $F_{1,104} = 7.812$, P-value $= 0.006$. Foals survived: $F_{1,104} = 7.698$, P-value $= 0.007$.
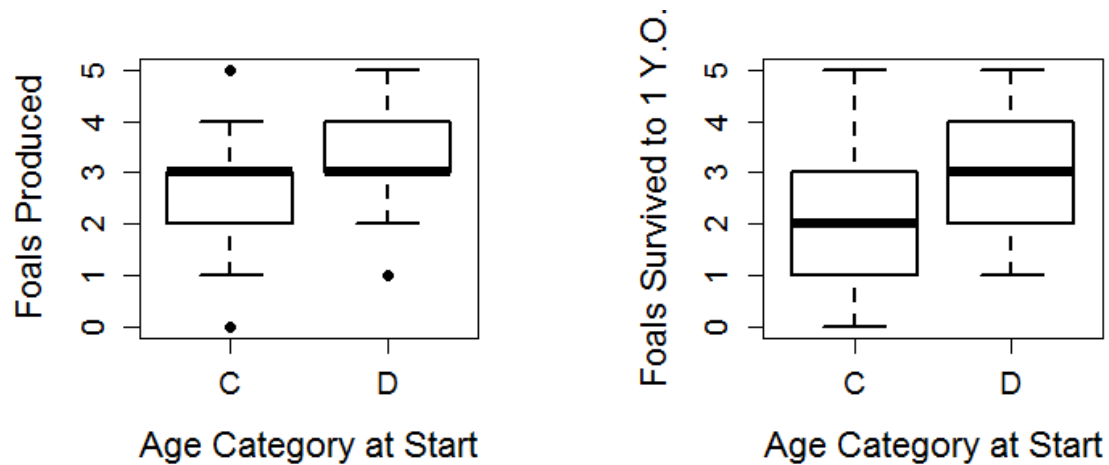
Figure 3.5. Five-year foal production by females (left) and 5-year foal survival (right) did have a tendency to be less for females who entered the 5-year period as 3 year olds (Age Category C) then for females who were 4 years old or older (Age Category D). Foals Produced: $F_{1,104} = 8.518$, *P*-value = 0.004. Foals survived: $F_{1,104} = 9.638$, *P*-value = 0.002.

3.4.5   Social Characteristics of Associations and Female Reproductive Success

Having a high number of associates over a 5 year period can significantly reduce foal survival and to a lesser extent, foal production (Table 3.4; Figure 3.6). Foal survival to one year of age tends to be higher when females consort with fewer stallions over the period of 5 years (Figure 3.7). Similarly, when a female remains within a band for a longer period of time or maintains associations with the same stallion for longer both have a positive influence on foal production and survival (Figures 3.8 and 3.9). What was surprising was that long-term associations between females with other band females did not have any notable correlation to offspring production and survival (Figure 3.10; GLMER with Age at start and year as random factors, production: $b = -0.022$, *P*-value = 0.703; survival: $b = -0.020$, *P*-value = 0.750)

Table 3.4. Results of generalized mixed-effects modelling on foal production and foal survival. Random variables were the year and age at which the female started the 5-year observation. Explained deviance results calculated as per (Zuur et al. 2009).

Model – GLMER	AIC_c	ΔAIC_c	*df* residual	Residual deviance	Explained Deviance
Foal Production					
Null with random factors (year.start)+(age.start)	368.0		103	362.0	
Associates	366.3	−1.7	102	358.3	1.02%
Stallions	367.0	−1.0	102	359.0	0.83%
Number of bands	367.3	−0.7	102	359.3	0.75%
Associates + Stallions	367.5	−0.5	101	357.5	1.24%
Associates + Number of Bands	367.8	−0.2	101	357.8	1.16%
Longest Stallion Association	368.2	+0.2	102	360.2	0.50%
Longest Female Association	369.8	+1.8	102	361.8	0.06%
Foal Survival					
Null with random factors (year.start)+(age.start)	357.9		103	351.9	
Associates	**355.5**	**−2.4**	**102**	**347.5**	**1.25%**
Stallions	356.7	−1.2	102	348.7	0.91%
Associates + Stallions	356.7	−1.2	101	346.7	1.48%
Associates + Number of Bands	357.3	−0.6	101	347.3	1.31%
Longest Stallion Association	357.6	−0.3	102	349.6	0.65%
Number of Bands	357.7	−0.2	102	349.7	0.63%
Longest Female Association	359.8	+1.9	102	351.8	0.03%
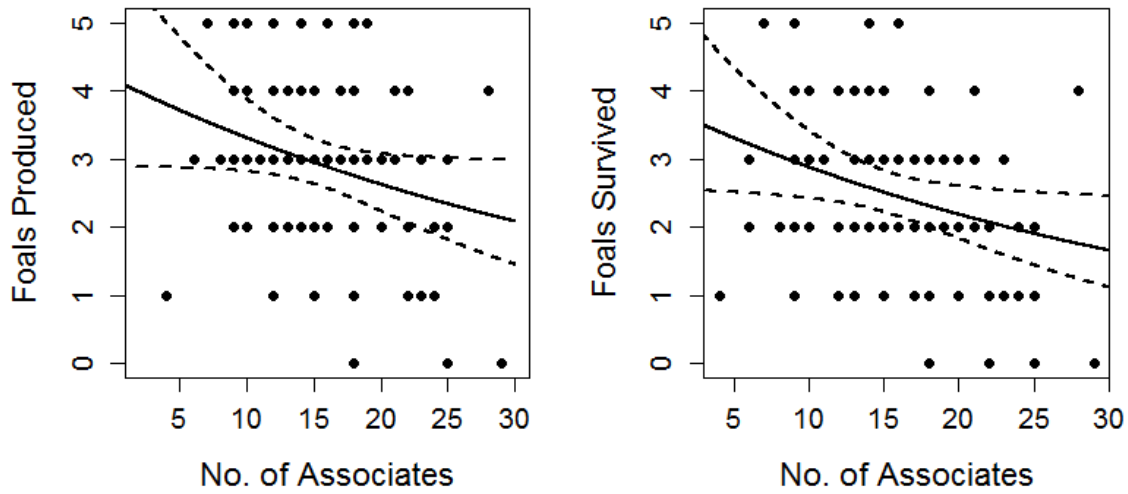
Figure 3.6. Having a high number of associates (both male and female) over a 5-year period may reduce female foal production (left) and offspring survival to one year of age (right). Model fit to generalized linear model fit with Poisson distribution ± 95% confidence intervals.
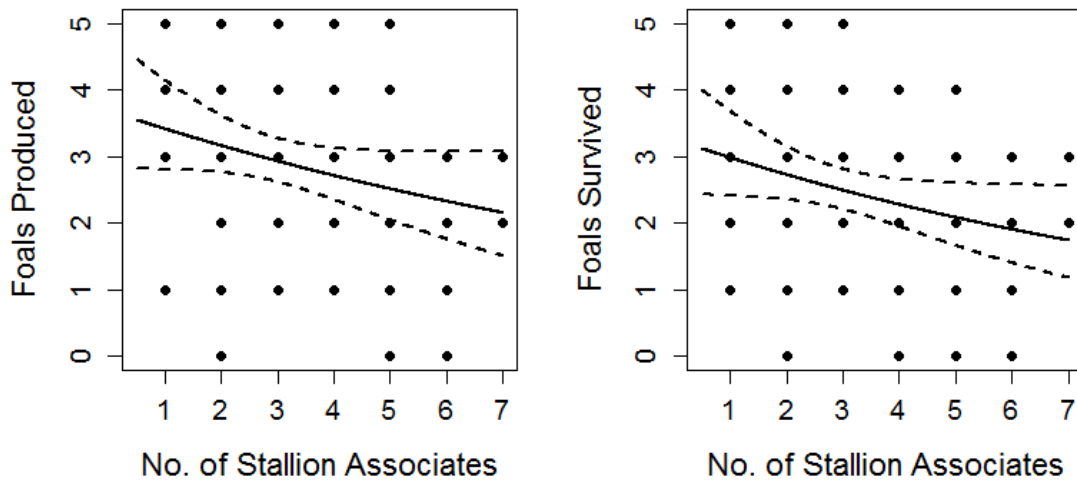
Figure 3.7. The number of stallions that a female will consort with over a five year period is slightly negatively associated with their production of foals and their foals surviving to one year of age. Model fit to generalized linear model fit with Poisson distribution ± 95% confidence intervals.
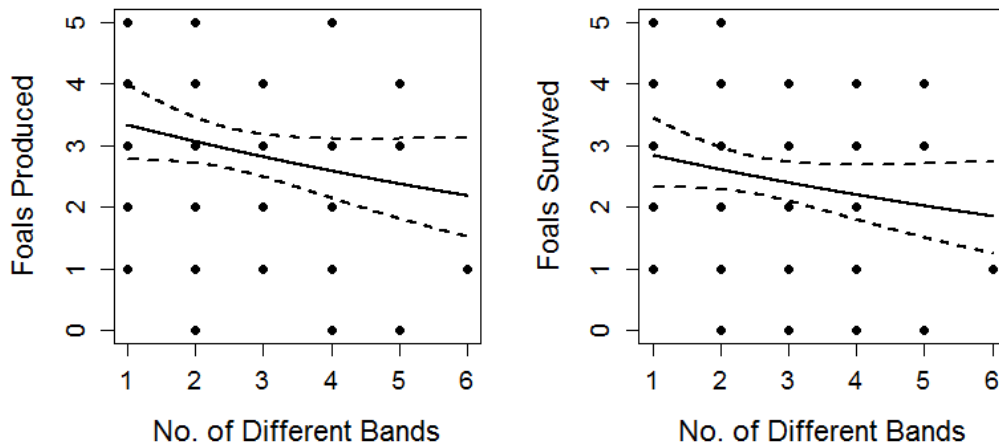
Figure 3.8. The number of different bands a female was observed with over 5 years and their production of foals and foals surviving to one year of age. A trend was observed that if a female associated with a lower number of bands over time their productivity was increased. Model fit to generalized linear model fit with Poisson distribution $\pm$ 95% confidence intervals.
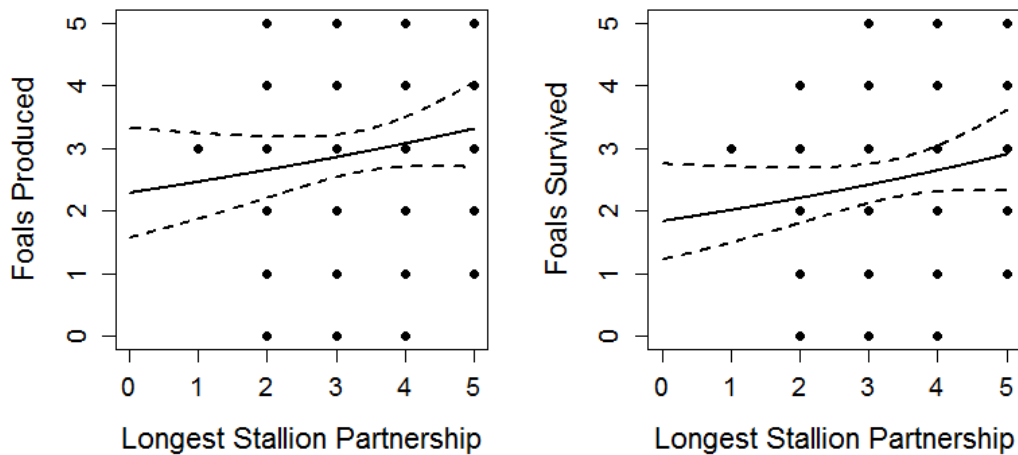
Figure 3.9. A trend was observed where foal production and survival was slightly increased based on how many years a female remained associated with a dominant male stallion. Model fit to generalized linear model fit with Poisson distribution $\pm$ 95% confidence intervals.
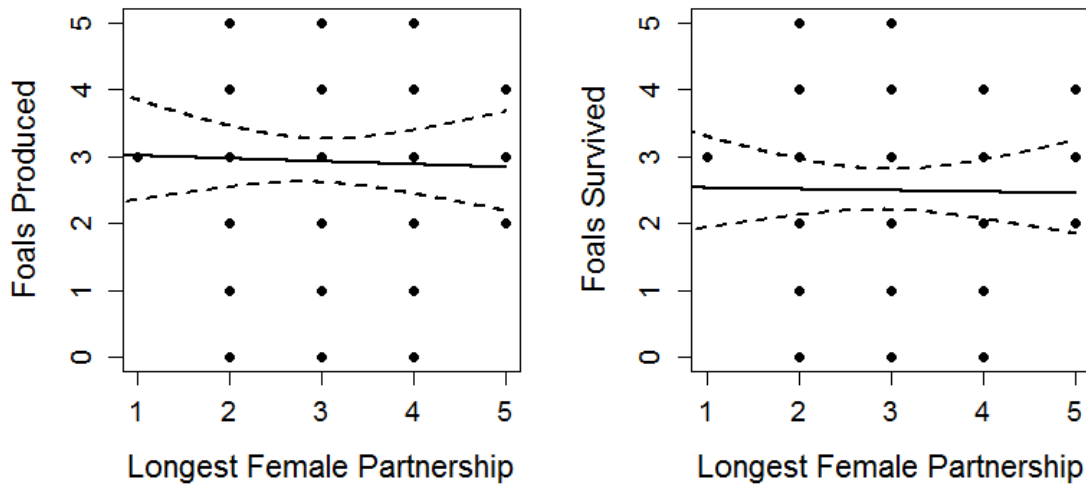
Figure 3.10. Maintaining long-term associations specifically with other females does not increase foal production or survival of offspring. Model fit to generalized linear model fit with Poisson distribution ± 95% confidence intervals.

3.4.6 Social Characteristics of Males Achieving Reproductive Roles in the Population

From data on males born between 2007 and 2010 we observed mean age of independence (natal dispersal) to be at 2.8 years of age. We observed males leaving bands at 1 year old, often when the mother died or if the mother was found in a new band the subsequent year. Very rarely we observed males stay with their band up to 5 years old. As we are still in the early stages of this long-term study we are unable to detect males that are late to take a position as dominant stallion; however, from those born in the early years of the study (2007–2009) we see a mean age of becoming a dominant band stallion ranging from 5.6-5.9 years of age (range 4–8; $n = 52$).

From 2008–2015 we observed 43 instances of males ($n = 49$), taking up the role as a tag or subordinate stallion; 17 (34.7%) of these tag males subsequently transitioned into a dominant band stallion position. Seven males went on to form bands with females from different bands, three formed new bands with a portion of the members from the band it was associated with, and seven successfully displaced or took over the current band from the dominant male. Other fates of tag males included remaining a tag male in the same band ($n = 4$), died before the next survey

($n = 14$), or returned to being bachelors ($n = 14$). Multi-male band structures make up a small proportion of the population but remain a common observed phenomenon on Sable producing a mean males per band of 1.11–1.25 over the 8 years of the study (Table 3.5). The mean age that males were first observed to take a position as a tag male is 4.52 years (range 3–7). It is also notable that the number of males per band tended to be higher when there were more females per band (and fewer bands).

Table 3.5. Social distribution of the adult feral horse population of Sable Island, NS.

YEAR	# of Males 4+	% as Bach	% in Bands	# of Bands	# of Multi-male bands	Mean Males per Band	# of Females 2+	Mean Females Per Band	ASR
2008	86	28.2	71.8	52	12	1.23	136	2.62	0.387
2009	114	35.9	64.1	58	12	1.25	147	2.53	0.437
2010	122	27.0	73.0	72	12	1.24	165	2.29	0.425
2011	123	30.9	69.1	72	8	1.18	147	2.04	0.456
2012	145	37.9	62.1	78	11	1.15	175	2.24	0.453
2013	177	41.8	58.2	86	13	1.19	178	2.07	0.499
2014	180	58.3	41.7	88	10	1.11	193	2.19	0.483
2015	182	59.9	40.1	81	13	1.16	150	1.85	0.548

3.4.7   Bachelor Social Networks

All annual networks for the bachelors proved to be significantly different from the networks created with the bootstrapped data suggesting their structure is not random (Figure 3.11).
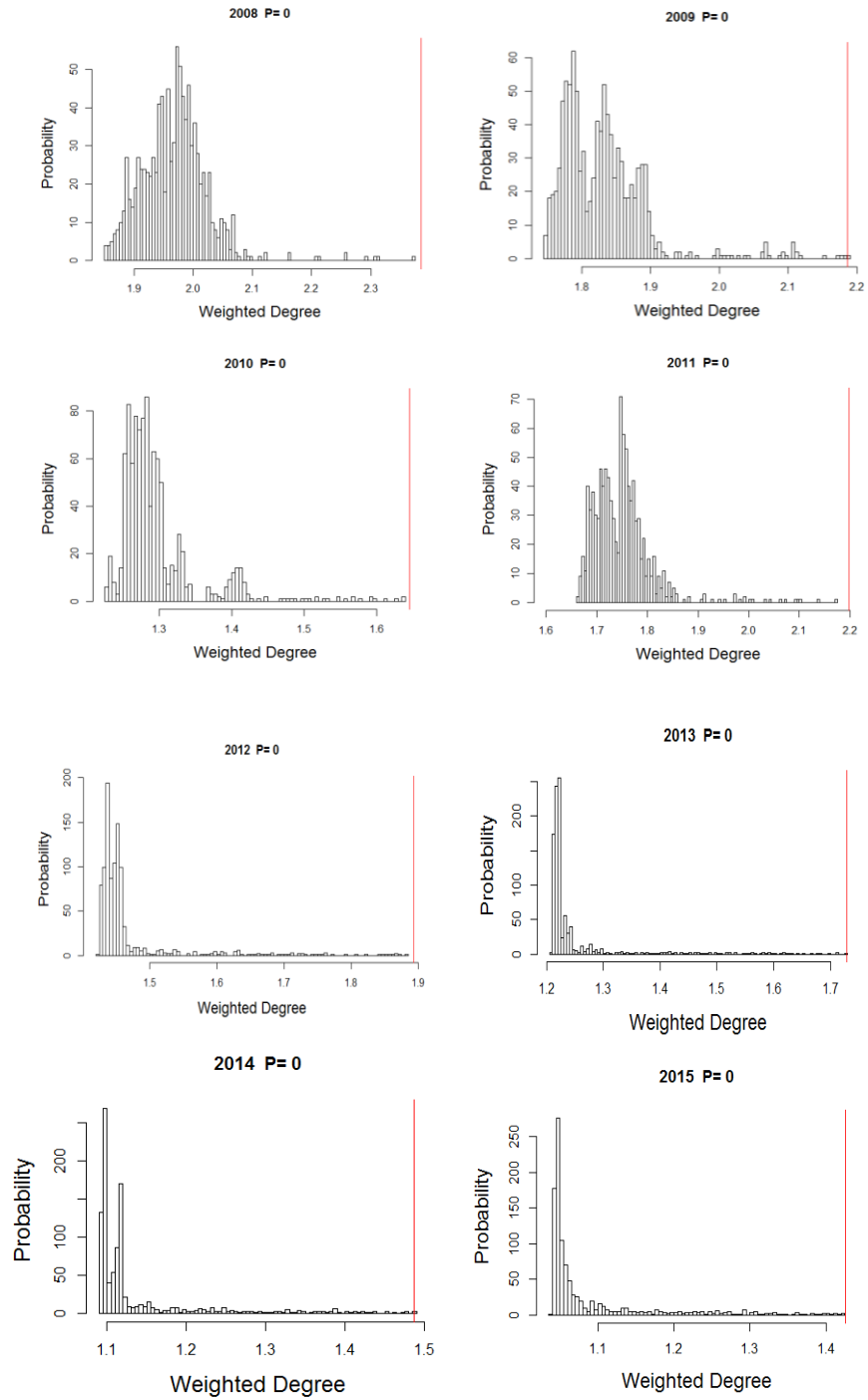
Figure 3.11. Results comparing the mean weighted degree (strength) of the observed annual social networks (red lines) to the mean weighted degree calculated from 1000 permutations of the network with individuals swapped between groups. Position of the red line indicates observed weighted degree is significantly higher then what would be expected by chance.

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There are some significant differences between the degree observed at different ages with bachelors aged 1 (intercept), 6 (P-value $= 0.606$) and 7 (P-value $= 0.084$) having significantly fewer associations (lower degree) then observed at ages 2 (P-value < 0.001), 3 (P-value $= 0.003$), and 5 (P-value $= 0.012$). Age 4 was almost considered significantly different at (P-value $= 0.061$). There appears to be a trend that number of other individuals a bachelor is associated with becomes lower as they mature (Figure 3.12). Bachelors also showed that the strength of the associations showed significant decreases with increasing age when looking at the difference between the model including age and the null model ($\chi^2_{(6)} = 704.72$, P-value < 0.001).
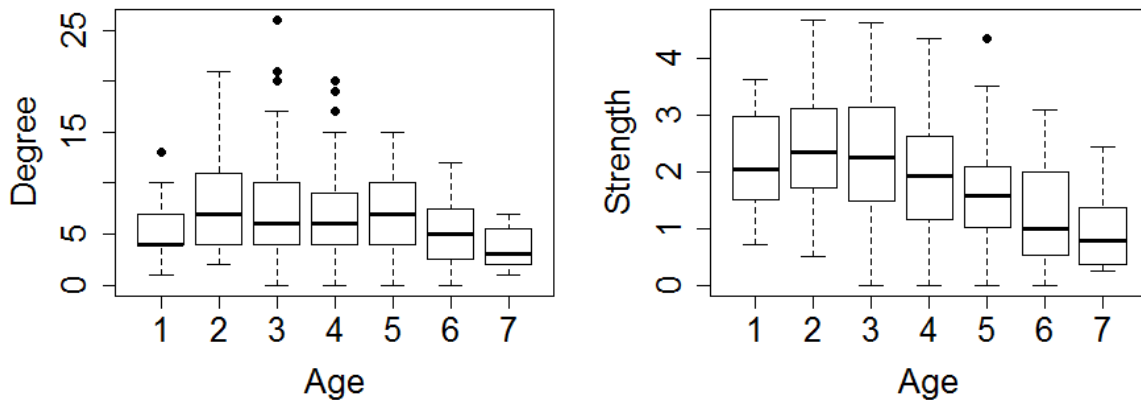
Figure 3.12. Degree (left) and strength (right) for bachelors males of known ages in the feral horse population of Sable Island, NS.

3.4.8   Social Network Measures of Males and Future Social Position

There was no clear relationship between the degree and the social position in which the individual was seen occupying the following year (Tag, Stallion, Dead, or Bachelor; Figure 3.13). Generalized mixed effect model, with Age and Horse ID used as random factors, did not reveal any significant differences in degree for tags, stallions, or dead individuals compared to those who remained bachelors (Bachelor: intercept $= 1.81697$; Tag: $b = -1.19331$ P-value $= 0.071$; Stallion: $b = -0.04285$, P-value $= 0.558$; Dead: $b = -0.09359$, P-value $= 0.473$) . Additionally, the model including the position next year has higher AIC$_c$ (2234.5) then the null

model (with the random factors; $AIC_c = 2232.3$) further suggesting very limited explanatory power. Similarly, linear mixed effect models of strength with Age and Horse ID as Random effects suggest very poor explanatory power of position next year and current strength with the null model having lower AIC_c (1057.3) then the model including position next year ($AIC_c = 1061.8$).

Figure 3.13. Social position of horses based on the degree (left) and strength (right) calculated from their previous year by age (1–7; in grey header). T = Tag, S = Stallion, D = Dead, B = Bachelor.

Comparing the degree, strength, and betweenness of bachelor males to whether or not they became dominant band stallions the following year did not reveal any predictive power (Figure 3.14; Degree: $b = -0.0131$, P-value = 0.775; Strength: $b = -0.2240$, P-value = 0.260; Betweenness: $b = 0.0037$, P-value = 0.870; variables age and horse ID used as random variables, 374 observations of 143 individuals, 7 age classes).

Figure 3.14. Probability of an adult male bachelor being a stallion in the following year does not appear to be dependent on the measure of degree, strength, or betweenness of an individual.

3.4.9   Group Size and Foal Production

As would be expected, having a larger harem size increased the potential number of foals born in a band (Figure 3.15: $b = 0.2585$, SE $= 0.0256$, P-value < 0.001). It was observed that harems of up to three females can, on occasion, have 100% foal production (that is all three females are observed with foals); however, harem size of four females and more have foal production consistently lower than 100% with no more than four foals observed even in harems of 7 females. Conversely, harem size of up to five females have had 0% foal production but overall larger harem sizes tend to have at least one foal produced. Band size was also positively correlated with foal production ($b = 0.158$, SE $= 0.0181$, P-value < 0.001).
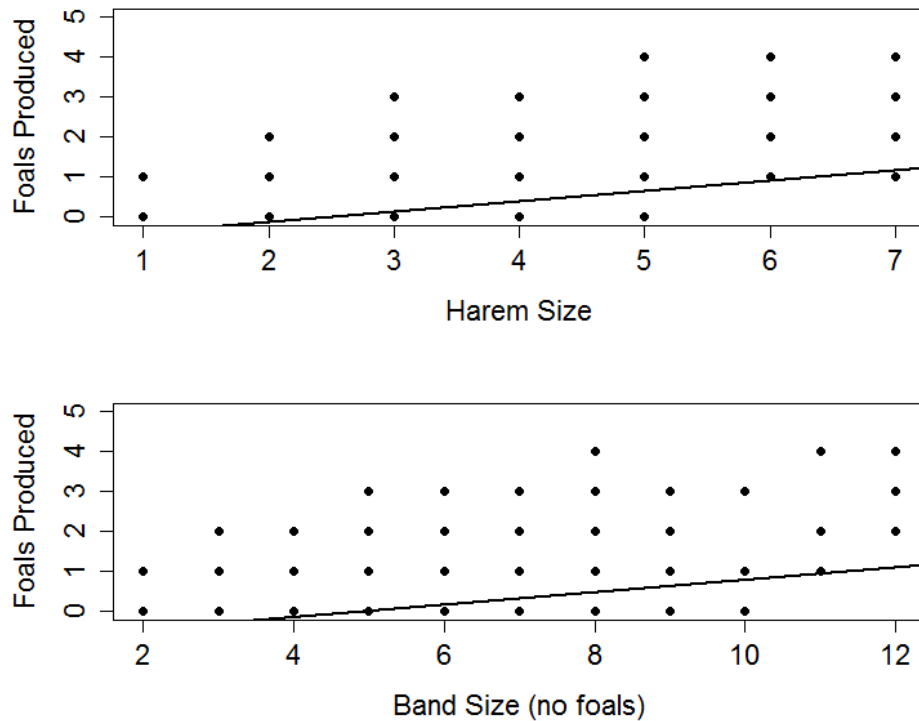
Figure 3.15. Total number of foals produced based on harem size (top; $b = 0.2585$, SE $= 0.0256$, *P*-value < 0.001) and band size (bottom; $b = 0.158$, SE $= 0.0181$, *P*-value < 0.001). 100% foal production has only been observed when harem size consists of 1–3 females.

3.5 Discussion

Lagged association rates and comparison of annual networks suggests that the social structure on Sable Island persists for approximately 5 to 6 years before being composed of nearly all new associations. The associations developed and maintained by bachelor males tend to break down more rapidly and possibly respond to variation in sex ratio. When looking at the number of associates over a 5-year period we see that the more individuals a female interacts with (i.e., stallions and other band members) the lower the female's foal production and fewer of those foals survived to one year of age. Clear reproductive advantages are gained when the number of associates stays small, specifically if the number of stallions (or bands) a female consorts with over time is minimized. When looking at the importance of maintaining long-term associations

with specific individuals we find no reproductive advantage among females maintaining long associations with other harem females but do see slight reproductive benefits in females maintaining long-term associations with stallions. Measures of sociality (degree, strength, or betweenness) of bachelors were not predictive to which males succeeded in acquiring harems the subsequent year.

Investigating lagged association rates shed light on similarities in social affinities between band members. Relationships between females in the bands are almost as lasting as relationships to the dominant band stallion(s), and follow a similar pattern of decay over time. However, the probability of females being observed together between years does remain lower than the probability that a female will remain with the stallion. The LAR between bachelor males shows an overall much lower probability of two males being found together the following year compared to the more stable bands. Of note, one of the longest lasting associations between two individuals was a small group of bachelors comprised of three individuals which went on to form a multi-male band. An association between two of the males persisted the entire 8 years (the third individual died in year 7). The social structure of feral horses may in fact differ from many of the fission-fusion mating systems where typically the associations between females tend to persist longer than those between females and males (e.g., red deer *Cervus* elaphus, Bonenfant et al. 2004; giraffe, *Girafffa cameloparadalis*, Carter et al. 2013).

Mantel test for similarity of annual networks suggest that it takes up to 5 years for the original social network to become unrecognizable, essentially reflecting what might be considered a 'social generation'. What is interesting with the mantel test is the potential ability to compare external factors to the variation seen annually in the carry-over of network similarity. For example, from 2008 to 2009 we see almost 2/3rd of the individuals maintain their associations, but in 2010 to 2011 only 1/3rd of the population maintains association ties. A portion of that variation between the annual networks would be related to loss of individuals due to high mortality years (e.g., high overwinter mortality in 2010–2011) however population dynamics such as sex ratio and density could also be influencing the similarity of the networks across years. Mantel tests of the bachelor networks also reveal the lower persistence of network structure over time with essentially two years until the majority of original associations no longer exist. However, among the bachelors we start to see longer persistence of the original network as

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the study progresses. This could reflect associations between bachelors persisting longer as the population becomes more male biased and as mating opportunities for males become more highly contested (and less available) as indicated by the increase in the percentage of males remaining bachelors in later years of the study (Table 3.5).

Looking at female social characteristics over the course of 5 years suggests there are benefits to maintaining a lower number of associates and to affiliate with fewer different stallions. Minimizing the number of associates can be maintained by either staying in the same band or by moving between smaller bands. Both year of entry and age of entry into the 5-year observation period were significant factors on the production of foals. Year-of-entry likely impacted production based on environmental or density-dependent factors influencing reproduction and offspring survival. Age was likely a factor as although females can produce their first foal at 3 years of age they do not all successfully reproduce at this age (Lucas et al. 1991). Maintaining a longer association with a specific stallion was also observed to improve reproductive success in other feral horse populations (Kaseda et al. 1995). Changes in band stallion either through females moving bands or new stallions taking over existing bands increases female protectiveness of offspring as there is risk of infanticide by new stallions (Linklater et al. 1999; Cameron et al. 2003; Gray et al. 2012). Females in multi-male bands also are known to receive more aggression (herding, chases, threats or bites) then females in single stallion bands (Linklater et al. 1999).

Harem-defense polygyny arises when females group together for use of habitat or for protection and where they range too vast an area for territoriality to prevail (Clutton-Brock 1989). A number of studies have remarked on how important female – female associations are in these harems (primates: Silk et al. 2003, Silk et al. 2010, Cheney et al. 2012; cetaceans: Frère et al. 2010) and specifically for horses (Cameron et al. 2009; Heitor and Vicente 2010*b*; Debeffe et al. 2015; Stanley et al. 2017). From an evolutionary perspective, females may have benefited by being in a group to detect or deter predators. There have been some suggestions that during inclement weather horses in large groups gain a thermal advantage over those in smaller bands (Welsh 1975). Affiliative interactions such as mutual grooming also occur among females which have positive physiological effects on the individuals (Kimura 1998; Feh and De Mazières 1993). Our data comparing longest female and male associations to the 5-year reproductive

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success suggests that there are seemingly no fitness benefits to remaining in association with specific female associates but that remaining with the same stallion does improves fitness. Over a three-year study of associations in feral ponies of the Carneddau Mountain range (N. Wales) it was observed that the strength (based on proximity of individuals) of the female–female bond was also slightly less than the relationship between females and stallions (Stanley et al. 2017). Despite this they conclude that the female–female associations form the basis of the network structure. There are inarguable benefits for female grouping but the data here (and arguably in Stanley et al. 2017) don't directly support that familiarity between females specifically improves reproduction. Instead long-term associations between females could remain an artifact of remaining with the same male and for females to avoid the costs associated with changing associations and having to reestablishing dominance hierarchies.

It might seem inappropriate to have split the population into bands and bachelors for analyzing sociality data; after all, there is not complete separation between bands and bachelors. There are instances where band stallions will temporarily leave harems and associate for brief periods of time with bachelors and bachelors may 'tag' or follow bands for hours or days in attempt to takeover or steal females (Medill, personal observations). These interactions may in fact be quite important particularly for males that are attempting to establish harems. Juvenile males may also temporarily leave their natal bands to associate with bachelors when they are in the proximity but may return to the natal band as opposed to dispersing permanently with the bachelor group. However, I feel it is justified to analyze the data in this way given our ability to detect these occasional forays equitably among all members of the population is limited by our survey methods. Considering the population in a single analysis could perhaps erroneously give too much weight on these too few observations.

Although there was observed variation in bachelor male social measures of strength and degree with age there was no apparent relationship between these measures and predicting success in becoming a dominant band stallion. Among bachelors aged five and older, we observe that both degree and strength measures decrease suggesting that they interact with fewer other bachelors and that they are less frequently observed with the same individuals. One explanation for this observation is that some members of their cohort would have obtained harems themselves and are no longer available to consort with. Another explanation is that this reflects a period of

transition where older bachelors have been observed to reduce the strength of their associations with other bachelors and prepare to attempt harem takeovers (Khalil and Kaseda 1998; Fischhoff et al. 2009). The measures used in this research also focus on a quantitative evaluation of the associations and not related to the type or quality (e.g., contest outcomes) that the bachelors may be engaging in. These later are likely to be better predictors of who will succeed in challenging and obtaining reproductive opportunities (Bourjade et al. 2009; except see Berger 1986).

As in most other feral horse populations we also see multi-male bands persist on Sable Island (Boyd & Keiper 2005). Within multi-male bands the stallions have different social roles. The dominant stallion obtains all or most of the reproductive opportunities with the adult females (Feh 1990; Kaseda and Khalil 1996; Asa 1999; Linklater and Cameron 2000; see Miller 1981 for divergent observation), while the subordinate(s) play a greater role in harem protection (Miller 1981; Berger 1986; Pacheco and Herrara 1997; Linklater and Cameron 2000). There is support that subordinate males are highly effective at keeping outside males from successfully mating with band females preserving reproductive opportunities for the dominant male and to a lesser degree, also for the subordinate (Feh 1999; Linklater and Cameron 2000). The reproductive opportunities for subordinates involved in multi-male bands have been reported as very limited (Berger 1986) to nearly 42% (Miller 1981). Both Miller (1981) and Feh (1999) have noted that subordinate stallions in multi-male bands do sire more offspring than bachelors who attempt to sneak copulations suggesting that strong benefits may be gained by these males which are not able to secure dominant position in a single stallion band (Berger 1986). Additionally, due to the opportunity to form social bonds with members of the band, a subordinate may find itself in a better position to take over all or part of the harem as the dominant stallion senesces (Berger 1986; Feh 1999). Over one third of the Sable Island males which undertook a subordinate stallion position were later observed to be dominant males of either members of the same band (or a portion of the females) or with different females.

Group size can influence reproductive success of feral horses (Welsh 1975; Kaseda and Khalil 1996; Curry et al. 2007). Kaseda and Khalil (1996) observed that a stallion's reproductive success was greatest when 5 mares were present in the band; over 5 mares and the number of foals produced dropped to an average of 2. Welsh (1975) observed 100% foaling rates when Sable Island bands contained 3 adult females, which is similar to what is currently also observed

in the population. Although a male's reproductive success may be increased by having more females in their harem it would appear that female reproductive success may suffer putting male and female interests at odds with one another.

3.6   Conclusions

Individual fitness has been tied to the ability to maintain long-lasting social ties among several social species. Lagged association rates for Sable Island bands suggests that many associations do not persist beyond a few years and that after 5 years the current network is virtually unrecognizable from the original. Understanding the rate of changes in the association network can have important consequences to the genetic diversity of the population. Additionally, focusing on associations between females and their 5-year reproductive success sheds light on how high numbers of associations as well as frequent band changes or interactions with more males can have negative impacts on individual fitness. Given that several authors claim stable female–female associations is important to individual fitness it was particularly interesting to see that females that maintained longer associations with other females did not have higher foal production or foal survival then those that did not. This could suggest that instead of the actual associations being important for female fitness it is avoidance of the harassment from both males and females that occurs when individuals change associations that makes maintaining female–female associations appear to be beneficial. There could be several opportunities where not maintaining female–female associations may be beneficial, such as leaving a band to obtain a higher position in another band's hierarchy, or dispersing from larger bands with high competition for resources. As well, there may be times where loss of the female–female association through mortality or partner emigration may have negligible or even positive benefits to the focal individual (e.g., change in hierarchical rank).

Investigating bachelor male social network measures suggested that neither degree, strength of associations, or betweenness proved to be predictive of whether an individual would become successful as a band stallion, nor suggest any change in their social position the following year. However, there was some evidence in the decrease in degree and strength that prime-aged (5-7 years old) bachelors do tend to reduce the number of associates and be seen less often with

cohorts as they approach the age they would be actively seeking position as a dominant stallion. Although using social network approaches to quantify associations in a population can help to improve our understanding of social patterns without additional behavioural information the full potentials of these techniques are not reached. Supporting some of the analyses with contest outcomes or hierarchal ranks would have permitted even more detailed understanding of how individuals attain and maintain positions within the social structure.

CHAPTER 4

HAIR CORTISOL CONCENTRATIONS IN AN UNMANAGED FERAL HORSE POPULATION LIVING IN NATURAL SOCIAL CONDITIONS

4.1 Abstract

Cortisol is a glucocorticoid hormone that is produced during activation of the hypothalamic-pituitary-adrenal axis (HPA) in response to psychological or physiological demands which require energy obtained by catabolism of reserves. High amounts of circulating cortisol can be found in individuals experiencing energy demanding physiological events such as lactation, injury, or starvation; but also in individuals who may have less obvious activation due to anxiety from social situations. The feral horse population on Sable Island provides an opportunity to look at hair cortisol concentration, as a proxy for circulating cortisol concentration, to better understand physiological correlates. The horse's complex social structure also allows us to look at how population and group structure may influence an individual's HPA activation. Hair samples were analyzed from 113 females and 136 males. Females with dependent offspring (foals or yearlings) had higher hair cortisol concentrations then those females without dependent offspring. Horses in poor body condition were also more likely to have higher hair cortisol, particularly among females which had a greater variation in body condition index, which was also correlated with reproductive state. There was weak support for higher hair cortisol concentrations to be found in individuals which changed bands within the year before sample collection compared to females which did not move. In general models describing female cortisol levels included age, body condition, presence of foal, as well as social measures such as

harem size and the number of bachelors in the vicinity. Models describing male cortisol level included age, body condition, and year only.

4.2   Introduction

Activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis is known to occur in response to both internal physiological regulations as well as in response to cues from the external environment leading to the production and release of glucocorticoids (Sapolsky et al. 2000; Boonstra 2013*a*). The glucocorticoid hormone cortisol is well known to have numerous effects on the body. Primarily it is intended to prepare the body for increased energy demands by activating the release of glucose from body reserves while diverting resources from other physiological processes such as reproduction and immunity (Sapolsky et al. 2000; Sapolsky 2004). In the last two decades, wildlife biologists have been interested in understanding the relationships between glucocorticoid concentrations and how that may infer knowledge on how an organism is coping with changes in its own physiology, its habitat, resources, or social environment (Koren et al 2002; Boonstra 2013*a*; Creel, et al. 2013; Dantzer et al. 2014; Kersey and Dehnhard 2014).

The merits and disadvantages of the various matrices used to determine hormone concentrations has been discussed thoroughly by others (Sheriff et al. 2011; Meyer and Novak 2012; Russell et al. 2012; Novak et al. 2013). Blood/serum, saliva, urine, faeces, hair, and claw/fingernail differ in several properties, but most importantly they vary in the period of time represented by the hormone recovered. Blood, saliva, urine and faeces reflect very recent circulating levels, and frequently affected by circadian or other biological rhythms, while slow growing matrices such as hair, claw or fingernails represent hormone accumulation over longer periods of time. Hair is an attractive matrix as it is minimally invasive to collect, robust to various storage conditions and exposure (Macbeth et al. 2010; González-de-la-Vara et al. 2011) and offers a prolonged retrospective view of an individual's endocrine activity (Kirschbaum et al. 2009; Meyer and Novak 2012; Russell et al. 2012; Stalder et al. 2012; Carlitz et al. 2014).

Hair hormone analysis has been used to look at differences between the sexes (Bechshøft et al. 2011; Fourie & Bernstein 2011; Fourie et al. 2015*a*), different ages or development periods (Laudenslager et al. 2012; Dettmer et al. 2014; Fourie et al. 2015*b*), access to resources or energy

requirements (Macbeth et al. 2012; Bechshøft et al. 2013; Bryan 2013; Bryan et al. 2013*a*), reproductive state (Macbeth et al. 2012; Dettmer et al. 2015), or physical injury (González-de-la-Vara et al. 2011; Malcolm et al. 2013; Carlitz et al. 2014; Cattet et al. 2014). Social correlates have also been identified such as changes in social group membership (Carlitz et al. 2014; Nuñez et al. 2014), and aggression given or received in relation to hierarchal rank (Laudenslager et al. 2013; Yamanashi et al. 2013; Carlitz et al. 2014; Yu et al. 2015). Additionally, hair cortisol concentration has also been used to infer effects of anthropogenic activities on wildlife populations (Brearley et al. 2012; Bourbonnais et al. 2013; Bryan et al. 2015; Fourie et al. 2015*a*).

The majority of the studies using hair hormone concentrations have used primates or carnivore species. There are very few hair hormone studies on wild ungulates with the exception of caribou and reindeer (*Rangifer tarandus* spp.; Ashley et al. 2011). There has been some work among feral horse populations with regard to serum cortisol concentrations of recently captured individuals (Kirkpatrick et al. 1979; Seal et al. 1985; Plotka et al. 1988), along with a body of work originating from domestically kept horses which shed light on equine HPA responses to various physiological and social stressors (injury and illness: Hoffis and Murdick 1970; Kirkpatrick et al. 1979; Merl et al. 2000; Rietmann et al. 2004; strenuous exercise: Jakubowska et al. 2010; novel experiences: Schmidt et al. 2010). Reproductive state of female horses has shown both negligible correlation to cortisol levels (Sinderbrand 2011) and also peak releases of cortisol prepartum (Nagel et al. 2012; Comin et al. 2012). Manipulating domestic groups of horses have shown that horses have an increased HPA axis activity when faced with new individuals or losing access to familiar associations (Alexander and Irvine 1998; Anderson et al. 1999; Erber et al. 2012). The one study which investigated faecal cortisol metabolites of feral horses suggested that when free-living females find themselves changing bands and having to form new associations that they will also have increased HPA axis activity (Nuñez et al. 2014).

The Sable Island feral horse population provides an opportunity to explore relative HPA pathway activation under natural situations in an ungulate with a complex social structure. Using hair as our recording matrix provides a long window of hormone accumulation which would be resilient against peaks of cortisol occurring from random events and indicate an individual's baseline or if an individual is subject to frequent or continuous stressors. The accompanying survey data

includes social and physiological information for the preceding, current, and subsequent years for each individual in the population.

This chapter of my thesis is largely exploratory, determining what correlates can be drawn between physiological and sociological states and hair hormone concentration (HCC). However, in Sable Island horses I do expect to see some differences in cortisol concentrations than those observed in domestically kept horses. The first of these differences is the challenges that reproducing individuals on Sable Island would face while having access to the same resources as non-reproducing individuals (i.e., no supplemental feeding as kept horses would have). I expect that female feral horses would have higher hair cortisol concentrations when producing foals or otherwise lactating. Along similar lines, horses in poor body condition and not obtaining adequate resources, who may potentially having underlying illness or injuries preventing them from acquiring sufficient resources, would have higher hair cortisol levels. Among males, social position may also influence energetic requirements. Stallions (dominant males which control a harem of females) must expend more energy defending females and travelling then their bachelor (non-harem holding males) counterparts and therefore have higher HCC levels. As in domestic horses and one other population of feral horses (Nuñez et al. 2014), I anticipate that changes in associations could lead to higher HCCs. This chapter concludes with building linear mixed effect models including the strongest physiological correlates (e.g., age, body condition, reproductive/social state) with sociological correlates which include both local population demographics (e.g., density, adult sex ratio, number of bands or bachelors in area) as well as variables characterizing an individual's social situation (e.g., degree, harem size). Models will be built separately for males and females to investigate how the sexes may be responding differently to various stressors in their environment.

4.3 Methods

4.3.1 Study Area and Species

Sable Island National Park Reserve is located 275 km east of Halifax, NS, Canada in the Atlantic Ocean. The population of feral horses has existed on the island since the mid-1700s and has long established a wild-type social structure. They have not experienced significant human

interference (e.g., additions or removal of individuals, or disruption to social groups) since their protection granted in the early 1960s under the Canada Shipping Act (2001) and now most recently, in 2013, through the Canada National Parks Act (2000) as it applies to wildlife. The island is approximately 42 km long and has a maximum breadth of 1.6 km, tapering to both ends resulting in a linear crescent shape. Vegetation on the island is predominantly marram (*Ammophila breviligulata*) grassland and heath; all growing on a substrate composed entirely of sand and what little organic material remains from vegetation or animal life. Being the only terrestrial mammal, there are no other competitors, nor predators, influencing the horse population.

4.3.2   Annual Survey

Each summer since 2008, the horses have been surveyed and data on their location, associations, reproductive status, and health collected. Individuals are readily identifiable using their natural markings, hair patterns, scars or other characteristics. Female reproductive status is determined by the presence or absence of a foal or yearling. Male social positions are identified based on behaviour (Linklater and Cameron 2000) with individuals classed during sampling as being a dominant band stallion, subordinate band stallion referred to here as a 'Tag' male (an adult male that is not the dominant stallion in a multi-male band) or as an adult bachelor (>5 years old) which is unassociated with reproductive aged females, or a juvenile (3–4 years old) that may be with its natal band or dispersed and living as a juvenile bachelor. Male horses may produce semen as young as two years old (Bennett and Hoffmann 1999) however, males that are less than 5 years old living in a natural social structures are rarely seen consorting with females with the intention to reproduce (Berger 1986; Asa 1999). For bands, total band size (a count of all individuals including foals) as well as harem size (number of females 2 years old and older) was calculated. Survival of an individual was based on their being observed in subsequent annual surveys.

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4.3.3 Body Condition Index

Body condition was determined by evaluating photographs taken during the field survey. Individuals would be graded on a scale of 0–5, zero being emaciated and 5 being very obese. Three body regions on an individual were rated, hips, rib cover, and backbone, half points were used when a difference was found between the three regions. This scale follows the criteria set out in Carroll and Huntington (1988). To eliminate bias from multiple observers, all body conditions for the individuals used in this analysis were determined or reviewed by S. Medill with no knowledge of the individual's hair hormone concentrations.

4.3.4 Median Location and Buffer Data

A median location for an individual is determined using all the observations of that horse during the survey year. From these median locations a buffer of either 2000 m, 4000 m or 8000 m which represent the 80th, 95th, and 99th percentile of annual movements of all horses (Laforge et al. 2015). Within these buffers we calculate measures of density (horses per vegetated km^2), sex ratio (see equation 2.1), or the number of bands or bachelors. These measures characterize the social landscape the individuals are living within upon the island.

4.3.5 Degree

Degree is a concept derived from social network analysis (see Chapters 3) which reflects the number of individuals a focal individual is observed to interact with. For horses which are within bands this is equivalent to band size (minus the individual). For bachelors, who may be observed with different associates during different days on the survey, this is a total number of unique individuals that horse was seen with for the entire annual survey period.

4.3.6 Sampling Method and Storage

In 2011 and 2012 rooted tail hairs were collected for microsatellite DNA analyses (unpublished data) and for hormone analysis (this study) under our institutional animal care permit (University of Saskatchewan UCACS #20090032). Collection methods included collecting samples that

were observed to be scratched off onto previously cleaned natural and artificial (velcro strips set out on known rub structures) hair snags, however the majority of samples were collected by directly pulling the hairs from the individual if they permitted approach. Hair collected in the field was stored at ambient temperature in envelopes and kept in the dark. Only hairs with the root attached were used in this analysis to ensure the most recent hair growth was used. We removed 4 mm of the root end to retain for future DNA analysis and used the next 5 cm segment to evaluate hormone concentrations. We limited this study to individuals three years and older as this represents a segment of the population which are sexually mature.

Tail growth rates of domestically kept horses have been reported as between 0.066 and 0.081 cm per day (Tracey et al. 2002; Dunnett and Lees 2003; Schlupp et al. 2004; West et al. 2004; Boyer et al. 2007; Šturm et al. 2015). The 5 cm segment of tail hair, based on these rates of growth, would represent the time up to 67–82 days prior to sample collection excluding the most recent 5–6 days associated with the 4 mm portion of hair root removed. Hair samples were collected during roughly the same time period in both years: between July 24rd and August 23rd in 2011 ($n = 49$) and July 20th to Aug 17th in 2012 ($n = 233$). We therefore captured the hormone profile within the hair shaft corresponding to a period of time in which peak parturition (May) and initiation of the breeding season occurred (Welsh 1975; Lucas et al. 1991).

4.3.7   Laboratory Analysis

We analyzed the hair samples following procedures described in Macbeth et al. (2010) with the following modifications relating to the use of hair segments. Methanol washes used to remove exogenous sources of hormones and contaminants (e.g., from sweat or faeces) were performed after the removal of the root end but before the 5 cm segment was isolated from the full hair shaft. Up to 25 mg of powdered hair sample was analyzed by extracting hormones with HPLC-grade methanol over 24 hours and reconstituted using the phosphate buffer provided. Cortisol concentrations were quantified using commercially available enzyme-linked immunosorbent assay kits (EA-65 Cortisol EIA kit, Oxford Biomedical, Oxford, MI, USA). Samples were run in duplicate however they resulted in a coefficient of variation over 15% then a third sample was run to achieve CV <15%. Intra-assay percent coefficient of variation (%CV) for cortisol 3.6% ($n = 6$), and inter-assay %CV for cortisol 5.8% ($n = 12$; Macbeth et al. 2010). The limit of

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detection for cortisol was 0.02 ng/ml, and parallelism of serially diluted hair samples was obtained ($R^2 = 0.997$, P-value < 0.001; Macbeth et al. 2010). Samples were processed for cortisol between Oct 2012 and May 2014.

4.3.8 Data Analysis

There are several variables related to both the sample collection and processing that can introduce variation in the data which is of little interest to this study. Biological considerations include year and Julian day of sample collection, hair colour, and for some analyses, age. While factors known to affect hair cortisol concentrations from a procedural point of view may be related to the initial mass of hair used for the extraction, hair coarseness, or date of the assay. Where possible I tried to use 25 mg of hair but some individuals had very few hairs collected and I was unable to meet that limit for 72% of the samples. Hair coarseness was measured as the total mass of 5 cm segments divided by the number of hairs giving us a mean mg per 5 cm segment. As some stages of the assay can be influenced by climate characteristics such as temperature or air pressure the date of assay was also evaluated. These characteristics were compared to hair cortisol results as single variable linear models in R (R Core Team 2013). Variance component analysis was also performed setting all variables as random variables in a linear mixed effect model and comparing the standard deviations. After evaluating how these biological and procedural effects may affect our data I evaluated separately for males and females which random variables have the strongest influence on hair cortisol concentration using AIC_c model selection (Burnham and Anderson 2002). The variables in the model which was simplest which remained within 2.0 of the lowest AIC_c were considered as random variables in subsequent models.

The influence of reproductive status of females was investigated using linear mixed effect models. Comparisons requiring generalized linear mixed effect models include the presence of a foal in the current year, the presence of a foal in the subsequent year, sex of the current foal, and whether a female was primiparous or known to be multiparous at the time of sampling. I further evaluated whether the hair cortisol concentration would reflect reproductive load by looking for trends in HCC and presence or absence of yearlings in addition to or instead of foals. Body condition as a strong influence on HCC was evaluated using linear mixed effect modelling.

Finally, biological variables, social variables, and a combination of top ranking variables were selected using AIC_c model selection to identify the what best explains the observed variation in HCC in females. Package lme4 in R was used to analyze the linear mixed effect models (Bates et al. 2014).

Among males my hypothesis focusses on social position and HCC as a proxy for reproductive effort and success. Again, linear models (with and without random effects) are used to evaluate the influence on HCC of age, social position, and body condition. I also look at how the current year's HCC relates to the social position of the individual the following year. As for females, we build models specifically to evaluate how biological and social factors may relate to HCC using AIC_c model selection.

4.4   Results

4.4.1   Cortisol Sample Characteristics

377 samples of hair from horses three years old or older were analyzed to determine the cortisol concentration. Forty of these samples returned values below detection limit, 11 more samples had coefficients of variation above 15% and were excluded from analysis. An additional 23 samples were removed from further analysis as they were used to test sample differences (i.e., mane samples [$n = 17$]), deemed outliers [$n = 3$], or procedural issues [$n = 7$]). 299 samples remain for testing further hypothesis. Values for cortisol (i.e., sample did not return a reading below detection limit) were obtained for ground mass as light as 2.59 mg, or from as few as 3 hairs. These samples contain 138 samples from female horses (113 individuals) and 161 male horses (136 individuals). Fifty-four of the samples were from 2011 and the remaining 246 samples collected in 2012. To avoid adding individual as a random factor within year repeated samples from within year [$n = 17$] were removed selectively by retaining the results from the sample with the highest ground sample mass. Hair cortisol concentrations were skewed to the right, but log-transformation produced a normal distribution for statistical analysis (Shapiro-Wilk test, $W = 0.99$, P-value $= 0.2479$).

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4.4.2 Biological Considerations

4.4.2.1 Sample Collection Year & Day

There were 49 hair samples collected in 2011 and 246 samples collected in 2012; for 34 individuals there was samples collected in both years (17 females and 17 males). Paired t-test of hair cortisol concentrations continued to show a significant year effect with a mean difference of –0.242 suggesting that cortisol levels were lower in 2011 than in 2012 (T = –2.474, *df* = 33, *P*-value = 0.019). Comparison of all the data from both years also shows an overall significant difference between years with high cortisol levels more common in 2012 (Figure 4.1). Although variation in year is worthy of exploration for the most part it is considered here as a random factor for subsequent analyses. For the 34 individuals that have samples from both years I chose not to consider ID as a random variable as they may have experienced a change in social status, reproductive status, or physiological change between years. Julian day of sample collection did not turn out to be a significant factor in hair cortisol level (Table 4.1; *b* = –0.002, df = 280, *P*-value = 0.68).
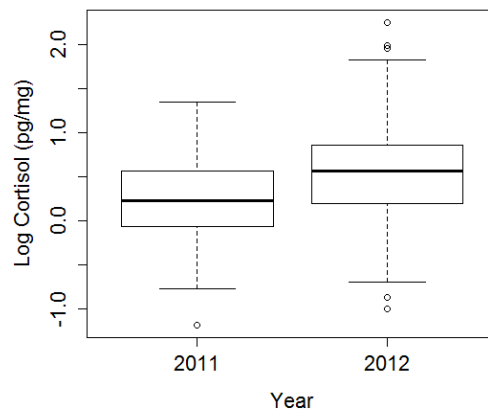
Figure 4.1. Cortisol concentrations from hair collected from feral horses sampled in 2011 (*n* = 49) and 2012 (*n* = 233; T = 3.861, *df* = 280, *P*-value < 0.001).

4.4.2.2   Sex and Age

The difference in cortisol levels between males and females is significant (T = 2.816, *df* = 280, *P*-value = 0.005; Figure 4.2). Males had a mean hair cortisol concentration of 2.098 pg/mg (*n* = 152, range 0.370– 9.502 pg/mg) while female mean was 1.630 pg/mg (*n* = 130, range 0.307– 5.329 pg/mg). The different reproductive and social demands between males and females warrants separate model construction for males and females and age may have to be looked at as a fixed or random effect. Age also shows significant differences in HCC with those individuals aged 5 (*b* = 0.290, *n* = 20, *P*-value = 0.032), age 6 (*b* = 0.549, *n* = 18, *P*-value <0.001), and aged 6 + (*b* = 0.369, *n* = 156, *P*-value < 0.001) tending to have higher hair cortisol levels then juvenile individuals aged 3 and 4 (Figure 4.2).
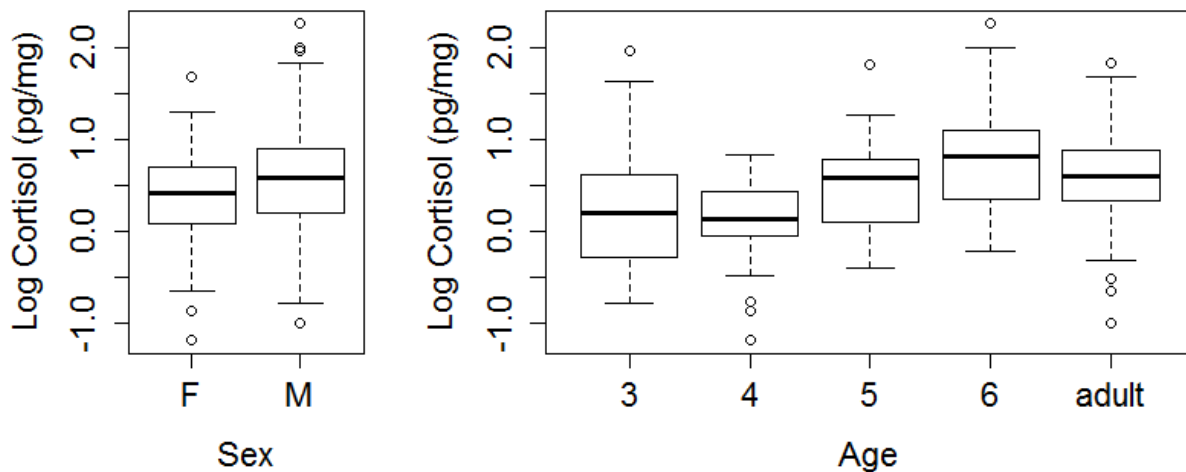
Figure 4.2. Hair cortisol concentration (HCC) of female (F; *n* = 130) and male (M; *n* = 152) feral horses (T = 2.816, *df* = 280, *P*-value = 0.005). Feral horses ages 5, 6, and those identified as adults (exact age unknown) have higher HCC then younger individuals. Sample sizes as follows: Age 3 [*n* = 55], Age 4 [*n* = 33], Age 5 [*n* = 20], Age 6 [*n* = 18], Adult (6+) [*n* = 156].

4.4.2.3 Median Location

No discernable correlation between hair cortisol concentration and median location on the island could be found (T = 0.5896, df = 280, P-value = 0.556, R^2 = 0.04)

4.4.2.4 Hair Colour

Hair colour is also a variable producing potential bias, however, there are much fewer flaxen (n = 3) and sorrel (n = 11) individuals than there are chestnut (n = 28), and especially black (n = 240), which has increased the potential for type 1 error (Figure 4.3; Table 4.1).
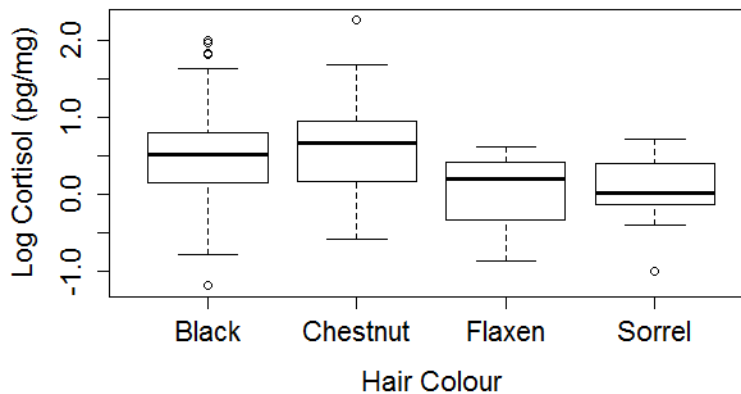
Figure 4.3. Hair colour and the observed hair cortisol concentration in horses. Sample sizes are as follows: Black [n = 240], Chestnut [n = 28], Flaxen [n = 3], Sorrel [n = 11].

4.4.3 Procedural Considerations

Procedural considerations included weight of the ground sample that underwent extraction, the date of the assay, and the coarseness of the hair (determined by dividing the weight by number of hair). All of these variables when compared in a linear model with HCC showed statistical significance (Table 4.1). There is some evidence that HCC levels were higher in samples which had the maximum 25 mg of powdered sample (Figure 4.4; b = 0.011, df = 280, P-value = 0.016). There is also some indication that the date of assay may also influence the cortisol

results but these two variables (ANOVA: $F_{8,\,273} = 4.85$, P-value < 0.001) are compounded by the fact that assays were run with increasing sample weights such that the later assays run for males (assay I) and females (assay D and part of E) would contain more samples with 25 mg of powdered hair.
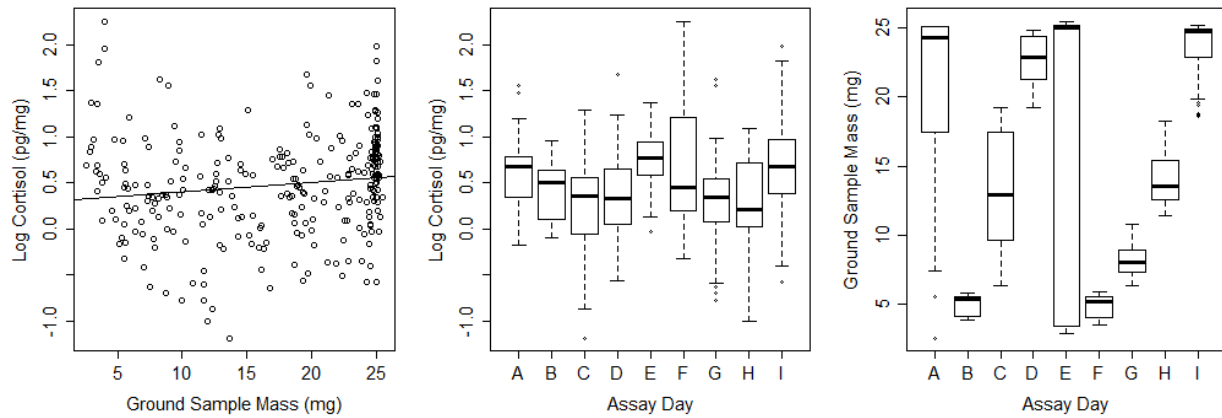
Figure 4.4. Hair cortisol concentration was found to be significantly higher in samples derived from higher sample mass (left panel; $b = 0.011$, $df = 280$, P-value $= 0.016$). Assay day was also observed to be significant (central panel; $F_{8,\,273} = 4.85$, P-value < 0.001); however, contributing to that is the fact that assays were run (with the exception of assay A) from lowest ground sample mass to highest ground sample mass and females followed by males so that more 25 mg samples were run in the final batch of assays (D & E for females and I for males; right panel).

Table 4.1. Comparison of procedural and biological variables as fixed variables to understand their influence on hair cortisol concentration.

	Variable	df	T-value	P-value	AIC_c	%VCA
Null model					467.52	
Biological						
	Year	280	3.861	< 0.001	454.89	8.7 %
	Day	280	-0.413	0.680	469.35	0.0 %
	Age	277	4.563	<0.001	433.51	14.9 %
	Hair Colour	278	-2.666*	0.008*	462.9	13.3 %
Procedural						
	Assay	273	-2.556*	0.011*	446.05	6.2 %
	Ground Mass	280	2.424	0.016	463.67	24.0 %
	Coarseness	280	3.014	0.003	460.52	12.4 %
Residual						20.5 %

* indicates the factor within the given variable with the strongest influence

4.4.4 Describing Cortisol Levels in Females

4.4.4.1 Establishing Random Variables for Females

Amongst females, the variable for hair colour has one flaxen individual which leads to statistical significance when testing this variable. Removal of this individual results in hair colour returning non-significant. This individual was omitted from further models. Model selection using AIC_c criteria was used to identify the variables with the strongest influence on the cortisol concentration that are not directly of interest to the hypotheses (Table 4.2). Although the model using year, coarseness, assay, day, and age produces the lowest AIC_c value, the next model (dropping day) and the subsequent model (dropping year) shows minimal change in AIC_c value and gains degrees of freedom simplify future models. Depending on our interest in the hypothesis, age may be included as a fixed effect in future models.

Table 4.2. Drop one model selection based on ΔAIC_c for potential random variables in females influencing hair cortisol concentrations.

MODEL	df	AIC_c	ΔAIC_c
FULL: Year + Coarseness + Assay + Ground_Mass + Day + Age	12	158.71	
Year + Coarseness + Assay + Day + Age	11	158.03	0.00
Year + Coarseness + Assay + Age	10	158.37	0.34
Coarseness + Assay + Age	*16*	*158.75*	*0.72*
Assay + Age	8	162.72	4.69

4.4.4.2 Hair Cortisol and Reproductive Effort in Females

Females with a foal ($n = 78$) have higher hair cortisol levels than females without a foal ($n = 52$) from observations of 112 individuals and 2 years of data. (Figure 4.5; GLMER with assay, coarseness, and age as random factors, P-value = 0.004; AIC_c = 182.8, Null model AIC_c = 191.0). Cortisol level was not a reliable predictor of the presence or absence of a foal in the subsequent year (Figure 4.6; GLMER with assay, coarseness, and age as random factors, P-value = 0.220; AIC_c = 184.0, Null model AIC_c = 183.5). The cortisol concentration of the female did not differ with the sex of the foal offspring (Figure 4.7; GLMER with assay, coarseness, and age as random factors, P-value = 0.770; AIC_c = 117.6, Null model AIC_c = 115.7). Hair cortisol concentration was also not significantly different between primiparous or multiparous females (GLMER with assay, coarseness, and age as random factor, P-value = 0.665, AIC_c = 33.2, Null model AIC_c = 31.5). It should be noted however that parity is strongly influenced by age, in that no primiparous females are found in the 6 and older age categories and similarly, no multiparous females are seen at age 3.
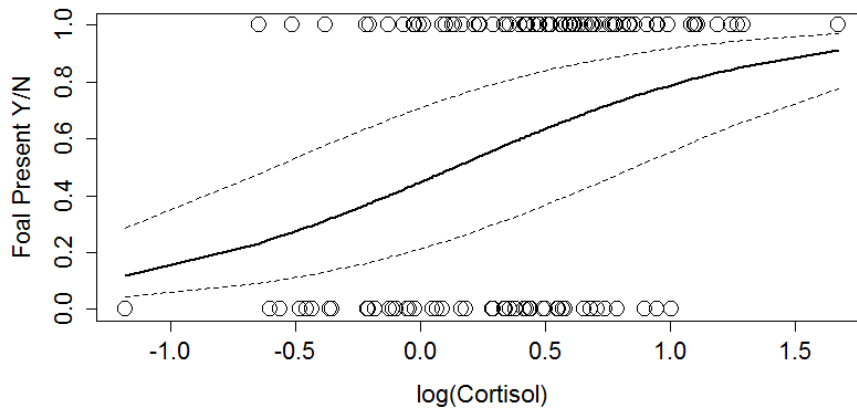
Figure 4.5. Log cortisol concentration (pg/mg) measured from hair in female horses and the presence (1) or absence (0) of a foal. Females with foals had significantly higher hair cortisol concentrations then females without foals (*P*-value = 0.004).
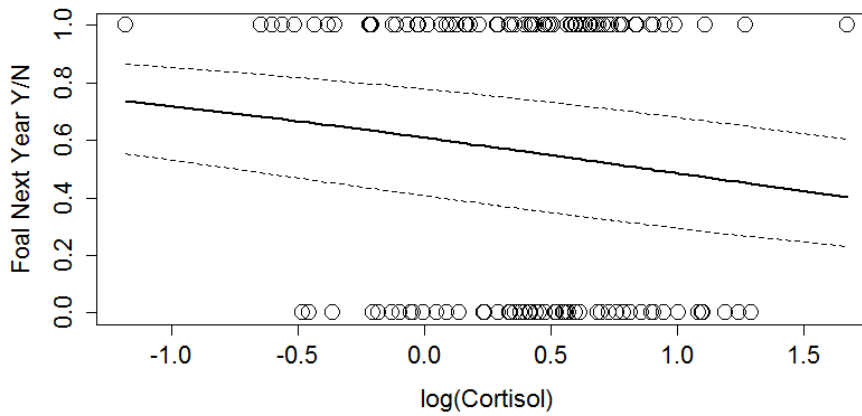
Figure 4.6. Log cortisol concentration (pg/mg) in hair of female horses and the presence (1) or absence (0) of a foal the following year (*P*-value = 0.220).
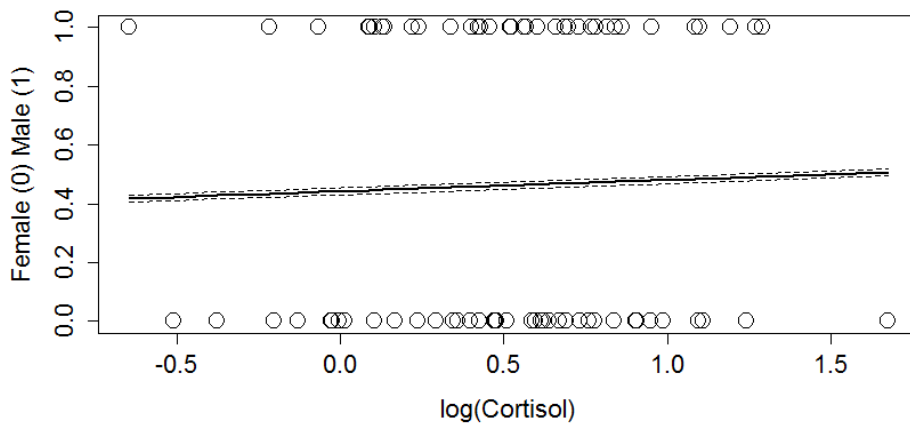
Figure 4.7. Gender of the foal and the log cortisol concentration (pg/mg) of the mother. Model suggests no observable difference in cortisol of females with male foals or female foals (*P*-value = 0.770).

When expanding the focus of reproductive load to consider females who may also be nursing yearlings or being accompanied by yearlings but have a foal also with them we find that females with no accompanying offspring have significantly lower hair cortisol concentrations then females that are accompanied by foals or by foals and yearlings. Females accompanied by yearlings tend to have only slightly higher HCC then those without foals. Hair cortisol concentrations for females with different reproductive loads rank from highest to lowest follows in the following order: Foal & Yearling > Foal Only > Yearling Only> No Dependent Offspring (Figure 4.8).
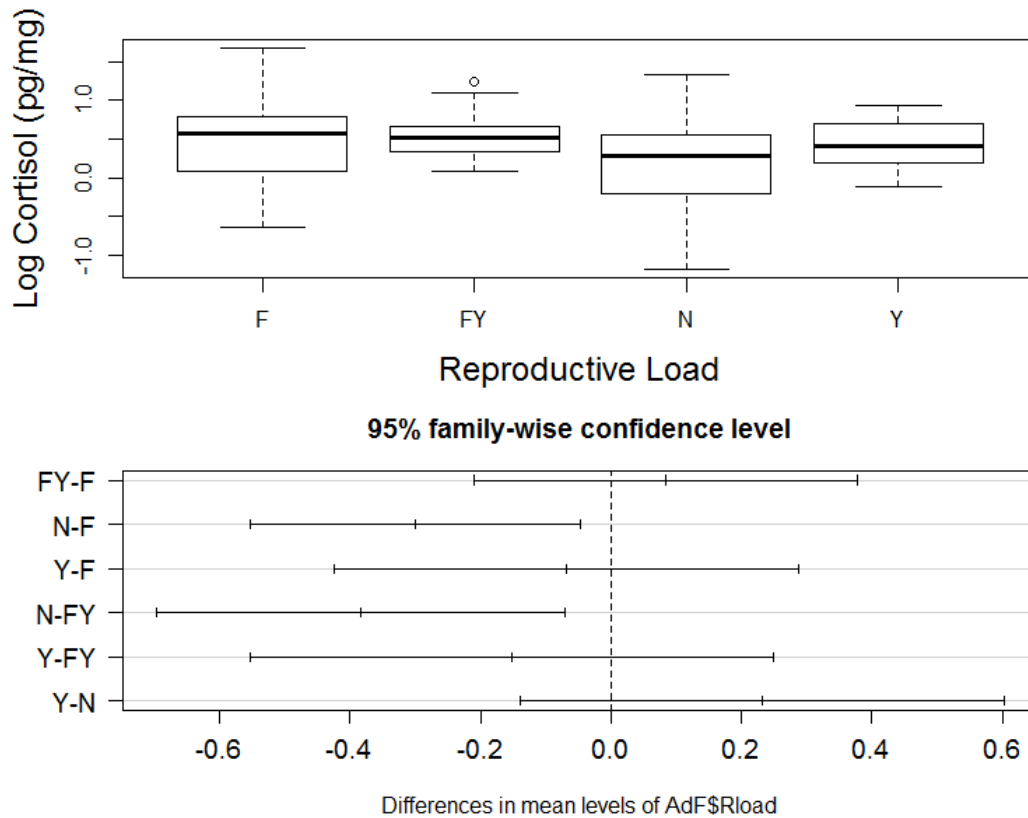
Figure 4.8. Upper: Log hair cortisol concentration (logHCC) of females with different reproductive loads (F – Foal only, FY – Foal and Yearling, N – None, Y – Yearling only). Lower: Difference between mean levels of logHCC in females with different reproductive loads and the 95% confidence interval. From highest logHCC to least: FY > F > Y> N.

4.4.4.3 Body Condition

Body Condition (BC) was also a significant variable describing cortisol level (GLM, $b = –0.459$, $df = 127$, P-value < 0.001, $AIC_c = 127.58$). Including the random variables assay, coarseness, and age improved model fit (LMER, $AIC_c = 124.87$). However, since body condition in females can be strongly tied to reproductive demands (ANOVA, $F_{1,127} = 5.451$, P-value $= 0.021$) the model including the presence or absence of a foal and body condition has the best fit (LMER; $AIC_c = 118.67$; Figure 4.9). The interaction term between body condition and foal presence/absence did not improve the model fil ($\chi^2 = 1.895$, $df = 1$, P-value $= 0.169$).
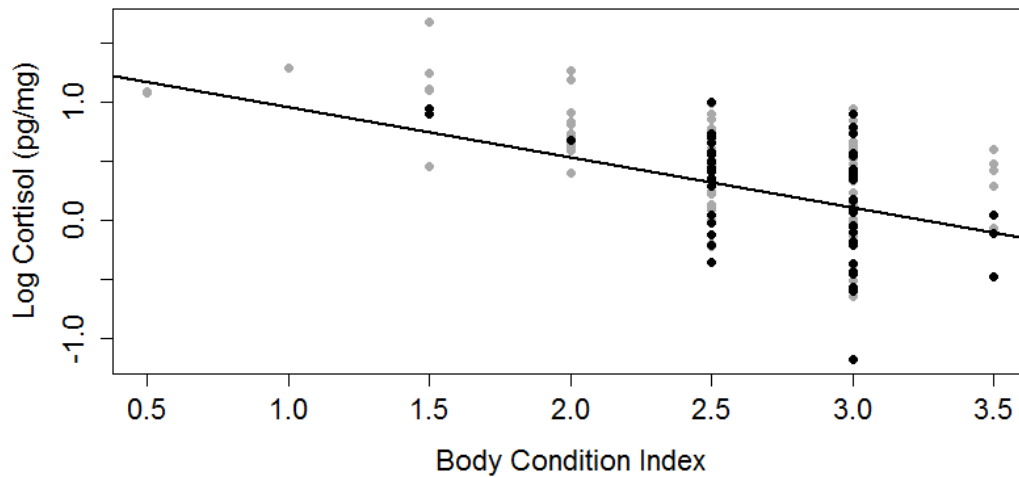
Figure 4.9. Log hair cortisol concentrations of females with foals (grey) and without foals (black) decreases with increasing body condition index (0 – very poor condition, 5 – obese condition; GLM, $b = -0.459$, $df = 127$, P-value < 0.001).

4.4.4.4   Band Changes and Structure

Female horses showed a trend where individuals which had changed bands over the period before the collection of the hair sample had higher hair cortisol concentrations which was significant at $\alpha = 0.10$ (GLMER, with assay day, coarseness, and age as random variables; $b = 0.7544$, SE $= 0.429$, P-value $= 0.079$). Comparing hair cortisol concentration to whether an individual was seen to change bands following sampling (before the next survey) suggested that individuals with low cortisol were more likely to change bands and that individuals with high HCC would more likely remain in the band they were observed in (Figure 4.10; $b = -1.049$, SE $= 0.567$, P-value $= 0.064$).
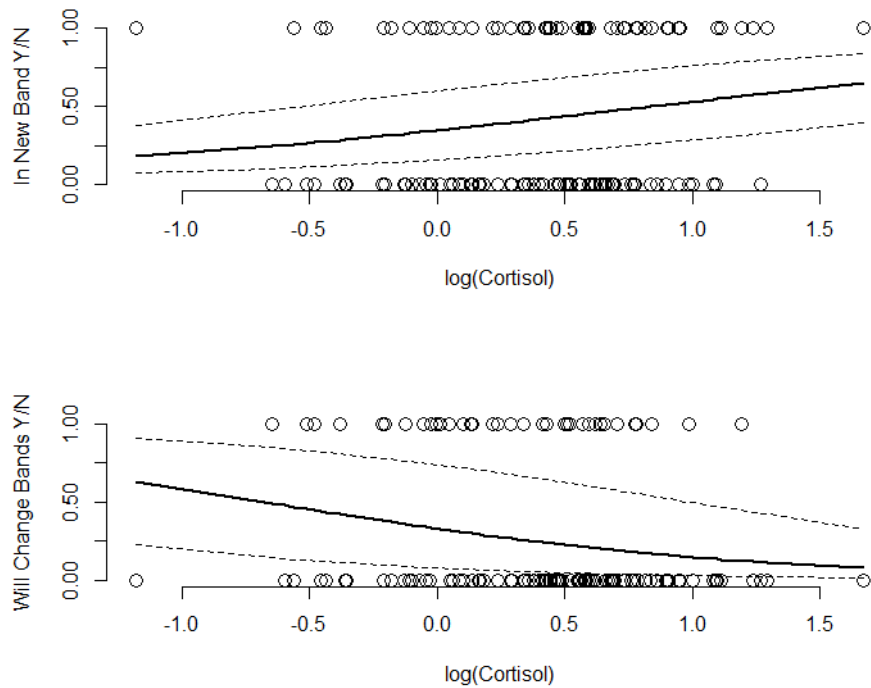
Figure 4.10. Log hair cortisol concentration in females that were observed in either the same band (0) or a new band (1) since the previous survey (upper frame); and females which proceeded to change bands (0 – did not change, 1 – female did change bands) between sampling and the next year's survey (lower frame). Both relationships were significant at $\alpha = 0.10$.

4.4.4.5 Biological Models Describing Female HCC

Variables linked to biological reasons for variation in cortisol were evaluated for their inclusion in the best model for explaining the observed variation in female hair cortisol levels. Body condition and foal presence/absence along with age were found to be in the top model (Table 4.3). An interaction term between age and foal presence was in the next model with $\Delta AIC_c = 0.30$.

Table 4.3. Modeling of log hair cortisol concentration to biological factors. Top models include body condition (BC), presence or absence of foal (Foal), age, year, as well as the interaction term between age & foal, and foal & body condition. Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Random factors coarseness and assay included in all models. Null model $AIC_c = 173.5$.

BC	Foal	Age	Year	Age*Foal	Foal*BC	df	AIC_c	ΔAIC_c	logLik
-	*	*				10	117.2	0.00	-47.67
-	*	*		*		14	117.5	0.30	-42.91
-	*	*			*	11	117.6	0.39	-46.67
-	*	*	*			11	118.8	1.55	-47.25
-	*	*	*	*		15	118.9	1.68	-42.32
-	*	*		*	*	15	119.0	1.80	-42.38
-	*	*	*		*	12	119.5	2.28	-46.40

4.4.4.6   Social Models Describing Female HCC

Social characteristics, as determined from the 2000, 4000, and 8000 m buffers around an individual's median location were compared to cortisol levels to find which buffer level explained the most variation for HCCs. The number of bachelors, bands, and density of horses (vegetation) were all strongest when measured at the 2000 m buffer. Adult sex ratio was strongest at the 8000 m buffer. Harem size and Band size that the individual occupied are inherently strongly correlated ($R^2 = 0.84$, *P*-value < 0.001) so harem size was chosen as it was the stronger correlate of the two predictor variables. These variables, along with all 2-way interaction terms were analyzed to determine their influence on hair cortisol concentrations (Table 4.4). Adult sex ratio, bachelor abundance, harem size, density as well as interaction terms of bachelor*harem size and density*harem size were all found in the top model.

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Table 4.4. Modeling of log hair cortisol concentration to sociological factors. Variables include: Adult Sex Ratio (ASR), abundance of bachelors in 2000 m (Bach), number of bands in 2000 m buffer (Band), Harem size individual is in (Harem), horse density in the vegetated region (Dens), as well as the two-way interaction terms between these variables. Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Random factors coarseness and assay included in all models. Null model $AIC_c = 173.5$.

ASR	Bach	Band	Harem	Dens	Bach* Harem	Harem *Dens	ASR* Dens	Band* Harem	ASR* Harem	df	AIC_c	ΔAIC_c	logLik
-	+		+	+	-	-				10	157.4	0.00	-67.76
-	+		+	+		-				9	157.4	0.01	-68.95
-	+	+	+	+	-	-		-		12	159.0	1.58	-66.14
-	+	+	+	+		-		-		11	159.0	1.59	-67.36
-	+		+	-	-	-	+			11	159.1	1.71	-67.42
-	+		-	+		-			+	10	159.3	1.94	-68.74
-	+	+	+	+	-	-				11	159.5	2.07	-67.61

4.4.4.7   *Best Fit Model Describing Female HCC*

The variables found in the top models for social factors and biological factors were combined to determine if an even stronger best fit model could be found to explain hair cortisol concentration in females. In all of the resulting top models the variables age, bachelors (number of bachelors in 2000 m buffer), harem size, body condition index and presence or absence of a foal combined to explain the greatest amount of variation (with coarseness and assay date at random variables; Table 4.5). Adult sex ratio and density of horses in 2000 m were also found in models with a $\Delta AIC_c < 2.0$.

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Table 4.5. Modelling of female log hair cortisol concentration (HCC) to combination of social and biological factors which were strong in previous models. Variables include age, adult sex ratio (ASR), abundance of bachelors in 2000 m (Bach), presence or absence of foal (Foal), harem size (Harem), body condition (BC) and horse density in the vegetated region in 2000 m (Dens). Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Random factors coarseness and assay included in all models. Null model $AIC_c = 173.5$.

Age	ASR	Bach	Foal	Harem	BC	Dens	df	AIC_c	ΔAIC_c	logLik
*		+	*	-	-		12	109.3	0.00	-41.32
*	-	+	*	-	-		13	110.0	0.64	-40.40
*	-	+	*	-	-	+	14	111.0	1.68	-39.66
*		+	*	-	-	+	13	111.5	2.19	-41.17
*		+		-	-		11	111.8	2.5	-43.05

4.4.5 Describing Cortisol Levels in Males

4.4.5.1 Establishing Random Variables for Males

Amongst males, the variable for hair colour has seven sorrel individuals which lead to statistical significance for the variable hair colour. There are 26 samples from males in 2011 and 126 samples collected from males in 2012. Seventeen of these individuals have samples collected in both years. Model selection using AIC_c criteria was used to identify the variables with the strongest influence on the cortisol concentration that are not directly of interest to the hypotheses (Table 4.6). The model using year, assay, age and hair colour produces the lowest AIC_c value. However, depending on the hypothesis being evaluated, age or year may be included as a fixed effect in future models.

Table 4.6. Drop one model selection based on ΔAIC_c for potential random variables in males influencing hair cortisol concentrations.

MODEL	df	AIC_c	ΔAIC_c
FULL: Year + Coarseness + Assay + Ground_Mass + Day + Age + Hair Colour	17	223.82	4.45
Year + Coarseness + Assay + Ground_Mass + Age + Hair Colour	16	221.98	2.61
Year + Coarseness + Assay + Age + Hair Colour	15	220.47	1.1
Year + Assay + Age + Hair Colour	*14*	*219.37*	*0.00*
Year + Assay + Age	11	223.74	4.37

4.4.5.2 Social Position of Males

Hair cortisol concentration of males occupying different social positions was not found to be different, except for juvenile individuals (Ages 3 and 4; Figure 4.11). This likely speaks more to an age effect with some significant difference in cortisol observed among different aged individuals (Figure 4.11). When a model using age as a random factor none of the social positions returned a statistically significant difference (LME; Intercept (Bachelor [$n = 31$]); Juvenile [$n = 43$, P = 0.980]; Stallion [$n = 69$, P-value = 0.285]; Tag [$n = 9$, P-value = 0.450]).
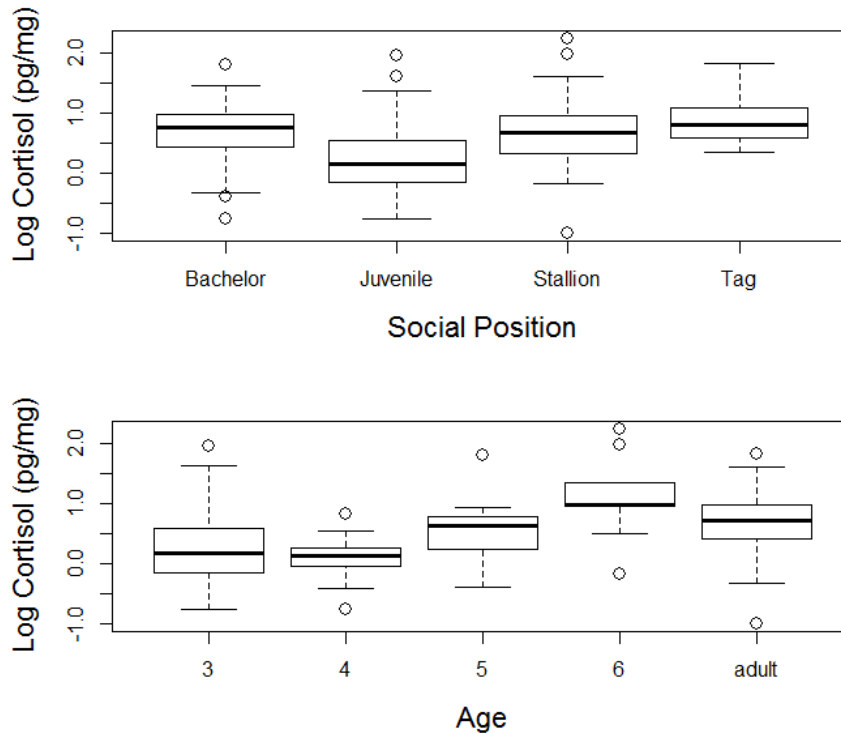
Figure 4.11. Upper: Log hair cortisol concentrations (HCC) of male horses in different social positions. Lower: Log HCC of males male feral horses on Sable Island for individuals of known age of 3 [$n = 30$], 4 [$n = 15$], 5 [$n = 11$], or 6 [$n = 9$], and adult individuals who are 6 years of age or older [$n = 87$]. When age is included as a random variable into the model of social position and cortisol concentration there are no differences between social positons.

4.4.5.3   *Cortisol and Survival or Social Position the Following Year*

Comparing the current year cortisol level to what social position the individual is in the following year (without including random variables) we see what appears to be a trend for cortisol to increase in the following order Juvenile < Tag < Bachelor < Stallion < Dead (Figure 4.12). Individuals which became (or maintained) their position of bachelor or stallion had almost equal cortisol levels while those that did not survive to the following year (Dead) appeared to have higher cortisol.
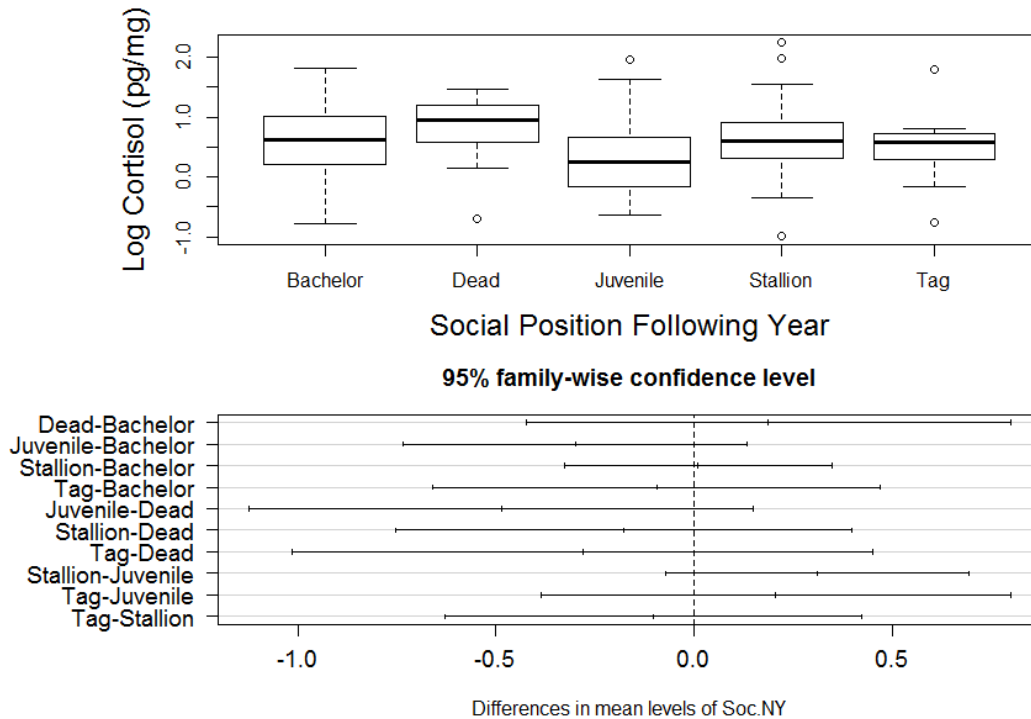
Figure 4.12. Log hair cortisol concentration of male horses and their social position or survival in the following year (upper panel). Difference between mean levels of log hair cortisol concentrations for males of different social positions in the following year and the 95% confidence interval (lower panel). Trend is for previous years hair cortisol concentration to increase in the following order Juvenile < Tag < Bachelor < Stallion < Dead.

4.4.5.4 Body Condition

Body condition index values in males ranged from 1.5 to 3.5 with few males at the extreme ends of this range (Figure 4.13). Age is a significant factor in body condition index with 3-year olds (n = 30, $\bar{x}$ = 2.63) and 4 year olds (n = 15, $\bar{x}$ = 2.83) having lower body condition index scores than 5 year olds (n = 11, $\bar{x}$ = 2.95, P-value = 0.015), 6 year olds (n = 9, $\bar{x}$ = 2.94, P-value = 0.029), and adults 6 years and older (n = 87, $\bar{x}$ = 2.89, P-value = 0.001). When age is taken into account as a random factor there is a significant decrease in cortisol in individuals with higher body condition index scores (LME with age as random factor; T = –2.558, df = 146, P-value = 0.012).

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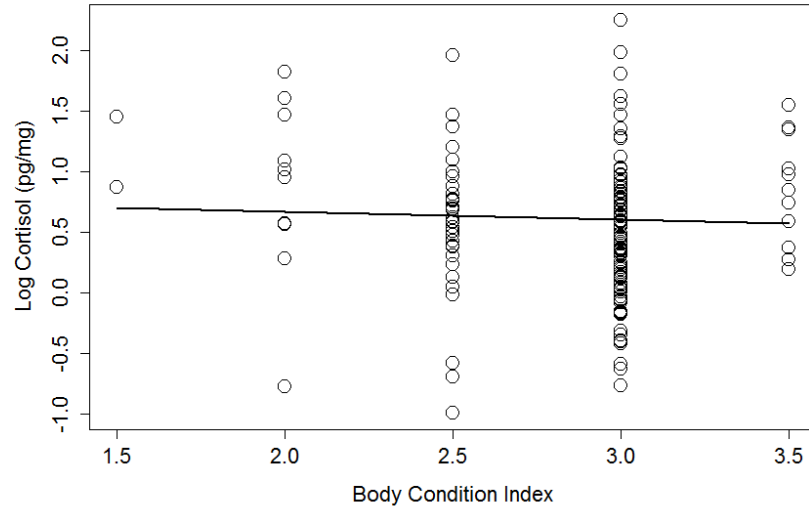
Figure 4.13. Log hair cortisol concentrations of male horses of Sable Island, NS in relation to body condition index (0 – very poor condition, 5 – obese condition). Line drawn representing the relationship between log cortisol and body condition with age added as a random factor (LME; T = –2.558, df = 146, P-value = 0.012).

4.4.5.5 Band Changes

The relationship between hair cortisol concentration and whether a male was in a new band (or social position) was not significant (GLMER with age, assay day, year and hair colour as random factors; b = 0.295, SE = 0.319, P-value = 0.355) but did trend towards higher cortisol in individuals that were in new social groups (Figure 4.14).
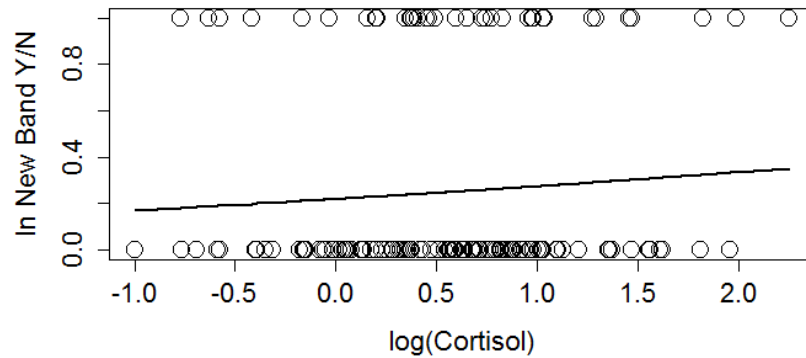
Figure 4.14. Log hair cortisol concentration of male horses which were either in the same band as last year (0) or found to be in a new band or social structure (e.g., bachelor to stallion or stallion to bachelor; 1).

4.4.5.6 *Biological Models Describing Male HCC*

Random factors of assay date and hair colour were included in all models but year and age were included as fixed effect as they are biologically relevant. Age, body condition and year showed up in all of the top models describing hair cortisol concentration (Table 4.7) as well as an interaction term between body condition and year in the second-place model.

Table 4.7. Modeling of log hair cortisol concentration to biological factors in male horses of Sable Island, NS. Variables include age, body condition (BC), social position (SocP), year, and all interactions terms were included. Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Random factors assay and hair colour included in all models; null model $AIC_c = 274.42$.

Age	BC	Soc P	Year	BC*Year	Age *Year	df	AIC_c	ΔAIC_c	logLik
*	-		*			10	228.1	0.00	-103.26
*	-		*	*		11	229.4	1.27	-102.74
*			*			9	231.7	3.60	-106.21
*	-	*	*			13	231.7	3.64	-101.55
*	-		*		*	12	232.2	4.08	-102.96

4.4.5.7 Social Models Describing Male HCC

As for females, the buffer size with the strongest direct relation to hair cortisol concentration was included in the model building to understand how social characteristics of the population can potentially be related to cortisol levels. Adult Sex Ratio in the 8000 m buffer, abundance of bachelors in 4000 m, number of bands in 2000 m buffer, horse density in the vegetated region in the 2000 m buffer, and degree (number of associates individual was observed with in the year) as well as the two-way interaction terms between these variables were included in the full model. The null model, not including any variables except for the random variables age, assay date, hair colour, and year was the top model. The next top model included the number of bands located within the 2000 m radii of the individual ($\Delta AIC_c = 0.50$), while the model just below that included the single variable of bachelors in the 4000 m buffer (Table 4.8).

Table 4.8. Modeling of log hair cortisol concentration to sociological factors in male horses of Sable Island, N.S. Variables include: Adult Sex Ratio (ASR) in the 8000 m buffer, abundance of bachelors in 4000 m (Bach), number of bands in 2000 m buffer (Bands), horse density in the vegetated region in the 2000 m buffer (Dens), and degree (number of associates individual was observed with in the year) as well as the two-way interaction terms between these variables. Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Random effects included age, assay, year, and hair colour; Null model $AIC_c = 243.9$.

ASR	Bach	Bands	Degree	Dens	df	AIC_c	ΔAIC_c	logLik
					6	243.9	0.00	-115.68
		+			7	244.4	0.50	-114.83
	+				7	245.0	1.07	-115.12
-					7	246.0	2.05	-115.61
			+		7	246.0	2.07	-115.62
				+	7	246.1	2.11	-115.64
	+		+		8	246.4	2.41	-114.66

4.4.5.8 Best Fit Model Describing Male HCC

Combining the top variables from the biological and social variables models suggests that the variation in hair hormone concentration in males is more directly under biological influence through age, body condition, or related to the characteristics of year (e.g. climate, resource availability, etc.; figure 4.9). The variable which takes into consideration the number of bands at the local scale suggests that cortisol may increase slightly with higher numbers of bands in the region.

Table 4.9. Generalized linear models using a combination of biological and social explanatory variables to explain log hair cortisol concentration in male feral horses. Included fixed factors are age, body condition (BC), year, number of bands in 2000 m buffer (Bands), and the interaction between year and body condition (BC*Year). Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Random Terms: assay date and hair colour, Null model $AIC_c = 274.7$.

Age	BC	Year	Bands	BC*Year	df	AIC_c	ΔAIC_c	logLik
*	-	*			10	228.1	0.00	-103.26
*	-	*		*	11	229.4	1.27	-102.74
*	-	*	+		11	229.5	1.42	-102.81
*	+	*	+	*	12	230.9	2.79	-102.32
*		*			9	231.7	3.60	-106.21

4.5   Discussion

Cortisol is a hormone that is regulated by a complex feedback system which is constantly responding to changes in physiological and psychological demands in animals and man. This leaves an endless number of opportunities in which to examine potential correlates. This body of research sought to focus on broad biological and social relations that occur in free-living feral horses. Using cortisol concentrations recovered from hair (HCC), I found that females rearing foals had higher levels of cortisol then females that were without dependent offspring or with older offspring which nurse less frequently. For males, reproductive success is strongly linked to their social position, with the majority of reproductive opportunities going to the dominant band stallions (Miller 1981; Berger 1986; Stevens 1990; Linklater and Cameron 2000); however, HCC in adult males were not found to be tied to social position. Overall, both female and male hair cortisol concentrations were more strongly linked to biological factors than it is to our measures of social experiences. However, the best models describing female cortisol levels do include social variables such as harem size and the number of bachelor males in the vicinity.

Females showed strong correlation between HCC and presence of dependent offspring. Even females with yearlings are potentially experiencing higher physiological demands then females without any dependent offspring based on HCC. Females accompanied by both foals and their offspring from the previous year have a slightly higher mean HCC then females with only the current year's foal accompanying. Although a female is not likely to be nursing the yearling at the same time she has a new foal, it can happen (Figure 4.15), and likely they were nursing the previous foal until the third trimester of their pregnancy (Berger 1986). Females often continue to nurse yearlings when new foals are either not born or are lost (Berger 1986) which accounts for why their cortisol levels likely remain higher then individuals with no dependent offspring. Cortisol in the current year was not useful to predict whether a female would successfully reproduce the following year though a trend was observed that suggested females with low HCC were more likely to have a foal in the following year. I had hoped to observe evidence of females show greater mobilization of resources into their raising male offspring by having higher HCC then mothers with female foals in support of the Trivers-Willard hypothesis (Trivers and Willard 1973; Cameron and Linklater 2000) but this was not detected among the samples.

Figure 4.15. A female concedes a nursing opportunity to both her foal and offspring from the previous year; a rare but notable sighting.

No significant differences were found in hair cortisol levels between the adult male social classes (Bachelor, Stallion, or Tag), however juvenile males (3 and 4 years old) tended to have lower hair cortisol concentrations. A decrease in hair cortisol concentrations among juvenile or late-pubescent males has been detected in other populations of feral horses (Plotka et al. 1988) as well as other species using hair hormone analysis, and is suggested to be linked to the inhibitory influence of testosterone on the HPA axis acting prior to the onset of stress induced by reproductive competition (Fourie and Bernstein 2011; Laudenslager et al. 2012). The lack of significant difference in cortisol between adult males occupying different social positions is not altogether surprising given the role of this hormone to assist in meeting a variety of energetic demands. Winning males (i.e., dominant band stallions) have high energy demands related to their reproductive roles but perhaps do not experience the rise in cortisol related to the social defeat that subordinate males and bachelors may experience (Mateos 2005; Creel et al. 2013).

A few studies directly compared sociality and rank to cortisol production of domesticated horses (Anderson et al. 1999; Sinderbrand 2011; Christensen et al. 2012). Anderson et al. (1999) observed domestic horses which were identified as being "social" (with other horses) had higher plasma cortisol concentrations than horses described as "loners". While horses which were considered dominant towards other horses had higher blood cortisol levels than those described as subordinate (Anderson et al. 1999). However, Christensen et al. (2012) observed the opposite; lower ranked horses produced higher concentrations of faecal cortisol metabolites. Christensen et al. (2012) concluded that horses followed the "stress of subordination" hypothesis where an access to resources was compromised by the individual's social standing leading to higher levels of physiological or psychological stress. Sinderbrand (2011) found no significant correlations between hierarchy ranks and concluded that domestic horses followed neither the stress of subordination nor stress of dominance hypotheses.

Hair cortisol in the current year was not predictive of the male's social position in the subsequent year but some trends were emerging. Predictively (and obviously), those individuals observed as 3-year old juveniles were observed as 4-year old juveniles and had low HCC. More interesting was the apparently higher HCC level of individuals that did not survive to the next survey season. Mortality in Sable Island horses has been predominantly attributed to eventual starvation, but may be preceded by illness or injury (Welsh 1975) which would lead to high levels of

cortisol in the months leading up to their death (Foley et al. 2001; Rogovin et al. 2003; Pride 2005*b*; Raouf et al. 2006; Ganswindt et al. 2010).

One surprising observation was that females in general had lower HCCs then males given that females were sampled at a time when many should have been experiencing heavy metabolic demands related to lactation, and our sample had a higher number of lactating than non-lactating females. Males would also have been experiencing high energy requirements through mating and harem defense for dominant band stallions and challenging for mating opportunities by bachelors. Both males and females should have been benefitting from it being late spring and early summer when vegetation is abundant and nutritious. Potentially there are physiological differences in HPA responses between the sexes. This observation should not be concluded as males are more stressed then females but that males and females could potentially have different baseline levels or respond differently to physiological circumstances.

Uneven sampling between years did not permit full exploration of annual effects which may have been linked to broad scale effects such as climate. Sable Island experienced lower precipitation during the summer of 2012 (Manning et al. 2015) which led to higher densities around water sources or increased the amount of time travelling between water and grazing areas. This may be one reason why we observe HCC in samples collected in 2012 to be generally higher than those collected in 2011. In addition to this, high overwinter mortality in 2010-2011 (Manning et al. 2015) would have perhaps improved access to resources for the remaining individuals in 2011.

Overall, none of the variables which represented the social landscape of the individual was considered in the best models for male HCC, while amongst females, harem size along with the number of bachelors in the local area (i.e., home range) appeared to explain some of the variation observed in female HCC. This could speak to differences in how females and males perceive and respond to population demographics and resulting social structures. This could also represent part of the physiological response leading to lower female reproductive success which is seen when the local sex ratio is more male-biased (Chapter 2) and with larger band sizes (Chapter 3). Models for both male and female HCC contained age and body condition as important fixed factors.

Stability in social relationships or group hierarchies can influence cortisol production (Alberts et al. 1992; Engh et al. 2006*a*; Sapolsky 2011; Nuñez et al. 2014). An increase in cortisol production has been attributed to the increased fighting and aggression which occurs during takeovers (Alberts et al. 1992). Engh et al. (2006*a*, 2006*b*) observed that instability within the baboon female hierarchy led to increased cortisol particularly amongst those females whose ranks were changing. Similar observations have been made for feral horses using faecal cortisol metabolites (Nuñez et al. 2014). When looking at HCC and whether a female is in a new band compared to the preceding year we observed that higher hair cortisol levels were more often observed in females in new bands. Female horses which change bands would find themselves having to establish their hierarchal position in the new group. Therefore, when harems are disrupted and/or individuals find themselves in new harems there is likely going to be an increase in cortisol production until the benefits of familiarity and hierarchical positions are recognized.

There were a number of variables that showed some influence on HCC results that were not particularly of interest but demanded some consideration in model design. The most critical of these was the coarseness of the hair which showed higher HCC levels from hair which was more coarse (higher mg per 5 cm strand). Second, the ground sample mass was also potentially adding bias and suggesting that more cortisol was recovered from larger original sample masses. However, this may also be confounded by a third factor, date of assay, due to the order the samples were run. It leaves unresolved the question of do we see a real difference between assay day? Or is it reflecting a real difference in cortisol results with higher sample mass? In both males and females, ground sample mass was removed as a potential random factor during model selection while retaining assay day which would also have some correcting affect if indeed higher sample masses have higher cortisol. Hair colour did show some indications of being an important factor but those colours which appeared to be lower in cortisol (flaxen and sorrel) had very low sample sizes, they were however trending towards being lower in cortisol than darker hair colours (black and chestnut).

4.6   Conclusion

The social and physiological data of the long-term individual-based research allowed for detailed investigations of hair cortisol correlates and patterns. Both male and female HCC is linked more strongly to physiological inputs such as body condition and age; and for females the presence of dependent offspring is also attributed to higher HCCs. The data suggests that the male and female HPA axis may respond differently to other cues, females in particular have more variation in hair cortisol concentrations relating to factors which describe their social experiences (e.g., harem size and bachelor abundance). This information could suggest the physiological link to what with what we have learned in previous chapters about female fitness responses to changes in social structure.

CHAPTER 5

HAIR TESTOSTERONE CONCENTRATIONS IN MALE FERAL HORSES: SOCIAL AND PHYSIOLOGICAL CORRELATES

5.1   Abstract

Steroid hormone concentrations derived from proximal hair segments provide a representation of circulating hormones over the concurrent period of growth. In a population of feral horses (Sable Island, Nova Scotia), we measured cortisol and testosterone concentrations from hair grown during the peak of the reproductive season from 99 males. Social position was determined based on the male's social role as either a dominant breeding stallion, subordinate male, adult bachelor (5 years old or older), or juvenile (3–4 years of age). There was no difference in hair cortisol concentration among any class of adult males; juveniles presented significantly lower concentrations than other groups. Hair testosterone concentrations were highly variable among the juvenile group, but among the three adult classes, dominant band stallions had significantly higher testosterone concentrations than did non-breeding subordinates or bachelors. Comparing hair testosterone and cortisol concentrations, different relationships were observed between breeding stallions and non-breeding bachelors: at low hair cortisol concentrations stallions had much higher hair testosterone levels than did bachelors; while at high hair cortisol concentrations the difference in hair testosterone levels between stallions and bachelors diminished.

5.2   Introduction

Generally, glucocorticoid levels rise when there is an actual or perceived (e.g., psychological stress) need for the mobilization of stored energy (Sapolsky et al. 2000; McEwen and Wingfield

2003; Goymann and Wingfield 2004), while success in acquiring mates is linked to the ability of males to develop and maintain costly ornamentation and display behaviours, which are associated with high circulating levels of testosterone (Folstad and Karter 1992; Ketterson and Nolan 1999; Wingfield et al. 2001; but see Barron et al. 2015). The hypothalamus-pituitary-gonadal axis (HPG; regulating testosterone production) and the hypothalamus-pituitary-adrenal axis (HPA; regulating glucocorticoid production) do not operate as independent systems, and can be activated by some of the same physiological and psychological stimuli (Lennartsson et al. 2012; Turan et al. 2015). Additionally, the two axes can have an inhibitory effect on one another and on other systems in the body. Testosterone is known to have an inhibitory effect on HPA function (Sapolsky 1987; Viau 2002; Rubinow et al. 2005) while other studies have shown that basal cortisol levels can influence the production of testosterone (Viau 2002; Wingfield and Sapolsky 2003; Hardy et al. 2005; Bartos et al. 2010; Mehta and Josephs 2010; Bedgood et al. 2014; Leary and Knapp 2014). Further, responsiveness of the HPA axis to stress during reproductive periods may be linked to life history and social structures (Wingfield and Sapolsky 2003; Boonstra 2013*b*).

Like glucocorticoids, testosterone levels are known to be modified by a number of physical and psychological processes (Mazur and Booth 1998; Goymann et al. 2007; Earley and Hsu 2008). Testosterone increases in preparation for contests and generally winners of these contests proceed to generate even greater amounts of testosterone (Wingfield et al. 1990, 2001; Mazur and Booth 1998; Hsu and Wolf 1999; Oyegbile and Marler 2005; Mcglothlin et al. 2008; Gleason et al. 2009; Lennartsson et al. 2012; Maruska 2015). Circulating testosterone levels also rise with an increase in sexual behaviour, particularly in mammals (Kirkpatrick et al. 1977; Borg et al. 1992; Lynch et al. 2002; Hirschenhauser and Oliveira 2006; Gleason et al. 2009; Khalil et al. 2009)' including those behaviours related to mate acquisition (Borg et al. 1992; Mills et al. 2009), and in anticipation of the breeding season (Goymann et al. 2007).

In the wild, horses are a polygynous species with successful males (dominant stallions) acquiring and defending their access to one or more breeding-age females (Welsh 1975; Berger 1986). Males which are unsuccessful in acquiring or retaining access to females (bachelors) will often form all-male groups or remain solitary. As an alternative male strategy, sometimes a subordinate male (or 'tag') will associate with a particular band (group composed of the

dominant male, mature females and their related offspring) and contribute to the band defense activities. These males may gain limited or opportunistic access to females of the group (Welsh 1975; Berger 1986; Asa 1999; Feh 1999; Linklater and Cameron 2000). Once established, band stallions may keep a harem for several years before being no longer able to defend against younger males. Based on previous literature regarding testosterone levels and the winner effect (Wingfield et al. 1990; McDonnell and Murray 1995; Oyegbile and Marler 2005; Gleason et al. 2009; Maruska 2015), and in relation to courtship opportunities (Kirkpatrick et al. 1977; Borg et al. 1992; Lynch et al. 2002; Hirschenhauser and Oliveira 2006; Gleason et al. 2009; Khalil et al. 2009) we predict that dominant band stallions should have higher levels of testosterone than bachelors as they monopolize the courtship and mating opportunities and obtain (or maintain) their position through winning contests.

Using hair as a matrix for hormone analysis provides the opportunity to understand the interaction of cortisol and testosterone over long periods of time and how it may be implicated with sexual selection and future fitness. Steroid hormones recovered from hair predominantly arise from passive diffusion of circulating free (unbound) hormone integrated during the hair growth, and provide a representation of long-term circulating hormone concentrations (Kirschbaum et al. 2009; Russell et al. 2012; Carlitz et al. 2014). Matrices such as blood serum, saliva, urine, or faeces reflect only the most current (minutes to hours) circulating levels (Sheriff et al. 2011). Most commonly, hair analysis has been used to determine cortisol concentrations (Davenport et al. 2006; Macbeth et al. 2010); recently, however, there has been an increased interest in examining other steroid hormones (Koren et al. 2006; Bryan et al. 2013*a*; Terwissen et al. 2014). Testosterone has previously been measured from horse hair in studies linked to detecting doping in the horse racing and breeding industry (Anielski et al. 2005; Boyer et al. 2007).

The feral horse is an excellent model to investigate given its harem-defense polygynous mating structure is maintained throughout the year and reproductive activity may extend for several months (Welsh 1975; Berger 1986). In this study, we determined hair testosterone and cortisol concentrations in a population of feral horses existing in a naturally derived social organization. Additionally, this population exists without predators or other outside sources of disturbances which could unknowingly influence an individual's hormone levels. We hypothesize that

dominant band stallions will have higher hair testosterone concentrations than males that were not successful in attaining or retaining a breeding position (i.e., bachelors). Additionally, males with high levels of cortisol should be more likely to have lower levels of testosterone due to the suppressive influence of HPA activity on HPG function. Conversely, males with high hair testosterone levels will have lower hair cortisol based on the suppressive effects of HPG activity on the HPA axis.

5.3 Methods

5.3.1 Study Area and Species

Sable Island National Park Reserve is located 275 km east of Halifax, NS, Canada, situated in the Atlantic Ocean. A feral population of horses has existed on the island since the mid-1700s and have long-since established a wild-type social structure. The population gained protection granted in the early 1960s under the Canada Shipping Act (2001) and now more recently through the Canada National Parks Act (2000) as it applies to wildlife. For the last half a century the population has lived without interference of humans (e.g., additions or removal of individuals, or disruption to social groups). Being the only terrestrial mammal on the island, other than humans, the horses live without predators or even other competitors which has made them an excellent population for an intensive study on individual contributions to population dynamics and evolution.

5.3.1.1 Annual Survey

Each summer since 2008, the horses are surveyed with data collected on their location, associations, reproductive status, and health. Individuals are readily identifiable using their natural markings, hair patterns, scars or other characteristics. Female reproductive status is determined by the presence or absence of a foal or yearling. Male social positions are identified based on behaviour and associations with other individuals (Linklater and Cameron 2000) with individuals classed during sampling as being a dominant band stallion, subordinate band stallion referred to here as a 'Tag' male (an adult male that is not the dominant stallion in a multi-male band) or as an adult bachelor (>5 years old) which is unassociated with reproductive aged females, or a juvenile (3–4 years old) that may be with its natal band or dispersed and living as a

juvenile bachelor. Male horses may produce semen as young as two years old (Bennett and Hoffmann 1999) however, males living in natural social structures that are less than 5 years old are rarely seen consorting with females in the wild (Berger 1986; Asa 1999).

5.3.1.2   Body Condition Index

Body condition was determined by evaluating photographs taken during the field survey. Individuals would be graded on a scale of 0-5, zero being emaciated and 5 being very obese. Three body regions on an individual were rated, hips, rib cover, and backbone, half points were used when a difference was found between the three regions. This scale follows the criteria set out in Carroll and Huntington (1988). To eliminate bias from multiple observers, all body conditions for the individuals used in this analysis were determined or reviewed by S. Medill.

5.3.1.3   Median Locations and Buffer Data

A median location for an individual is determined using all the observations of that horse during the survey year. From these median locations a buffer of either 2000m, 4000m or 8000m which represent the 80th, 95th, and 99th percentile of annual movements of all horses (Laforge et al. 2015). Within these buffers we calculate measures of density (horses / vegetated km^2), sex ratio (Males 4+ / Males 4+ & Females 2+; see equation 2.1), or the number of bands or bachelors. These measures characterize the social landscape the individuals are living within upon the island.

5.3.1.4   Degree

Degree is a concept derived from social network analysis (see chapters 2 and 3) which reflects the number of individuals a focal individual is observed to interact with. For horses which are within bands this is equivalent to band size (minus the individual). For bachelors, who may be observed with different associates during different days on the survey, this is a total number of unique individuals that horse was seen with for the entire survey period.

5.3.2 Sampling Method and Storage

In 2011 and 2012 rooted tail hairs were collected for microsatellite DNA analyses (unpublished data) and for hormone analysis (this study) under our institutional animal care permit (University of Saskatchewan UCACS #20090032). Sampling methods included collecting hairs that were observed to be scratched off onto previously cleaned natural and artificial (velcro strips set out on known rub structures) hair snags, however the majority of samples were collected by directly pulling the hairs from the individual if they permitted approach. Hair collected in the field was stored at room temperature in envelopes and kept in the dark. Only hairs with the root attached were used in this analysis to ensure that only the most recent hair growth was used. We removed 4 mm from the root end to retain for future DNA analysis and used the next 5 cm segment to evaluate hormone concentrations. We limited this study to individuals three years and older as this represents a segment of the population which are sexually mature.

Tail growth rates of domestically kept horses have been reported as between 0.066 and 0.081 cm/day (Tracey et al. 2002; Dunnett and Lees 2003; Schlupp et al. 2004; West et al. 2004; Boyer et al. 2007; Šturm et al. 2015). The 5 cm segment of tail hair, based on these rates of growth, would represent the time up to 67–82 days prior to sample collection excluding the most recent 5–6 days associated to the 4 mm portion of hair root removed. Two samples were collected in 2011 (on Aug 10 and 22), and 46 samples between July 29th and Aug 17th in 2012. We therefore captured the hormone profile within the hair shaft corresponding to a period of time in which peak parturition (May) and initiation of the breeding season occurred (Welsh 1975; Lucas et al. 1991). Highest plasma levels of testosterone were observed in May among a population of feral horses in the Pryor Mountains (Montana – Wyoming, USA; Kirkpatrick et al. 1977).

5.3.3 Laboratory Analysis

We analyzed the hair samples following procedures described in Macbeth et al. (2010) with the following modifications relating to the use of hair segments as opposed to whole hairs. Methanol washes to remove exogenous sources of hormones and contaminants (e.g., from sweat or faeces) were performed after the removal of the root end but before the 5 cm segment was cut from the full hair shaft. Testosterone samples were evaluated secondarily to the cortisol samples. Where

possible, left over powdered hair samples (up to 25 mg) were analyzed by extracting hormones with HPLC-grade methanol over 24 hours and reconstituted using the phosphate buffer provided with the testosterone enzyme immunoassay kit (ADI-900-065 Testosterone EIA kit, Enzo Life Sciences, Plymouth Meeting, PA, USA). However, several of the samples processed for cortisol, and reconstituted with the cortisol EIA enzyme extraction buffer (EA-65 Cortisol EIA kit, Oxford Biomedical, Oxford, MI, USA) provided sufficient volume to be analyzed for testosterone content and we evaluated whether this approach would produce similar results by having a number of replicates using both approaches. As some of the samples came from a very small mass of powdered hair we adjusted the dilution factor accordingly, samples less than 6 mg were not diluted, 6-12.5 mg of hair were diluted 3:1, and samples between 12.5 and 25.0 mg were diluted 5:1 in order to keep samples within a detectable range.

Intra-assay percent coefficient of variation (%CV) for testosterone was 4.7% ($n = 6$) and for cortisol 3.6% ($n = 6$), and inter-assay %CV for testosterone was 10.5% ($n = 12$) and for cortisol 5.8% ($n = 12$). The limit of detection for testosterone was 6.71 pg/ml, and serially diluted samples were parallel with standards ($R^2 = 0.983$, P-value < 0.001). The limit of detection for cortisol was 0.02 ng/ml, and parallelism of serially diluted hair samples was obtained ($R^2 = 0.997$, P-value < 0.001; Macbeth et al. 2010).

5.3.4   Data Analysis

There are several variables related to both the sample collection and processing that can introduce variation in the data which were not the direct interest of this study. These include biological variables such as hair colour, year (and it associated influence on biology), Julian day of sample collection, and depending on the question at hand, age. Additionally, procedural variables such as assay date, original mass of ground sample, and hair coarseness (measured as mean mg per 5 cm segment) are also potential bias to our results. Testosterone results will be compared using linear models to determine the relative influence of these factors and AIC_c model selection criterion will be used to determine if any need to be considered as random (or fixed) factors in future models.

I used general linear models to compare hair testosterone concentrations (HTC) with male social position in the current year and social position and survival in the subsequent year. To more clearly understand testosterone levels in males capable of successfully reproducing (or competing for reproductive opportunities) some analyses are restricted to bachelors and stallions older than 5 years of age. Many analyses will also include hair cortisol concentrations (HCC) measures, more information on cortisol can be found in chapter 4, but in brief, cortisol concentrations were log transformed for analysis due to a skewed distribution. As for cortisol I also built linear models to best explain observed testosterone variation and used AIC_c model selection criteria of $\Delta AIC_c < 2.0$ to identify top models and most influential variables. Analyses were performed using R statistical software (R Core Team 2013) including the package lme4 (Bates et al. 2014).

5.4   Results

5.4.1   Testosterone Sample Summary

I was able to test 109 samples from either left over powdered hair or samples reconstituted with the cortisol extraction buffer, however 10 of the samples produced results with CV values > 15% and removed from analysis. This left 51 samples previously reconstituted with the cortisol buffer and 48 samples which were processed direct from hair powder. Comparison of concentrations between those processed with the cortisol buffer and those extracted with the buffer provided in the testosterone kit was possible for 27 individuals. There was a significant difference in the results produced by the two processing approaches which suggests that we cannot consider the methods equal (Paired T-test; T = 6.38, *df* = 26, *P*-value < 0.001). A correction for samples processed from cortisol extraction buffer was attempted by using linear regression however tests of the corrected data continued to be significantly different from samples processed from powder ($F_{1, 70}$ = 8.39, *P*-value = 0.005). Samples processed from powder and reconstituted with the testosterone assay buffer had a normal distribution (Shapiro-Wilk test; W = 0.9826, *P*-value = 0.689). Greater variability for further analysis and therefore hypotheses were tested using the 48 samples of testosterone extracted from powdered hair. Mean testosterone concentration for males derived from these 48 samples was 2.310 pg/mg (range: 1.211–3.494 pg/mg).

The analysis of hair for testosterone appears to be influenced less by some of the variables which caused variation in cortisol (see Chapter 4). Biological variables such as year, day, age or hair colour were not strongly correlated to hair testosterone concentrations, nor were the procedural variables of assay date or weight of the powdered sample used for the extraction (Table 5.1). Hair coarseness was found to have a significant negative relationship to the measured level of testosterone (Figure 5.1) but as it is a continuous variable and we have so few samples it is more appropriate to include it as a fixed effect in future models.

Table 5.1. Influence of biological and procedural variables on the observed hair testosterone concentration determined by single factor regressions.

	Variable	df	T-value	P-value	AIC_c
Null model					80.48
Biological					
	Year	46	−0.047	0.963	82.48
	Julian Day	46	0.440	0.662	82.28
	Age	43	−1.758*	0.086	79.06
	Hair Colour	46	1.692	0.097	79.59
Procedural					
	Assay	45	−0.961*	0.342*	83.33
	Ground Mass	46	0.622	0.537	82.08
	Coarseness	46	−3.307	0.002	72.25

*result from strongest level of factor
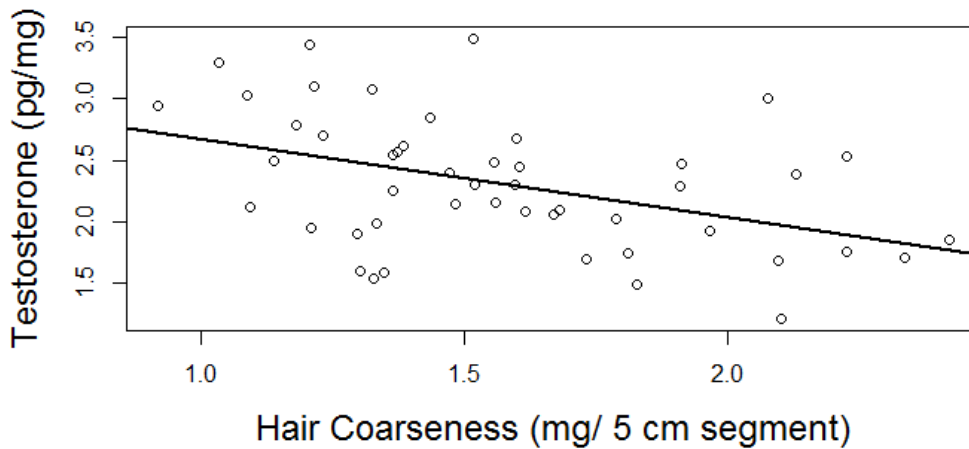
Figure 5.1. Hair coarseness, measured as mass per 5 cm segment of hair, was negatively associated with the testosterone concentration of hair (T = –3.307, *df* = 46, *P*-value = 0.002).

5.4.2 Testosterone and Age

Small and uneven sample size between age classifications makes it difficult to make accurate inferences on testosterone concentrations from hair. Three years olds ($n = 12$, $\bar{x} = 2.346$ range = 1.602–3.494) and adults six and older ($n = 28$, $\bar{x} = 2.435$ range = 1.545–3.444) were the best represented; age four ($n = 2$, $\bar{x} = 1.656$ range = 1.211–2.100), five ($n = 4$, $\bar{x} = 1.839$ range = 1.495–2.02), and known aged six-year-olds ($n = 2$, $\bar{x} = 1.940$, range = 1.585–2.295) much less so (Figure 5.2). Including age, with the interaction of hair coarseness, in the model to describe hair testosterone (AIC$_c$ = 77.70) is a better fit then the null model (AIC$_c$ = 80.48) however none of the levels appear to be significantly different from the others. Figure 5.2 suggests a high level of testosterone in males 3 years of age and a lull during pre-adulthood before a wide range of testosterone levels amongst adults. Coarseness also followed an almost mirror imaged relationship where samples from the individuals aged 4, 5, and 6 were seemingly coarser then the samples from three year olds and adults.

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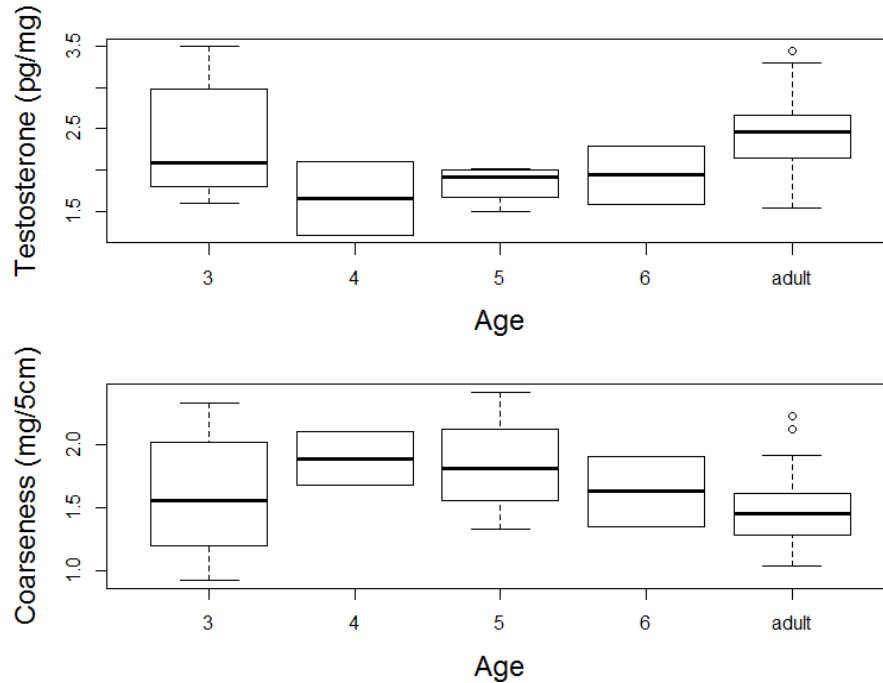
Figure 5.2. Hair testosterone concentration (upper) and the observed hair coarseness (mean mass of 5 cm segment of hair; bottom) of male Sable Island horses of different ages (Age 3 [*n* = 12], Age 4 [*n* = 2], Age 5 [*n* = 4], Age 6 [*n* = 2], and Age 6+ [*n* = 28]).

5.4.3 Testosterone and Social Position

Mean hair testosterone concentration was highest in harem stallions (*n* = 21, $\bar{x}$ = 2.507, range = 1.699–3.444), followed by juveniles (*n* = 14, $\bar{x}$ = 2.247 range = 1.211–3.494) then tag males (*n* = 4, $\bar{x}$ = 2.263, range = 1.759–2.621) and finally bachelors (*n* = 9, $\bar{x}$ = 1.97, range = 1.495–2.573] who appeared to have the lowest testosterone levels (Figure 5.3). Fitting a linear model with both social position and hair coarseness as fixed variables returned a significant interaction term between stallions and hair coarseness (T = –2.239, *df* = 40, *P*-value = 0.031) as well as a significant difference between hair testosterone levels of bachelors and stallions (T = 2.647, *df* = 40, *P*-value = 0.012). Considering testosterone tends to decrease with increased hair coarseness it is remarkable to see that stallions, which tend to have higher hair testosterone concentrations also had less coarse hair (Figure 5.3).
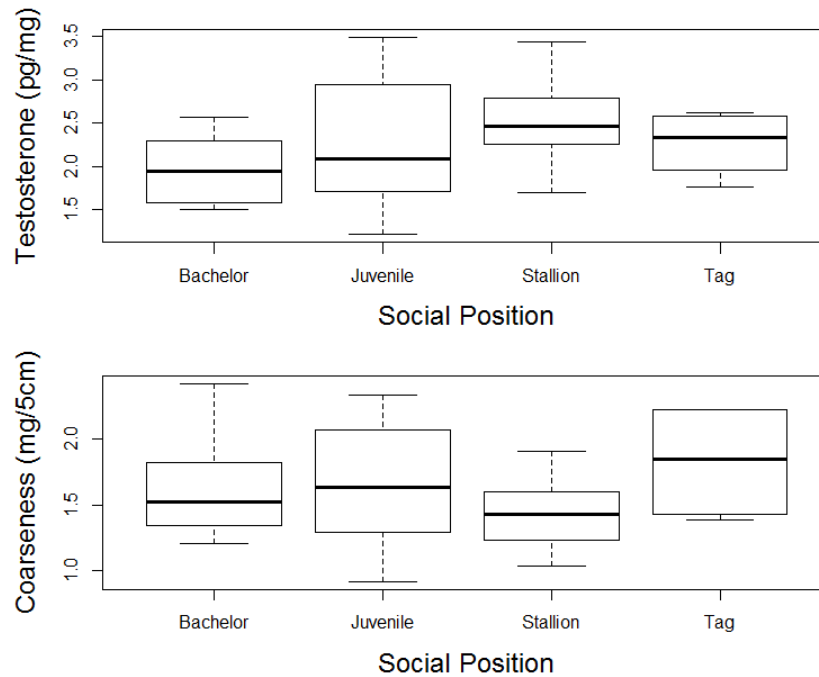
Figure 5.3. Hair testosterone concentration (upper) and the observed hair coarseness (mean mass of 5 cm segment of hair; bottom) of male Sable Island horses of different social positions (Bachelor [$n = 9$], Juvenile [$n = 14$], Stallion [$n = 21$], and Tag [$n = 4$]). A significant difference in testosterone concentration was observed between stallions and bachelors (T = 2.647, $df = 40$, P-value = 0.012) and the interaction term between stallions and hair coarseness was also significant (T = –2.239, $df = 40$, P-value = 0.031).

5.4.4 Testosterone and Cortisol in Sexually Mature Individuals

Using individuals with both cortisol and testosterone data ($n = 28$) I investigated the potential for a significant interaction between hair cortisol level and social position amongst adult males (Sollberger and Ehlert 2016). This analysis was explored amongst stallions and bachelors only, as we had insufficient data from tag males. We observed a large difference in hair testosterone levels between bachelors and stallions when the hair cortisol concentration was low (Fig. 5.4). At high cortisol concentrations the difference in testosterone between stallions and bachelors was reduced. High cortisol in stallions tended to be associated with lower testosterone levels, whereas in bachelors, high cortisol was also associated with higher levels of testosterone; however, the

interaction term (log cortisol × social position) was not significant (T = −1.382, df = 26, P-value = 0.179).
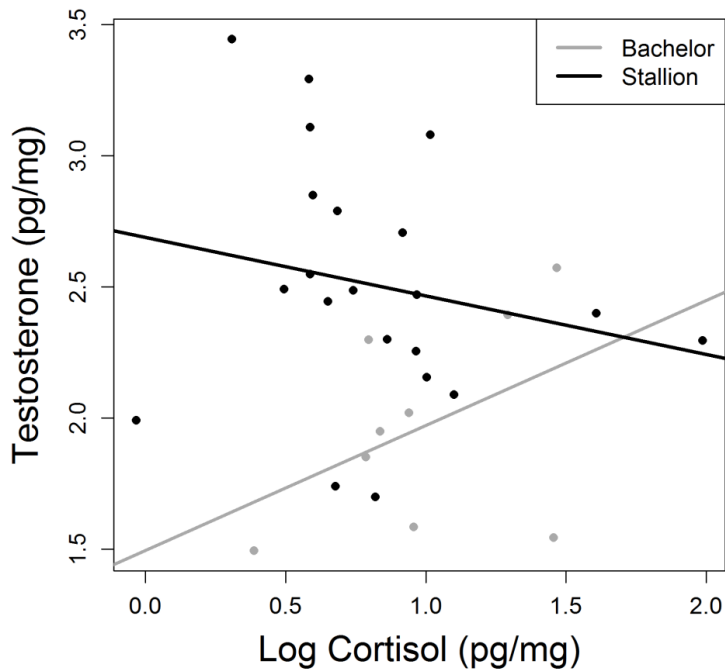
Figure 5.4. Hair testosterone concentrations for non-breeding adult males (Bachelor, grey; $n = 8$, $R^2 = -0.201$) and dominant breeding males (Stallion, black; $n = 21$, $R^2 = 0.435$) in relation to hair cortisol concentrations. Interaction term is not statistically significant (glm; Log Cortisol: P-value = 0.296, Social Position: P-value = 0.029, Log Cortisol × Social Position: P-value = 0.179).

5.4.5   Testosterone and Future Social Position or Survival

Hair testosterone concentration in the current year does not have a correlation to the social position (or vitality) of the individual in the following year (Figure 5.5). Despite an apparently low testosterone concentration in hair collected from individuals which eventually passed away ($n = 8$) there were no significant differences found between the groups (anova, $F_{4,43} = 1.211$, P-value = 0.320).
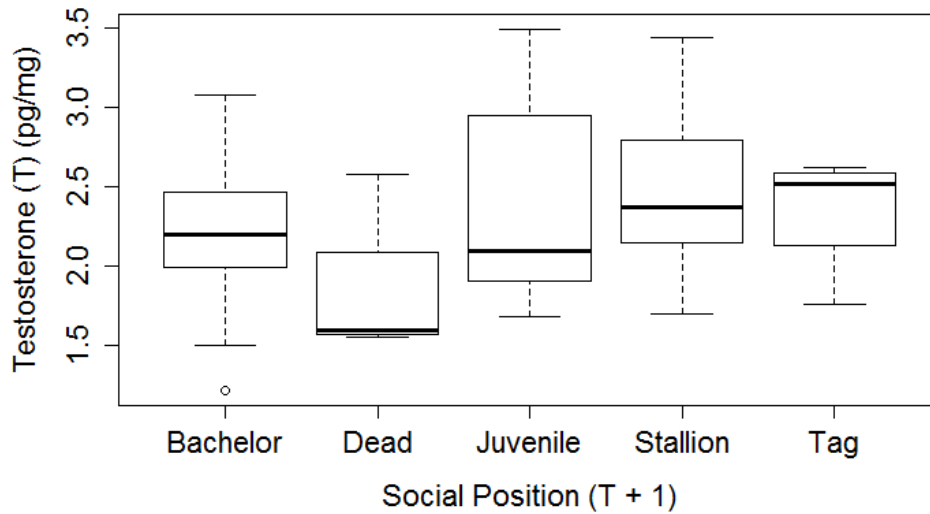
Figure 5.5. Hair testosterone concentration in the current year (T) was not a strong predictor of the social position or survival of the individual the following year (T + 1; ANOVA; $F_{4,43}$ = 1.211, *P*-value = 0.32). Sample sizes for social position at T + 1: Bachelor (*n* = 26), Dead (*n* = 8), Juvenile (*n* = 20), Stallion (*n* = 39) and Tag (*n* = 6).

5.4.6   Hair Testosterone in Dominant Band Stallions

Looking at the twenty-one samples from band stallions I looked specifically at their band structure to see if there is a relationship between their hair cortisol and hair testosterone concentrations. Although not found to be significant we see both an increase in cortisol levels and a slight decrease in testosterone with an increase in harem or band size (Figure 5.6).
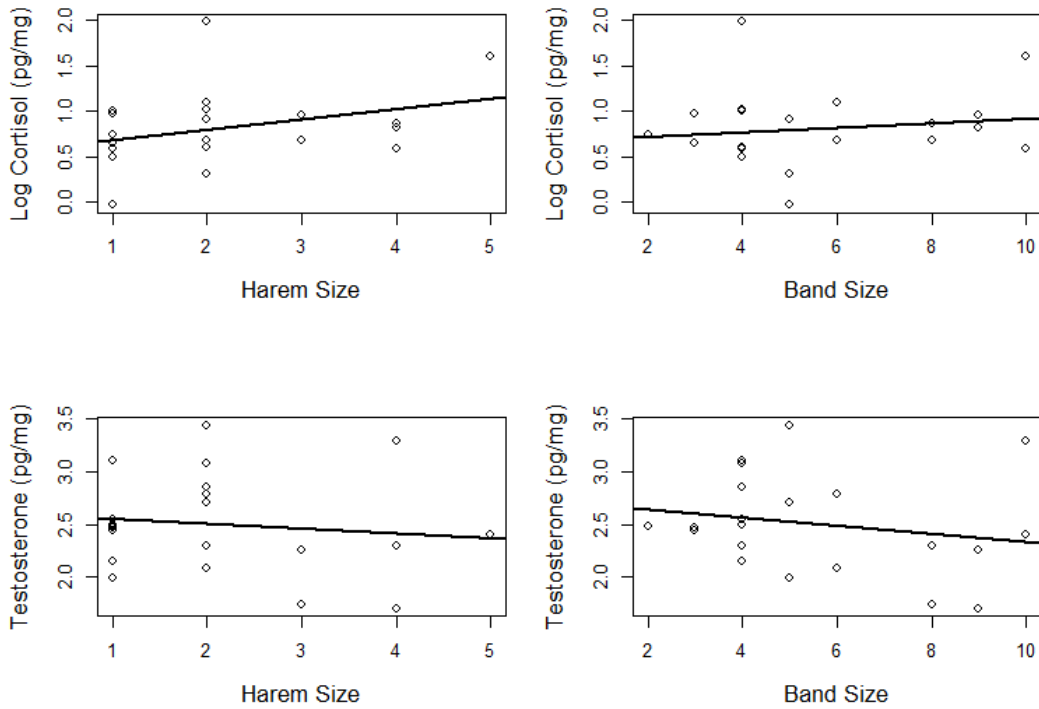
Figure 5.6. Log hair cortisol concentrations (upper) and hair testosterone concentrations (lower) for both harem size (left) and band size (right) for dominant male stallions on Sable Island. None of the relationships are statistically significant.

5.4.7 Testosterone and Body Condition

Body condition was not reflected in hair testosterone concentration with coarseness (T = -0.105, $df = 44$, P-value = 0.917) or without (T = 1.018, $df = 46$, P-value = 0.314) included in the model.

5.4.8 Testosterone and Location on Island

There was no indication that the median location of the individual was correlated with the concentration of testosterone recovered from the hair without accounting for coarseness (T

$= -0.597$, $df = 46$, P-value $= 0.553$; $AIC_c = 82.113$) or with ($AIC_c = 75.997$) though the model was improved with the inclusion of coarseness.

5.4.9 Modelling Hair Testosterone Concentration in Males

5.4.9.1 Biological Models Describing Male HTC

Using factors of interest to build models to explain the variation in testosterone concentrations in hair suggested that the strongest of the four factors included in the model (age, body condition [BC], hair coarseness [Coarse], hair cortisol concentration [HCC], or social position [Soc Pos]) was hair coarseness, and model fit was slightly improved when body condition was also included (Table 5.2). No interactions terms were observed in the top models.

Table 5.2. General linear model results of biological variables used to explain hair testosterone concentration in Sable Island horses. Variables include age, body condition (BC), hair coarseness (Coarse), hair cortisol concentration (HCC), and social position (Soc Pos). Interactions terms were included in the model design but none were found to be in the top models. Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Null Model $AIC_c = 80.48$.

Age	BC	Coarse	HCC	Soc Pos	df	AIC_c	ΔAIC_c	LL
	+	-			4	72.1	0.00	-31.57
		-			3	72.8	0.73	-33.12
	+	-	-		5	74.5	2.48	-31.56
			-		4	74.9	2.85	-32.99
		-		*	6	75.1	3.01	-30.52

5.4.9.2 Social Models Describing Male HTC

Comparing hair testosterone concentrations to social conditions on the island we find stronger correlations for all variables (number of bachelors, numbers of bands, horse density and adult sex ratio) when using values calculated in the 8000 m buffer. Checking variables for collinearity suggested strong correlation between the number of bands and the adult sex ratio (ASR; Figure 5.7, $R^2 = 0.84$). For this reason, the variable ASR was not included in the model but the number of bachelors and the number of bands (which are components of ASR) were retained. Coarseness, due to its high influence on testosterone is also included in the model but without interaction terms. The strongest model explaining hair testosterone showed the negative influence of hair coarseness, the number of bands in the 8000 m radius of the individual as well as horse density on hair testosterone (Table 5.3, $AIC_c = 69.70$).
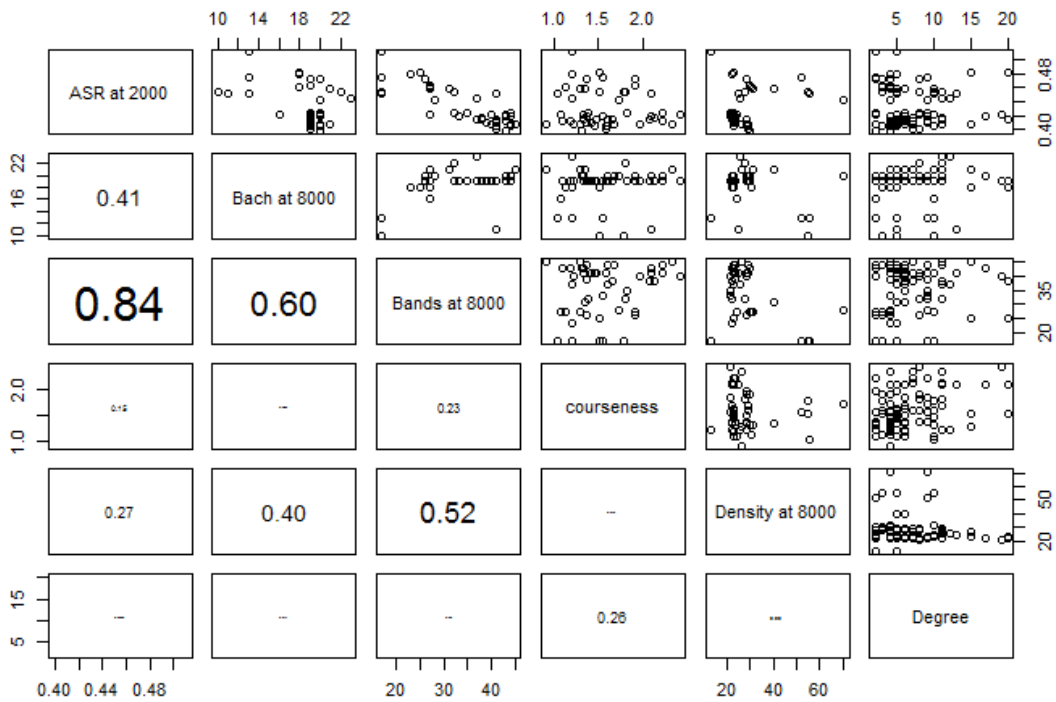
Figure 5.7. Scatterplot (upper right panes) and Pearson correlation coefficients (lower left panes) of candidate covariates used to develop models describing social influences on male testosterone in feral horses. Variable names appear in the diagonal and include adult sex ratio (ASR), abundance of bachelors (Bach), number of bands (Bands), hair coarseness (coarseness), horse density in vegetated habitat (Density), and degree. ASR and Bands are strongly correlated ($R^2 = 0.84$).

Table 5.3. General linear model results of sociological variables used to explain hair testosterone concentration in Sable Island horses. Variables include number of bachelors (Bach), bands, and horse density in the vegetated habitat (Dens) calculated in the 8000-m buffer around individuals' median locations, along with the degree and hair coarseness (Coarse). Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Null Model $AIC_c = 80.48$.

Bach	Bands	Coarse	Degree	Dens	Bach* Bands	Bands* Degree	Bands* Density	df	AIC_c	ΔAIC_c	LL
	-	-		-				5	69.7	0.00	-29.13
-	-	-		-				6	71.4	1.75	-28.70
	-	-	+	-			-	7	71.8	2.06	-27.48
-	+	-	+	-			-	8	71.8	2.12	-26.06
-	-	-		-	+			7	71.8	2.12	-27.51
	-	-		-			-	6	72.0	2.31	-28.98
	-	+	-					6	72.1	2.43	-29.04

5.4.9.3   Best Fit Model to Describe Male HTC

Combining the variables with the strongest biological and social effects we determine that the top model remains that which only included the number of bands and horse density in the 8000 m surrounding the individual's location along with body condition, hair cortisol concentration, and hair coarseness as well as an interaction term between bands and hair cortisol concentration ($AIC_c = 68.9$; Table 5.4).

Table 5.4. General linear model results of variables investigated to explain hair testosterone concentration in Sable Island horses. Variables include number of bachelors (Bach), Bands, and Density (Dens) calculated in the 8000-m buffer around individual's median locations, along with log hair cortisol concentration (HCC), social position (Soc Pos), and hair coarseness (Coarse). Plus or minus sign indicate direction of effect and an asterisk indicates use of factor variable in model. Null Model $AIC_c = 80.48$.

Bach	Bands	BC	Coarse	HCC	Soc Pos	Dens	Bands* HCC	BC* HCC	Dens* HCC	df	AIC_c	ΔAIC_c	LL
	-	+	-	-	-	+				8	68.9	0.00	-24.60
	-	+	-	-		+				7	69.0	0.08	-26.08
	-	-	-	-			+	+		8	69.6	0.68	-24.94
	-	-			-					5	69.7	0.81	-29.13
	-	-	-	-	-		+	+		9	69.8	0.96	-23.55
	-	-	-			+				6	70.0	1.07	-27.95
	-	-	-		-	+				7	70.1	1.21	-26.65
	+	-			-					6	70.5	1.59	-28.21
	+	-	-		-	+			+	9	70.7	1.82	-23.98
-	-	+	-	-	-	+				9	70.9	1.99	-24.07

5.5 Discussion

Evidence was found that hair testosterone concentrations were greater in dominant breeding band stallions compared to adult bachelors. Males that have established themselves as dominant band stallions have done so through successfully winning contests or by defending their social position against other males; whereas those males which are bachelors have either lost, or not attempted, these contests. Bachelor males also have little or no access to reproductive opportunities (Berger 1986; Feh 1999; Linklater and Cameron 2000) though in some populations bachelors or subordinate males do sneak some reproductive opportunities (Miller 1981; Kaseda et al. 1982; Kaseda and Khalil 1996; Asa 1999). Although the sample size to draw conclusions

on the hair-hormone profiles of males choosing an alternative mating strategy was low (Tag males $n = 4$) it is interesting to note that their hormone profiles fell into an intermediate range between non-breeding adult bachelors and dominant breeding males. Our sample sizes for the various ages were not suitable for making conclusions on HTC and age. In our best represented groups, 3 years old and adults, we did not see a difference in HTC. The poorly represented ages in between appeared to have lower testosterone.

As well documented for cortisol (Sapolsky et al. 2000; Sheriff et al. 2011), circulating testosterone levels can change rapidly in response to sexual or social triggers such as the winning or losing of contests (Wingfield et al. 1990, 2001; Mcglothlin et al. 2008; Karubian et al. 2011; Lennartsson, et al. 2012; Maruska 2015;). In many species, courtship behaviours or intercourse may lead to an increase in testosterone production (Borg et al. 1992; Gleason et al. 2009). The combination of repeated experiences that dominant band stallions have both defending their harem and their reproductive activities gives plausible support to our observations of higher testosterone concentration in the hair of dominant band stallions. Stallions defending access to females from a group of bachelors showed a rapid increase in serum testosterone at the start of their tenure and a rapid decrease when they were removed from that position (McDonnell and Murray 1995). Bison which tended (guarded) cows during the reproductive season were also more likely to have higher testosterone levels than males that did not tend (Mooring et al. 2004). Additionally, through non- or minimal-contact posturing, band stallions can bring an early end to approaches by subordinates and bachelors without escalating to injurious fighting (Berger 1986). Anticipation of a contest or fight can result in elevated testosterone production (Mazur et al. 1992; Salvador et al. 1999) and it is possible that a stallion defending a harem through posturing behaviour could also be effective at raising systemic testosterone levels. Non-physical contests in humans have revealed that winners may show elevated testosterone levels (Mazur et al. 1992) and can even lead to reduced cortisol production in individuals with high testosterone (Mehta et al. 2008).

Only one other study in horses was found that measured testosterone (from faeces) in relation to harem size in free-living feral horses (Khalil et al. 2009). That study observed an increase in testosterone with an increase in harem size. Our study failed to find a relationship between harem or band size and hair testosterone or cortisol concentration. However, we did see a trend of

increased cortisol along with a decrease in testosterone in males with larger harem size suggesting a possible reflection of the inhibitory influence of cortisol on testosterone when reproductive or social demands are above average (Viau 2002; Wingfield and Sapolsky 2003; Hardy et al. 2005; Bartos et al. 2010; Mehta and Josephs 2010; Bedgood et al. 2014; Leary and Knapp 2014).

The interaction between cortisol and testosterone levels on an individual's performance has led to the development of the dual-hormone hypothesis (Hermans et al. 2008; Mehta and Josephs 2010). This hypothesis suggests that the effectiveness of testosterone is related to the level of glucocorticoids. Glucocorticoids have been shown to have a moderating effect on androgen based behavioural or phenotypic traits which can in turn influence sexual selection (Leary and Knapp 2014) and conspecific interactions (Virgin and Sapolsky 1997). When glucocorticoids are low, the enhancing effects of testosterone are more pronounced and when glucocorticoid levels are high the relationships between high testosterone and dominance or aggressive behaviours tend to break down (Hermans et al. 2008; Mehta and Josephs 2010; Mehta et al. 2015; Mehta and Prasad 2015).

Here, among adult males with low cortisol levels there were clear differences in social position where the hair testosterone concentrations for stallions was much higher than for non-breeding bachelors (Figure 5.4). Interestingly, among individuals with higher levels of hair cortisol the difference in testosterone between stallions and bachelors was minimal. This could reflect the previously described inhibitory effect of cortisol upon the production of testosterone. Comparing this from a different point of view, bachelors with the lowest cortisol also have the lowest testosterone while those with higher cortisol have the highest testosterone which might suggest that these later bachelors are perhaps more actively competing for mating opportunities and are investing more energy in these behaviours. Unfortunately, we lack the concurrent behavioural data to test these hypotheses.

When building a model to examine the strongest influences on HTC we observe a top model which includes number of bands in the vicinity, body condition, density, and HCC, and an interaction term between number of bands and HCC. The number of bands within the 2000m buffer of the individual was included in one of the top models ($\Delta AIC_c = 1.42$) for describing male hair cortisol levels (Chapter 4). The inclusion of a negative interaction term between HCC

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and bands is perhaps expected. A landscape of high conflict or competition for resources with other bands could explain both an increase in cortisol which, as already established, can have a negative impact on testosterone production. Additionally, the majority of samples were collected in 2012, a year that has been identified as a drought year that caused bands and bachelors to be clustered in areas with accessible water (Manning et al. 2015) which increased local density. Body condition did not have a strong significant relationship to HTC when investigated on its own; however, it does appear to have enough influence to find it in our top model.

Although the use of hair for hormone analysis is very attractive for understanding endocrine states in wildlife because of its ease of collection and storage, there are still some problematic aspects to its use. Variation in growth rates between individuals (Šturm et al. 2015), or the potential for positive influence of testosterone on hair growth (Giltay and Gooren 2000; Dunnet and Lees 2003) could influence both the exact period of time represented in the hair segment and it is unknown how a variation in growth rate may affect the incorporation of hormones into the hair. For this reason, researchers often use a shave-reshave method to ensure that only hair actively grown over the time period of interest is analyzed (Davenport et al. 2006; Sheriff et al. 2011). This would be particularly critical when sampling body hair which is often produced rapidly and has a long telogen phase consisting of no new growth (Geyfman et al. 2015). Mane and tail hair of horses are nearly continuously growing with a large proportion of the hairs in active growth at any given time (Dunnet and Lees 2003; Geyfman et al. 2015; Šturm et al. 2015). Although some degree of variation in hair growth is expected among our individuals (Šturm et al. 2015), this variation likely remains within the prolonged active breeding season of feral horses (Welsh 1975; Berger 1986).

The observed influence on hair coarseness and testosterone is interesting and unexpected. Grind times were calculated based on the total weight of the hair sample to be ground in order to obtain a consistent grind across samples with additional time added if a uniform grind was not achieved. However, this approach did not account for differences in the texture of the hair where some samples were much coarser. Interestingly, hair coarseness was not a strong factor in hair cortisol concentrations (Chapter 4) so there must be some interaction between testosterone molecules and how they are incorporated into the hair that make them potentially more difficult to extract from hair.

5.6 Conclusions

The potential applications of using tissues, such as hair, that are capable of recording hormone levels over long periods of time are gaining interest in wildlife endocrinology. Many tissues used in hormone analysis require frequent collections (blood, saliva, faeces, urine) to establish baseline hormone levels and are prone to random events or circadian rhythms. Hair, particularly continuously growing hair found in the manes and tails of many mammalian species, provides an opportunity to evaluate long-term hormone concentrations with fewer sampling periods required. Individual based, longitudinal studies are rare among wild populations, but we show the potential for hair hormone analysis to be used to reflect endocrine state as an individual transitions between juvenile, pubescent, breeding, and senescent stages of their life. We have shown here that testosterone concentrations derived from hair revealed that males which have won breeding contests and maintain an active role in reproduction have higher testosterone than males that failed to obtain or maintain breeding opportunities. Our data also suggest an interesting relationship between hair cortisol concentration and social position and hair testosterone concentrations. Considering that the effectiveness of testosterone in developing the phenotypes or behaviours related to sexual selection can be dependent on cortisol levels we may find that a critical balance of these (and other hormones) at a specific point of time in an individual's life could be critical for achieving future reproductive success.

CHAPTER 6

GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

The results presented in the last four chapters provides a useful background for future work using the growing body of data coming from the research program on Sable Island horses. I managed to establish that not only do we continue to observe density-dependent changes in social structure and affiliations (see also Marjamäki et al. 2013) but also that social structure changes in response to variation in adult sex ratios, as do rates of adult female movements between groups (secondary dispersals). Variation in the frequency of adult female band changes can have interesting repercussions on genetic variation, particularly in a small closed population like Sable Island horses. Understanding the factors which influences group size and stability is critical when a species maintains associations year-round and when reproductive success is intimately linked with their group compositions. While the population as a whole experienced a transition from female-biased to a more male-biased sex ratio over the 8-year study we saw an increase in the percentage of males that remained a bachelor suggesting that the opportunities for maturing males to obtain harems becomes even more limited under male-biased sex ratios.

A recent article commented on how overlooked sex ratios are when contemplating questions in evolutionary biology or modelling populations (Gerber and White 2014; Székely et al. 2014). The increasingly male-biased sex ratio we are detecting on Sable Island may be important to monitor given the isolation of the population and risk of a genetic bottleneck. There is some indication that adult female survival amongst the horses of Sable Island is lower when the sex ratio is more male-biased (significant at $\alpha = 0.10$). There is also evidence of male-biased sex ratios having a negative impact on adult female reproduction with females producing fewer foals

in areas with more males. Both these effects prolong negative feedback on female recruitment and perpetuate the trend of increasingly male biased adult sex ratios.

There are plenty of reasons to study the influence of adult sex ratio variation on sexual selection in various mating systems. In some mating systems a male biased sex ratio can result in reduced male-male competition for access to females where females become less choosy which resulted in them to mate with lower quality males merely to avoid additional harassment (Dreiss et al. 2010). The mating system of horses may in fact have stronger selective pressure for quality males by having proportionally fewer mating opportunities for the 'excess' males resulting in only the competitively superior males securing harems. What is missing from this research is the accompanying behavioural data on antagonistic interactions between males along with establishment of paternity; does a male's reproductive success in fact decrease in male-biased populations? There is much variation in the literature regarding the percentage of the foals in a band are in fact sired by the dominant band stallion (ranging from 100–51% of foals) versus those sired by subordinates or even roaming bachelors (Miller 1981; Kaseda et al. 1982; Kaseda and Khalil 1996; Asa 1999) this has not been looked at in the context of population demographics influencing sexual selection and group structure or stability.

Not evaluated in this study but a critical component to investigate is why the population was trending to a higher proportion of adult males. Likely culprits in this population are biased mortality rates for either juveniles or adults, given a usual equal sex ratio at birth for horses. There was a slight trend towards adult female mortality observed at higher ASR. Despite the fact that adult females appear less likely to change bands we do observe that bands sizes are decreasing with an increasingly male biased sex-ratio while at the same time observe an increased number of bands (Table 2.2). One explanation could be that juvenile females are likely to form associations with bachelors creating the newer and smaller bands. This could have interesting implications on population growth rates. For example, does consorting with 'new' stallions versus joining existing bands have reproductive or survival costs to natally dispersing females? Does a male-biased population have other impacts such as age of natal dispersal, age of first reproduction, body condition, or tenure of males?

More information on male reproductive success will also be critical in improving our understanding of adult sex ratio and its role in sexual selection in this population. Males take

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longer to reach their reproductive potential and have been reported to have highest reproductive success at 6-10 years of age (Berger 1986; Feh 1990). At the time of this study we are just starting to have an idea of patterns of male entry into the reproductive population with not quite enough data to examine how population parameters may influence individual male reproductive success. The successful males on Sable Island are usually obtaining a position as a dominant stallion at age 5 or 6.

The second research chapter explores social associations among the horses and whether or not they play a role in influencing reproductive success. Evaluation of social networks of bands showed that adult associations among Sable Island horses are not quite as long lasting as expected, with only around 55 % of male–female associations remaining intact between annual surveys. That said, some associations will persist with the longest male–female association lasting 7 years. Comparing annual social networks of adults, we see that it takes approximately 5 years for the network to be considered different (with statistical significance) from the original arrangement. What is perhaps more interesting is the variation in the correlation statistic with the annual changes in the networks. A large portion of this variation is likely from adult mortality (making individuals unavailable) and the recruitment of new individuals providing new association opportunities. It may be possible to explore this variation against other measures such as environmental or population demographics. The fact that the bachelor networks remain similar longer during the later years of the study may be in relation to the more male biased sex ratio, fewer opportunities for males to establish harems, and bachelors remaining associated with known individuals longer.

Female lifetime reproductive success does appear to be negatively affected by the number of associations they have, and in particular the number of stallion associates. By remaining in the same band females successfully minimize the number of both other female and stallion associations over time. It was interesting to see that although maintaining long periods of associations with specific band stallions improved female reproductive success, a similar analysis of associations between female band members did not reveal any effect of association duration on foal production or survival. The importance that female–female associations for improved individual fitness has been described for other species with harem-defense polygyny mating systems (Silk et al. 2003; Frère et al. 2010; Silk et al. 2010; Cheney et al. 2012) and even

for horses (Cameron et al. 2009; Heitor and Vicente 2010*b*; Stanley et al. 2017). Although there are definitely fitness benefits gained by minimizing the number of associates and avoiding band changes the actual associations between two specific females may not be as critical to maintain in feral horses but rather an artifact of females staying with preferred males or avoiding the physiological effects of having to reestablish their position in a new band hierarchy. To further support that band changes can lead to negative physiological or psychological responses there is evidence of higher hair cortisol levels among females found to be in new bands compared to the previous year's association.

Other informative correlations were found between hair cortisol concentration and physiological or sociological situations of feral horses. First it was surprising to see that male horses had overall higher HCC then females. This does not likely represent that males are more 'stressed' then females but rather a different physiological baseline in the sexes or could even be a physiological difference in hair growth (mediated by testosterone perhaps). However, as the hair we sampled was grown over a period of time concurrent with the most breeding activity it is possible that cortisol levels were elevated in males to ensure they met the energetic requirements to perform mate guarding and reproductive activities among stallions or mate acquisition attempts among bachelors.

In females we see that higher HCC is strongly linked to the presence of a foal. This would be expected from individuals performing energy demanding processes such as lactation. Females with high cortisol were slightly less likely to have a foal in the following year although this was not found to be a statistically significant trend. High cortisol, and prolonged activation of the HPA axis, are known to have an inhibitory effect on the HPG axis reducing reproductive success (Sapolsky 2004).

Age and body condition was a significant factor correlating to HCC in both females and males. Horses in poor body condition tended to have higher HCC levels. Greater variation in body condition in females (i.e., more individuals in very poor condition) likely made this relationship more prominent in this sex. Only when the different ages of males was taken into consideration was body condition considered a significant correlate with HCC. The fast growing 3 and 4 year-olds tended to have lower body condition scores than older horses, and tended to have lower HCC values. Throughout this study, I focused more on the activities and interactions of adults

and therefore chose to only analyze samples from individuals aged 3 or older. Including younger individuals would have been informative as to how cortisol or testosterone trends in the ontogeny of feral horses. Future work may even consider focusing on collecting annual samples from specific individuals to track hormone profiles through their lifetime. This would be particularly interesting for males to see if the transition from juvenile to bachelor to band stallion can be traced in their hormone profiles. The inclusion of hormone research in longitudinal field studies are fairly limited (Boonstra 2013*b*) and would be incredibly informative in understanding links between endocrinology and life history.

When using model selection to better understand female hair cortisol levels we see the variables age and body condition are significant in all the top models along with the presence or absence of a dependent foal. Also in all the top models we see that harem size had a negative influence on HCC while the number of bachelors in the local area was linked to increased HCC. Both these factors represent the social component of the population. A higher number of bachelors would increase the local ASR and higher ASR was shown to have a tendency to lead to smaller harem sizes. We are potentially seeing the result that the important function of the harem to reduce male harassment may be lost when there are fewer intraband females for the dominant male to attend to. For males, surprisingly we see almost no social variables in the top models describing HCC. Age and body condition were again important factors describing male HCC but for males the variable year was also important. Does this difference in what may drive the observed hair cortisol values in males versus females suggest different sensitivity to social structure?

Looking at the testosterone concentration in males did reveal a predicted pattern of higher testosterone in hair collected from stallions than in hair from bachelors. The use of hair in comparing the cortisol and testosterone levels showed an interesting difference in the relative level of these two hormones among males which were actively reproducing and those which were not. Stallions tended to have high testosterone and lower cortisol and a negative relationship between the two hormones. Bachelors, on the other hand, that had high testosterone were likely to also have much higher cortisol then their low testosterone counterparts. One possible explanation is that the bachelors with the high HTC and HCC are those that are actively trying to establish their position in the breeding population (i.e., challenging stallions). Unfortunately, we lack the behavioural data to support this explanation, but it does possibly point

to using hair hormone concentrations to better understand the endocrine changes behind the ontological transition from bachelor to stallion or even potentially stallion tenure.

The analyses which look at non-target variables that may introduce variation in our cortisol and testosterone results suggest that some changes in methodology may be appropriate if continuing to use hair from horses to monitor hormone concentrations. First, we had fairly good success with regards to using samples of different masses for testosterone but detected a bias for higher cortisol concentrations recovered from heavier samples. However, this was also potentially a bias stemming from the day the assay was performed. When establishing which factors should be controlled for by inclusion as random factors in the model ground mass was selected out for both sexes. Hair coarseness however was found significant for both cortisol and testosterone. Strangely, there was a positive relationship between hair coarseness and cortisol (higher cortisol in more coarse hair) while testosterone had a negative relationship (lower testosterone in more coarse hair). So, although it would be easy to say that poor grinding resulted in poor recovery of testosterone it doesn't explain why we observed higher levels of cortisol. The methods used were originally developed for hair collected off of the body of carnivores or ungulates (Macbeth 2013) which would have fairly uniform textures between subjects. Moving forward, similar to how grinding time is calculated by sample mass it is likely that hair coarseness will also have to be considered when calculating grind time. The tail hair collected for this study, although all pulled from the base of the tail, showed quite a variation in coarseness and may have been a mixture of outer hairs which may have been exposed to higher UV levels or damaged from rubbing or the elements, while more protected internal hairs may have been coarser. Being more selective during collection or inclusion into the study may also help to limit the error introduced by variation in hair coarseness.

Overall, this research has shown that the structure and stability of feral horse associations is linked to changes in population density and sex-ratio. This is in fact one of the first studies to look at adult sex ratio as a factor in social structure for this species and indeed found some reasons why sex ratio may need to be closely monitored to avoid potential genetic bottlenecks or threats to population persistence. Factors leading to increased female secondary dispersals or increase the number of associations with other individuals have negative impacts on female reproductive success. A possible pathway for that reduced reproductive success may come from

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the physiological response of the HPA axis resulting in increased cortisol inhibiting successful reproduction as suggested by our observations of higher hair cortisol in females who have changed bands. Although the results don't necessarily prove that last point directly we do observe that females hair cortisol concentrations are more strongly influenced by social variables then are males.

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