

**SITE SELECTION FOR PARTURITION BY GRAVID FEMALE RATTLESNAKES
(*CROTALUS OREGANUS*) IN OSOYOOS, BRITISH COLUMBIA**

by

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Abstract

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Knowledge of habitat use and resource selection behaviours are critical to understanding the population ecology of any animal species. In some species, genders and age classes exhibit different preferences for foraging, reproduction, and shelter. The Western Rattlesnake (*Crotalus oreganus*) is a good example of a species that shows variation in habitat use between genders, mainly due to different reproductive life histories. My research focussed on identifying and describing gestation sites referred to as rookery sites used by gravid female rattlesnakes for parturition. Radiotelemetry was used to monitor twelve gravid females from April to September 2017 in Osoyoos, British Columbia. I assessed vegetation cover and other habitat features at the rookery sites across three different spatial scales (1 m, 3 m, 10 m radius plots). To compare rookeries to random habitat plots, I used a matched case-control study design. Conditional logistic regressions revealed that at the 1 m scale, females selected sites with higher surface temperature, whereas at the 3 m scale, they selected for higher air temperature and the sufficient cover of rocks. At the largest scale, 10 m radius plot, average air temperature once again was selected for by gravid females. Overall, rookery sites had $\approx 80\%$ rock cover and were found on south-facing slopes (on average 26% slope incline). Information on the location, habitat structure and use of rookeries provides important insight into understanding the ecology of Western Rattlesnakes and facilitating their conservation.

Keywords: rattlesnakes, rookery, telemetry, site selection, habitat analysis, conditional logistic regression, *Crotalus oreganus*

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Introduction

Site Selection

Patterns of habitat use as shown by animals is a key factor in understanding the basic ecology of species. Heterogeneity of resource distribution, competition, environmental pressures and disturbances on the land can influence aspects of niche selection demonstrated by individuals in a population (Cornell and Lawton 2016). Site selection is evident when animals choose a certain habitat type more often than expected by overall availability (Koper and Manseau 2012). Available habitat includes all habitat types found within an area that are used or not used by the species (Jones 2001). Selected habitat can be characterized by specific vegetation cover, sun exposure, prey availability, and other landscape features within available habitat (McLoughlin et al. 2002). Resource selection is assessed within the existing home range of an animal, whereas site selection can be interpreted as the selection of a home range in an area defined by the entire population (Aebischer et al. 1993). Since habitat variables, structure, and resource availability differ depending on the size of the assessed area, resource selection could vary based on spatial scale (Jones 2001). For example, large spatial scale could refer to a patch of forest with a heterogenous stand structure, whereas a small scale could incorporate an area within that forest patch where individual tree features are assessed. Investigating site selection at different spatial scales is important because it may demonstrate varying criteria used by animals at different levels, such as broader habitat range or within a microsite (Jones 2001).

Selectivity of resources or sites is a tactic expressed by individuals to increase overall fitness and survivability (McLoughlin et al. 2006). Differences in habitat use and resource selection within one population are often observed due to the biological characteristics of individuals such as age class, reproductive status or gender. For example, studies show that habitat use can differ between juvenile and adult fish of the same population due to the ontogenetic differences in energy needs for growth, competition and predation pressures (Bay et al. 2001, Hofmann and Fischer 2002, King 2004). Habitat needs also may vary between reproductive females and males, for

example, in site selection for breeding and nesting in avian populations. Male birds tend to select breeding sites that provide resources and opportunity for acquiring food, mates, and appropriate acoustic environment for communication (Barg et al. 2006). On the other hand, female birds often utilize habitat in close proximity to males to increase chances of copulation (Mennill et al. 2004). In addition, sexual segregation can arise from higher energetic costs for females associated with reproduction where energy demands increase during lactation, gestation, and replenishment of body mass after giving birth. Depending on reproductive mode, energy costs to embryo development and parturition would be different. For example, egg-laying (oviparous) animals might have to allocate less energy for reproduction due to external embryonic development of their young (Shine 2003). However, this strategy poses higher risks to the development of the eggs, since they are fully dependent on the environmental conditions at the point of oviposition (Shine 2003). On the other hand, energetic costs of live-bearing (viviparous) animals that rely on the internal embryonic development are much higher, and, therefore, may restrict activity of gravid females and greatly exploit their energy reserves (Macartney and Gregory 1988, Shine 2003). Viviparous strategy makes reproductive females more susceptible to predation and extreme environmental conditions compared to males (Barten et al. 2001, McLoughlin et al. 2002). These costs are counterbalanced in viviparous animals by improved protection of embryos from unpredictable environment, predation, microbial attacks and pathogens (Blackburn 1999).

Gravid, viviparous females require habitat that provides protection (refuge) from predators, while at the same time affording opportunities to replenish energetic expenditures (Barten et al. 2001, McLoughlin et al. 2002). Locations where animals give birth are often referred to as ‘rookery’ sites (nest sites), particularly for birds and reptiles (Sage and Vernon 1978, Graves and Duvall 1993a, Kasprzykowski 2003, Anderson et al. 2005). However, characteristics of rookery sites can vary between endothermic and ectothermic animals due to their metabolic needs. Since ectotherms (e.g. reptiles) rely on external sources of heat, rookery habitat intuitively should provide conditions for efficient thermoregulation during embryonic development in reproductive females (Johnson et al. 2016). Temperature, along with sufficient cover that provides shade and refuge for snakes, are therefore likely two critical characteristics of snake rookeries

(Graves and Duvall 1995, Johnson et al. 2016). Environmental temperature is of particular interest because gravid female snakes often exhibit higher body temperatures compared to males and non-gravid females (Johnson et al. 2016). Overall, few studies have assessed or tested for temperature and cover habitat characteristics predicted for rookery sites used by snakes.

From a conservation standpoint, identifying selected sites such as rookeries is essential they likely provide habitat critical to animal needs. If a wildlife species is at risk, these selected habitat types can be protected and monitored, securing essential resources for species persistence.

Western Rattlesnakes Reproductive Ecology

Western Rattlesnakes are only found within Thompson-Nicola and Okanagan-Similkameen regions of British Columbia (BC) (COSEWIC 2015). They are viviparous (live-bearing) and have a biennial or triennial reproductive cycle (Macartney 1985). Rattlesnakes become sexually mature at the age of seven or nine years old. Energy reserves can pre-determine the success and timing of the consequent mating events (Macartney and Gregory 1988). An average litter size ranges from two to eight neonates. Gestation sites are typically known to be within a short distance from the hibernation sites (Macartney 1985). Overall, limited information is available on reproduction of the Western Rattlesnake in BC (Macartney and Gregory 1988). In general, the movements and behaviour of males and non-gravid females have been studied more extensively than reproductive females (Macartney 1985, Gomez 2007, Lomas 2009, Harvey 2015). This historic bias has been possibly caused by the complicated life history of mature females (Macartney 1985) and a desire to eliminate variants in movement patterns.

Population Status

The Western Rattlesnake (*Crotalus oreganus*) is listed as threatened under the Committee on the Status of Endangered Wildlife in Canada (SARA registry 2015). Habitat loss, anthropogenic mortality and destruction of their hibernacula are major contributors to population decline of rattlesnakes in British Columbia (COSEWIC 2015). Slow

population replenishment from biennial/triennial reproductive cycles and high juvenile mortality also challenge stability of the vulnerable populations (COSEWIC 2015).

Objectives

The objectives of my research were (1) to locate rookery sites utilized by gravid female rattlesnakes, and (2) to evaluate habitat characteristics associated with these sites at three spatial scales. In addition, gravid females were monitored over the summer to identify behaviour associated with rookery use, changes in body condition during gestation and post-parturition periods.

The goal of this study is to apply site selection modelling to habitat use by gravid (pregnant) female Western Rattlesnakes in Osoyoos. Ultimately, identifying critical habitat for rattlesnake parturition can be used to craft informed management strategies that promote population stability and growth.

Materials and Methods

Study Site Description

My study population of Western Rattlesnakes was centered on the land of the Okanagan Indian Band (OIB) in Osoyoos, British Columbia (49.0°N, 119.4°W). I collected data over one field season from April to September, 2017. The study area lied within semi-arid Okanagan-Similkameen grasslands with the vegetation community dominated by bunchgrasses and dryland shrubs such as *Artemisia tridentata* (Big sagebrush) and *Purshia tridentata* (Antelope brush) (Iverson et al. 2004). This region is characterized by hot and dry summer climate with limited precipitation and a prolonged midsummer drought (Iverson et al. 2004).

Snake Capture and Assessment

During spring emergence from denning (hibernation) sites, rattlesnakes were opportunistically captured and processed, allowing me to identify reproductive (gravid) females. These individuals could be recognized by a robust body condition and enlarged ovarian follicles. A gentle ventral palpation of gravid female rattlesnakes allowed to

detect these follicles (Parker and Anderson 2007). Twelve gravid female rattlesnakes of relatively large body mass were selected for this study from three different hibernation sites. Each gravid female was uniquely tagged with a Passive Integrated Transponder (PIT) to provide permanent identification (Gibbons and Andrews 2004).

Telemetry

All twelve females were surgically implanted with radio transmitters to allow telemetry monitoring. Surgeries were performed by a professional veterinarian in an Osoyoos veterinary clinic. Also recorded was an assessment of snake body condition measuring weight and a snout-ventral length (SVL) for each individual following standard methodology (Jenkins et al. 2009, Lomas 2009, Johnson et al. 2016).

After surgery, gravid females were kept in the lab for two days to ensure rehabilitation after transmitter implantation. Females were released at their original point of capture and each female was regularly tracked 3-4 times per week throughout the field season. To inspect whether surgical incisions were healing properly, and to monitor changes in body weight, we attempted brief recaptures of the focal females once each month. This information was later used to compare changes in body mass over gestation and post parturition time periods.

Each time a snake was located, I attempted to check for the presence of other snakes within rookeries. After females gave birth, I also estimated the number of neonates from each female based on neonatal shed counts and visual observations of neonates in rookeries. In general, I tried to minimize disturbance to gravid females during telemetry by remaining distant from the tracked snakes and spending minimum time at the rookeries.

Snake handling methods duplicated those previously used on this site and were approved by an animal ethics committee (Harvey 2015). During field work I was paired with a partner to meet safety requirements.

Rookery Identification & Habitat Measurements

When long-distance movements ceased, and each gravid snake was observed sedentary at a particular site for at least 10 days, I assumed that site was a rookery and established habitat plots at each rookery site and two associated reference sites. I marked the centre of each rookery by the rock under which a female gave birth. To place the centre of the reference sites, I randomly generated two independent compass bearings and followed each 40 m distance from the center of a rookery site. The distance between plot centers was set to 40 m to avoid overlap between the largest plot size (scale). Using these locations, I then established three plots of different radii (1 m, 3 m and 10 m radius) on each site, allowing me to assess habitat characteristics at three spatial scales. Identical parameters were measured within detection sites and reference sites at each scale. Environmental variables and habitat characteristics that I recorded at each site were 1) topographic, 2) vegetative and 3) thermal parameters (Column 1 in Table 1).

I recorded slope, elevation, aspect, and site location. Every time a natural underground retreat was found on the site, I recorded substrate material and depth of the cavity using a measuring tape. At reference sites, where underground cavities appeared absent, I assumed 0 cm cavity depth.

Secondly, due to the observed association between snakes and plant cover in previous studies, I estimated the relative cover (%) of grasses, shrubs, trees and rocks (Johnson et al. 2016). Vegetation percentages were assessed in layers using a canopy cover technique. Estimation of each vegetation layer was estimated based on the vertical projection of its canopy. For example, cover of shrubs was assessed as the proportion of ground floor covered by shrubs within a plot. I directly conducted visual estimation of plant and rock cover at all scales in person to ensure consistency in measurements. However, if other people with previous experience of cover assessments were present at the site, I consulted them for a second opinion.

Finally, fluctuations in air, surface and cavity temperatures were monitored with temperature dataloggers (iButtons model DS1922, accuracy +/-0.5 °C, Hindman et al. 2006). I placed 3 dataloggers in the center of each habitat plot (rookery and random) to

Table 1. Summary of explanatory environmental variables assessed at three scales (column 2) and identified significant variables (column 4) for individual scales. See text for explanation of variables. Modelling of habitat variables was conducted in COXREG (IBM SPSS Statistics).

Recorded Habitat Variable	Scale	Putative Regression Variables	Significant Variables
Cavity depth, (m)	1 m radius	Cavity depth, (m)	Minimum surface temperature, (C°) <i>P</i> =0.022
Grass cover, (%)		Shrub cover, (%)	
Shrub cover, (%)		Average surface temperature, (C°)	
Tree cover, (%)		Maximum surface temperature, (C°)	
Rock cover, (%)		Maximum air temperature, (C°)	
Minimum air temperature, (C°)	3 m radius	Minimum surface temperature, (C°)	Average air temperature, (C°) <i>P</i> =0.020
Average air temperature, (C°)		Cavity depth, (m)	
Maximum air temperature, (C°)		Shrub cover, (%)	
Minimum surface temperature, (C°)		Maximum surface temperature, (C°)	
Average surface temperature, (C°)	3 m radius	Minimum surface temperature, (C°)	Rock cover, (%) <i>P</i> =0.025
Maximum surface temperature, (C°)		Average air temperature, (C°)	
Minimum cavity temperature, (C°)		Rock cover, (%)	
Average cavity temperature, (C°)		Maximum cavity temperature, (C°)	
Maximum cavity temperature, (C°)	10 m radius	Minimum air temperature, (C°)	Average air temperature, (C°) <i>P</i> =0.020
		Average cavity temperature, (C°)	
		Grass cover, (%)	
		Maximum cavity temperature, (C°)	
		Average air temperature, (C°)	
		Average cavity temperature, (C°)	
		Minimum air temperature, (C°)	

record cavity, air and surface temperatures. Only 3 dataloggers were used per plot due to constraints with the availability of iButtons. All temperatures were recorded every 40 min throughout a three-month period (mid June-mid September). To protect the air and surface dataloggers from UV radiation and potential overestimation of temperature value, I placed each of them in a Ziploc bag with a reflective foil cover and punctured holes for passive ventilation. For air temperature dataloggers, I made double cone shields out of reflective material to ensure little sun penetration and allow for maximum exposure of a datalogger to air (Tarara and Hoheisel 2007). A Ziploc pouch with an air logger was taped inside the top cone. Two cones were attached to each other with four plastic cable ties allowing 3-5 cm distance between cone edges (Appendix A). Air loggers were tied to shrub branches 1 m above ground in a partial shade. Each surface datalogger was attached to the ground using metal stakes, or it was duct-taped on the rock surface, if rocks were found in the centre of a plot. Cavity loggers were placed inside a single underground hole located within 0.5 m of a plot center. I selected potential natural cavities based on their size making sure that a snake could fit inside and that it was shaded from sun. This was primarily done for reference sites where snakes using cavities were not observed. If natural cavities were absent on the reference sites, I placed cavity loggers at the stem base of dense shrubs ensuring no direct sun exposure.

As parturition occurred in August for all snakes (see Results), I used a subset of average daily temperatures to represent air, surface, and cavity conditions. To compare extremes in occurred temperatures within rookeries and reference sites, I calculated 24 hr maximum and minimum temperatures for air, surface and cavities that were then averaged between the sites for August (31 days). This allowed me to compare an average maximum temperature measured in rookery sites and in reference sites in time period from the 1st to 31st of August. I repeated the same calculation method to compare minimum temperatures and mean temperatures between the sites.

Statistical analysis

Modelling of resource selection function is frequently generated with a binary logistic regression (McLoughlin et al. 2006). In study designs that have one main and several

associated reference sites, conditional (paired) logistic regression is used (Duchesne et al. 2010, Chan 2011).

Conditional logistic regression (COXREG) is most commonly associated with drug resistance studies, hazard and survival analysis in medicine (Chan 2011, Koletsi and Pandis 2017). However, COXREG was adopted in ecology for multivariate datasets addressing habitat selection based on the presence or absence of the studied event or animal and an associated set of predictor variables recorded for each detection site paired with multiple reference sites (Farmer et al. 2006, Thaker et al. 2011, Maag 2017).

Differences between rookery and reference sites were analyzed using conditional logistic regressions following a COXREG procedure in IBM SPSS Statistics (version 24.0)(Chan 2011, IBM Software Group 2013). I selected forward conditional data entry for modelling with 0.5 entry and 0.1 removal stepwise probability. Associations between individual rookery plots and their reference plots were included in the models through an additional numeric variable (i.e., all three plots shared the same identifying number). This technique is standard for analysis of paired sampling designs (Chan 2011, IBM Software Group 2013). To avoid feeding autocorrelated variables into the regression analyses, I tested *a priori* for correlation between all environmental variables (Pearson correlation, Minitab version 18). The strength of the correlation between vegetation cover, rock cover and cavity depth varied based on plot (scale) size, therefore, I combined these variables in different sets for each scale ensuring no significantly-correlated variables occurred in each set. Firstly, I listed variables that were correlated (Appendix B) and then excluded pairs of correlated variables within one scale from the list of all habitat measurements. Ultimately, only uncorrelated sets of variables (Column 3 Table 1) were used for logistic regression modelling at each scale.

Results

Snake Capture and Assessment

Gravid female rattlesnakes were captured at their hibernation sites between the 15th and the 25th of April 2017. Average SVL of the studied females was 60.9 cm (SE \pm 1.3cm, $n=12$), and average weight of captured snakes immediately after emergence from hibernacula was 222.5 g (SE \pm 64.2 g, $n=12$). Overall trend shows a slight (12%) increase in body weight a month after emergence (May), (Figure 1). Post parturition, females' weight dropped by an average of 46% (from 224.3 g to 126.3 g) relative to their weight in April (Figure 1).

Tracked female rattlesnakes settled down at their rookery sites in mid-June and showed little movement within the rookery area during the rest of summer. From the 16th to the 31st of August, nine out of twelve telemetered females successfully gave birth with litter size ranging from 2 and 5 neonates per snake. I could not identify whether the remaining 3 females gave birth or aborted as no neonates or any products of abortion were found. These females left the sites that they occupied during mid-Summer and moved to other areas presumably to feed. No signs of body abnormality, sickness or injury were detected in these females that could suggest failed reproduction.

Females that gave birth spent on average a minimum of 5 days in the proximity of their newborn neonates, and then dispersed downslope (August 23-August 31). Neonate sheds were found 2-3 days prior to when their mothers left rookery sites.

Location and Rookery Description

Nine rookery sites in total were located on the south and southwestern slopes of my study area (Figure 2). The average elevation for all nine sites was 447 m (SE \pm 20 m) with 26% slope incline (SE \pm 3%, $n=9$). The mean distance between where females hibernated (denned) and their rookery sites was 142.0 m (range 5.0 m to 384.9 m). On average, predominant vegetation cover on the rookery sites was grasses (49%), and shrubs (8%) (Figure 3). Tree cover was minimal (\approx 3%). Rookeries were represented by rock piles covering underground cavities. Depth inside these cavities ranged between 6.2 cm and

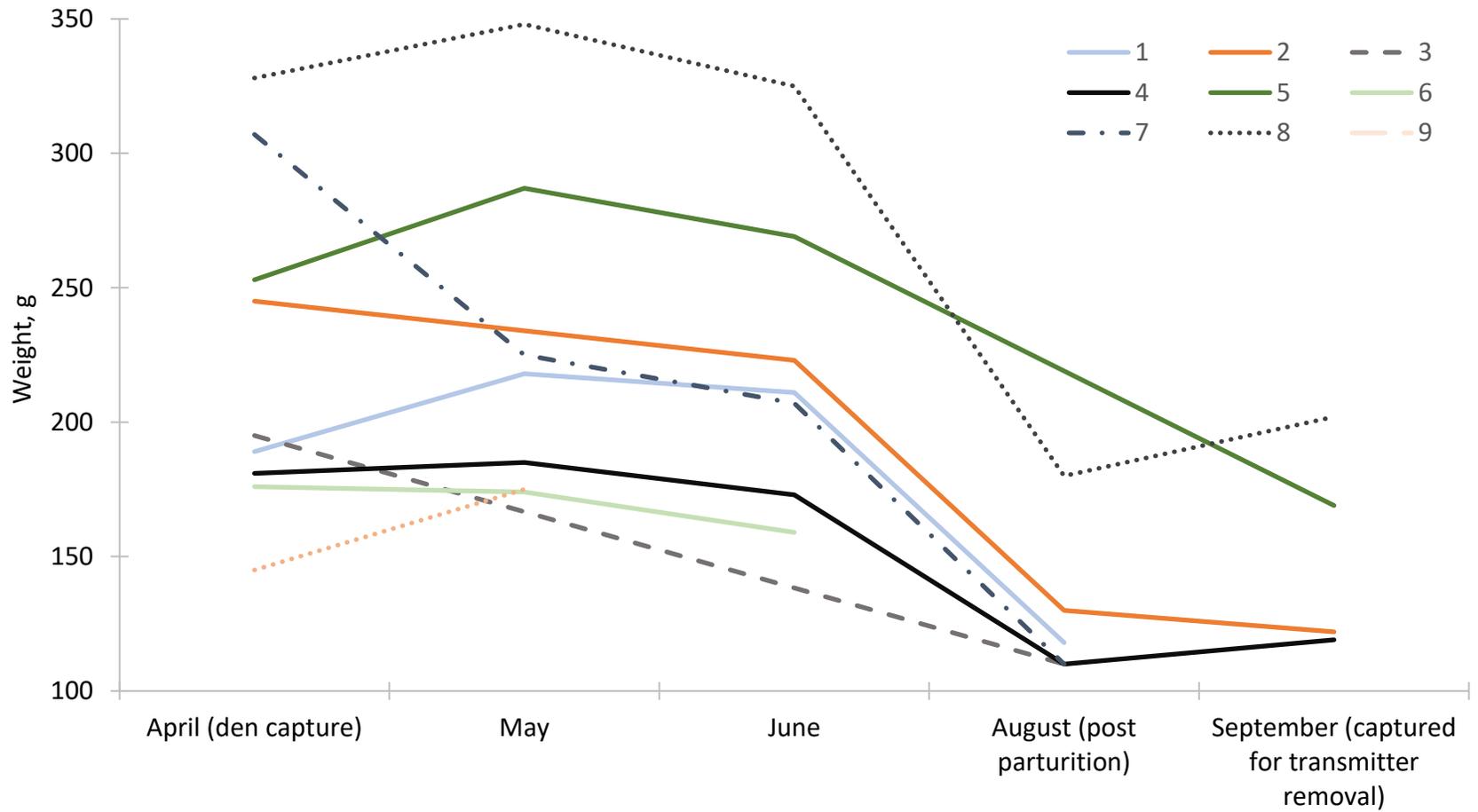


Figure 1. Changes in body weight shown for gravid female *Crotalus oreganus* in Osoyoos, 2017. Each female is depicted separately from one another (1-9).

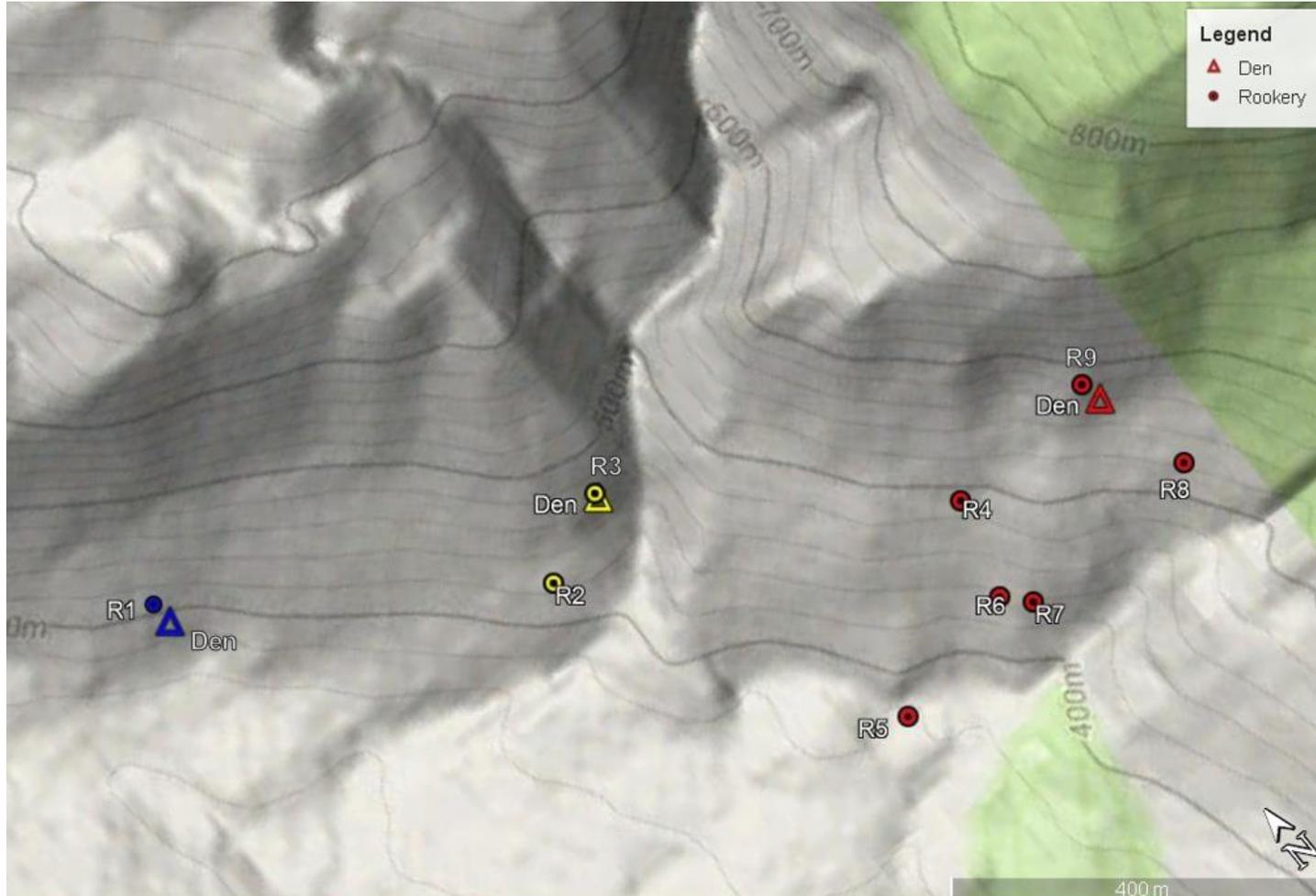


Figure 2. Location of nine identified rookery (©) and three den (Δ) sites within bunchgrass zone in Osoyoos, British Columbia. Colour coding was used to show location of rookery sites used by female rattlesnakes relative to the den sites from which they emerged. Average distance traveled by a gravid female rattlesnake from her den to her rookery site was 142.0 m (SE ± 41.4 m).



Figure 3. Four rookery sites (☆) used by gravid Western Rattlesnakes for parturition in August, Osoyoos, 2017.

227.2 cm (\bar{x} =108.0 cm, n =9). Substrate material at all rookery sites was sand with a small amount of plant debris.

Temperature

Fluctuations in air temperature during the summer revealed a slight decrease in ambient temperature during the parturition period (Figure 4). Fluctuations in cavity temperature had the lowest amplitudes compared to air temperature (Figure 5). Overall trend suggests that daily average maximum air and surface temperatures in rookery sites were higher than in reference sites (Figure 5). On the other hand, maximum cavity temperature exhibited in rookery sites tended to be lower than recorded in reference sites (Figure 5).

Site Selection

Since the majority of putative explanatory habitat variables were correlated with one another (Appendix A), four combination sets of uncorrelated variables were used for the regression analysis (Column 3 in Table 1). Three variables associated with the location of a rookery site were identified based on the spatial scale.

At the 1 m scale, minimum surface temperature was strongly associated with the presence of a rookery site ($P=0.022$, $n=27$). At the 3 m scale, average air temperature and rock cover (Figure 6) showed a strong predictive relationship with the presence of rookeries ($P=0.020$ and 0.025 respectively, $n=27$). On the largest scale (10 m radius), the presence of a rookery site was positively associated with average air temperature ($P=0.020$, $n=27$). None of the vegetation cover classes (grasses, shrubs, trees) were associated with the rookery sites.

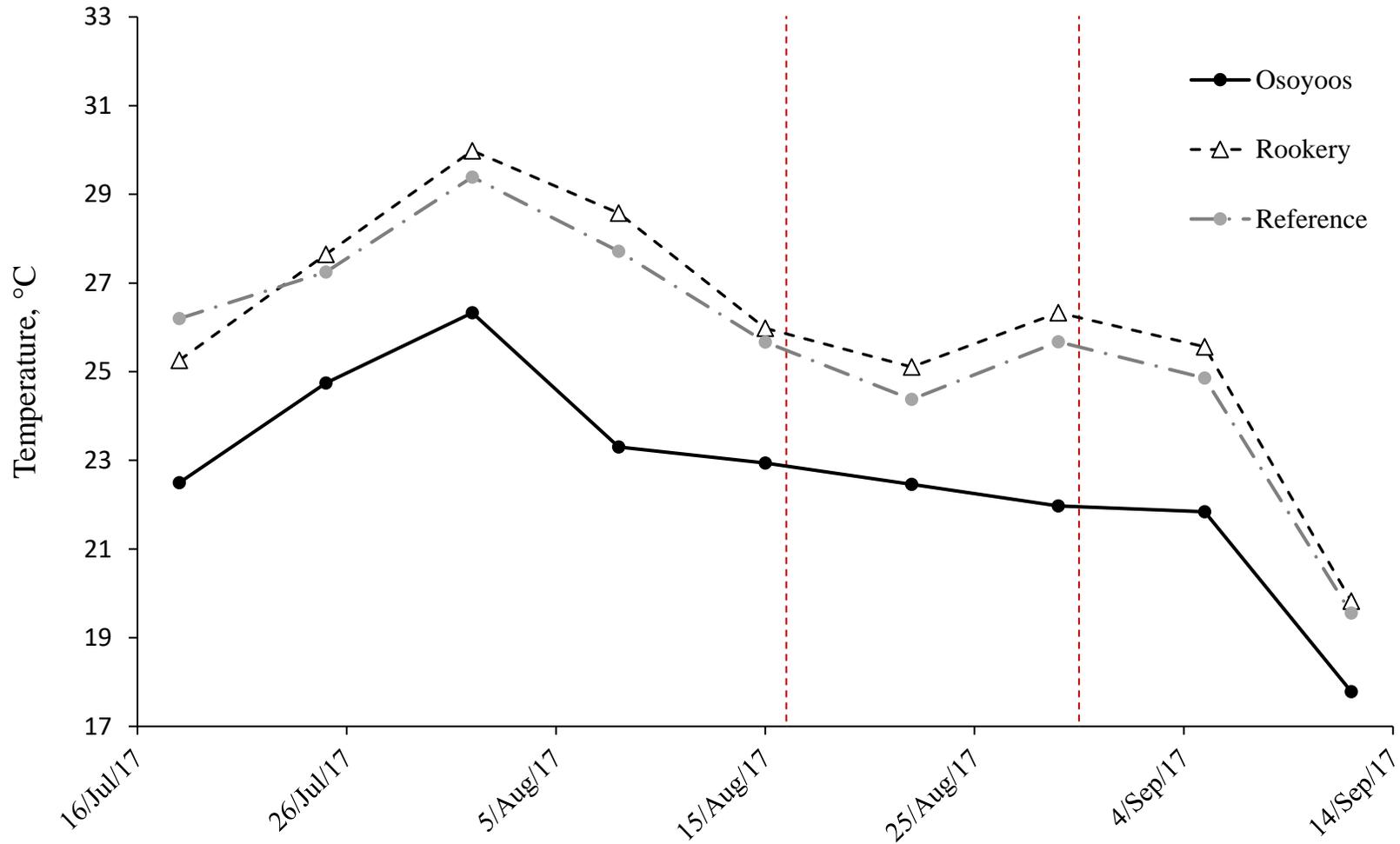


Figure 4. Comparison between average air temperature in Osoyoos retrieved from the Osoyoos Weather Station and the air temperature at the rookery sites and the reference sites recorded with air loggers in 2017. The period of snake parturition is outlined in red.

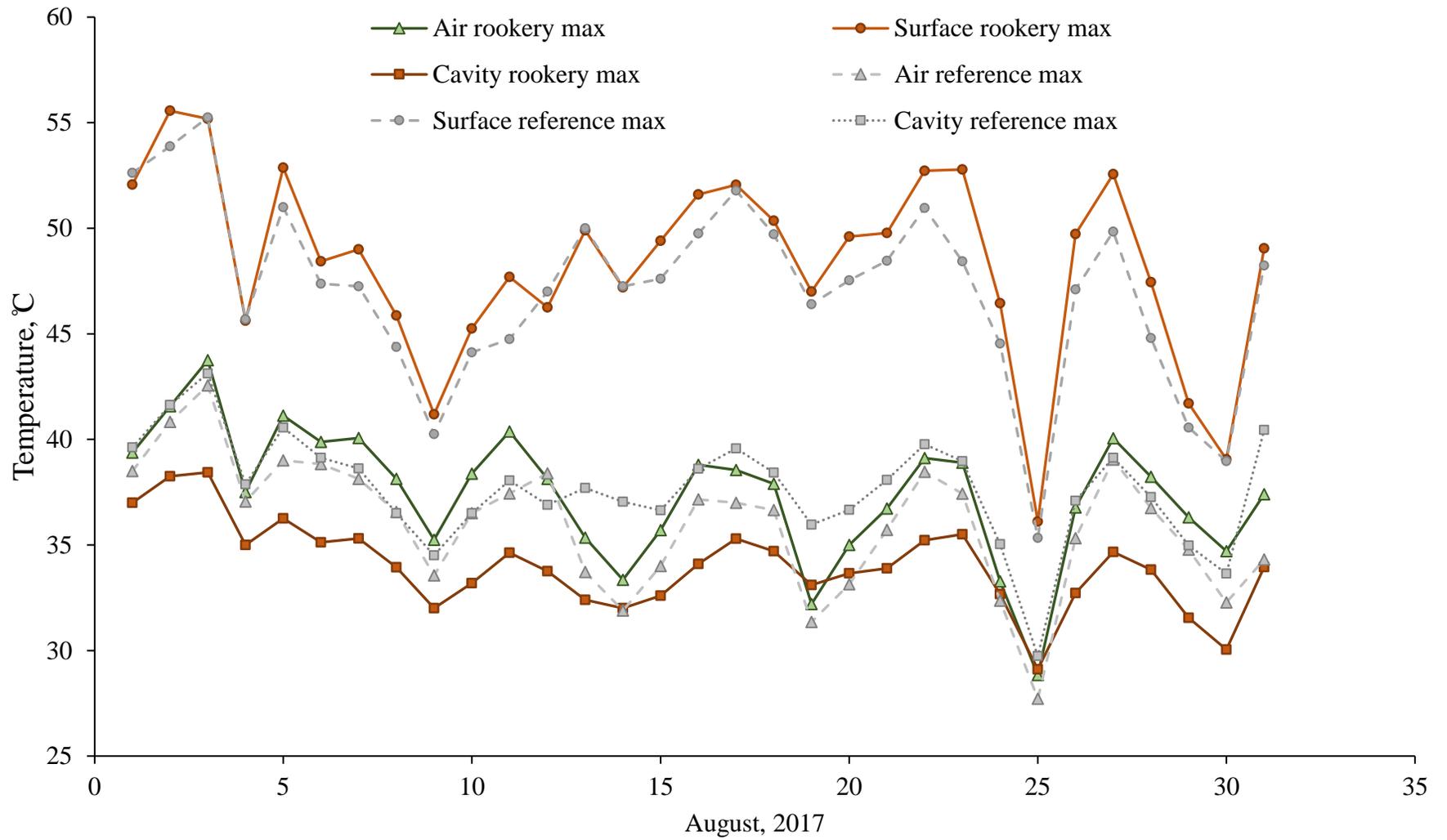


Figure 5. Fluctuations in 24 hr maximum air, surface and cavity temperatures between the rookery sites and reference sites in August, 2017.

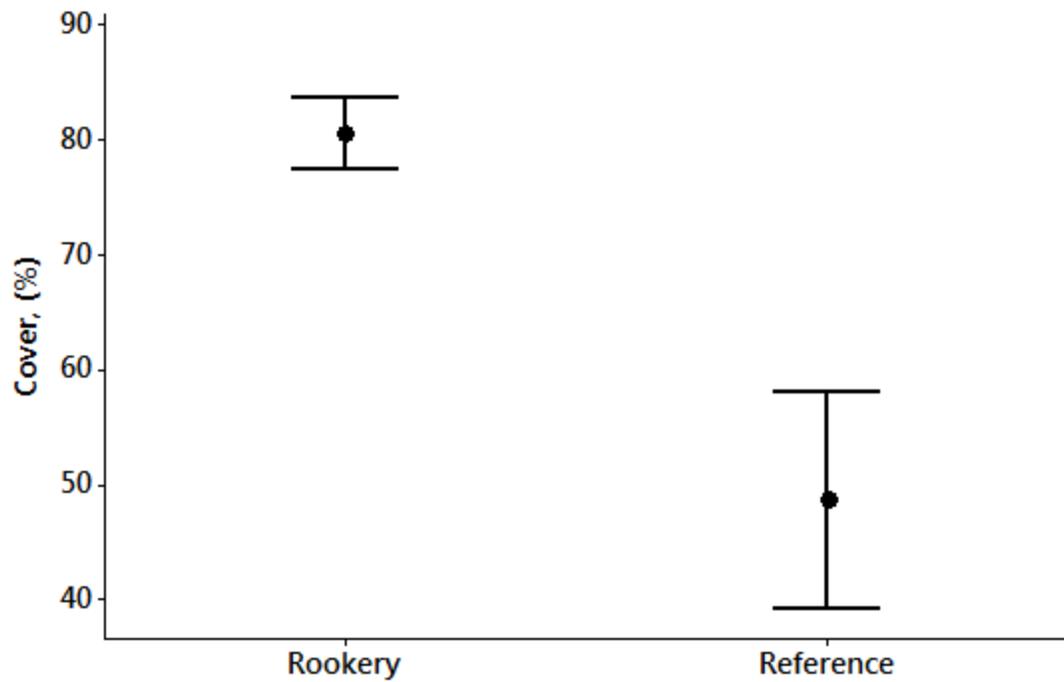


Figure 6. Estimated rock cover on rookery sites was significantly higher (80%) than on reference sites (50%) at 3 m scale (depicting SE for each site).

Discussion

This is the first study that monitored and evaluated habitat characteristics of the rookery sites used for parturition by Western Rattlesnakes in British Columbia. Similar habitat use studies were conducted on other snake species in North America, however, focus was made more on males and non-gravid females (Graves and Duvall 1993, Parker and Anderson 2007, Jenkins et al. 2009, Maag 2017). In general, little information is available on activity, behaviour and habitat use for gestation and parturition by gravid *Crotalus oreganus*. My research provides important information on timing, reproduction and habitat use by gravid rattlesnakes.

Observed timing of parturition and the behaviour of neonate attendance by a mother were consistent with other rattlesnakes populations (Parker and Anderson 2007, Maag 2017). None of the observed snakes shared the same rookery site which contradicts existing literature that identified communal rookeries (Graves and Duvall 1993a, Parker and Anderson 2007). For those three females that failed to reproduce, no abnormal behaviour, body condition or external factors were observed that could have negatively impacted their reproduction. Other studies have reported that reproductive failure and follicle resorption can occur in the wild rattlesnake populations due to environmental pressures (Graves and Duvall 1993, Parker and Anderson 2007).

In the second part of my study, I evaluated located rookery sites and identified characteristic habitat variables at three spatial scales. Estimated cover of vegetation was not directly associated with the rookery sites. When rookery and reference sites were compared, air and surface temperatures and the cover of rocks showed stronger association with the rookeries than other recorded habitat features.

On the smallest scale (1 m surrounding the rookery), higher minimum surface temperature had a strong association with the location of a rookery site. This could be that females preferentially seek out relatively warm sites to ensure the thermal requirement for gestation (Maag 2017). Since gravid females need to maintain higher body temperatures during gestation (Johnson et al. 2016, Maag 2017), it is likely that they would select for microsites that exhibit higher surface temperatures.

On a medium scale (3 m around a rookery), cover of rocks was relatively high and was a significant predictor of rookery sites. Since gravid females are more vulnerable to predation due to the decreased activity and mobility (Johnson et al. 2016), refugia (the cover of rocks) may be vital for their survival. Rock cover of 80% may provide protection from predators and create cooler environment within underground cavity (Gomez 2007). Cover of rocks also can play a role in structural diversity on the landscape providing refuge for small mammal prey (Tews et al. 2004). Other studies suggest that prey availability can predetermine reproductive success in females; therefore, it could be beneficial for gravid females to stay within high prey density areas (Jenkins et al. 2009). At present we lack information on prey abundance around rookeries, much less the predilection of gravid females to opportunistically feed, so this theory is primarily conjecture at this point. I recommend further investigations into rookery characteristics by conducting small mammal trappings in the immediate area of a rookery site, to determine whether the abundance of prey may influence rookery site selection.

On the large scale (10 m), average air temperature was the only variable positively associated with rookery sites. Higher air temperatures were reached on the rookery sites, likely through higher solar radiation that would be an aspect and cover-dependent (Weiss et al. 1988). If rookery sites are positioned on south-facing slope (true for all nine rookeries assessed herein) this site would be exposed directly to solar radiation in the Northern hemisphere. As stated earlier, the more heat is available, the more likely gravid females would meet their energetic demands (Johnson et al. 2016). However, it is hard to draw conclusions from these results: no replication of temperature was done throughout the plot which likely lead to an underrepresentation of temperature of the entire plot area. This issue could be resolved by sampling systematically air, surface and cavity temperatures at several random locations within each 10m plot. Similar method could be also used for a 3m plot to ensure more accurate estimation of temperatures at that scale.

All told, my analysis reveals an intuitive shift in characteristics of rookeries according to scales. Gravid females seem to select their environment to meet their energetic demands, hide from predators, and to have an immediate access to sun radiation for thermoregulation. Such information asks interesting questions on how reproductive

females find rookeries with such characteristics in the first place and whether these rookeries are regularly used for reproduction by other snakes in an area. Ongoing research that currently takes place in the Osoyoos study site may provide answers to these questions and address the idea of rookery fidelity by females.

Understanding the location of rookery sites and their associated habitat characteristics likely will provide an important tool for managing species habitat. Because gestation habitat plays a major role in providing sites for females' protection, embryo development, and parturition, this habitat can be considered integral for the recovery of Western Rattlesnake population (Southern Interior Reptile and Amphibian Working Group 2016). Since the majority of rookery sites lied within 142 m downslope from hibernacula, a buffer zone of 100 m-150 m radius around den sites could incorporate these rookeries. However, this recommendation for rattlesnake conservation cannot be extrapolated to other sites without parallel information being collected. Longer-term data at my study site also is warranted, and is in progress (Eye et al, unpubl.). If similar patterns are observed, conservation initiatives might be improved by establishing these buffer zones and minimizing human disturbance around hibernation and rookery areas.

This pilot study is an important first step in providing insight into this crucial but unstudied aspect of Western Rattlesnake life history. By understanding habitat use and site selection by gravid female rattlesnakes for parturition, sound management strategies could be developed to locate and protect their sensitive rookeries. I hope my research enables future work to be continued to aid in the conservation of this iconic and fascinating grassland species.

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Appendix A

Image of a protective shield for dataloggers used to measure air temperature in habitat plots



Figure A 1. A prototype of a double cone used for air temperature loggers. Distance between top and bottom cone edges is 3-5cm. An air logger was placed inside the top cone secured in a plastic Ziploc bag. Cone design was adopted from literature (Tarara and Hoheisel 2007).

Appendix B

Results of Pearson's correlation analysis for environmental variables

Table B 1. Summarized correlations for air, surface and cavity temperatures collected at rookery sites and reference sites (Pearson's correlation, n=27, Minitab).

Temperature 1	Temperature 2	Correlation coefficient (r-value)	Significance of correlation (p-value)
Average air	Average surface	0.482	0.013
Average air	Maximum air	0.664	0.000
Average surface	Average cavity	0.837	0.000
Average surface	Maximum air	0.477	0.014
Average surface	Maximum surface	0.750	0.000
Average surface	Min surface	0.398	0.044
Average surface	Minimum cavity	0.374	0.060
Average cavity	Maximum air	0.375	0.059
Average cavity	Maximum surface	0.696	0.000
Average cavity	Minimum surface	0.365	0.067
Average cavity	Minimum cavity	0.430	0.028
Maximum air	Maximum surface	0.425	0.030
Maximum surface	Maximum cavity	0.369	0.064
Maximum cavity	Minimum cavity	0.673	0.000
Minimum air	Minimum surface	0.642	0.000
Minimum surface	Minimum cavity	0.552	0.003

Table B 2. Pairs of strongly correlated explanatory habitat variables measured at rookery sites and associated controls (Pearson's correlation, n=27, Minitab).

Scale	Variable 1	Variable 2	Correlation coefficient (r-value)	Significance of correlation (p-value)
1 m	Cavity depth	Rock cover	0.529	0.005
	Shrub cover	Grass cover	0.475	0.012
	Shrub cover	Rock cover	0.615	0.001
	Grass cover	Rock cover	0.546	0.003
3 m	Shrub cover	Grass cover	0.443	0.021
	Shrub cover	Rock cover	0.588	0.001
	Grass cover	Rock cover	0.376	0.053
10 m	Grass cover	Rock cover	0.608	0.001