

Alarm calling, stress, and fitness in central versus peripheral territories  
of Richardson's ground squirrels (*Urocitellus richardsonii*)

by

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## Abstract

Selfish herd theory predicts that as predators usually approach from the periphery of a group, survivorship and reproductive output of peripheral group members should be decreased. Non-lethal encounters with predators may affect prey through the costs associated with increased activation of the hypothalamic-pituitary-adrenal axis. Richardson's ground squirrels (*Urocitellus richardsonii*) defend territories within their colonies and thus may experience differential fitness outcomes based on the location of their territory. I recorded *U. richardsonii* alarm vocalizations with Wildlife Acoustic SM3 audio recorders at centre and edge locations of a colony to estimate predation pressure and predator type (airborne vs. terrestrial), quantified faecal glucocorticoid metabolite (FGM) concentration as a measure of stress, and tracked offspring production and survivorship of females and their young for a year. The results obtained suggest that individuals occupying centre and edge areas experience different levels of predation pressure, but not FGM concentration, survival, or offspring production.

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## Introduction

Animals display a diverse array of grouping and social strategies, ranging from solitary lifestyles to permanent aggregations of large numbers of individuals (Lott, 1991; Lee, 1994; Parrish & Hamner, 1997). The proximate cause of group formation ranges from physical factors causing conspecifics to aggregate in the same location, attraction of conspecifics to the same environmental stimuli, or through the attraction of individuals to the cues of conspecifics (Parrish & Hamner, 1997). The extent of social interaction and cooperation within a group also ranges widely (Parrish & Hamner, 1997). Furthermore, the genetic relatedness of individuals within a group varies, often influencing the frequency and types of social interactions within a group (Hoogland, 1995, Parrish & Hamner, 1997). While cooperation can be driven by inclusive fitness, cooperation also occurs between non-related individuals (Trivers, 1971, Brown, 1983; Wilson & Sober 1994, Seyfarth & Cheney, 1984).

Group-living can be expected to evolve and persist when the benefits outweigh the costs. Group-living provides benefits for animals such as the dilution of predation risk (Wrona & Dixon, 1991), increased probability of the detection of predators (Lima, 1995; Fairbanks & Dobson, 2007), increased ability to defend against predators (Arnold, 2000), increased ability to defend territories (Mosser & Packer, 2009), decreased predator attack success (confusion effect; Landeau & Terborgh, 1986), increased foraging efficiency (Pitcher *et al.*, 1982), and increased thermoregulatory capacity (Ebensperger, 2001). The costs of group-living include increased transmission of parasites (Van Vuren, 1996) and diseases (Langwig *et al.*, 2012), competition for resources (West-Eberhard, 1978), encounters with aggressive conspecifics (Huchard & Cowlshaw, 2011), infanticide (Van Schaik, 1996), competition for reproductive opportunities (Møller & Birkhead, 1993), and the attraction of predators (Parrish & Edelstein-Keshet, 1999).

Selfish herd theory (Hamilton, 1971) predicts that these benefits are not enjoyed equally by group members, but rather that individuals at the centre of a group benefit disproportionately over more peripheral group members, particularly in the face of terrestrial predators that approach from the periphery. Empirical evidence supporting selfish herd theory can be gleaned from a wide range of taxa including fish (Parrish, 1989), insects (Foster & Treherne, 1981), crustaceans (Viscido & Wetthey, 2002), birds (Quinn & Cresswell, 2006), and mammals (King *et al.*, 2012), and can be applied to parasitic interactions as well as predation (Mooring & Hart, 1992).

Behavioural predictions based on the selfish herd have also been tested, and more often than not, are borne out by empirical data. For example, King *et al.* (2012) demonstrated that individual sheep tended to move towards the centre of the flock when approached by a threatening stimulus (a sheep dog), and Krause (1993) demonstrated that minnows exposed to alarm cues of conspecifics tended to move towards the centre of the shoal. Rattenborg *et al.* (1999) found that mallards (*Anas platyrhynchos*) sleeping on the edge of their flock increased their use of unihemispheric slow-wave sleep, allowing the eye facing away from the centre of the group to remain open and vigilant to predators. The difference in predation risk predicted by the selfish herd theory should be especially pronounced in organisms that live in permanent aggregations, and where territorial individuals can maintain a central location over long periods of time.

Among many species, such as eastern grey kangaroos (*Macropus giganteus*; Rieucou *et al.*, 2012), impalas (*Aepyceros melampus*; Blanchard *et al.*, 2008), and common starlings (*Sturnus vulgaris*; Jennings & Evans, 1980), individuals on the periphery of the group spend more time scanning the environment than central individuals which suggests that predation risk



is higher for peripheral individuals. Measurement of predation risk also supports increased predation risk on the periphery of groups. Balmford and Turyaho (1992) found that male Uganda kob (*Kobus kob thomasi*) at the edge of leks experience a higher risk of predation. Coyotes (*Canis latrans*) consistently attacked mule deer (*Odocoileus hemionus*) on the periphery of groups (Lingle, 2001). However, coyotes did not preferentially attack peripheral white-tailed deer (*O. virginianus*), which unlike mule deer do not respond to predator presence by merging groups and bunching together, suggesting that behaviour can mediate the presence or absence of benefits of central locations. However, predation risk does not vary for all species between centre and edge. The survival of damselfish (*Stegastes planifrons*) did not differ between individuals with central or edge territories (Meadows, 2001).

Richardson's ground squirrels (*Urocitellus richardsonii*) defend burrow clusters within their colonies (Michener, 1979a), so individuals that inhabit central territories are likely recipients of the benefits predicted by the selfish herd theory. Squirrels in central locations should experience lower predation risk when compared to individuals in edge locations. Central individuals, however, may experience a trade-off between increased competition for resources and decreased predation risk. Conversely, squirrels with territories on the edge of the colony may experience higher predation pressure, but perhaps lower intraspecific competition. Blanchard *et al.*, (2008) found that impalas in central locations experience more competitive interactions with conspecifics than peripheral individuals. However, Meadows (2001) found that damselfish in central territories had greater body sizes and growth rates than edge individuals, potentially due to the lower costs of territory defence, as central fish defended smaller territories and lost less food to intruders.

Richardson's ground squirrels are a moderately social, semi-fossorial, sciurid (Michener & Koepl, 1985). Previously described in the genus *Spermophilus*, Richardson's were re-described in the genus *Urocitellus* (Helgen *et al.*, 2009). Richardson's ground squirrels are native to North America, residing in short grass prairies ranging from southern Alberta to southern Manitoba in Canada, and then southward into Montana, North Dakota, north-eastern South Dakota, western Minnesota, into northern Iowa in the United States of America (Hoffman *et al.*, 1993). Richardson's ground squirrels are prey to many species including coyotes, domestic dogs (*Canis lupis familiaris*), red foxes (*Vulpes vulpes*), domestic cats (*Felis catus*), American badgers (*Taxidea taxus*), long-tailed weasels (*Mustela frenata*), mink (*M. vison*), striped skunks (*Mephitis mephitis*), red-tailed hawks (*Buteo jamaicensis*), ferruginous hawks (*B. regalis*), Swainson's hawk (*B. swainsoni*), northern harriers (*Circus cyaneus*), prairie falcons (*Falco mexicanus*), bald eagles (*Haliaeetus leucocephalus*), snowy owls (*Nyctea scandiaca*), great horned owls (*Bubo virginianus*), and black-billed magpies (*Pica pica*) (Michener & Koepl, 1985). While ground squirrels cause economic damage in some agricultural systems (Johnson-Nistler *et al.*, 2005; Whisson *et al.*, 1999), Richardson's ground squirrels have been reported to reduce the abundance of invasive plant species, increase the abundance of cattle (*Bos taurus*) forage species as well as plant species diversity, and influence available nitrogen in the soil of cattle pastures in aspen parkland pastures (Newediuk *et al.*, 2016).

Predation risk varies not only spatially, but also temporally as predators respond to changes in the environment (Lucas *et al.*, 1996; Werner, 1986). The number of Richardson's ground squirrels present in a colony varies cyclically throughout the year (Michener & Koepl, 1985). Population density peaks during juvenile emergence from nest burrows, which occurs in May to June (Michener, 1979b; Michener, 1974). Predation risk should increase during juvenile

emergence, as predators take advantage of the appearance of large number of juveniles (Schmutz *et al.*, 1980). The number of squirrels active above ground then wanes as juveniles are depredated, and adults enter into hibernation (Michener & Koepl, 1985). Predation pressure should subsequently decrease as the number of individuals active above ground decreases over the rest of the summer.

Richardson's ground squirrels have a complex alarm communication system, producing functionally referential calls specific to both terrestrial and avian threats (Davis, 1984). They produce whistle calls, which are longer in duration, repeated, and relatively constant frequency calls, in response to threats on the ground (Davis, 1984). Airborne predators however, elicit chirps, which are shorter, more frequency modulated, and unrepeated calls (Davis, 1984).

Richardson's ground squirrels can extract information regarding caller location and predator movement from alarm calls (Sloan *et al.*, 2005; Thompson & Hare, 2010). Individual squirrels discriminate among callers as individuals (Hare, 1998), using that ability as adults to enumerate the number of callers in a multi-caller bout (Sloan & Hare, 2008), and to weight their response according to the past reliability of individual alarm signallers (Hare & Atkins, 2001). Because alarm calls are emitted in the presence of presumptive predators, the incidence of those calls provides an estimate of the number of encounters with threatening stimuli perceived by the ground squirrels.

Predation influences ecosystems through the removal of prey, and there is little doubt as to its influence on the evolution of prey species morphology, physiology and behaviour (Krebs *et al.*, 2001; Lima, 1998). While research historically has focused on the effects of mortality caused by predation, more recent work has focused on the indirect effects of predators on prey populations, revealing potent effects like those imposed by direct effects of predation (Schmitz *et*

*al.*, 1997; Krebs *et al.*, 1995). The ecology of fear hypothesis suggests that predator presence, even in the absence of fatal predation events, can affect prey demography (Lima, 1998). A meta-analysis of experiments involving predator presence suggests that predator presence alone has effects at least as great as direct, predator-induced mortality does on prey populations (Preisser *et al.*, 2005).

The perception of predation risk can affect prey morphology (Vamosi & Schluter, 2004) and behaviour, involving both changes in vigilance (Childress & Lung, 2003; Bachman, 1993) and habitat use (Karels & Boonstra, 1999). Experiments where predators are prevented from killing prey have shown that the presence of predators can influence prey demography (Peckarsky *et al.*, 1993; Nelson *et al.*, 2004; Sheriff *et al.*, 2009). Indeed, perceived predation risk (in the absence of actual predators) has been demonstrated to reduce the number of offspring produced by forty percent in song sparrows (*Melospiza melodia*; Zanette *et al.*, 2011). Furthermore, the non-lethal effects of a predator on prey can carry over across seasons (Elliott *et al.*, 2016). In fruit flies (*Drosophila melanogaster*), exposure to predation pressure in the non-breeding season can carry-over to affect offspring production in the breeding season (Elliott *et al.*, 2016).

Predator presence affects prey populations through the costs associated with the physiological and behavioural responses of prey to the perceived threat of predators despite their continued survival (Clinchy *et al.*, 2013; Sheriff *et al.*, 2009). One proposed mechanism through which predator presence affects prey is through the activation of the stress axis (Sheriff *et al.*, 2009). ‘Stress’ can be defined as set of neural and endocrine responses that occur in response to departure from homeostasis, which in the context of predation is precipitated by the perception of threat (Sapolsky, 1987). One of the most commonly discussed components of the stress

response among vertebrates is the hypothalamic-pituitary-adrenal (HPA) axis, which is ultimately responsible for the secretion of glucocorticoids (Sheriff *et al.*, 2009; Sapolsky, 1987). The release of glucocorticoids facilitates processes which aid individuals in either escaping or responding to dangerous situations, while suppressing non-essential processes (Wingfield *et al.*, 1998). Chronic activation of the stress axis, however, decreases individual fitness (Boonstra *et al.*, 1998), supporting the notion that stress responses provide a mechanism via which predators can negatively affect prey indirectly.

Mechanisms beyond chronic stress, however, may also contribute to the indirect effects of predators on their prey populations (Clinchy *et al.*, 2013). Predator presence affects the foraging habits of many species (Cowlshaw, 1997; Kotler, 1984). When predation pressure is high, prey may spend more time being vigilant (Creel & Christianson, 2008). In some instances, declines in birth rate associated with predator presence may be attributable to changes in foraging behaviour and subsequent decreases in body condition, rather than heightened stress levels (Creel *et al.*, 2009).

Further, predation pressure is not the only factor that may influence baseline glucocorticoid levels. Baseline levels vary seasonally in many species with breeding and mating status (Coe & Levine, 1995; Boonstra *et al.*, 2001; Nunes *et al.*, 2006; Chauke *et al.*, 2011). Body condition and resource availability also affect baseline, with lower food availability and body condition correlating with decreased baseline glucocorticoids (Kitaysky *et al.*, 1999). An individual's baseline glucocorticoid can also vary with age (Elliott *et al.*, 2014). Stable behavioural traits ("personality") can also affect baseline levels (Montiglio *et al.*, 2012; Clary *et al.*, 2014).

Glucocorticoid levels of mothers can influence offspring through pre- and post-natal programming (Love *et al.*, 2013). Natal programming can affect offspring sex (Cameron *et al.*, 2008, Ryan *et al.*, 2012), growth rate (Dantzer *et al.*, 2013), and neuromotor development (Schneider & Coe, 1993). Past work on Richardson's ground squirrels has demonstrated that a portion of the stress response is heritable (Bairos-Novak *et al.*, 2017). Pre-natal programming may cause highly stressed mothers to produce offspring that are more physiologically responsive to external stressors (Kanitz *et al.*, 2003; Love *et al.*, 2013). However, post-natal care can attenuate the effects of a highly stressed mother on offspring (Bókony *et al.*, 2009; McGhee & Bell, 2014).

In polygynous species, where low quality males experience lower reproductive success, it is adaptive for high quality parents to produce high quality sons. High quality sons should have higher potential fitness payoff than high-quality daughters, as high-quality females should experience smaller benefits when compared to low-quality females. Conversely, parents in poor condition should produce more daughters (if low quality daughters are still likely to reproduce) over poor quality sons that are unlikely to successfully reproduce. This is known as the Trivers-Willard hypothesis (TWH; Trivers & Willard, 1973). The HPA axis, and its associated hormones, is a potential system through which mothers may manipulate the sex ratio of their litters based on their body condition (Wingfield & Sapolsky, 2003).

In Richardson's ground squirrels, squirrels with higher FGM concentration during gestation produced more male-biased litters (Ryan *et al.*, 2012, Ryan *et al.*, 2014). Females that produced proportionately more males had smaller litter sizes (Ryan *et al.*, 2012, Ryan *et al.*, 2014). In polygynous species, the TWH predicts that females in poor condition should produce more females. However, in a species where females are philopatric, but males are not, producing

a small litter size with a high proportion of males may benefit poor quality females as fewer offspring will remain in the natal area to compete for resources with the mother in the future (Ryan *et al.*, 2012; Ryan *et al.*, 2014; Myers, 1978). Additionally, females remaining within the colony without a high-quality mother and kin should be pushed to the edge of colony, or experience greater competition for space and resources if they remain in the centre (Ryan *et al.*, 2014).

Gardiner (2010) reported that faecal cortisol levels of Richardson's ground squirrels were higher in central areas immediately before and after parturition, though this trend reversed as the summer continued. This shift in the relationship between location and stress could be caused by decreases in conspecific competition as squirrels enter hibernation, and an increased risk of predation in edge areas due to the decreasing number of vigilant individuals (Gardiner, 2010; Kildaw, 1995). In that the existence of more-or-less stable selfish herds in territorial species would promote an uneven landscape of fear within groups, I predict that Richardson's ground squirrels living in different areas of a colony should experience different encounter rates with predators, and that predator encounter rate should be positively correlated with stress levels, and negatively correlated with survivorship and reproductive success. These predictions can be reduced to several testable hypotheses:

H<sub>01</sub>: Stress levels of Richardson's ground squirrels do not correlate with an individual's location within the colony, with central and peripheral individuals not differing in stress levels.

H<sub>a1</sub>: Location is predictive of Richardson's ground squirrel's stress, with central individuals having lower stress levels on average than individuals residing in peripheral areas.

H<sub>02</sub>: Location within a colony is not correlated with alarm calling rates of Richardson's ground squirrels, with central individuals not differing in alarm calling rates from peripheral individuals.

H<sub>a2</sub>: Location within a colony is correlated with alarm calling rates, with central areas having lower alarm call incidence than peripheral areas.

H<sub>03</sub>: Territory location within a colony is not correlated with fitness of Richardson's ground squirrels, with central and peripheral individuals not differing in fitness.

H<sub>a3</sub>: Territory location within a colony is correlated with fitness of Richardson's ground squirrels, with central individuals enjoying greater fitness than those on the periphery.

I examined how predation pressure, as measured by rates of alarm calling, and the activation of the HPA axis, as measured by concentrations of faecal glucocorticoid metabolites (Hare *et al.*, 2014), vary between central versus peripheral areas of a colony across different periods of the squirrel's active season. Spatial and temporal variation in predation risk should lead to differential activation of the HPA axis, and ultimately lead to different fitness outcomes for squirrels. To assess this, I also determined how location, faecal glucocorticoid metabolite concentrations, and the incidence of anti-predator calling relate to variation in survivorship and reproductive success of individuals. These results provide insight into the factors promoting the evolution and maintenance of sociality, as well as quantifying indirect effects of predator presence on this social, group-living species.



## Methods

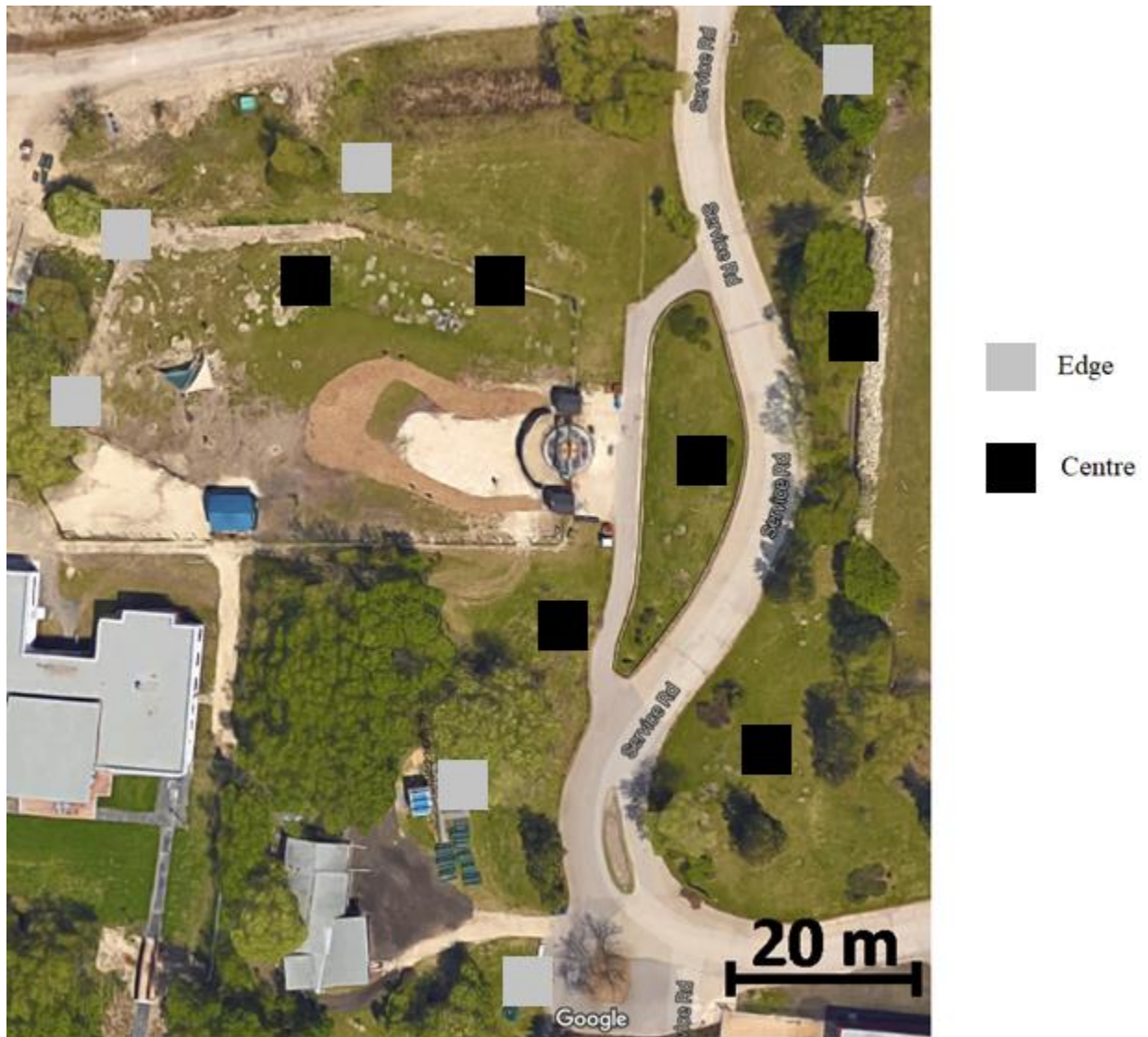
I gathered data from 25 May through 17 July 2015 to estimate predation pressure, stress, and fitness among individuals occupying centre versus edge areas within a populous Richardson's ground squirrel colony at Winnipeg, Manitoba's Assiniboine Park Zoo (49.86° N, 97.23° W). Work was performed under Manitoba Conservation Wildlife scientific permit WB14952, and protocol number F12-014 approved by the University of Manitoba Fort Garry Campus Animal Care Committee. Squirrels in the study area were each ear-tagged with a uniquely numbered metal ear-tag (National Band and Tag Co., Monel #1, Newport, KY, USA) pierced through the right pinna at first capture for year-to-year identification of individuals. During the field season squirrels were given unique dye marks (Clairol Hydrience. No. 52, Pearl Black, Stamford, CT, USA) on their dorsal pelage for rapid visual identification. Squirrels in the study area have been marked in this manner since 2003. Squirrels were captured using Tomahawk live traps (Model 201; Tomhawk Live Trap Co., Hazelhurst, WI, USA) baited with No Name® peanut butter (Loblaw Companies Ltd., Brampton, ON, Canada) between 09:00 and 17:30 hrs CDT. Juvenile squirrels were trapped as they emerged from their natal burrows to determine their mother, allowing for data to be collected on matriline year-to-year (Michener 1985).

The study site is comprised of maintained grassy knolls that is bordered by both tree-lines and additional parkland. Walking paths for zoo patrons divide the Richardson's ground squirrel colony into smaller neighbourhoods. The site is also divided by several types of fences, but Richardson's ground squirrels could cross all fence types. Some of the fenced subdivisions housed zoo animals, such as caribou (*Rangifer tarandus*), kangaroos (*Macropus rufus*), emus (*Dromaius novaehollandiae*), and pelicans (*Pelecanus erythrorhynchos*). Additionally, the site contained other man-made structures such as signs, an amusement ride, and buildings including a

zoo classroom, staff building, and a seasonally-enclosed butterfly garden. Potential ground squirrel predators observed at the site included Cooper's hawks (*Accipiter cooperii*), hawks (*Buteo* spp.), common ravens (*Corvus corax*), American crow (*Corvus brachyrhynchos*), merlin (*Falco columbarius*), red foxes (*Vulpes vulpes*), dogs (*Canis lupus familiaris*), American mink (*Neovison vison*), and fisher (*Pekania pennanti*).

To estimate predation pressure, I placed six SM3 Song Meter™ (Wildlife Acoustics Inc., Maynard, MA, USA) recorders in edge locations of the colony, and six in central locations of the colony (Fig. 1). Whether a recorder was categorised as a “centre” or “edge” was determined by the presence of active ground squirrel burrow systems (I considered burrow systems active if excavated dirt was present and debris was absent from the aperture of the burrow, or if I observed ground squirrels using the burrow) at the time of recorder placement. Quadrants were established in a 360° field around each presumptive recorder location based on the cardinal compass directions. If a recorder had active burrows within 10 meters in each quadrant, it was classified as “centre”, while recorders having active burrows in three or less quadrants were classified as “edge” areas. Recorders were mounted at 1.3 m above ground level using a combination of plastic zip ties and screws to existing structures (such as fences and buildings), or to metal fence posts pounded into the ground when no suitable pre-existing structures were available. The recorders were set to record from sunrise to sunset to capture instances of alarm calling (16-bit PCM; sampling rate = 32000 Hz) from 25 May 2015 until 17 July 2015, which roughly coincided with the onset of juvenile emergence through to the immergence of the last yearling and adult individuals into hibernation. Once a week, each recorder had its batteries replaced and audio recordings from its memory cards (Kingston® 32GB SDHC cards; Kingston

**Figure 1.** Satellite image (imagery ©2018 Google) of study site at Assiniboine Park Zoo marked with recorder locations.



Technology, Fountain Valley, California, U.S.A) transferred to a hard drive (Seagate™ Backup Plus; Seagate Technology PLC, Cupertino, California, U.S.A).

To assign squirrels to a recorder, I used either the nearest recorder to their nest burrow entrance (burrows from which juveniles were first seen to emerge, which diagnostically have a tighter aperture and more vertical entrance when compared to the wider, more gradually sloping

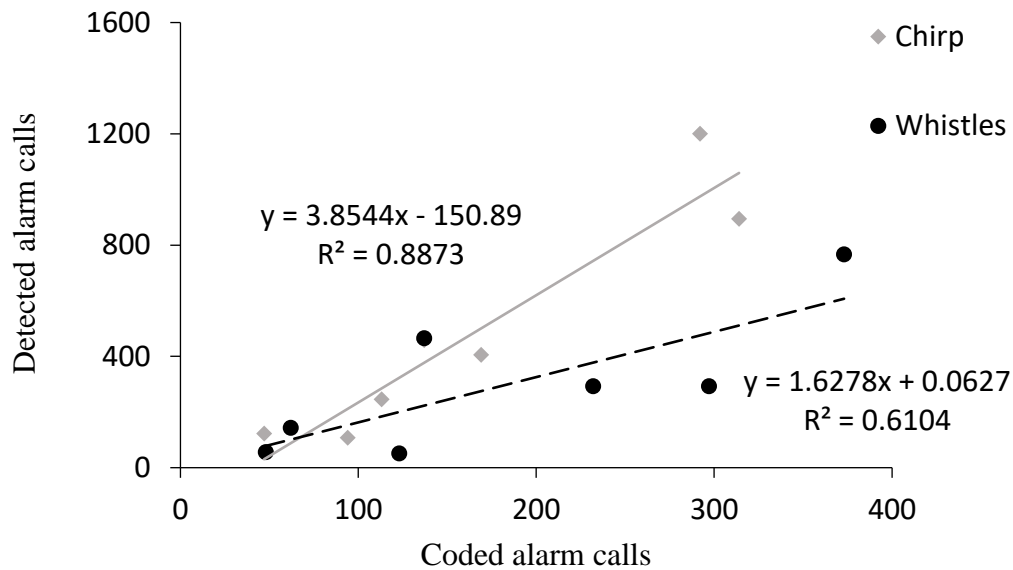
entrances into the larger escape burrow system), or from the centre of their defended territory. Territories were estimated based on behavioural observations of aggressive interactions between squirrels performed by Angela Freeman, which were being made in the course of her concurrent Ph.D. research (Freeman, 2016; Freeman *et al.*, 2018) on the same Richardson's ground squirrel population.

To efficiently code and quantify Richardson's ground squirrel alarm calls from the 9540 hours of audio recordings obtained, I produced recognizers to automatically detect alarm calls in my recordings in Song Scope<sup>TM</sup> (Wildlife Acoustics Inc., Maynard, MA, USA). Recognizers are produced by providing the program with previously recorded call exemplars, which then identify common features among the call exemplars. I used Richardson's ground squirrel calls recorded in the context of natural predator encounters in 2007 and 2008 by Dr. J. Hare at Assiniboine Park Zoo, as well as calls recorded in 2015 by the SM3 Song Meters as representative training exemplars. I created separate recognizers for chirp and whistle calls. Whistles have a longer duration, are repeated, and are relatively constant frequency calls that are produced in response to threats on the ground (Davis, 1984). Chirps are calls produced in response to aerial threats that are shorter, more frequency modulated, and unrepeated (Davis, 1984). Ultimately, I designed the whistle recognizer to detect whistle syllables, and not repeated whistle bouts, as I could not produce a recognizer that could reliably recognize the variation present in the repeated syllables of whistle bouts.

To test the veracity of the recognizers, I compared the number of calls identified by a human coder to the number of calls identified by the recognizer. The correlation between the chirps detected by a human coder and the chirps identified by the automated recognizer had an

$R^2$  of 0.89 ( $F_{1,5} = 7.93$ ,  $p = 0.038$ , Fig. 2), while the correlation between whistle calls detected by the whistle recognizer and a human coder had an  $R^2 = 0.61$  ( $F_{1,5} = 39.36$ ,  $p = 0.002$ , Fig. 2). I

**Figure 2.** Richardson’s ground squirrel alarm calls identified by automated recognizers versus calls identified by a human coder.



determined the active space of the recorders by playing back Richardson’s ground squirrel alarm calls at 80 dB<sub>A</sub> at 1 m from source at 10 m intervals from a SM3 recorder using a Genexxa Pro LX5 loudspeaker (InterTAN Canada Ltd., Barrie, ON, Canada), and then ran the recognizers to detect whistle and chirp calls. The recognizers reliably detected alarm calls played back at the 20 m interval, but not at the 30 m interval for both chirps and whistles. These automated recognizers thus provide us with an estimate of the number of alarm calls produced by squirrels no further than 30 m from each recorder, and thus allow a meaningful comparison of the relative incidence of the two call types in centre versus edge areas. To account for increases in the number of calls caused by larger numbers of squirrels calling to the same presumptive predators in central areas as compared to edge areas, I examined the number of calls produced per squirrel per day as my index of local predation pressure.

Faecal samples were collected from live-trapped squirrels, allowing us to assign them to the squirrel that produced them. When initially placing a trap, or resetting it, I cleared any faecal matter present where the trap was to be set to prevent accidentally assigning faeces to the wrong squirrel. I collected only fresh-looking faeces (fresh faeces have a slight sheen) from underneath the trap that were not contaminated by urine (Mateo and Cavigelli, 2005). When checking traps, I determined a squirrel's identity either through their unique dye-mark or ear tag. Scats were collected at least four days after the previous sample, as FGM concentration peaks after 3-5 days after a stressful event (Hare *et al.* 2014). Squirrels were targeted for trapping based on the location of the nearest recorder, rotating through recorder locations. Order of locations was determined haphazardly at the beginning of the field season based on ease of trapping different areas concurrently. Trapped squirrels were weighed once daily with a Pesola spring scale (Pesola AG, Chaltenbondenstrasse 4A, Schindellegi, Switzerland) to the nearest 5 g, and any squirrels needing a dye mark or ear tag were marked or tagged.

I estimated physiological stress using radioimmunoassays (RIA) to quantify faecal glucocorticoid metabolite (FGM) concentrations using methods similar to those described by Hare *et al.* (2014) for Richardson's ground squirrels, whose methods were based on those published by Mateo and Cavigelli (2005) for Belding's ground squirrels. After I weighed faecal samples, I dried them overnight at 60 °C (Fisher ISOTEMP<sup>®</sup> Oven 200 Series Model 230F, Fisher Scientific International, Inc., Hampton, NH, USA). I homogenized samples using a mortar and pestle, after which I added 0.75 ml of 95% ethanol (Commercial Alcohols, Toronto, ON, Canada) to 0.2 g of dried faecal matter and then vigorously mixed the sample. I centrifuged (Fisherbrand<sup>™</sup> ACCUSPIN<sup>™</sup> Micro 17R microcentrifuge, Fisher Scientific International, Inc., Hampton, NH, USA) the samples for 20 minutes at 2500 G at 4 °C. I pipetted off the supernatant

into a new microcentrifuge tube, the weight of which I had recorded prior to loading. I then added 0.75 ml of ethanol to the original faecal sample, centrifuged as previously, and added the new supernatant to the supernatant from the original extraction. This departure from previous methods was to accommodate microcentrifuge vials which could not hold the faecal sample in addition to 1.5 ml of ethanol. While I used larger microcentrifuge vials later, I continued to use the same extraction method for consistency.

I dried down supernatants using a sample concentrator (Thermo Scientific™ Savant™ ISS110 SpeedVac™ Concentration, Thermo Fisher Scientific, Waltham, MA, USA) on the low setting for an hour. The resulting sample was re-suspended in a buffer solution (0.1 M phosphate buffer, 0.9% NaCl (w/v) and 0.5% bovine serum albumin (w/v)) before measurement through RIA. I measured samples in duplicate, and calculated the average of the two values, while I ran cold cortisol standards (Steraloids, Newport, RI, USA) in triplicate.

To begin the assay, I combined 100 µl of the sample (or 100 µl of known concentration cortisol standard) with 100 µl of cortisol specific antibody (1:16000 dilution; Fitzgerald Industries International, Acton, MA, USA), and 100 µl of cortisol labelled with tritium (approximately 5000 dpm; PerkinElmer, Inc., MA, USA). Fitzgerald Industries reports the cross reactivity of the antibody to be 100% for cortisol, 36.0% for prednisolone, 5.7% for 11-desoxycortisol, 3.3% corticosterone, and < 0.7% for cortisone. Next, I incubated assay tubes at room temperature for 1 hr, and then overnight at 4 °C. To stop the reaction, I added 100 µl of dextran-coated charcoal to all assay tubes, which I then vigorously mixed and incubated at 4 °C for 15 minutes.

After stopping the assay, I centrifuged the samples for 30 minutes at 2500 G at 4 °C in a Heraeus™ Multifuge™ X3R centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) to

separate ligands bound by the antibody from unbound ligands that were absorbed by the charcoal. I decanted the supernatant of each vial into a scintillation vial and added 4 ml of scintillation fluid (Ultima Gold™ AB, PerkinElmer, Inc., MA, USA). I used a liquid scintillation counter (Tri-Carb® 3110TR Liquid Scintillation Analyzer, PerkinElmer, Inc., MA, USA) to measure the radioactivity of the samples. I calculated FGM concentration by interpolating from a standard curve of the known concentrations of the cold cortisol samples, and then correcting for changes in dilution and mass of faecal sample. If not otherwise stated, chemicals were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

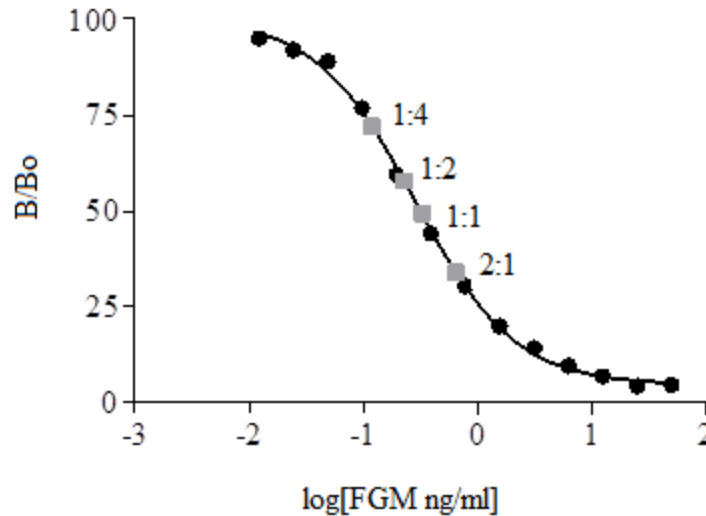
Inter-assay variation can be calculated as the average coefficient of variation (CV) of a pooled sample measured in different assays. Using 18 samples run in different assays, I calculated my inter-assay CV to be 14.6%. Intra-assay variation can be calculated as the CV among duplicates of samples. I calculated my intra-assay variation to be 10.9%. Previous work examining FGM in Richardson's ground squirrels reported inter-assay and intra-assay variations of 10.9 +/- 6% and 15.3% respectively (Ryan *et al.*, 2012; Clary *et al.*, 2014; Hare *et al.*, 2014). To assess parallelism, I ran concentrations and dilutions of a pooled sample (Fig. 3). Extraction efficiency was calculated to be 110%.

The survivorship of individuals living in central versus edge areas was determined by returning to the field site in spring 2016 and live-trapping all remaining individually-marked squirrels from 2015 occupying the site. Females are largely philopatric, so a female not being present typically represents mortality (Michener & Michener, 1997). As males typically disperse from natal areas, their absence is not indicative of mortality and as such I excluded them from my analyses (Michener & Michener, 1997). In November of 2016 I took measurements of other



geographical variables at the site. The variables I measured were distance to nearest tree (vegetation taller than head height that could constitute a suitable perch for avian predators),

**Figure 3.** Parallelism of serial dilutions of a pooled sample of Richardson’s ground squirrels’ faecal material.



human walking path, and edge (area of contiguous vegetative cover at least 0.5 m in height that might conceal approaching predators) from each recorder.

### Statistical Analysis

Detected chirps per squirrel per day were not normally distributed (Shapiro-Wilk,  $W = 0.737$ ,  $p < 0.001$ ), and variances between centre and edge were not equal (Levene’s,  $F_{1,573} = 10.503$ ,  $p = 0.001$ ). As such, I compared the aligned rank transformation (ART) of chirps per squirrel per day with a repeated measures analysis of variance (ANOVA; Zar 1991, Wobbrock *et al.*, 2011), as a Friedman’s test comparing only two categories does not account for the interaction between the measured variable and the repeated measures (Wobbrock *et al.*, 2011). I compared the ART of whistles per squirrel per day with a repeated measures ANOVA, as whistles per squirrel per day were not normally distributed (Shapiro-Wilk,  $W = 0.766$ ,  $p < 0.001$ ), although I failed to reject the assumption that variances were equal (Levene’s,  $F_{1,588} =$

1.022,  $p = 0.312$ ). Chirps per squirrel per day and whistles per squirrel per day among recorder locations were compared with a Kruskal-Wallis test, as neither were normal, and variances of chirps per squirrel per day and whistles per squirrel per day were not equal (Chirps, Levene's,  $F_{11,563} = 14.022$ ,  $p < 0.001$ ; Whistles, Levene's,  $F_{11,578} = 67.014$ ,  $p < 0.001$ ; Zar, 1991; Kruskal & Wallis, 1952). I examined the correlation between chirps and whistles per squirrel per day and the distance to the nearest tree, edge, walking path using linear regression, including an F-test to assess the significance of any departure of the slope of the best-fit line from zero (Zar, 1991).

Mean adult female FGM concentration was not normally distributed ( $W = 0.567$ ,  $p < 0.001$ ), but I failed to reject that variances were equal between centre and edge locations ( $F_{1,43} = 0.974$ ,  $p = 0.329$ ). The logarithmic ( $\log_{10}$ ) transformation of average adult female FGM concentrations were normally distributed (Shapiro-Wilk,  $w = 0.966$ ,  $p = 0.213$ ), and I failed to reject that variances of centre and edge locations were equal ( $p = 0.213$ ; Levene's,  $F_{1,43} = 1.855$ ,  $p = 0.180$ ). As such, I used a Welch's t-test to compare mean FGM concentration for centre versus edge adult females (Zar, 1991; Welch, 1947). I failed to reject that the variances among recorder locations were equal for the  $\log_{10}$  transformation of mean adult female FGM concentrations (Levene's test,  $F_{11,43} = 1.9$ ,  $p = 0.7607$ ). To compare mean adult female FGM concentrations among recorder locations I used an ANOVA (Zar, 1991).

The distribution of all FGM concentrations were not normal (Shapiro-Wilk,  $W = 0.344$ ,  $p < 0.001$ ), but I failed to reject that variances between centre and edge were equal (Levene's,  $F_{1,280} = 0.239$ ,  $p = 0.625$ ). I compared them using a repeated measures ANOVA after an ART. I selected 4-day blocks as the time frame for repeated measures as it was the shortest time period that allowed me to meet the requirement for a balanced designed. Longer blocks required that I

exclude samples from analysis to prevent individuals from having multiple samples within a block.

The  $\log_{10}$  transformation of average female FGM concentration before and after juvenile emergence were normally distributed (Shapiro-Wilk,  $W = 0.986$ ,  $p = 0.494$ ), but variances were not equal between those (Levene's,  $F_{1,81} = 4.131$ ,  $p = 0.045$ ). I used a Welch's t-test to compare average female FGM concentration before versus after juvenile emergence. A  $\log_{10}$  transformation of mean FGM concentration of adult females for May, June, and July did not result in normality (Shapiro-Wilkes,  $W = 0.944$ ,  $p < 0.001$ ), so I used a Kruskal-Wallis test to compare mean FGM of adult females for May, June, and July.

I used linear regression analyses followed by F-tests to examine the correlation between adult female mean FGM concentration and the distance to the nearest tree, edge, and walking path. Similarly, I used linear regression to examine the correlation between a squirrels' FGM concentration and the mean number of whistles per squirrel per day, chirps per squirrel per day, and the combined total of chirp and whistles calls per squirrel per day over the preceding three days. I compared FGM to the mean number of calls over the preceding three days as FGM concentration may be affected by the squirrels' experiences over the past three days (Bamberg *et al.*, 2001; Mateo and Cavigelli, 2005).

I compared overwinter survivorship of adult females in centre and edge areas with a Fisher's exact test (Zar, 1991; Fisher, 1922). To compare the number of female offspring surviving until 2016 in centre versus edge locations I used a Mann-Whitney U test as the data were not normal (Shapiro-Wilk,  $W = 0.642$ ,  $p < 0.001$ ; Levene's,  $F_{1,42} = 3.19$ ,  $p = 0.081$ ; Zar, 1991; Mann & Whitney, 1947). I also used a Fisher's exact test to compare the sex ratio of litters weaned from nest burrows in centre versus edge locations. To test for any possible correlations

between the sex ratio of litters and FGM concentration of mothers, the probability of adult female survival and mean whistles and chirps per squirrel per day of recorder locations, or survivorship and distance to nearest tree, edge, and walking path, I used linear regression and the F-test. I used a logistic regression to examine the correlation between adult female survivorship and their mean FGM concentration.

## Results

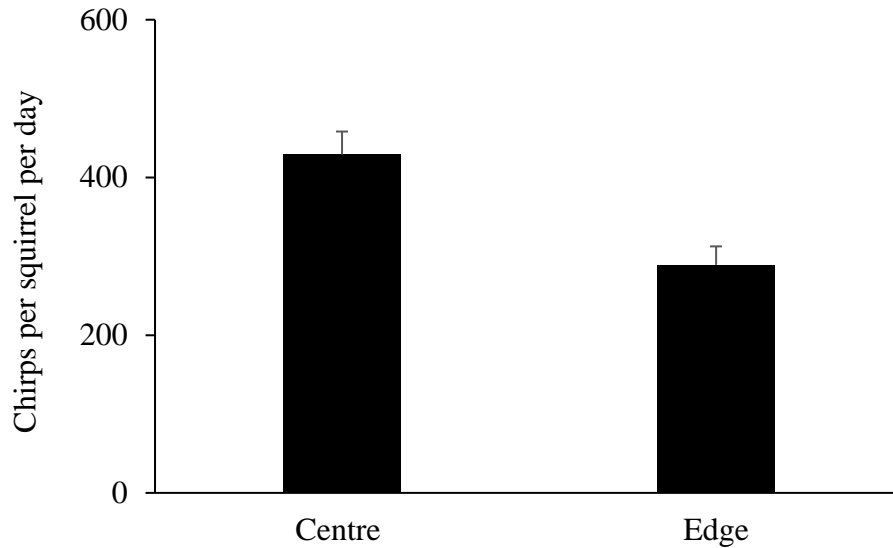
### Alarm Calls

Repeated measures ANOVA on the ART of chirps per squirrel per day detected a significant difference between centre and edge locations ( $F_{1,573} = 16.885$ ,  $p < 0.001$ ), with no significant interaction between date and chirps ( $F_{1,573} = 1.124$ ,  $p = 0.290$ ). Centre locations had more mean chirps per squirrel per day than edge locations (Fig. 4) from 30 May through 13 July 2015. Similarly, a repeated measures ANOVA on the ART of whistles per squirrel per day found a significant difference between centre and edge locations ( $F_{1,588} = 20.092$ ,  $p < 0.001$ ), though fewer mean whistles per squirrel per day were detected in centre than edge locations (Fig. 5). There was no significant interaction between date and whistles ( $F_{1,588} = 0.342$ ,  $p = 0.559$ ). Chirps per squirrel per day differed among recorder locations (Kruskal-Wallis,  $\chi^2_{11} = 384.71$ ,  $p < 0.001$ , Fig. 6), as did whistles per squirrel per day (Kruskal-Wallis,  $\chi^2_{11} = 410.55$ ,  $p < 0.001$ , Fig. 6). The average chirps per squirrel per day was positively correlated with a neighbourhood's distance to the nearest tree ( $R^2 = 0.348$ ,  $F_{1,10} = 6.06$ ,  $p = 0.034$ , Fig. 7a), but not with the distance to the nearest edge ( $R^2 = 0.004$ ,  $F_{1,10} = 0.040$ ,  $p = 0.846$ , Fig. 7b) or nearest walking path ( $R^2 = 0.026$ ,  $F_{1,9} = 0.238$ ,  $p = 0.637$  Fig. 7c). For whistles per squirrel per day, no correlations with a neighbourhood's distance to nearest tree ( $R^2 = 0.003$ ,  $F_{1,10} = 0.034$ ,  $p = 0.858$ , Fig. 8a), edge ( $R^2 = 0.001$ ,  $F_{1,10} = 0.006$ ,  $p = 0.938$ , Fig. 8b), or walking path ( $R^2 = 0.198$ ,  $F_{1,10} = 2.226$ ,  $p = 0.170$ , Fig. 8c) were evident.

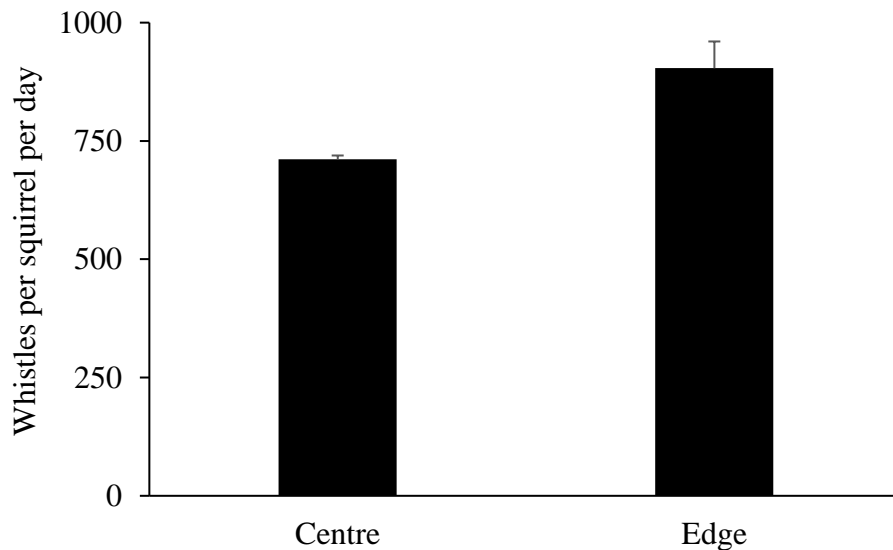
### Stress

Using a Welch's t-test I found no significant difference between the  $\log_{10}$  of average adult female FGM concentration for centre and edge locations ( $t_{43} = 0.181$ ,  $p = 0.857$ ). However, ANOVA revealed a significant difference in average adult female FGM concentration among

**Figure 4.** Mean + SE chirps per Richardson’s ground squirrel per day for centre versus edge locations of the colony at Assiniboine Park Zoo.

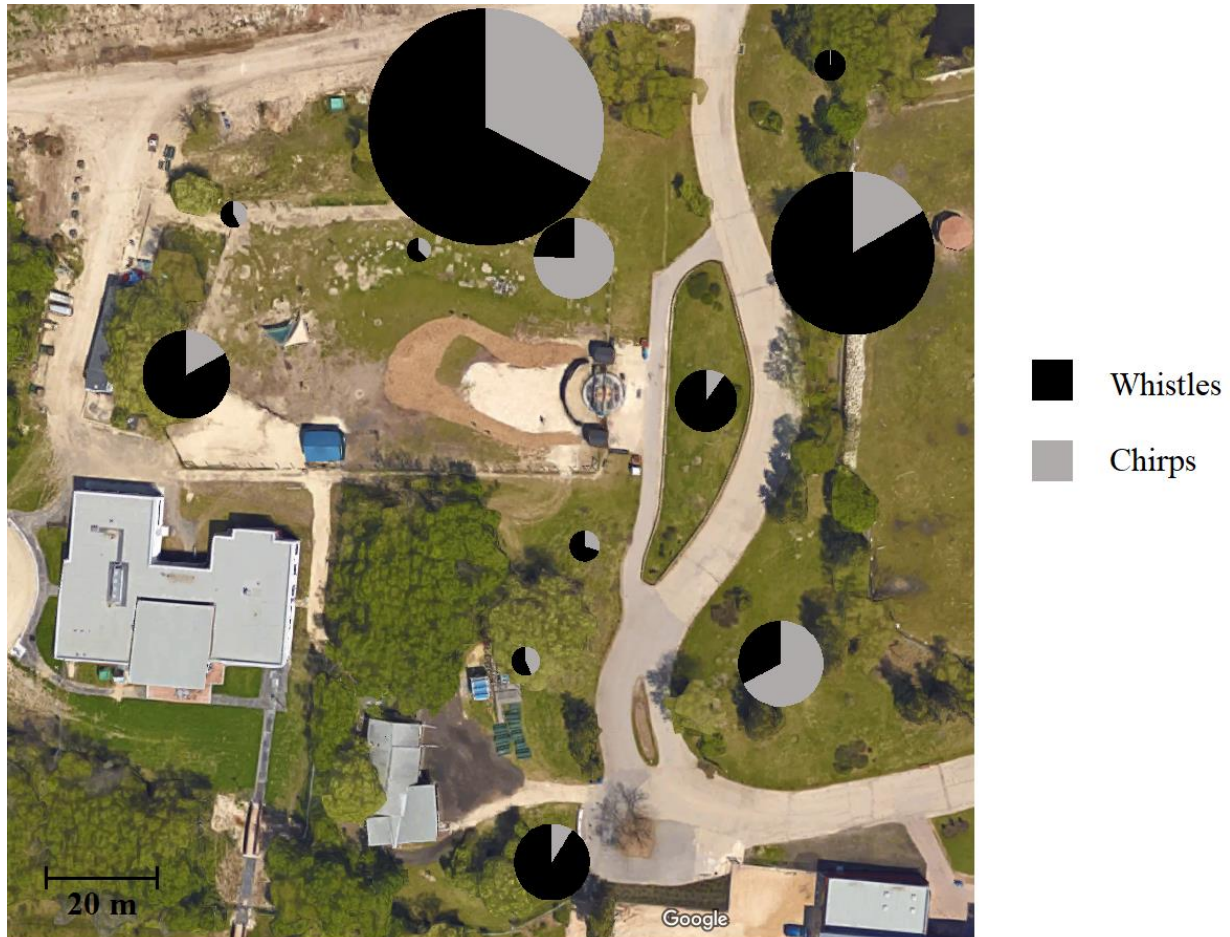


**Figure 5.** Mean + SE whistles per Richardson’s ground squirrel per day for centre versus edge locations of the colony at Assiniboine Park Zoo.



recorder locations ( $F_{11,31} = 2.647$ ,  $p = 0.015$ , Fig. 9). The distance of a squirrel’s neighbourhood to the nearest path was negatively correlated with their average FGM concentration ( $R^2 = 0.104$ ,  $F_{1,41} = 4.785$ ,  $p = 0.034$ , Fig. 10a), but not with distance to the nearest tree ( $R^2 = 0.001$ ,  $F_{1,41} = 0.034$ ,  $p = 0.854$ , Fig. 10b), or to the nearest edge ( $R^2 = 0.05$ ,  $F_{1,41} = 2.155$ ,  $p = 0.150$ , Fig. 10c).

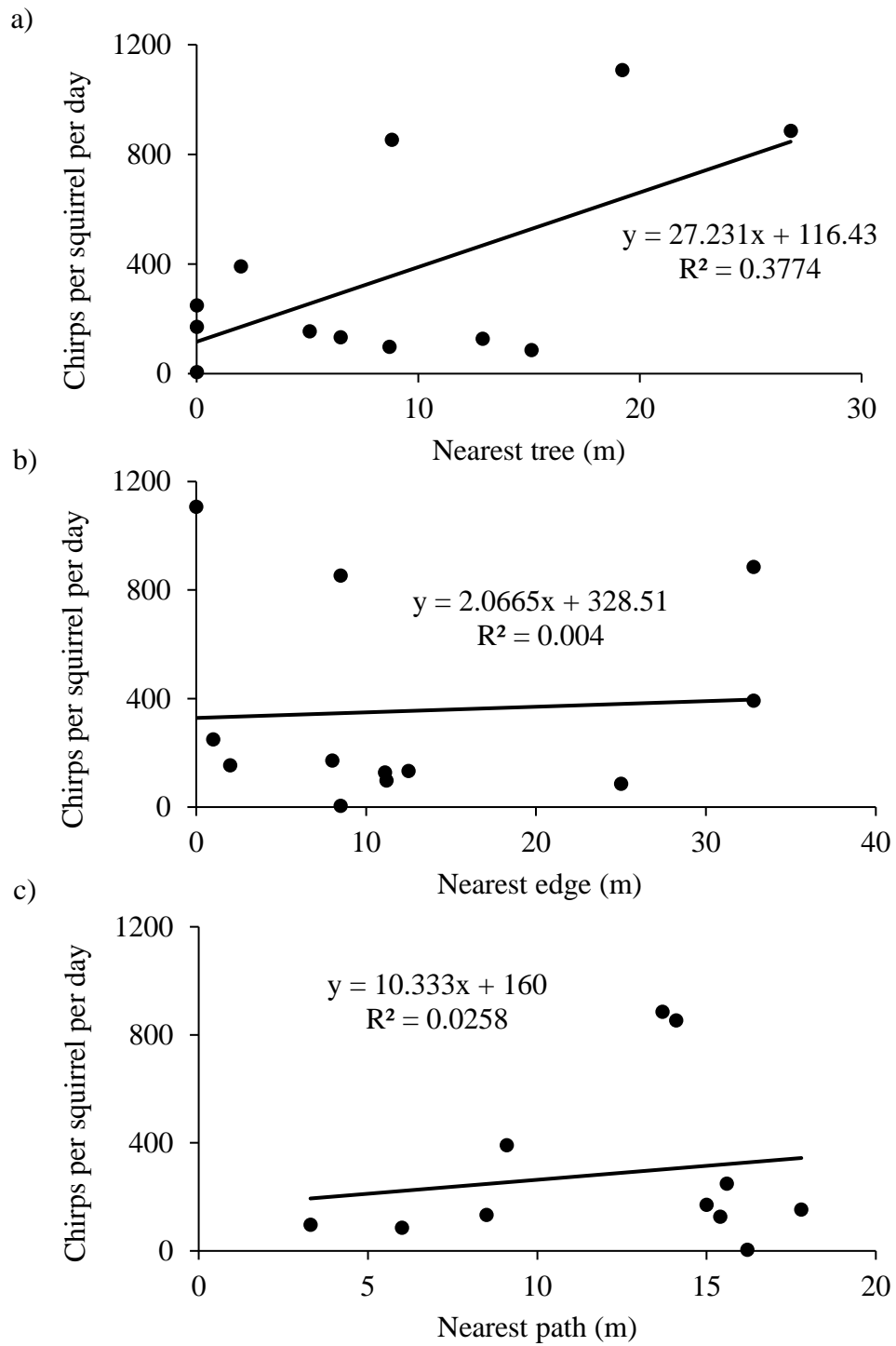
**Figure 6.** Mean number of calls per squirrel per day at each of 12 recorder locations for Richardson’s ground squirrels at Assiniboine Park Zoo. Diameter of pie graphs is proportional to the numbers of calls per squirrel per day (sum of whistle and chirp calls; Imagery ©2018 Google).



A squirrel’s FGM concentration was not correlated with the mean number of chirp calls per squirrel per day ( $R^2 < 0.001$ ,  $F_{1,266} = 0.003$ ,  $p = 0.987$ , Fig. 11a), the mean number of whistle calls ( $R^2 < 0.001$ ,  $F_{1,272} = 0.006$ ,  $p = 0.934$ , Fig. 11b), or the combined total of chirp and whistle calls ( $R^2 < 0.001$ ,  $F_{1,266} = 0.005$ ,  $p = 0.941$ , Fig. 11c) over the preceding three days.

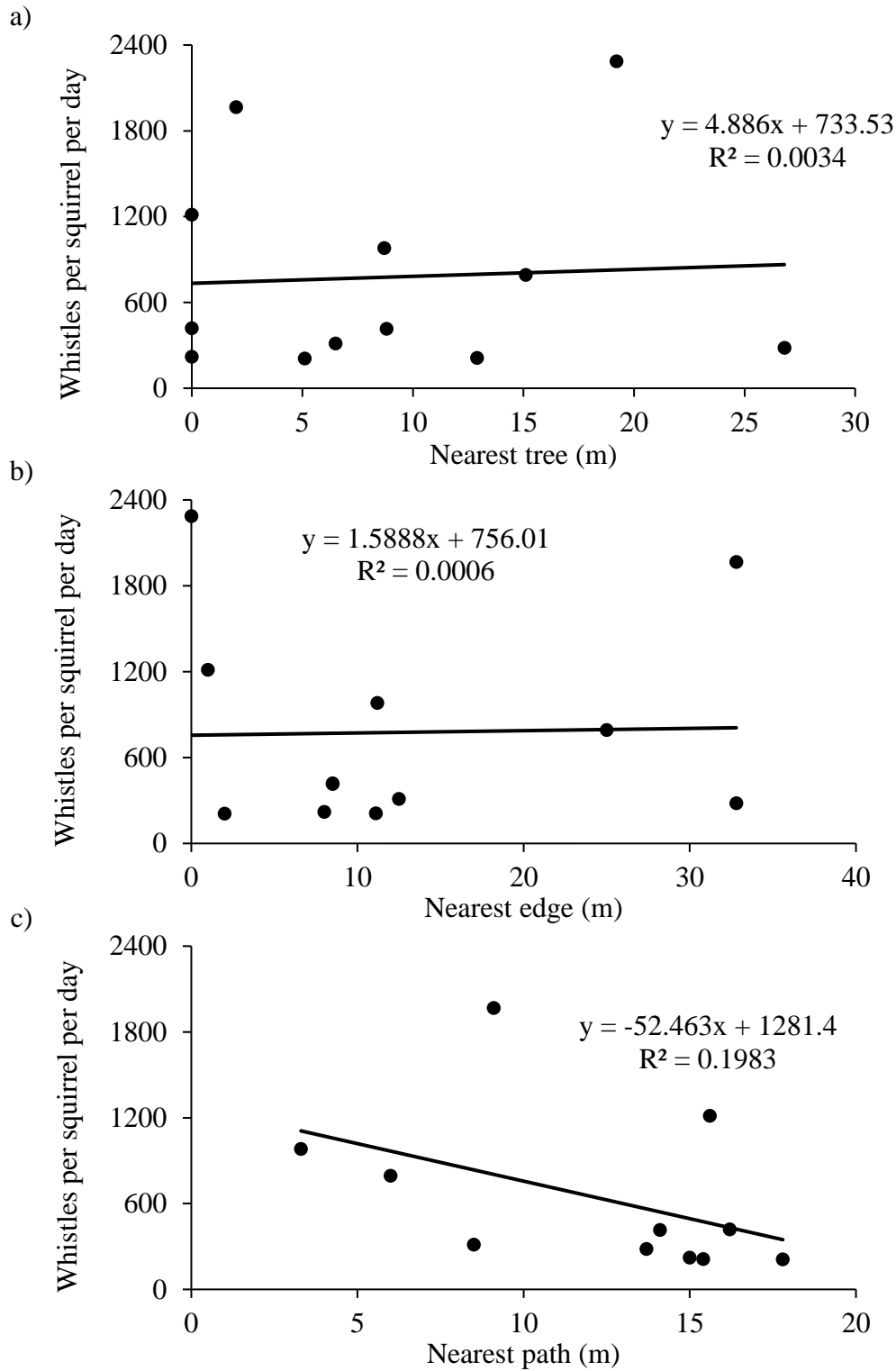
No significant difference was detected between the ART of FGM concentration of centre versus edge locations with a repeated measures ANOVA ( $F_{1,280} = 0.001$ ,  $p = 0.973$ ). Further, I

**Figure 7.** Mean number of chirps per Richardson’s ground squirrel per day versus the distance (m) to; a) the nearest tree (N = 12), b) edge (N = 12), and c) walking path (N = 12) of squirrel neighbourhoods at Assiniboine Park Zoo.





**Figure 8.** Mean number of whistles per Richardson’s ground squirrel per day versus the distance (m) to; a) the nearest tree (N = 12), b) edge (N = 12), and c) walking path (N = 11) of squirrel neighbourhoods at Assiniboine Park Zoo.

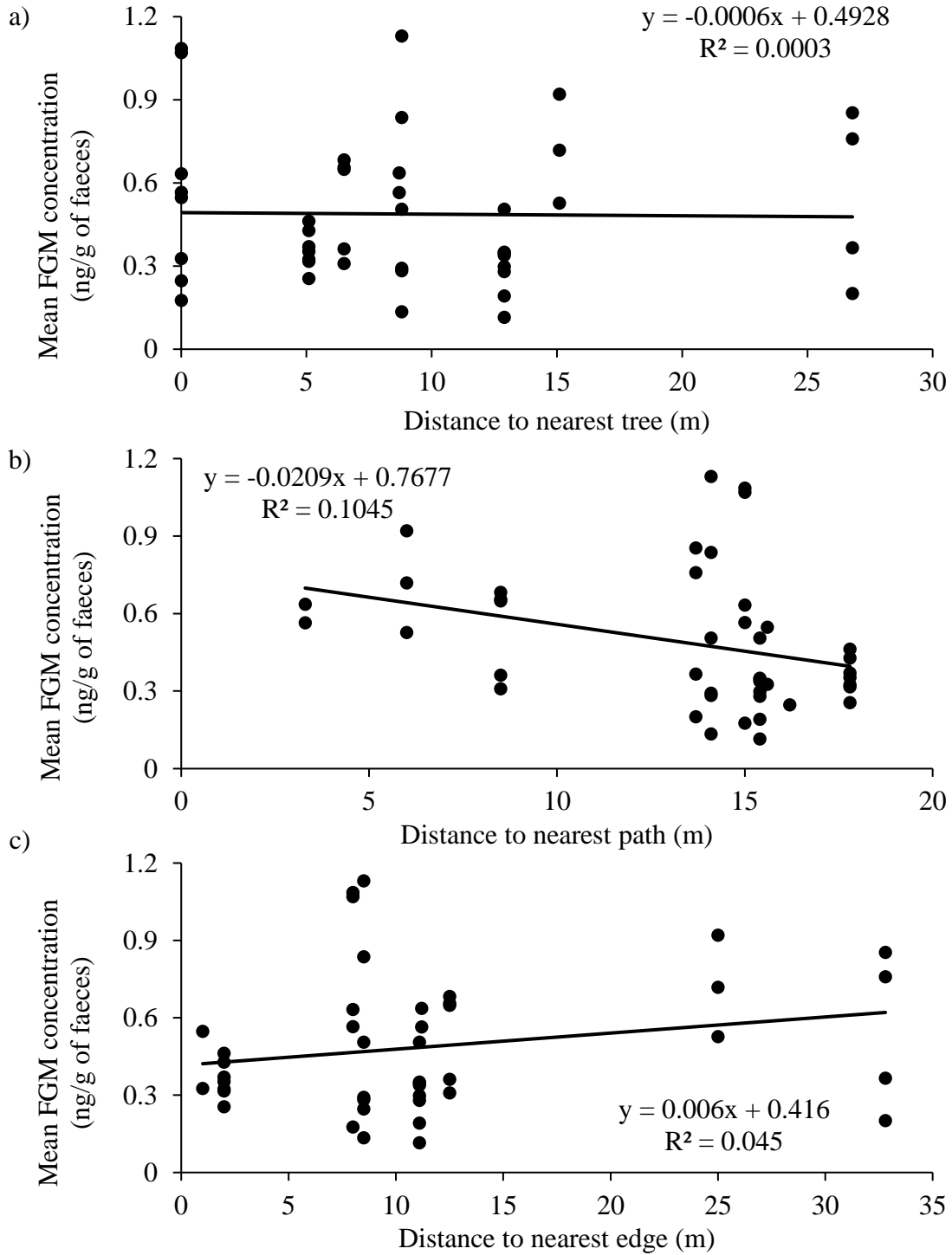


**Figure 9.** Mean faecal glucocorticoid metabolite concentration (ng/g of faeces) of adult, female Richardson’s ground squirrels among recorder locations at Assiniboine Park Zoo. Mean concentration represented by the size of dot with larger dots representing higher concentrations (Imagery ©2018 Google).

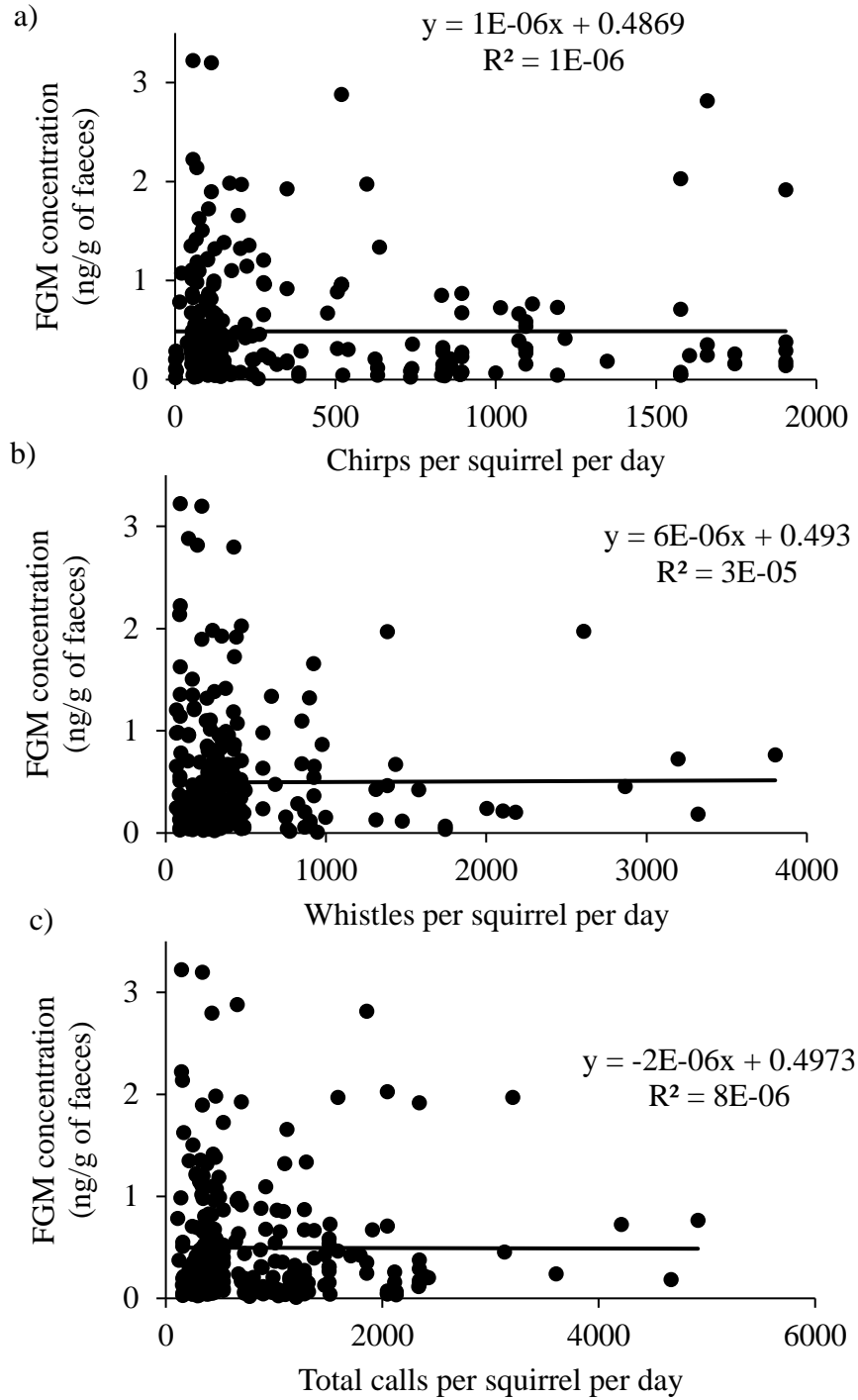


did not detect a significant relationship between FGM concentration and date ( $F_{1,280} = 0.010$ ,  $p = 0.921$ ). Finally, I detected no significant difference between the  $\log_{10}$  of average female FGM concentration during versus after juvenile emergence (Welch’s t-test,  $t_{81} = 0.394$ ,  $p = 0.695$ ), and

**Figure 10.** Mean faecal glucocorticoid metabolite concentration (ng/g of faeces) of adult, female Richardson’s ground squirrels and the distance to; a) the nearest tree (m; N = 42), b) walking path (m, N = 42), and c) edge (m, N = 42) of their neighbourhoods at Assiniboine Park Zoo.

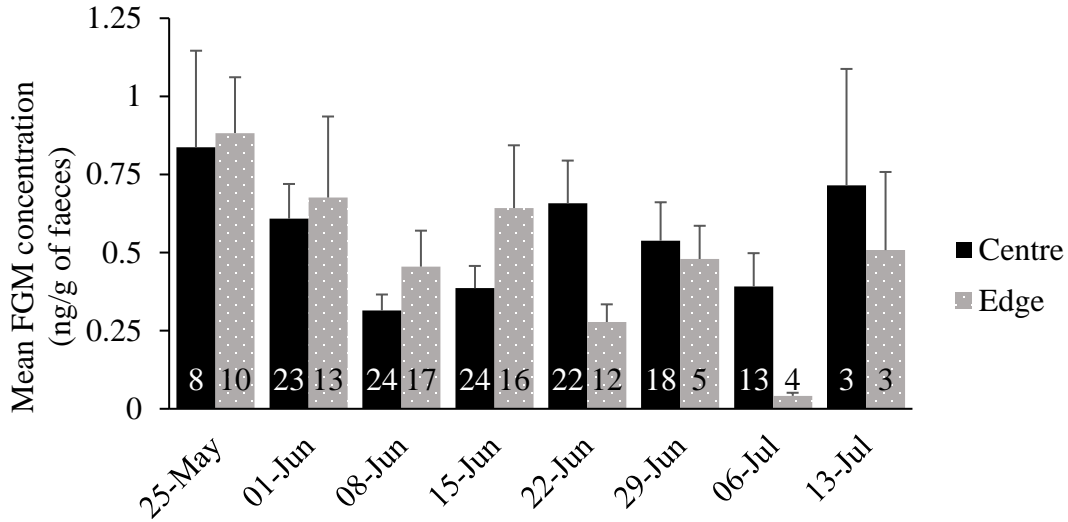


**Figure 11.** Faecal glucocorticoid metabolite concentration (ng/g of faeces) of adult, female Richardson’s ground squirrels and mean a) chirps per squirrel per day over the preceding three days (N = 268), b) whistles per squirrel per day over the preceding three days (N = 274), and c) total calls per squirrel per day over the preceding three days at Assiniboine Park Zoo (N = 268).



no significant difference among average squirrel FGM concentrations for May, June, and July (Kruskal-Wallis,  $\chi^2_2 = 5.286$ ,  $p = 0.07$ , Fig. 12).

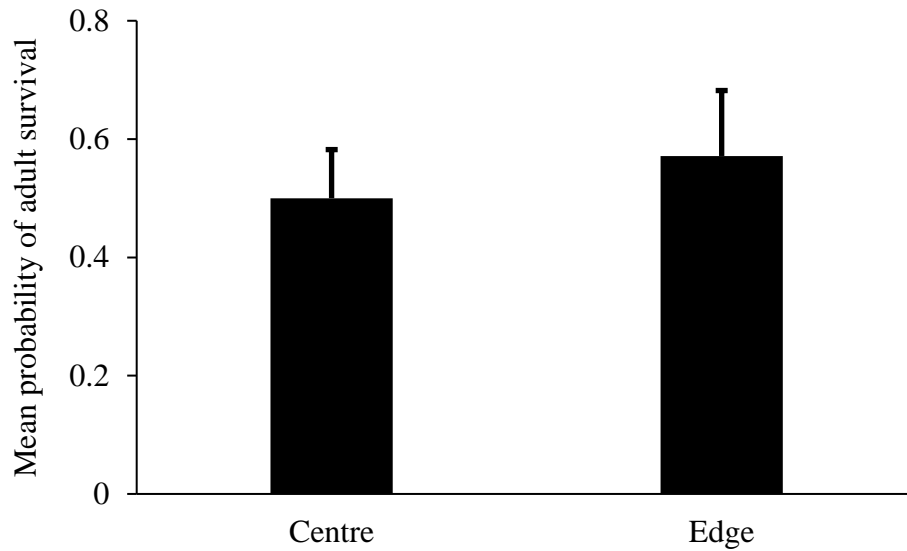
**Figure 12.** Mean + SE faecal glucocorticoid metabolite concentration (ng/g of faeces) of adult, female Richardson’s ground squirrels for centre and edge locations at Assiniboine Park Zoo from 25-May to 15-July. Number within bars reports the number of individuals scats for that period were derived from.



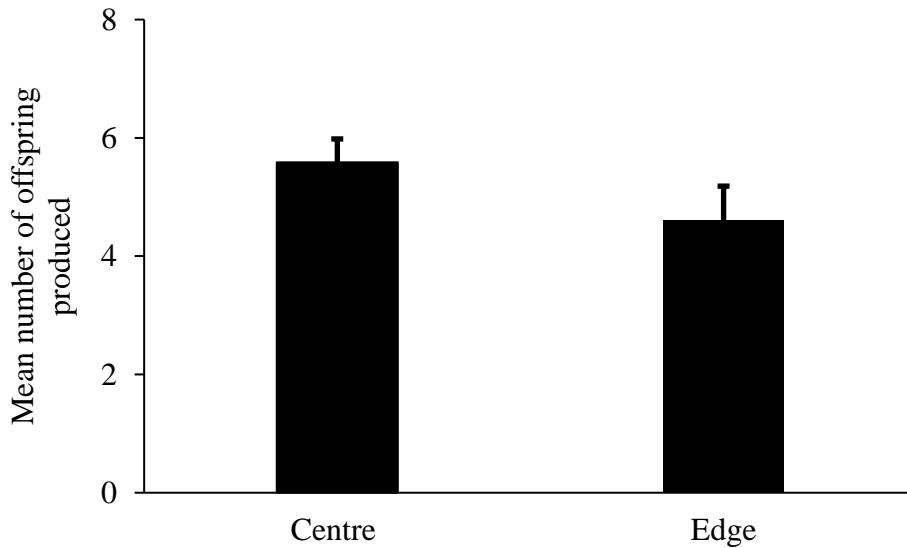
### Survivorship

I returned to the field site in the spring of 2016 to live trap Richardson’s ground squirrels to determine which squirrels from 2015 survived so I could explore whether survivorship and reproductive output were correlated with centre versus edge occupancy. No difference in overwinter survivorship of adult females was detected between centre and edge locations (Fisher’s exact test,  $p = 0.79$ , Fig. 13). While mean survivorship of female juveniles approached 80% for edge locations and was under 40% for centre locations, no statistically significant difference was detected ( $Z = 1.17$ ,  $p = 0.24$ , Fig. 14). I found no significant difference in the number of offspring produced in centre versus edge locations ( $W = 268$ ,  $p = 0.211$ , Fig. 15), or in the sex ratio of litters weaned from nest burrows in centre versus edge locations ( $Z = 0.72$ ,  $p = 0.47$ , Fig. 16). I found no correlation between survivorship of adult females and the mean

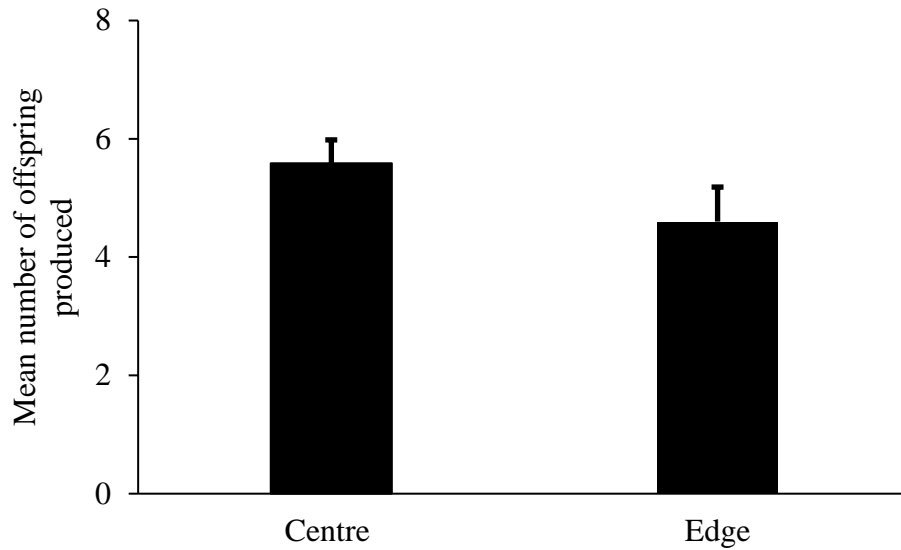
**Figure 13.** Mean + SE probability of adult, female Richardson's ground squirrel survival for centre and edge locations of the colony at Assiniboine Park Zoo ( $N_{\text{centre}} = 38$ ,  $N_{\text{edge}} = 21$ ).



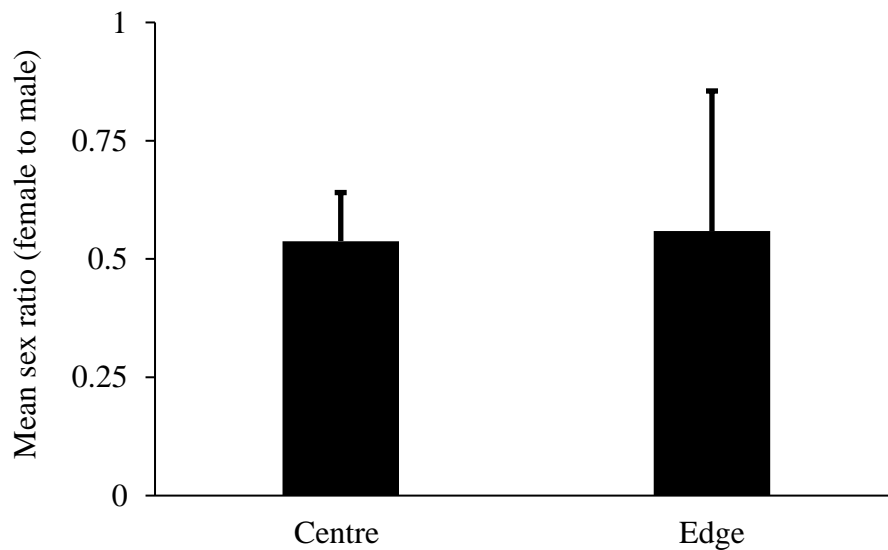
**Figure 14.** Mean + SE number of female offspring that survived to 2016 of female Richardson's ground squirrel's that produced offspring in 2015 in centre versus edge areas of the colony at Assiniboine Park Zoo ( $N_{\text{centre}} = 29$ ,  $N_{\text{edge}} = 14$ ).



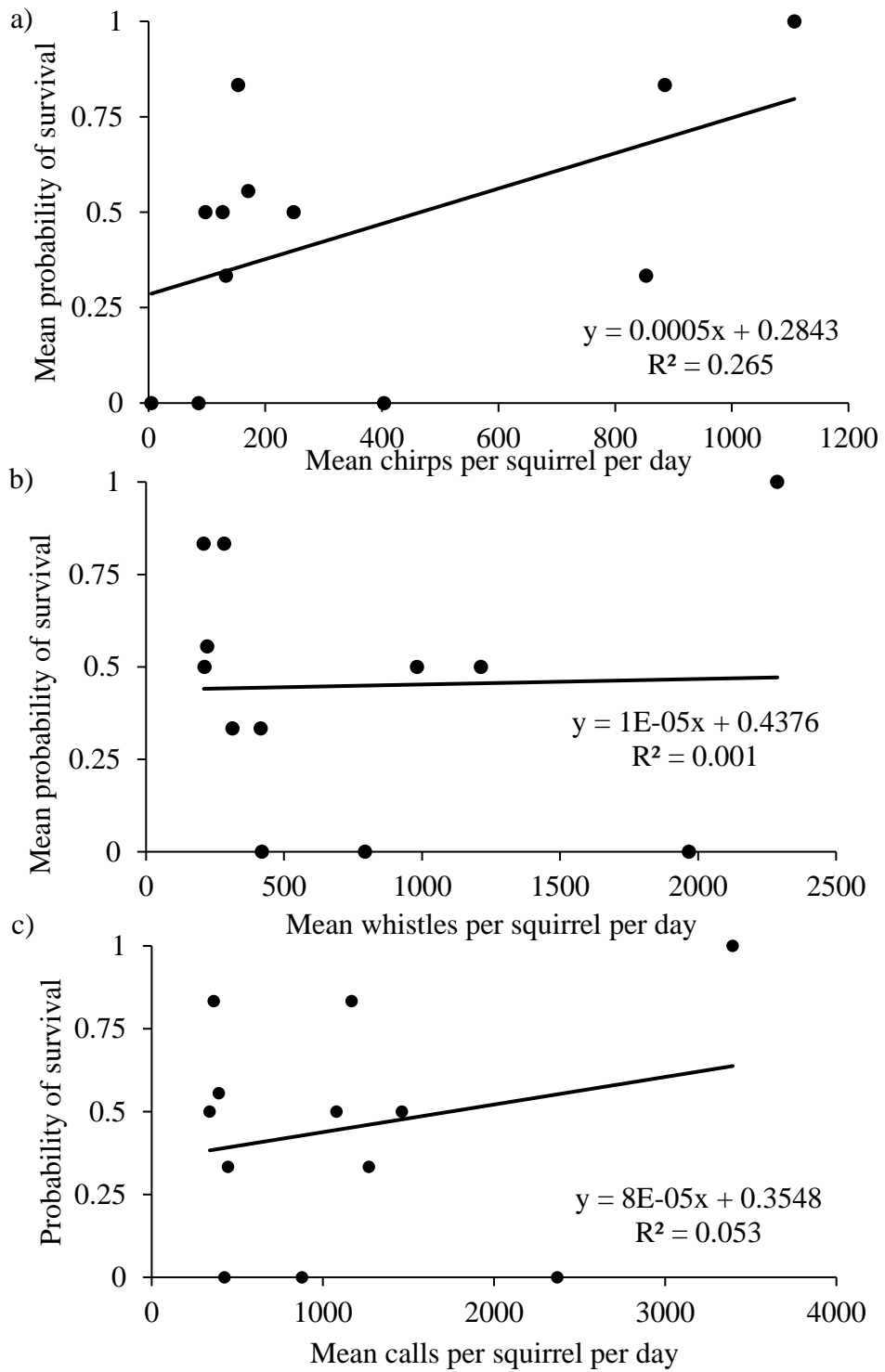
**Figure 15.** Mean + SE number of offspring in litters that emerged above ground produced by Richardson's ground squirrels in centre versus edge areas of the colony at Assiniboine Park Zoo ( $N_{\text{centre}} = 29$ ,  $N_{\text{edge}} = 15$ ).



**Figure 16.** Mean + SE sex ratio of litters that emerged above ground produced by Richardson's ground squirrels in centre versus edge areas of the colony at Assiniboine Park Zoo ( $N_{\text{centre}} = 29$ ,  $N_{\text{edge}} = 15$ ).



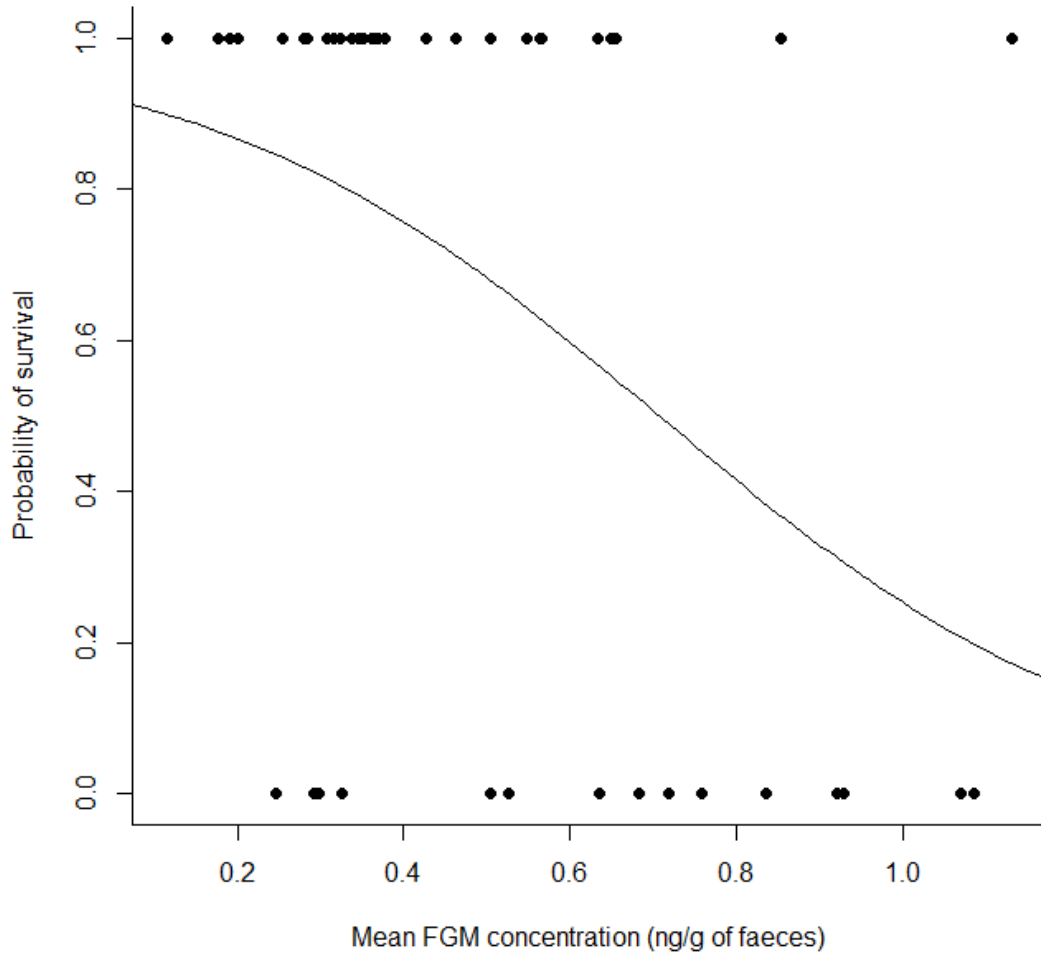
**Figure 17.** Mean probability of survival for adult, female Richardson’s ground squirrels versus a) mean chirps per squirrel per day (N = 12) and b) mean whistles per squirrel per day (N = 12) of squirrel neighbourhoods at Assiniboine Park Zoo.



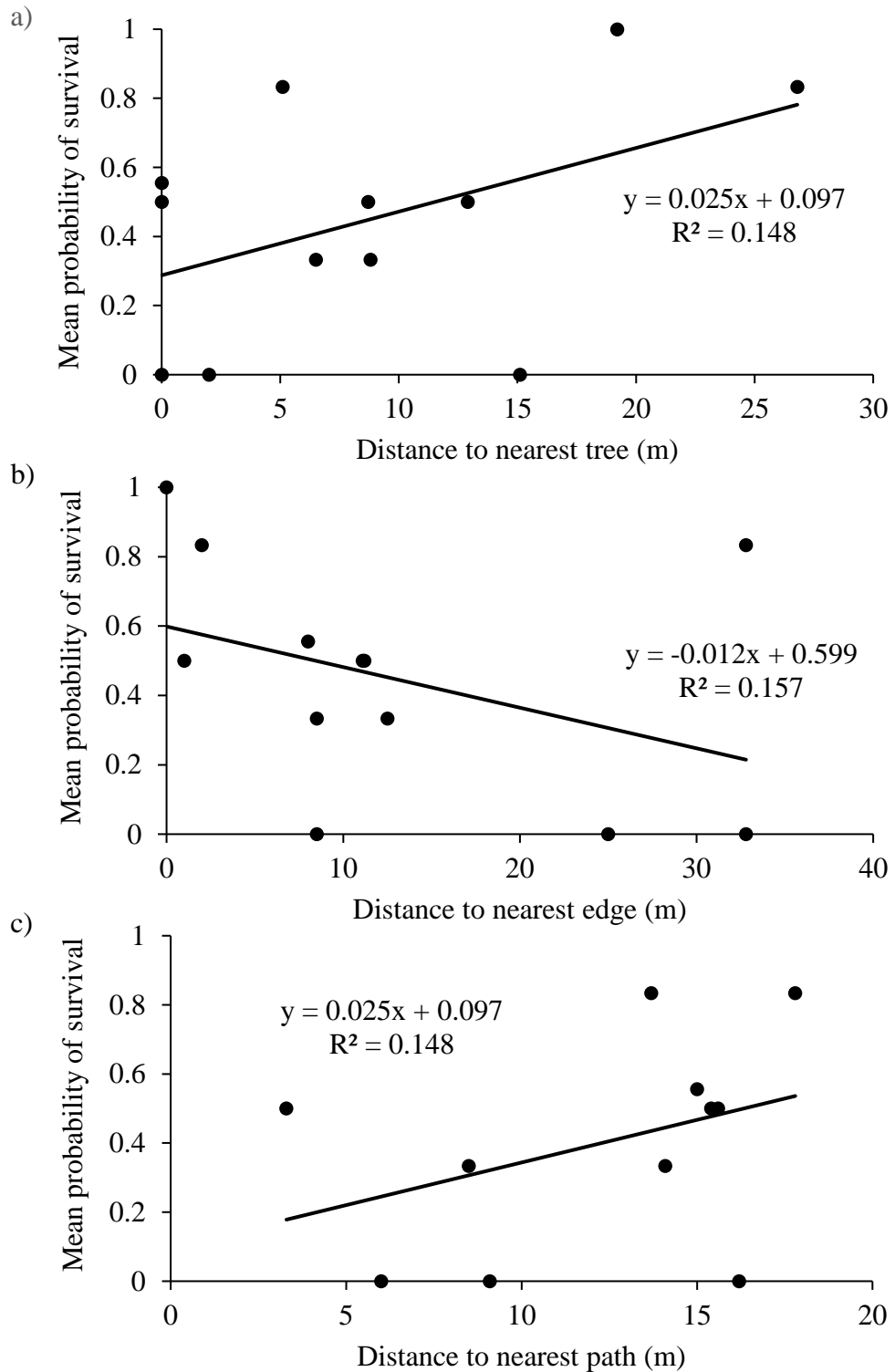


number of chirp calls per squirrel per day ( $R^2 = 0.265$ ,  $F_{1,10} = 3.605$ ,  $p = 0.087$ , Fig. 17a), mean number of whistle calls per squirrel per day ( $R^2 = 0.001$ ,  $F_{1,10} = 0.010$ ,  $p = 0.992$ , Fig. 17b), or mean of the total number of whistle and chirp calls per squirrel per day ( $R^2 = 0.053$ ,  $F_{1,10} = 0.562$ ,  $p = 0.471$ , Fig. 17c). I found that survivorship was inversely correlated with mean FGM for adult females ( $Z = -2.56$ ,  $p = 0.01$ , Fig. 18). Further, I found no correlation between sex ratio and average FGM concentration calculated for each breeding female ( $R^2 = 0.034$ ,  $F_{1,42} = 1.513$ ,  $p = 0.226$ ), between survivorship and distance to nearest tree ( $R^2 = 0.214$ ,  $F_{1,10} = 0.104$ ,  $p = 0.130$ , Fig. 19a), or between survivorship and the distance of a female's nest burrow to the nearest edge ( $R^2 = 0.1574$ ,  $F_{1,10} = 1.867$ ,  $p = 0.202$ , Fig. 19b). There was, however, a positive correlation between distance to nearest path and survivorship ( $R^2 = 0.374$ ,  $F_{1,10} = 5.964$ ,  $p = 0.035$ , Fig. 19c).

**Figure 18.** Survivorship (1 = present, 0 = absent) of adult, female Richardson's ground squirrels from 2015 to 2016 versus their mean FGM concentration (ng/g of faeces) at Assiniboine Park Zoo.



**Figure 19.** Mean probability of survival for adult, female Richardson’s ground squirrels versus the distance (m) to the nearest; a) tree (N =12), b) edge (N =12), and c) walking path (N = 11) of squirrel neighbourhoods at Assiniboine Park Zoo



## Discussion

Predation pressure associated with terrestrial predators appears to follow the predictions of selfish herd theory for Richardson's ground squirrels, with central locations having lower rates of whistle calls per squirrel per day than edge locations. The lower rate of whistle calling in central locations indicates that central individuals may benefit via a reduced risk of predator-induced mortality relative to those residing in more peripheral locations (Hamilton, 1971). Individuals occupying edge locations should experience higher risk due to their proximity to vegetative cover, which could conceal predators. Predators that employ ambush strategies preferentially spend more time in habitat where prey catchability is higher (containing good cover or camouflage) as compared to areas with high prey abundance but low prey catchability (Hopcraft *et al.*, 2005). Despite increased whistle calling in edge locations, I detected no relationship between whistle calls per squirrel per day and distance to closest edge (vegetative cover). Further, I detected no relationship between whistles and distance to the nearest tree, but as trees do not necessarily represent a platform from which terrestrial predators would launch an attack, I would not predict a correlation between the distance to the nearest tree and whistle calls.

Opposite to the effect detected for whistles, central locations had higher rates of chirps per squirrel per day than edge locations, suggesting that central individuals may be experiencing increased predation pressure from avian predators relative to individuals in peripheral locations. Unlike terrestrial predators, avian predators can strike at the centre of the colony relatively quickly, and might fit the circumstances where selfish herd theory does not predict central individuals to accrue benefits as they can enter the group without encountering individuals on the edge first (Hamilton, 1971). The structure of Richardson's alarms supports this general interpretation in that avian predators strike quickly, and thus chirp calls are shorter and unrepeated, while whistle calls are longer and repeated (Davis, 1984, Sloan *et al.*, 2005). If edge

areas impose increased predation pressure from avian predators, I would expect to see a negative correlation between a neighbourhood's distance to nearest tree and chirps per squirrel per day as trees can act as perches from which avian predators may strike. However, I detected a positive correlation between the distance of a neighbourhood to the nearest tree in mean rate of chirps per squirrel per day. Due to their proximity to the threat, squirrels nearer to trees may be alarm calling less often to avoid rendering themselves conspicuousness to presumptive predators, or prioritizing escape down a burrow over emitting an alarm call. As not all vegetative cover provides suitable perches for avian predators, I neither predicted, nor detected, a correlation between chirps per squirrel per day and the distance to nearest vegetative cover. I also detected no correlation between chirps and distance to nearest human walking path, but as Richardson's ground squirrels are unlikely to perceive humans as an airborne threat, such a relationship was not predicted.

Contrary to my prediction that squirrels in centre areas should exhibit lower FGM concentration, and the results of Gardiner (2010) who found that squirrels in central locations had lower FGM concentration after juvenile emergence, I detected no significant difference in FGM concentration between centre and edge locations (both in mean FGM over the sample period and in repeated measures throughout the overall sample period). Similarly, I detected no difference in survival or offspring production. Given that central locations appear to be at increased risk of avian predation, and edge locations appear to impose an increased risk of terrestrial predation, it may be that the squirrels are experiencing a trade-off between the two sources of mortality. Alternatively, predation pressure of both types may not differ between centre and edge, but instead, the propensity of the squirrels to alarm call may differ according to the area they reside in.

The Trivers-Willard hypothesis has seen support in Richardson's ground squirrels, with more highly-stressed dams during early gestation producing smaller, more male-biased litters (Ryan *et al.*, 2012, Ryan *et al.* 2014). I detected no significant difference in litter size and sex ratio between dams nesting in centre versus edge areas, but as I detected no difference between centre and edge for FGM concentration, I would not expect litter size or sex ratio of litters produced in centre versus edge locations to differ. That said, I did not examine FGM concentration of females during gestation, the period where a relationship between FGM concentration with litter size and sex ratio has been documented (Ryan *et al.*, 2012, Ryan *et al.*, 2014).

An important attribute of the study site is the routine presence of humans. Brenner *et al.* (2017) reported that human disturbance leads to increased FGM concentration in juvenile and adult male and juvenile female European ground squirrels, yet I found that the distance from a walking path was negatively correlated with adult females' mean FGM concentration for the study period. Brenner *et al.* (2017) did not find an increase in FGM for adult females with increased human disturbance, though they did report an increase in progesterone. Further, my data for Richardson's ground squirrels at the Assiniboine Park Zoo reveal that as the distance from the nearest walking path increased, survivorship of adult females increased. The decrease in FGM and increase in survival as the distance from the closest walking path increases, suggests that humans may still be perceived as threats by the Richardson's ground squirrels. Alternatively, another characteristic of walking paths may be detrimental. For example, wolves take advantage of human modification to the environment (such as roads) to move more quickly (Dickie *et al.*, 2017), and it is possible that predators of Richardson's ground squirrels at the zoo do the same. That FGM was not correlated with the distance to the nearest tree, or the distance to the nearest

edge, supports the notion that human presence is an important factor for Richardson's ground squirrels at the Assiniboine Park Zoo.

If human disturbance is the cause of increased FGM and decreased survivorship of squirrels near walking paths, we would expect to see a negative correlation between distance to walking path and mean whistles per squirrel per day. While whistles per squirrel per day decreased with increasing distance from the nearest walking path, the association was not statistically significant. It is possible that the ubiquity of humans at the site has caused squirrels to attenuate alarm call production in response to the presence of humans, even though they perceive human proximity as threatening. Some species attenuate their stress response to human presence (e.g. king penguin, *Aptenodytes patagonicus*, Viblanc *et al.*, 2012; marine iguanas, *Amblyrhynchus cristatus*, Romero & Wikelski, 2002). However, a study of hoatzins (*Opisthocomus hoazin*) found that hoatzin juveniles were sensitive to human presence (Müllner *et al.*, 2004). The same study found that adult hoatzin at tourist-visited sites had startle distances half of those at sites undisturbed by tourism. It is also possible that any relationship between walking path and whistles and between edge and whistles are obscured by the fact that edges and walking paths tend to be on opposing sides of the colony, so as a squirrel moves away from one they get closer to the other.

Consistent with the importance of non-lethal effects of predation, I detected a negative correlation between survivorship and mean FGM concentration. Previous work has shown that chronic activation of the stress axis can lead to decreases in survivorship (Boonstra *et al.*, 1998; Wingfield & Romero, 2001). However, others have suggested that chronic but mild activation of the stress axis can prove beneficial. Cote *et al.* (2006) found that experimental increases in corticosterone lead to increased energy expenditure, daily activity, food intake, and changes to

behaviour that lead to increases survival in adult male common lizards (*Lacerta vivipara*; Cote *et al.*, 2006). Pravosudov (2003) caught wild mountain chickadees (*Poecile gambeli*), and found that individuals implanted with corticosterone cached and consumed more food, and were better able to remember cache locations than non-implanted chickadees. Cabezas *et al.* (2007) found that in European wild rabbits (*Ortycolagus cuniculus*) exposed to long-term stress, moderate blood corticosterone and FGM were correlated with decreased body condition, but increased survival when released into the wild. Unlike these latter examples, the stress experienced by Richardson's ground squirrels in close proximity to walking paths appears to be detrimental.

I predicted that alarm calling should be correlated with FGM, as encountering threatening stimuli should lead to activation of the HPA axis, leading to increases in FGM concentration, though neither the incidence of chirps nor whistles were correlated with FGM concentration. It could be that FGM concentration is determined primarily by other factors, such as food availability (Kitaysky *et al.*, 1999), body condition (Heath & Dufty, 1998), conspecific interaction (Foley *et al.*, 2001), maternal history (stress-axis priming; Love *et al.*, 2013), or behavioural traits (Montiglio *et al.*, 2012; Clary *et al.*, 2014). Alternatively, FGM concentration may be largely determined by genetics rather than environmental factors, as a portion of the Richardson's ground squirrel stress response is heritable (Bairois-Novak *et al.*, 2017).

During juvenile emergence I expected to see increased predation pressure as predators should take advantage of the increased number of squirrels, and in particular, the abundance of relatively naïve and easy to handle juveniles. I did not detect any relationship between alarm calling and date, suggesting that predation pressure is not as variable over time as expected. However, in that I did not have recorders out early enough to capture alarm call recordings prior to juvenile emergence, I cannot compare predation pressure during and after juvenile emergence



to predation pressure preceding juvenile emergence. Similarly, mean FGM concentration of adults did not differ significantly during juvenile emergence and thereafter. Temporal variation in predation pressure is not uncommon for other species (Scharf & Schlicht, 2000; Lode, 2000; Schmutz *et al.*, 1980). It is possible that predation pressure increases during juvenile emergence, and does not decrease over the summer as the colony still contains a greater proportionate representation of juvenile ground squirrels as adults enter hibernation. Alternatively, the colony may represent a clumped resource patch with higher prey concentration than the surrounding landscape for the portion of the year that the ground squirrels are above ground.

While my results are meaningful, several limitations must be considered in drawing inferences from those. First, my measure of predation pressure is an estimation based on rates of alarm calling. Squirrels may not alarm call during every predator encounter, or, in some cases, alarm call when a predator is absent. Additionally, my measurement of alarm calling is subject to any biases that exist in the recognizers I produced and used to search through my recordings to quantify calling rates. Both recognizers estimate the number of alarm calls in an area on average. I examined calls per squirrel per day to account for the fact that areas with more squirrels would likely have more alarm calls. However, in that squirrels in this free-living population move about the site, I have no knowledge of the number of squirrels within the active recording space of a recorder for any specific period. Similarly, stress response was not measured directly, instead I used the concentration of glucocorticoid metabolites in faecal material, which presumably reflects activation of the HPA axis of Richardson's ground squirrels over the preceding few days (Hare *et al.* 2014). Lastly, the zoo environment of the study population could be influencing both the Richardson's ground squirrels and their predators.

The lack of centre-edge differences in FGM concentration, survival, and offspring production suggests that trade-offs mitigate any clear-cut advantage of central over edge territories, counter to the predictions of selfish herd theory. Squirrels may still benefit disproportionately owing to the location of their territory, as I found that factors such as distance to human walking paths affects fitness outcomes of squirrels. As much of the historical environment of Richardson's ground squirrels is now highly modified by humans (Hoffman *et al.*, 1993; Sampson & Knopf, 1994), how human presence and habitat modification influence their populations' demographics is an important consideration.

Future work could examine how different landscape features created by humans, such as roads and/or utility corridors affect Richardson's ground squirrels. It would also be valuable to investigate if Richardson's colonies in more natural areas show different trends, as the effects of predation pressure may be more pronounced in areas with lower human presence (Tella *et al.*, 1996; Bowers & Breland, 1996; Fischer *et al.*, 2012). A comparison among areas with no human presence or environmental modification, environmental modification but low or no human presence, and sites with both high environmental modification and human presence (such as the zoo) would be required to ascertain whether human presence is indeed influencing the squirrels. Examining how survivorship and FGM concentration vary with location at a finer spatial scale (e.g. location of territory as opposed to recorder location) may also better resolve how environmental factors influence Richardson's ground squirrels. Additionally, long term monitoring of survivorship and offspring production may reveal difference in centre and edge that were not detectable over a single year. Finally, examining alarm calling and stress response throughout the entire above-ground portion of the Richardson's ground squirrel's annual cycle

may reveal seasonal differences that were not evident in comparing only juvenile emergence to post-emergence.

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