

Can Florida Softshell Turtles (*Apalone ferox*) Use Nest Site Choice to Offset
Climate Change Effects on Developing Embryos in Nests?

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Conservation Biology
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Date of Approval:
3/13/20

Keywords: behavioral adaptation, hemispherical photography,
Gap Light Analyzer[®], canopy cover, linear regression modeling

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Acknowledgements

I would like to thank my advisor, Dr. Sean Doody, for assisting me on each and every step of this project. I could not have completed this thesis without his advice and support. I would also like to thank my other committee members, Dr. Deby Cassill and Dr. Alison Gainsbury, for their knowledge and advice throughout this process. I would like to thank George Heinrich for introducing me to nesting areas at Boyd Hill Nature Preserve and his continued assistance throughout the project. I would like to thank Colin Shae for his assistance with linear regression modelling in R. I would like to thank Boyd Hill Nature Preserve, Myakka River State Park, and Sawgrass Lake Park for allowing me to study the Florida softshell turtle nests throughout each site. A big thank you to all of the volunteers who helped me survey Boyd Hill Nature Preserve: Ide Messmore, Michelle Mark, Jeremiah Paddock, Alek Bussell, Kelsi Jackson-Smith, Helen Payne, and Alexis Montero. Lastly, I would like to thank all of my family and friends who have made it through the last three years with me.

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Abstract

Over the next 100 years, climate change will impact nearly all species. To survive climate change, species will need to shift their ranges, or develop adaptations that offset rising temperatures. To prevent rising temperatures from influencing nest temperatures, oviparous ectotherms such as freshwater turtles will need to adapt their physiology (i.e., raise the range of temperatures suitable for developmental processes) or their behavior (i.e., through nest site choice). Theory suggests that behavior is more evolutionarily labile than physiology, generating the prediction that turtles will use nest site choice to offset climate warming. In the present study, I quantified nest site choice in the Florida softshell turtle (*Apalone ferox*) across three sites in Central Florida to test the hypothesis that the species can use nest site choice to counter climate change by nesting in more shaded areas that receive less incident solar radiation and experience lower nest temperatures. Canopy openness and incident radiation intensity at each nest site and potential nest site were calculated using hemispherical photography and Gap Light Analyzer[®]. To establish links among canopy openness, incident radiation intensity, and nest temperatures, continuous nest temperatures were recorded for a subset of nests and potential nests using Thermochron[®] i-button data loggers. The nesting season of *A. ferox* ranged from late March to late July. Canopy openness ranged from 6.84 - 70.89%, and had a significant, positive relationship to incident radiation intensity. Likewise, nest

temperatures had a significant, positive relationship to incident radiation intensity. Potential nest sites in shade were abundant at all study sites and at all nesting areas within those sites. These potential nest sites were significantly less open, received less incident radiation, and exhibited lower temperatures (at nest depth) than chosen nest sites. *A. ferox* utilized a wide range of canopy cover, and could offset increasing nest temperatures by nesting in more shaded areas with cooler potential nest temperatures, suggesting there is scope for softshell turtles to use nest site choice to counter, at least to some extent, current and future climate warming. Although we cannot assess the rate and ability of turtles to evolve nest site choice behavior against the rate of current and future climate change, our study provides evidence that natural selection for nesting in more shaded areas is a plausible mechanism turtles possess in their repertoire for responding to climate change.

1. Introduction

1.1 Climate Change

1.1.1 Climate Change Background

Rapid, human-induced climate change is impacting global ecosystems and affecting nearly every species through shifting temperature extremes. Anthropogenic greenhouse gas (GHG) emissions have steadily increased since the pre-industrial era (Pachauri et al., 2014). From 2000 to 2010, GHG emissions were the highest in recorded history. Nearly 80% of the increase seen in GHG emissions can be traced to carbon dioxide (CO₂) from fossil fuels. Forty percent of the anthropogenic CO₂ accumulated since 1750 remains in the atmosphere, while natural sinks in both the soil and the ocean have absorbed the rest. Despite the implementation of climate change mitigation policies, fossil fuel emissions continue to increase every year (Pachauri et al., 2014; Rogelj et al., 2016).

There are a multitude of anthropogenic GHG effects on the climate (Pachauri et al., 2014; rogelj et al., 2016). Excess CO₂ in the atmosphere is the primary cause of rising temperatures. The CO₂ in ocean sinks has led to ocean acidification and ocean warming. Rising temperatures, both in the ocean and the air, have contributed to a loss of ice mass at the north and south poles and throughout glaciers globally. Loss of mass in ice formations has led to rising sea

levels. These effects are compounded by an increase in extreme weather patterns (Pachauri et al., 2014; Rogelj et al., 2016).

While there are many interacting factors, the primary issue in climate change is still increasing temperatures. Projections indicate within 90% probability that temperatures could rise by as much as 4.4 °C by 2100 (Rogelj et al., 2016). The United Nations Framework Convention on Climate Change (UNFCCC) created a set of Intended Nationally Determined Contributions (INDCs) comprised of targets and actions each country can implement to address climate change. If these targets are reached, global temperatures will increase by up to 3.7 °C by 2100 when scaled to contain a 90% probability (Rogelj et al., 2016).

1.1.2 Climate Change and Extinction

Currently, 7.9% of all species are predicted to become extinct due to climate change, while almost all species will be impacted in some way (Urban, 2015). Regional distribution, small ranges, and localized or endemic populations leave certain species predisposed to disproportionate climate change impacts (Pacifci et al., 2015). Coastal regions will have a higher rate of species extinction due to rising sea levels. This will be compounded in areas where dispersal from the coastline is limited (Urban, 2015). Ecosystems with high temperature extremes, such as deserts and tropical rainforests, will have an increased likelihood of seeing temperature increases outside of the survivable range. Species in these climates will need to migrate away from, or adapt to, these extremes (Urban, 2015). For specific species, regional distribution is

compounded by a small range of distribution. Species with smaller ranges have a higher rate of extinction from catastrophic local events (Urban, 2015). Localized and endemic species compound the issues found in small range species. Without multiple populations, local population extinctions can result in species' extinction (Urban, 2015). Outside of direct effects, climate change will also compound extinction events caused by other environmental factors (Pacifi ci et al., 2015).

With rapid climate change, species realistically have two mechanisms for survival: range shifts and evolutionary adaptation (Moritz & Agudo, 2013). Range shifts to areas that are trending towards the climatic niche is the most likely adaptation (Moritz & Agudo, 2013). As conditions become hostile in the local ecosystem, species will need to migrate to a range that still represents their natural climatic conditions. The ability to move away from their native range varies by species. Species with limited mobility may be unable to shift their range fast enough to keep up with the rate of climate warming. Moreover, some species may be unable to shift their range to adapt to factors such as rising sea levels if the inland habitat has been repurposed for industrialization or agriculture (Pacifi ci et al., 2015). Species unable to shift their range will need to evolve adaptations to compensate for a shifting climate. To adapt to climate change, species must have the appropriate heritable traits available in their gene pool (Moritz & Agudo, 2013).

1.2 Regulating Temperature

1.2.1 Regulating Body Temperature

Reptiles and other ectotherms will be uniquely impacted by climate change due to how they regulate their body temperature (Deutsch et al., 2008). Endothermic species are able to regulate their body temperature to a wide variety of external conditions through the use of metabolic heat (Porter & Kearny, 2009). When an endotherm is in a temperature range that is near their ideal body temperature they maintain a basal metabolic rate (BMR) which can be viewed as the baseline rate of their metabolism. When the external temperature becomes significantly lower or higher than their ideal body temperature, the metabolic rate of the individual endotherm will rise to maintain an ideal temperature (Porter & Kearny, 2009).

Ectotherms are unable to regulate their body temperature internally through metabolic heat (Bogert, 1959). To maintain an ideal body temperature, ectotherms utilize behavioral adaptations to maintain thermoregulation. Methods for thermoregulation in ectotherms vary primarily due to the species' environment. For terrestrial ectotherms, the primary method for increasing body heat is basking. Reptiles often utilize terrain angled directly toward the sun's rays and position themselves at a 90 degree angle to the rays. When their body temperature becomes too warm, they primarily regulate it by moving away from the sun, often underground (Bogert, 1959).

Thus, ectothermy leaves species uniquely vulnerable to climate change as a shift in temperature extremes will affect their body temperature and therefore

their physiology (Sinclair et al., 2016). Ectotherms adjust to seasonal climatic changes through behavioral responses that allow them to exploit their environment (Hutchison & Maness, 1979). As temperatures climb to impactful or lethal limits, ectotherm species must adapt. Those unable to shift their range must develop behaviors that will allow them to persevere in harsher climates (Woods et al., 2015). To ensure these behaviors continue, the traits must be heritable by future generations (Moritz & Agudao, 2013).

1.2.2 Regulating Incubation Temperature

Most ectotherms are oviparous. Laying eggs leaves a species' offspring vulnerable to external climates. To adapt, species can utilize microclimate conditions to adjust the incubation temperature of their nests. Microclimates can be adjusted through nest site construction, phenology, or nest site choice (Doody et al., 2006a). Nest site construction primarily affects egg temperatures through nest depth (Georges, 1989). For example, nest temperatures in the top and bottom of pig-nosed turtle nests differ by as much as 3.5 °C (Georges, 1989). By adjusting nest depth, nesting females can theoretically affect temperatures of incubating eggs (Georges, 1989; Georges, 1992). Phenology, in the form of seasonal timing of nesting, can affect nest temperature due to variations in external temperatures throughout the nesting season (Doody et al., 2004). Typically, nest temperatures earlier in the season are cooler than those later in the season (e.g., Doody, 1995). Nest site choice includes attributes actively chosen by nesting females such as canopy cover/openness, slope, aspect, ground cover, and substrate type. Ultraviolet (UV) radiation is a product of the

canopy openness above a nest site. Painted turtles (*Chrysemis picta*) use vegetation cover to determine where to nest during oviposition to ensure offspring sex through temperature sex determination (TSD) (Janzen, 1994).

Increasing temperatures due to climate change will have a greater effect on oviparous species due to immobile, developing embryos. In olive ridley sea turtles (*Lepidochelys olivacea*), incubation temperatures over 35°C are 100% lethal to developing embryos (Valverde et al., 2010). Oriental garden lizards' (*Calotes Versicolor*) hatching success at 33°C is only 3.4%, with hatchlings emerging significantly smaller than embryos incubated at 27°C and 30°C respectively (Ji et al., 2002). If increasing temperatures are not compensated for during nest site choice, oviparous species will face a greater risk of extinction (Telemeco et al., 2009).

1.3 Project Proposal

Ectotherms can use nest site choice to achieve optimal or suitable incubation conditions, thereby preventing detrimental or lethal temperatures (Adolph & Porter, 1993). Multiple attributes affect the incubation temperature of the nest, such as season, depth, and canopy cover, which in turn are influenced by behavioral choices of the nesting female. As environmental temperatures increase under future climate warming, species may need to adjust one or more of these factors, or adjust the physiology of developing embryos. Theory suggests that behavior would be more evolutionarily labile than physiology in a climate change scenario (Huey et al., 2003).

But are nesting females able to compensate for climate change by adjusting nest site choice behavior across generations? In theory, to offset climate warming, nesting females can achieve cooler incubation temperatures by nesting earlier, deeper, or in more shaded areas (Ewert et al., 2005; Doody et al., 2006a). However, this requires (1) environmental variation available to nesting females in nature. For example, do reptile nesting females have access to more shaded microhabitats or deeper friable soils to offset a warming climate? And, (2) how well does this variation in nest site choice attributes (e.g., canopy cover) and nest temperatures during nest site choice predict those same attributes and temperatures during the entire incubation period? And, finally, (3) is there heritable variation in those behavioral traits manifested in the choice of a nest site? Janzen and Morjan (2001) found that while 207 painted turtle (*Chrysemys picta*) nests had a range of canopy cover from 0.5% to 90.6%, individual females consistently nested under very similar levels of canopy cover. This suggests adaptive potential in the use of canopy cover (Janzen & Morjan, 2001).

1.3.1 Objectives

I studied nest site choice and nest temperatures in three populations of the Florida Softshell Turtle, *Apalone ferox*, in southwestern Florida. Specifically, I tested the following hypotheses: (1) nesting female turtles currently use a wide range of canopy cover, and this is significantly positively related to incident (solar) radiation and nest temperatures; (2) a range of potential nest sites with higher canopy cover and cooler 'nest' temperatures are available to nesting females; and (3) nest temperatures increase with the seasonal increase in air

temperatures during the South Florida spring and summer, rather than decrease due to an increase in cover that affects nest temperatures.

2. Materials and Methods

2.1 Study Organism

The study species was the Florida softshell turtle (*Apalone ferox*). With a carapace length of up to 41 cm, *A. ferox* are the largest species of the North American softshell turtles (Iverson & Moler, 1997), with females reaching masses that are 3-5 times larger than their male counterparts (Meylan, 2006). Softshell turtles are named after their large cartilaginous shell which is covered in a layer of soft skin rather than hard scutes, and they have lost their marginal bones. They are also identifiable from their long neck and unique tubercle snout. *A. ferox* are distinct from other species of *Apalone* due to their larger size and a section of well-developed low tubercles on the anterior portion of their carapace. Their native range starts as far north as South Carolina and Southern Alabama expanding south to Southern Florida (Ernst & Lovich, 2009). Although the Florida Keys are not part of their native range, there is an introduced population on Big Pine Key (Meylan, 2006). Most commonly found in waters less than one meter deep, they utilize a wide range of freshwater habitats including ponds, lakes, rivers, canals, and watersheds (Ernst & Lovich, 2009). The species spends nearly all of its time either in the water, basking at the surface of the water, or on floating vegetation. Individuals rarely leave the line of sight of a freshwater source, even during nesting (Meylan, 2006). Analyzing the stomach contents of

96 individuals, Dalrymple (1977) found the majority of their diet includes fish, insects, crayfish, and snails. Nest predation by raccoons, foxes, skunks, and crows is common (Hamilton, 1947), with raptors also preying on hatchlings (Woodin & Woodin, 1981). Analyzing the stomach contents of American Alligators throughout Florida, *A. ferox* were not identified (Delaney et al., 1988).

The Florida softshell turtle was chosen for the present study due to its nesting habits. Eggs from *A. ferox* are easily identifiable from other species as they are spherical rather than elongated with a hard, brittle shell on the outside. The eggs are white and a sample of 317 eggs had a mean maximum diameter ranging from 24.5 mm to 30.5 mm (Iverson & Moler, 1997). This allows for species identification from egg shells alone, which was important in the present study because of the high rate of nest predation by raccoons (see Results). Other freshwater turtle species situated throughout the study range have elongated eggs that lack the hard, brittle outer layer found on the eggs of *A. ferox*.

Gopherus polyphemus (gopher tortoise) also lay spherical eggs with a hard outer coating, however, the mean maximum diameter of 183 *Gopherus polyphemus* eggs ranged from 40.5 mm to 53.2 mm (Landers et al., 1980) allowing eggs to be differentiated by size. Moreover, most gopher tortoise eggs are deposited in the apron (mound) near the mouth of the burrow. Florida softshells nest primarily in sandy soils (Ehrenfeld, 1979) with a narrow neck not much larger than the size of one egg (Meylan, 2006). Nest chambers have been recorded at 13 cm deep and 10 cm wide (Hamilton, 1947), and 14 cm deep and 9 cm wide (Heinrich & Richardson, 1993). However, nests of *A. ferox* have been found in alligator nests

(Enge et al., 2000) and one was found in the apron of a gopher tortoise burrow (Heinrich & Richardson, 1993). Although quantitative data are lacking, nests are generally deposited between the end of March and the end of July (Goff & Goff, 1935; Iverson, 1985). The mean clutch size of *A. ferox* is 20 eggs with clutches ranging in size from nine to 38 eggs (Iverson & Moler, 1997). Females usually lay 3-5 clutches each year but can lay as few as two and as many as seven (Iverson & Moler, 1997). Clutch mass is positively correlated to body mass in females, with individual clutches deposited roughly three weeks apart (Iverson & Moler, 1997). The incubation period ranges between 56 and 80 days (Lardie, 1973; Heinrich & Richardson, 1993). A study on *Apalone mutica* (smooth softshell turtles) found that incubation length was negatively proportional to incubation temperature (Janzen, 1993), a common pattern in reptile eggs (Ewert, 1985). Janzen (1993) demonstrated that the sex of the hatchlings is independent of the incubation temperature in the the congener *Apalone mutica*. The presence of sex chromosomes in another congener, *Apalone spinifera* (Badenhorst et al., 2013), suggests that Florida softshells also possess genetic sex determination.

2.2 Study Sites:

I studied nest site choice in three populations. The primary study population was at Boyd Hill Nature Preserve (BHNP) in Pinellas County, Florida (27.7245°N, 82.6499°W). A 1.5 km trail running along Lake Maggiore served as the study transect. Lake Maggiore is a shallow, 146.901 hectares lake surrounded by neighborhoods on one side and BHNP on the other side. Nesting

habitat at BHNP ranges from hammock to sandy scrub to pine flatwoods, with transitional habitat between each type evident throughout the study site.

The second study population was in Myakka River State Park (MRSP), a 150 square km park in Sarasota County (27.1883°N, 82.2623°W). An 800 m long study site at MRSP follows a fire track. The fire track is bordered by watersheds on each side fed by the Myakka River. The watersheds fluctuate in depth and connectivity seasonally. On either side of the watersheds are hammocks. The fire track is elevated in between the two watersheds.

The third study population inhabited Sawgrass Lake Park (SLP), which is located in northern Pinellas County (27.8413°N, 2.6723°W). The study site is a 365 m path that borders three separate ponds. The path is bordered by wetland hardwood habitat.

2.3 Nest Surveys

Nest surveys were conducted on foot at each site. The majority of nests were discovered by finding eggshells from eggs eaten by predators (mostly by racoons). Nearly all nest predation of freshwater turtle species occurs within 24 hours of nest construction (e.g., Spencer, 2002). Although my sample was largely depredated nests, I assumed that the large sample size and large study transect would yield a sample representative of the nesting effort at the sites. In other words, I assumed that nest site choice attributes did not differ between depredated and intact nests. In support, there has been little evidence for nest site choice influencing the probability of nest predation in turtles (Burke et al., 1998; Doody et al., 2003). Furthermore, the major predator was the Raccoon

(*Procyon lotor*), an olfactory-driven predator that may prey randomly on turtle nests with respect to vegetation and other nest site attributes.

As the primary site, BHNP was surveyed six days a week (no nest surveys were conducted on Mondays as the park was closed). Near-daily nest surveys ensured that I obtained lay dates at BHNP. The site was surveyed for a minimum of one hour each day between sunrise and sunset from 26 March to 18 August, after which bi-weekly surveys were conducted for one more month; daily surveys were stopped after no new nests were found for three weeks.

MRSP was surveyed two days each week with no more than four days between surveys, allowing lay date of depredated nests to be estimated to within four days. Surveys were conducted bi-weekly from 26 March to 19 August to coincide with surveys at BHNP. Surveys were conducted between sunrise and sunset and lasted a minimum of one hour.

SLP was surveyed every three weeks, making the estimation of lay date less accurate. The first survey at SLP was conducted on 29 May and the last survey was conducted on 8 August.

2.4 Data Collection

For each nest I recorded the estimated lay date, the distance to the nearest water source, and the type of water body. Lay date was estimated as the median date between consecutive nest surveys. Because raccoons mainly hunt at night and prey upon nests within 24 to 48 hours of the lay date (Burger, 1977), depredated nests discovered were considered to have been deposited the previous day during surveys on consecutive days. The distance to water was

calculated to the nearest 0.5 m for each nest site. To quantify canopy cover, I took hemispherical photos from the nest site. At BHNP and MRSP additional hemispherical photos were taken 30 and 60 days after the estimated lay date. These photos were used to calculate the percentage of canopy openness above the nest site and the incident radiation intensity at the nest site. At BHNP and MRSP ground cover photos were also taken at each nest site. These photos were taken immediately after a nest was deposited as well as at 30 and 60 days after the estimated lay date. Ground photos were used to calculate ground cover within one square meter around each nest and determine the primary ground cover type.

At BHNP and MRSP females typically deposited nests in clusters along the trails, with gaps existing with no nests between those clusters. These clusters were labeled as separate nesting areas. To determine the range of canopy covers available to nesting turtles, I created a fake nest at the most open (sunniest) and at the most closed (shadiest) sites within 50 m of the nesting area. At each of these potential nests the same nest site data was collected as was collected for the actual nests.

2.4.1 Intact Nests

A Thermochron[®] i-button temperature data logger was placed at the bottom of the nest chamber in intact nests. Data loggers were employed soon after laying and were collected after nests hatched. The number of eggs in the nest, depth to top egg, and depth to bottom egg was collected for all intact nests. Nest depths were measured to the nearest mm. Intact nests were then caged to

prevent predation, with cages removed after 55 days with the range of incubation of 56-80 days (Meylan, 2006). Cages were constructed using the below-ground method from Riley and Litzgus (2013) using 1 cm hardware cloth designed to be 30 cm long, 30 cm wide, and 20 cm deep. The cage was buried just below the surface. There was no base to the cage.

2.4.2 Canopy Openness and Incident Radiation Intensity

Canopy openness and incident radiation intensity were analyzed following Doody et al., (2006b). Hemispherical photos were taken using a Nikon Coolpix 4500 with a Nikon fisheye converter FC-E8 0.21x, allowing for 180 degree fisheye photos with a 360 degree view of the horizon (wide-angle). The photos were taken by placing the camera level on the nest site with the camera facing due north as located on a compass. The photos required uniform light which means they had to be taken either on a uniformly cloudy day or during the first hour or last hour of light (reflections from leaves during bright conditions can create an error in the calculation of canopy openness, and thus, incident radiation intensity). All photos were taken within one week of the date the nest was deposited. Photos taken 30 and 60 days after the nest was deposited were taken within one week of the 30 and 60 day dates. Photographs from potential nest sites were taken once, when the fake nest was made.

Photographs were analyzed using Gap Light Analyzer[®] (hereafter GLA; Frazer et al., 1999). Uploaded images were registered in the program with Due North specified. The elevation and latitude of the site and the horizontal position of the photo (not taken at an angle) were input into the configuration settings.

The date the nest was laid and an estimated hatching date were also entered in order to determine how many days incident radiation intensity should be measured. To allow for all nests to be compared to each other, all hatching dates were estimated as 60 days after the estimated lay date, which is the earliest a nest would hatch (Meylan, 2006). This information, along with the photograph, allowed GLA to calculate canopy openness and to track the path of the sun across the openness, thus calculating incident radiation intensity. Incident radiation intensity was calculated in MJ/m²/day (Frazer et al., 1999). All potential nests had the same data input, except that the lay date was listed as the mean lay date of all nests: 7 June. In this way, all potential nests could be compared directly without any bias introduced by seasonal effects.

2.4.3 Ground Cover

Ground cover was estimated by photographing the area around each nest or potential nest site. Photos were taken using an iPhone XR. Photos were taken of a one square meter quadrat around each nest site using two measuring tapes. Photos taken 30 and 60 days after the nest was deposited were taken within one week of the 30 and 60 day dates. Ground coverage was calculated to the nearest 10 per cent for the amount of ground cover inside of the one square meter quadrat. Ground cover was calculated as none, grasses, or understory. None was only used if ground cover was zero percent. Understory was considered to be any foliage that was not grass. If both types of ground cover were present, the type that was most prevalent was used. All photos were taken within one week of the lay date. Ground photos of potential nests were taken toward the end of the

nesting season so potential nests with new growth of understory or grasses could be documented.

2.5 Nest temperatures

Thermochron[®] i-button (Maxim) data loggers were used to record continuous nest temperatures. Data loggers were collected and uploaded using ExpressThermo. Data loggers were set to record temperature every 90 minutes, which provided up to 128 days of continuous nest temperature data for each logger. All data loggers at BHNP were removed on 26 September, 60 days after the last nest was deposited. All data loggers at MRSP were removed on 27 September, also corresponding with the date the last nest was deposited. No data loggers were employed at SLP. Data loggers were placed in all potential nests to create a baseline of temperatures available at each nesting area. An intact nest found early in the season had a depth of bottom egg of 20.7 cm, deposited by a large female. Two prior recorded nest depths were 13 cm deep (Hamilton, 1947) and 14 cm deep (Heinrich & Richardson, 1993). Data loggers were thus placed at the mean depth of these three nests: 16 cm. On 15 July it became clear that not enough intact nests would be found to quantify a robust sample size of nest temperatures. At this point, data loggers were placed in depredated nests that were deposited within the last 10 days before 15 July, and all depredated nests deposited after this date. Data loggers were placed in new depredated nests within one week of the nest being deposited.

2.6 Statistical Analysis

All data analyses were completed in R 3.6.1 (R Core Development Team, 2019) with the packages 'FSA,' 'PMCMR,' 'lme4,' 'MuMIn.' I used a Shapiro-Wilk test to analyze normality and Levene's test to compare homogeneity of variance for data both between sites and data collected throughout the 60-day estimated incubation period. When analyzing data throughout incubation, a non-parametric Friedman test was used in place of a repeated measures Anova with the posthoc Friedman conover test. To test whether the canopy openness and incident radiation intensity at nest sites were significantly different than those at potential nests (sunny and shaded sites), I used two-tailed t-tests. I tested whether canopy openness differed between nest sites using a Kruskal-Wallis one way analysis of variance with Dunn's multiple comparison test as the post-hoc test. The Kruskal-Wallis one way analysis of variance and Dunn's multiple comparison test were used with the complete data sets, as well as with a random sample of 27 nests, from BHNP so that each site had an equal sample size.

I used a linear regression model to assess the differences in incident radiation intensity at individual nests between lay date, nest site, and percent openness. I fit six regression models with different combinations of lay date, nest site, and percent openness. One of the models expressed lay date as a quadratic in case lay date was significantly different in the middle of the season, compared to the beginning and end of the season. I used Akaike's Information Criterion with a small sample bias adjustment (AICc) to assess the best model. The lower the

AICc, the more accurate the model. I used 95% confidence intervals to assess the precision of the parameters of the model.

Linear analysis was used to test whether canopy openness and incident radiation intensity were related to mean, maximum, and minimum temperatures. The test was run across all 35 data loggers, as well as separately for nest sites, potential sunny nest sites, and potential shaded nest sites.

3. Results

I found 156 total nests across the three sites, all of which were destroyed by predators (Table 1). Of these, 102 were found at BHNP, 27 at MRSP, and 27 at SLP (Table 1). There were 15 sightings of *A. ferox* on land searching for a nest site, but only one intact nest was found. Fourteen of the sightings, including the one intact nest, were at BHNP, while one sighting was at MRSP. There were no *A. ferox* sightings of turtles looking for a nest site at SLP.

3.1 Seasonal Timing of Nesting

The nesting season for *A. ferox* during the present study was late March to late July (Fig. 1). Early surveys on 16 February at MRSP and 2 February at BHNP revealed no nests. The first survey at BHNP on 26 March revealed one depredated nest with eggshells still wet with yolk, indicating the nest was very recently deposited. The first survey at MRSP on 29 March revealed one depredated nest that had been deposited in the prior six weeks, since the last survey at the state park. The final Nest at BHNP was found on 30 July, and the final nest at MRSP was found on 26 July. One nest was discovered during the 8 August survey at SLP. At BHNP, 44 of 102 nests (43%) were deposited in June (Table 1). At MRSP, April and May had the most nests with eight (30%) deposited each of those months (Table 1). At SLP, of the 27 nests, 14 nests (52%) were deposited before 29 May (Table 1).

3.2 Nest Site Choice

3.2.1 Intact Nest Sites

One female was discovered in the act of nesting at BHNP on 2 April 2019. She laid 25 eggs, with a depth to top egg of 6.5 cm and a depth to bottom egg of 20.7 cm. Although the nest was caged to prevent predation, a predator (apparently a raccoon) dug under the corner of the cage and took the eggs on 6 April, after attempting to do so on previous nights. The data logger employed in the nest was lost (apparently also taken by the predator).

3.2.2 Canopy Openness

Females in the current study nested in areas with intermediate canopy cover; most nests were deposited in relatively open areas, but not generally in full shade or in the most open areas. Mean canopy openness differed significantly among study populations, both with all nests at BHNP (Fig. 2: $X^2=23$, $df=2$, $p<0.0001$) and with a random sample of 27 nests from BHNP ($X^2=18.5$, $df=2$, $p<0.0001$). BHNP and SLP had no significant difference in canopy openness (all nests: $p=0.375$; BHNP sample: $p=0.365$). Canopy openness at MRSP was significantly different from BHNP (all nests: $p=0.0001$; BHNP sample: $p=0.0014$) and SLP ($p=0.0002$). Mean openness of all nests was $36.9\% \pm 13.92$ (Table 2). Mean openness of 102 nests at BHNP was $35.33 \pm 14.55\%$, compared to $46.80 \pm 10.04\%$ for 27 nests at MRSP and $32.65 \pm 10.07\%$ for 27 nests at SLP (Table 2).

Canopy openness at potential nest sites in the most open areas (sunny potential nest sites) was not significantly higher than openness of nest sites at

BHNP, but was significantly higher at MRSP ($t=2.60$, $df=30$, $p=0.0145$) (Fig.'s 3 and 4). Mean canopy openness of the sunny potential nest sites at all study sites was $46.46 \pm 17.3\%$ (Table 3). Canopy openness at potential nest sites in the most shaded areas (shaded sites) was significantly lower than openness of nest sites at both BHNP ($t=11.262$, $df=110$, $p<0.0001$) and MRSP ($t=4.89$, $df=30$, $p<0.0001$)(Fig.'s 3 and 4). The mean canopy openness of the shaded potential nest sites was $17.44 \pm 7.59\%$ (Table 3).

3.3 Consequences of Nest Site Choice

3.3.1 Incident Radiation Intensity

The mean incident radiation intensity for all nests combined was 5.64 ± 2.06 MJ/m²/d (Table 2). Model selection results based on AICc shows increased support for only one model (Table 4). The model (Fig. 5; weight=0.873) shows that incident radiation intensity was significantly explained by canopy openness (Fig. 6; $t=26.487$, $p<0.0001$), lay date (table 5; $t=-2.927$, $p=0.0040$), and nesting area (Fig. 2; MRSP; $t=2.203$, $p=0.0292$)(Fig. 5). This model was 15.32 times ($0.873/0.057$) more probable than the next best model (Table 4). Incident radiation intensity at BHNP and SLP were not significantly different, while MRSP had significantly higher incident radiation intensity than the other two sites (Fig.'s 2 and 5). Incident radiation intensity was significantly higher during the middle of the season than at the beginning or the end of the season (Table 5; Fig. 5). There was a significant positive relationship between canopy openness and incident radiation intensity (Fig. 6; $R^2=0.8476$, $F_{1,54} = 836.0$, $p<0.0001$).

3.3.2 Available Range of Incident Radiation Intensity

Mean incident radiation intensity of the most open potential nest sites (sunny sites) at all study sites combined was 7.39 ± 1.92 MJ/m²/day (Table 3). Mean incident radiation intensity of sunny potential nest sites was not significantly higher than that of nest sites at BHNP, but was significantly higher at MRSP ($t=2.65$, $df=30$, $p=0.01282$) (Fig.'s 3 and 7). Mean incident radiation intensity of potential shaded nest sites was 2.27 ± 1.41 SD/SE MJ/m²/d (Table 3). Mean incident radiation intensity of potential nest sites in the most shaded areas (shaded potential nest sites) was significantly lower than that of nest sites at both BHNP ($t=10.74$, $df=110$, $p<0.0001$) and MRSP ($t=6.13$, $df=30$, $p<0.0001$) (Fig.'s 3 and 7).

3.3.3 Nest Temperatures

Across all (35) potential sunny nest sites, potential shaded nest sites, and nests, mean temperature had a significant relationship to incident radiation intensity (Fig. 8; $R^2=0.82$, $F_{1,33}=154.1$, $p<0.0001$) and percent canopy openness (Fig. 9; $R^2=0.65$, $F_{1,33}=64.88$, $p<0.0001$). Maximum temperature had a significant relationship to incident radiation intensity (Figure 10; $R^2=0.61$, $F_{1,33}=53.12$, $p<0.0001$) and percent canopy openness (Figure 11; $R^2=0.49$, $F_{1,33}=33.03$, $p<0.0001$). Minimum temperature was not significantly related to incident radiation intensity ($R^2=0.041$, $F_{1,33}=0.03$, $p=0.8403$) or canopy openness ($R^2=0.131$, $F_{1,33}=0.03$, $p=0.7199$) across all potential and depredated nest sites. Figure 12 shows temperature readings taken every 90 minutes from six data loggers at nest area B6 (BHNP), and includes four nests, one shaded potential

nest site, and one sunny potential nest site. Figure 13 shows the one shaded potential nest site and one sunny potential nest site at nest area B6 (BHNP). Figure 14 shows one shaded potential nest site and one sunny potential nest site at nest area M1 (MRSP).

As expected, temperatures in nest sites and at potential nest sites increased markedly as the nesting season moved from early to late spring, with a more subtle decrease in temperatures later in summer, presumably due to increased cloud cover and rainfall during the South Florida summer wet season (Fig. 12). The grand mean nest temperature of 10 nests at BHNP was significantly positively related to incident radiation intensity (Fig. 15; $R^2=0.56$, $F_{1,8}=12.23$, $p=0.0081$), as were grand mean maximum nest temperatures (Fig. 16; $R^2=0.36$, $F_{1,8}=6.027$, $p=0.0396$), but not grand mean minimum nest temperatures ($R^2=0.11$, $F_{1,8}=0.134$, $p=0.7237$). Grand mean nest temperatures were not significantly positively related to canopy openness ($R^2=0.27$, $F_{1,8}=4.349$, $p=0.0705$), nor were grand mean maximum temperatures ($R^2=0.03$, $F_{1,8}=0.700$, $p=0.4275$) nor grand mean minimum nest temperatures ($R^2=0.1$, $F_{1,8}=0.181$, $p=0.6816$). Mean temperatures at these nest sites ranged from 27.00 °C to 29.78 °C (Table 6). The lowest minimum temperature across all ten nest sites was 23.5 °C and the highest maximum temperature was 32.5 °C (Table 6).

Grand mean temperatures of 12 shaded potential nest sites at BHNP and MRSP were significantly positively related to incident radiation intensity (Fig. 15; $R^2=0.66$, $F_{1,10}=22.61$, $p=0.0008$), as were grand mean maximum temperatures (Fig. 16; $R^2=0.37$, $F_{1,10}=7.351$, $p=0.0219$), but not grand mean minimum

temperatures ($R^2=0.1$, $F_{1,10}=0.28$, $p=0.87$). The grand mean temperature of 12 shaded potential nest sites at BHNP and MRSP was significantly positively related to canopy openness (Fig. 17; $F_{df}=54.08_{1,10}$, $R^2=0.83$, $p<0.0001$), as was grand mean maximum shaded potential nest sites (Fig. 18; $R^2=0.61$, $F_{1,11}=18.24$, $p=0.0016$) but not grand mean minimum shaded potential nest sites ($R^2=0.1$, $F_{1,10}=0.043$, $p=0.8393$). Mean shaded potential nest site temperature ranged from 25.7-28.7°C; the lowest temperature was 20°C (Table 6).

The grand mean temperatures of 13 sunny potential nest sites at BHNP and MRSP were significantly positively related to incident radiation intensity (Figure 15; $R^2=0.47$, $F_{1,11}=11.63$, $p=0.0058$), as were grand mean maximum temperatures (Figure 16; $R^2=0.38$, $F_{1,11}=8.437$, $p=0.0143$), but not grand mean minimum temperatures ($R^2=0.13$, $F_{1,11}=1.763$, $p=0.1246$). The grand mean temperatures of 13 sunny potential nest sites at BHNP and MRSP was significantly positively related to canopy openness (Figure 17; $R^2=0.36$, $F_{1,11}=7.707$, $p=0.018$), as was grand mean maximum shaded potential nest sites (Figure 18; $R^2=0.35$, $F_{1,11}=7.408$, $p=0.0199$) but not grand mean minimum shaded potential nest sites ($R^2=0.21$, $F_{1,11}=4.128$, $p=0.067$). Mean temperatures across all 13 potential sunny nest sites ranged from 27.82 °C to 30.94 °C (Table 6). The highest maximum temperature was 39 °C (Table 6).

3.4 Scope for Nesting Females Predicting Incubation Temperatures

3.4.1 Ground Cover

At MRSP and BHNP nests had a mean ground cover of 25% within a one square meter of the nest (Table 2). At BHNP there was a significant increase in

ground cover throughout incubation (Table 7; Fig. 19; $X^2=98.215$, $df=2$, $p<0.0001$). Ground cover increased significantly between day 0 and day 30 ($p<0.0001$) and between day 30 and day 60 ($p<0.0001$). At MRSP, ground cover increased significantly throughout incubation (Table 7; Figure 19; $X^2=11.277$, $df=2$, $p=0.0036$). Ground cover increased significantly between day 0 and day 30 ($p=0.0053$) and between day 30 and day 60 ($p<0.0001$). Across both sites, the number of nests with bare ground decreased from 17% at day 0 to 4% at day 60. Nests with a majority of grasses across both sites decreased from 46% to 41% of nests across 60 days of incubation. The amount of nests with a majority understory increased from 37% to 55% across 60 days.

3.4.2 Canopy Openness and Incident Radiation

Canopy openness decreased significantly throughout incubation at BHNP (Table 7; Fig. 20; $X^2=53.33$, $df=2$, $p<0.0001$). Nests had a significant decrease in percent openness between day 0 and day 30 ($p<0.0001$) and between day 30 and day 60 ($p<0.0001$). Incident radiation intensity at BHNP decreased significantly throughout incubation (Table 7; Fig. 20; $X^2=30.62$, $df=2$, $p<0.0001$). Incident radiation intensity did not decrease significantly between day 0 and day 30 ($p=0.83$). Between day 30 and day 60 incident radiation decreased significantly ($p<0.0001$). Throughout incubation at MRSP there was no significant decrease in canopy openness ($X^2=4.38$, $df=2$, $p=0.11$) or in incident radiation intensity ($X^2=1.46$, $df=2$, $p=0.48$).

4. Discussion

My study provides compelling evidence that nesting *A. ferox* females could use canopy openness at nest sites to adapt to current, and perhaps future, climate change. Nest sites chosen by females exhibited considerable variation in openness both within and among nesting areas and study sites, and there were direct consequences of those choices in incident (solar) radiation intensity and nest temperatures. Canopy openness of nests in my study did not shift markedly over the (predicted) incubation period, suggesting that openness during nest site choice can predict openness and its consequences in incident radiation and nest temperatures throughout incubation. Importantly, all nesting areas in all three study areas contained an abundance of more shaded areas that could not only be chosen by nesting females, but would result in mean nest temperatures 1.5-4 °C lower than temperatures currently experienced in nests (Fig.'s 8, 9, and 12; Table 6). My conclusion rests upon four assumptions, however: (1) depredated nests formed a representative sample of the nesting population; (2) variation in the choice of openness is heritable; (3) the rate of climate warming does not exceed the rate at which the populations can respond; and, (4) metabolic heat during incubation does not significantly increase nest temperatures.

Canopy cover has not previously been recorded for the nests of *A. ferox*. In Southcentral Louisiana, the species' closest relatives, *A. mutica* and *A.*

spinifera, nested primarily in areas with little to no shaded cover (Doody 1995). Canopy openness in *A. ferox* varied from 6.8% to 70.9% across three populations, with a mean canopy openness of $36.85 \pm 13.92\%$ (Table 2). Canopy openness at MRSP was significantly higher than canopy openness at BHNP and SLP (Fig. 2). High levels of variance, both within and among populations, shows that nesting females have the ability to choose shadier or sunnier nest sites (Fig. 4). This variation in canopy openness directly and positively influenced incident radiation intensity (Fig. 6). A nesting female's choice of canopy cover has a direct effect on the amount of UV radiation on that nest site. For example, Mitchell et al., (2015) showed that nests exposed to higher amounts of solar radiation had significantly higher mean nest temperatures. My study revealed similar results, with a significant positive relationship between incident radiation intensity and mean and maximum temperatures recorded at the nest site (Fig.'s 8 and 10). Thus, a nesting female's choice of canopy cover has a direct effect on the mean and maximum temperatures of the nest site (Fig.'s 9 and 11). I propose that nesting females can thus use canopy cover to compensate for among-year changes in air temperatures; more specifically, *A. ferox* mothers could nest in more shaded areas as the climate continues to warm.

Were canopy openness and the resulting incident radiation during nest site choice a good predictor of those attributes over the entire incubation period? Across a 60 day incubation period, canopy openness and incident radiation decreased significantly (Table 7, Fig. 20). However, this decrease in mean canopy openness was only 3.8%, reflected by a decrease in incident radiation

intensity of only 0.36 MJ/m²/day. This variation in incident radiation intensity is less than the variation in incident radiation intensity caused by seasonal lay date (Table 5). While nests trend towards increased canopy cover throughout incubation, the variation had no effect on the incubation temperature. Thus, canopy openness of the nest site when the nest is deposited is a good predictor of the canopy openness, incident radiation intensity, and temperature of the given nest site throughout incubation. Across the 60 day incubation period, most nests had an increase in percent ground coverage within one square meter of the nest (Table 7, Fig. 19). This vegetation has the ability to decrease the openness calculated by Gap Light Analyzer[®] due to the vegetation becoming visible in the periphery of the hemispherical photographs.

Each nesting area contained an abundance of shaded potential nest sites, with significantly more canopy cover than the nest sites chosen by *A. ferox* (Fig.'s 3 and 4). Currently, based on the sample of ten nest sites, mean temperatures of *A. ferox* nests range from 27.0 °C to 29.8 °C (Table 6). If temperatures rise by as much as 4.4 °C by 2100 (Rogelj et al., 2016), maximum nest temperatures could rise to fatal levels if mothers continue to nest in the same areas with similar openness and incident radiation. All shaded potential nest sites in my study, except for one outlier, had mean temperatures cooler than 27.0 °C (Table 6; Fig. 17). It therefore appears that *A. ferox* mothers can offset the prediction offered above by laying their eggs in potential nest sites with more shaded (cooler) nest temperatures.

Doody et al., (2006a) showed that *Physignathus lesueurii*, a wide ranging lizard with temperature sex determination (TSD), used canopy cover to maintain sex ratios across latitude and elevation. The effects of canopy cover on sex ratios was also observed with *Chrysemys picta*, a turtle with TSD, when canopy cover significantly predicted offspring sex, but did not significantly predict survivability (Janzen & Morjan, 2001). This was corroborated by a common garden experiment using *Chrysemys picta*. The canopy cover, incubation regimes, and sex ratios across nest sites remained constant, while nesting phenology and nest depth varied (Refsnider & Janzen, 2012). If mothers continue to nest in open areas (Petrov et al., 2018; Leger, 2019; Patricio et al., 2018) , a rise in air temperature of 4.4 °C could lead to high nest mortality due to lethal nest temperatures. Conversely, mothers may be able to offset the imminent increase in air temperatures by nesting in more shaded areas (Esteban et al., 2018; Patricio et al., 2017).

The putative claim that softshell turtles can use nest site choice to compensate for the effects of climate change on nest temperatures rests upon four assumptions. The first of these is heritability of nest site choice behavior. Individual females maintain consistency in canopy openness across nest sites, leading theoretical models to indicate that maternal behavioral choice of thermal qualities at nest sites has the potential to be heritable (Janzen & Morjan, 2001; Patricio et al., 2018). Freshwater turtle species with TSD have used increased canopy cover to ensure viable offspring sex ratios. This strategy has enough

variation to compensate for climate change and has been shown to be potentially heritable.

A second assumption is that the rate of microevolutionary change can keep pace with the rate of climate warming. Across a 10 year period, nesting female *Bassiana duperreyi* in Southeastern Australia adjusted nest depth and seasonal timing of oviposition to compensate for climate change, however, they were unable to compensate entirely for the increasing temperatures (Telemeco et al., 2009).

4.1 Seasonal Effects of Nest Site Choice

In the present study *A. ferox* apparently nested 4-5 times per year, though clutch frequency can be 2-7 times per year (Iverson & Moler, 1997). Goode (1983) reported a nesting interval of 13 days in a captive female *A. ferox*, though it is thought that they nest closer to every three weeks based on the rate of appearance of sets of corpora lutea (Iverson & Moler, 1997). Oviductal eggs appear as early as 3 March and as late as 23 July (Iverson & Moler, 1997), matching our findings of nesting between the end of March and the end of July (Table 1; Fig. 1). Male *A. ferox* undergo postnuptial spermatogenesis, producing spermatozoa in late fall and mating in February or March (Meylan et al., 2002). This matches the reproductive cycle of females in my study. At BHNP the first nest was found on 26 March and the last nest was found on 30 July. Utilizing the three week interval, a nesting female could lay at most six nests. Based on an average of five nests per season (Iverson & Moler, 1997) there were roughly 20 nesting females at BHNP. Lay date significantly affects incident radiation

intensity with nests deposited in April and July receiving less incident radiation intensity even when canopy openness is equivalent (Table 5; Fig. 5). Nests with higher canopy openness had more variation in incident radiation intensity. Incident radiation intensity under equivalent canopy openness shows a steep drop off for nests deposited at the end of July or the beginning of August, which could partially explain the rapid decline in new nests throughout the month of July.

4.2 Limitations and Assumptions

For the present study I assumed the distribution of depredated nest sites is equivalent to the distribution of surviving nest sites. At BHNP, nests were surveyed from the trail to the water. Individuals utilizing the sand scrub across the trail are assumed to have nested in equivalently shaded nest sites. With the broad variability of canopy cover throughout the nesting areas between the lake and the trail, the sand scrub habitat did not reveal unique nesting habitat. By using depredated nest sites, I was unable to address the effects of metabolic heating. Massey et al. (future publication) found that there was a significant difference in the temperature at nest sites of *Chelydra serpentina* when compared to the temperature of the surrounding soils. To test this, one Thermochron[®] i-button data logger was placed in the center of a clutch, while a second data logger was placed five centimeters away from the clutch, in the surrounding soils and at the same depth. The greatest mean difference between Thermochron[®] i-button data loggers placed in the center of the clutch and data loggers in the surrounding soil was 0.2768 °C (\pm 0.064 SE), indicating metabolic

heat adds a minimal difference to mean temperature. Both depredated and potential nest sites contained no eggs, so comparisons between groups were not affected by the lack of metabolic heat. Nest site choice in the present study was assumed to be heritable. Theoretical models indicate the possibility that offspring can inherit nest site choice behaviors (Janzen & Morjan, 2001). The current study also assumes that climate change will not outpace nesting females' ability to adapt canopy cover.

Ground cover had a minor impact on canopy openness. Photos were taken with the camera placed flat on the nest site. When ground cover, especially grasses, around the nest reached over six inches in height, the ground cover was picked up on the peripheral of the hemispherical photos. As this only affected the far edges of the photo, the differences caused in canopy openness and incident radiation intensity were minor.

4.3 Implications for Future Studies

As climate changes, the ability to understand how species will respond is critical to conservation. Refsnider and Janzen (2012) showed canopy cover is the behaviorally plastic method utilized by *Chrysemys picta* to control nest temperatures and to maintain sex ratios through TSD. Over the next 100 years, climate projections will not only affect TSD species, but will reach a point where they can affect hatchling survival of species with genetic sex determination (GSD). Tracking nest site choice across multiple freshwater turtle species over time will be the only way to develop an understanding of how freshwater turtles will respond to climate change. More research needs to be conducted on species

without TSD. While TSD will be affected before hatchling survival is impacted, species with GSD may respond to impacts through different methods.

Gap light analyzer[®], or a similar program, needs to be utilized to catalog percent canopy openness and incident radiation of nest sites in a broad range of species. Knowing how canopy openness varies between species, latitudes, and elevations will increase the ability to understand whether canopy openness can actually be used to behaviorally adapt nest site choice to climate change.

4.4 Conclusions

Rapid increases in anthropogenic GHG emissions, primarily due to increased levels of atmospheric CO₂, has caused climate change (Pachauri et al., 2014). Climate change is currently projected to impact nearly all species and lead to the extinction of 7.9% of all species (Urban, 2015). Species will need to compensate for climate change through range shifts or evolutionary adaptation (Moritz & Agudo, 2013). As ectotherms, freshwater turtles will be impacted more by climate change due to their use of behavior to thermoregulate, which is further compounded due to ovipary (Deutsch et al., 2008). Nesting females will need to utilize behavioral adaptations of nest site choice to combat climate change.

The present study shows the direct effects of canopy cover on nest temperatures in freshwater turtle nests. Potential nest sites with increased canopy cover have significantly lower mean temperatures during incubation (Fig.'s 17 and 18). Nesting females are not utilizing the full range of canopy cover available to them during nest site choice, leaving the possibility for nesting

females to utilize increased canopy cover to adapt to climate change (Fig.'s 3 and 7).

Species will utilize a wide variety of methods to compensate for climate change and understanding these methods allows scientists to assist in the conservation of each species effectively. Certain species may require habitat corridors, while others may require the facilitation of behavioral adaptations. If canopy cover is the method freshwater turtles use to compensate for climate change, land managers and restoration ecologists should ensure the preservation or restoration of shaded habitat along nest sites to ensure nesting females continue to have suitable nesting habitat.

Tables

Table 1: Seasonal timing of nesting.

| Location | March | April | May | June | July | August | Total Nests |
|-----------|-------|-------|-----|------|------|--------|-------------|
| All Nests | 2 | 20 | 47* | 55* | 31* | 1* | 156 |
| BHNP | 1 | 12 | 25 | 44 | 20 | 0 | 102 |
| MRSP | 1 | 8 | 8 | 6 | 4 | 0 | 27 |
| SLP | N/A | N/A | 14* | 5* | 7* | 1* | 27 |

Nests at SLP were surveyed on 29 May, 17 June, 10 July, and 8 August only.

Table 2: Mean canopy openness, incident radiation intensity, and ground cover at each site.

| Location | Variable | Mean | Standard Deviation | Range |
|-----------|---|-------|--------------------|---------------|
| All Sites | Canopy Openness (%) | 36.85 | 13.92 | 6.84 - 70.89 |
| All Sites | incident radiation intensity (MJ/m ² /d) | 5.64 | 2.06 | 0.85 - 9.83 |
| All Sites | Ground Cover (%) | 25.19 | 21.13 | 0 - 100 |
| BHNP | Canopy Openness (%) | 35.33 | 14.55 | 6.84 - 67.82 |
| BHNP | incident radiation intensity (MJ/m ² /d) | 5.41 | 2 | 0.85 - 9.02 |
| BHNP | Ground Cover (%) | 22.84 | 21.11 | 0 - 90 |
| MRSP | Canopy Openness (%) | 46.80 | 10.04 | 21.44 - 70.89 |
| MRSP | incident radiation intensity (MJ/m ² /d) | 7.29 | 1.31 | 3.64 - 9.83 |
| MRSP | Ground Cover (%) | 34.07 | 18.71 | 10 - 100 |
| SLP | Canopy Openness (%) | 32.65 | 10.07 | 19.27 - 53.3 |

| | | | | |
|-----|--|------|------|-------------|
| SLP | incident radiation intensity (MJ/m ² /d) | 4.83 | 2.09 | 1.67 - 8.42 |
|-----|--|------|------|-------------|

Table 3: Mean canopy openness and incident radiation intensity of nests and potential nests at each site.

| Location | Group | Variable | Mean | Standard Deviation | Range |
|-----------|-------|---|-------|--------------------|---------------|
| All Sites | Nests | Canopy Openness (%) | 36.85 | 13.92 | 6.84 - 70.89 |
| All Sites | Nests | incident radiation intensity (MJ/m ² /d) | 5.64 | 2.06 | 0.85 - 9.83 |
| All Sites | Sun | Canopy Openness (%) | 46.46 | 17.3 | 20.15 - 70.56 |
| All Sites | Sun | incident radiation intensity (MJ/m ² /d) | 7.39 | 1.92 | 3.41 - 9.43 |
| All Sites | Shade | Canopy Openness (%) | 17.44 | 7.59 | 10.41 - 41.98 |
| All Sites | Shade | incident radiation intensity (MJ/m ² /d) | 2.27 | 1.41 | 1.27 - 6.53 |
| BHNP | Nests | Canopy Openness (%) | 35.33 | 14.55 | 6.84 - 67.82 |
| BHNP | Nests | incident radiation intensity (MJ/m ² /d) | 5.41 | 2 | 0.85 - 9.02 |
| BHNP | Sun | Canopy Openness (%) | 39.91 | 16.64 | 20.15 - 66.18 |
| BHNP | Sun | incident radiation intensity (MJ/m ² /d) | 6.64 | 1.94 | 3.41 - 9.06 |
| BHNP | Shade | Canopy Openness (%) | 14.64 | 3.22 | 10.41 - 21.25 |
| BHNP | Shade | incident radiation intensity (MJ/m ² /d) | 1.90 | 0.74 | 1.19 - 3.19 |
| MRSP | Nests | Canopy Openness (%) | 46.80 | 10.04 | 21.44 - 70.89 |
| MRSP | Nests | incident radiation intensity (MJ/m ² /d) | 7.29 | 1.31 | 3.64 - 9.83 |
| MRSP | Sun | Canopy Openness (%) | 59.55 | 9.31 | 47.81 - 70.56 |
| MRSP | Sun | incident radiation intensity (MJ/m ² /d) | 8.88 | 0.47 | 8.28 - 9.43 |
| MRSP | Shade | Canopy Openness (%) | 22.47 | 10.15 | 13.83 - 41.98 |
| MRSP | Shade | incident radiation intensity (MJ/m ² /d) | 2.93 | 1.97 | 1.25 - 6.53 |

Table 4: Linear regression model results for incident radiation intensity.

| Model | K | AICc | ΔAICc | Weight |
|--|----------|-------------|--------------------------------|---------------|
| Canopy Openness Nest Site Date (Quadratic) | 3 | 371.3 | 0.00 | 0.873 |
| Canopy Openness Date | 2 | 379.0 | 7.70 | 0.019 |
| Canopy Openness | 1 | 379.2 | 7.89 | 0.017 |
| Canopy Openness Nest Site Date | 3 | 377.8 | 6.47 | 0.034 |
| Canopy Openness Nest Site | 2 | 376.8 | 5.46 | 0.057 |

Table 5: Changes in incident radiation intensity due to lay date.

| Canopy Openness (%) | Incubation Start Date | Incubation End Date | Incident Radiation Intensity |
|--------------------------------|----------------------------------|--------------------------------|---|
| 13.46 | April 1 | May 31 | 2.79 |
| 13.46 | May 1 | June 30 | 2.86 |
| 13.46 | 1 June | July 31 | 2.88 |
| 13.46 | July 1 | August 30 | 2.87 |
| 13.46 | August 1 | September 30 | 2.53 |
| 36.2 | April 1 | May 31 | 6.17 |
| 36.2 | May 1 | June 30 | 6.27 |
| 36.2 | 1 June | July 31 | 6.25 |
| 36.2 | July 1 | August 30 | 6.2 |
| 36.2 | August 1 | September 30 | 5.68 |
| 66.34 | April 1 | May 31 | 8.4 |
| 66.34 | May 1 | June 30 | 8.8 |

| | | | |
|-------|----------|--------------|------|
| 66.34 | 1 June | July 31 | 8.89 |
| 66.34 | July1 | August 30 | 8.36 |
| 66.34 | August 1 | September 30 | 7.22 |

Table 6: Mean, Minimum, and Maximum temperatures from each data logger.

| Data Logger Location | Start Date | End Date | Incident Radiation Intensity | Max Temp | Min Temp | Mean Temp |
|-----------------------------|-------------------|-----------------|-------------------------------------|-----------------|-----------------|------------------|
| B1 Sun | 16 July | 26 September | 6.88 | 31.5 | 24.5 | 28.90 |
| B1 Shade | 16 July | 26 September | 1.19 | 28 | 25.5 | 26.56 |
| B2 Sun | 24 April | 30 August | 8.43 | 36 | 23 | 30.60 |
| B3 Sun | 11 June | 26 September | 6.83 | 33.5 | 25 | 29.26 |
| B3 Shade | 11 June | 26 September | 1.82 | 33 | 24 | 26.27 |
| B4 Sun | 11 June | 26 September | 5.27 | 33.5 | 25 | 29.25 |
| B5 Sun | 20 April | 25 August | 8.33 | 32.5 | 20 | 28.07 |
| B5 Shade | 20 April | 25 August | 3.08 | 28.5 | 20.5 | 26.24 |
| B6 Sun | 19 April | 25 August | 8.99 | 34 | 20 | 29.45 |
| B6 Shade | 20 April | 25 August | 2.07 | 27.5 | 20 | 25.73 |
| B7 Sun | 1 June | 26 September | 3.41 | 30 | 26 | 27.82 |
| B7 Shade | 1 June | 26 September | 1.27 | 28 | 25 | 26.46 |
| B8 Sun | May 29 | 26 September | 5.61 | 34.5 | 26 | 28.97 |
| B8 Shade | May 29 | 26 September | 3.19 | 28.5 | 25 | 26.73 |
| B9 Sun | 16 July | 26 September | 4.08 | 33.5 | 24.5 | 28.36 |
| B9 Shade | 16 July | 26 September | 1.27 | 27.5 | 24.5 | 26.18 |
| M1 Sun | May 23 | 27 September | 8.78 | 38 | 23 | 30.65 |
| M1 Shade | May 23 | 27 September | 1.54 | 30.5 | 23 | 26.43 |
| M2 Shade | May 23 | 27 September | 3.55 | 31 | 23 | 26.89 |
| M3 Sun | May 27 | 27 September | 8.48 | 34 | 26.5 | 29.56 |
| M3 Shade | May 23 | 27 September | 1.25 | 32 | 23.5 | 26.52 |
| M4 Sun | 10 June | 27 September | 8.28 | 36.5 | 26 | 30.49 |
| M4 Shade | 10 June | 27 September | 1.76 | 28.5 | 24 | 26.60 |

| | | | | | | |
|-----------|---------|--------------|------|------|------|-------|
| M5 Sun | 10 June | 27 September | 9.43 | 39 | 24.5 | 30.94 |
| M5 Shade | 10 June | 27 September | 6.53 | 36.5 | 24.5 | 28.66 |
| Nest B90 | 16 July | 26 September | 3.04 | 30 | 26 | 27.92 |
| Nest B91 | 16 July | 26 September | 3.85 | 32.5 | 23.5 | 27.94 |
| Nest B92 | 16 July | 26 September | 5.07 | 31 | 25 | 27.46 |
| Nest B94 | 16 July | 26 September | 7.43 | 34 | 25 | 29.08 |
| Nest B96 | 16 July | 26 September | 4.69 | 31 | 25.5 | 27.81 |
| Nest B97 | 16 July | 26 September | 3.73 | 31 | 25 | 28.19 |
| Nest B98 | 16 July | 26 September | 7.61 | 31.5 | 24.5 | 28.69 |
| Nest B99 | 16 July | 26 September | 7.33 | 32 | 25.5 | 29.78 |
| Nest B100 | 23 July | 26 September | 3.78 | 30 | 25 | 27.00 |
| Nest B101 | 27 July | 26 September | 4.11 | 30 | 26 | 27.68 |

Table 7: Mean canopy openness, incident radiation intensity, and ground cover throughout incubation.

| Location | Group | Variable | Mean | Standard Deviation | Range |
|-----------|--------|---|-------|--------------------|---------------|
| All Sites | Day 0 | Canopy Openness (%) | 36.85 | 13.92 | 6.84 - 70.89 |
| All Sites | Day 0 | incident radiation intensity (MJ/m ² /d) | 5.64 | 2.06 | 0.85 - 9.83 |
| All Sites | Day 0 | Ground Cover (%) | 25.19 | 21.13 | 0 - 100 |
| BHNP | Day 0 | Canopy Openness (%) | 35.33 | 14.55 | 6.84 - 67.82 |
| BHNP | Day 0 | incident radiation intensity (MJ/m ² /d) | 5.41 | 2.00 | 0.85 - 9.02 |
| BHNP | Day 0 | Ground Cover (%) | 22.84 | 21.11 | 0 - 90 |
| MRSP | Day 0 | Canopy Openness (%) | 46.80 | 10.04 | 21.44 - 70.89 |
| MRSP | Day 0 | incident radiation intensity (MJ/m ² /d) | 7.29 | 1.31 | 3.64 - 9.83 |
| MRSP | Day 0 | Ground Cover (%) | 34.07 | 18.71 | 10 - 100 |
| All Sites | Day 30 | Canopy Openness (%) | 35.78 | 14.53 | 8.69 - 66.74 |
| All Sites | Day 30 | incident radiation intensity (MJ/m ² /d) | 5.63 | 2.05 | 1.12 - 9.24 |
| All Sites | Day 30 | Ground Cover (%) | 37.20 | 28.86 | 0 - 100 |

| | | | | | |
|-----------|--------|---|-------|-------|---------------|
| BHNP | Day 30 | Canopy Openness (%) | 33.37 | 14.78 | 8.69 - 66.74 |
| BHNP | Day 30 | incident radiation intensity (MJ/m ² /d) | 5.24 | 2.05 | 1.12 - 8.7 |
| BHNP | Day 30 | Ground Cover (%) | 38.08 | 31.1 | 0 - 100 |
| MRSP | Day 30 | Canopy Openness (%) | 45.15 | 8.45 | 28.75 - 64.45 |
| MRSP | Day 30 | incident radiation intensity (MJ/m ² /d) | 7.15 | 1.13 | 4.4 - 9.24 |
| MRSP | Day 30 | Ground Cover (%) | 33.85 | 17.56 | 0 - 70 |
| All Sites | Day 60 | Canopy Openness (%) | 33.06 | 14.54 | 8.69 - 66.74 |
| All Sites | Day 60 | incident radiation intensity (MJ/m ² /d) | 5.28 | 2.22 | 0.44 - 9.06 |
| All Sites | Day 60 | Ground Cover (%) | 51.95 | 32.16 | 0 - 100 |
| BHNP | Day 60 | Canopy Openness (%) | 30.27 | 14.74 | 3.81 - 61.12 |
| BHNP | Day 60 | incident radiation intensity (MJ/m ² /d) | 4.79 | 2.19 | 0.44 - 9.06 |
| BHNP | Day 60 | Ground Cover (%) | 53.24 | 33.76 | 0 - 100 |
| MRSP | Day 60 | Canopy Openness (%) | 43.69 | 6.85 | 33.74 - 59.82 |
| MRSP | Day 60 | incident radiation intensity (MJ/m ² /d) | 7.11 | 1.04 | 4.94 - 8.99 |
| MRSP | Day 60 | Ground Cover (%) | 46.92 | 24.3 | 10 - 100 |

Table 8: F_{df} , R^2 , and P-value for linear regressions of the relationship between mean, maximum, and minimum temperatures to canopy openness and incident radiation intensity separated by nest site type.

| Parameter | X | Y | F_{df} | R^2 | P |
|-----------|------------------------------|------|-----------------------|-------|--------|
| Sun | Incident Radiation Intensity | Mean | 11.63 _{1,11} | 0.47 | 0.0058 |
| Sun | Incident Radiation Intensity | Max | 8.437 _{1,11} | 0.38 | 0.0143 |
| Sun | Incident Radiation Intensity | Min | 1.763 _{1,11} | 0.13 | 0.1246 |
| Sun | Canopy Openness | Mean | 7.707 _{1,11} | 0.36 | 0.018 |

| | | | | | |
|-------|------------------------------|------|-----------------------|-------|---------|
| Sun | Canopy Openness | Max | 7.408 _{1,11} | 0.35 | 0.0199 |
| Sun | Canopy Openness | Min | 4.128 _{1,11} | 0.21 | 0.067 |
| Shade | Incident Radiation Intensity | Mean | 22.61 _{1,10} | 0.66 | 0.0008 |
| Shade | Incident Radiation Intensity | Max | 7.351 _{1,10} | 0.37 | 0.0219 |
| Shade | Incident Radiation Intensity | Min | 0.028 _{1,10} | 0.1 | 0.87 |
| Shade | Canopy Openness | Mean | 54.08 _{1,10} | 0.83 | <0.0001 |
| Shade | Canopy Openness | Max | 18.24 _{1,10} | 0.61 | 0.0016 |
| Shade | Canopy Openness | Min | 0.043 _{1,10} | 0.1 | 0.8393 |
| Nest | Incident Radiation Intensity | Mean | 12.23 _{1,8} | 0.56 | 0.0081 |
| Nest | Incident Radiation Intensity | Max | 6.027 _{1,8} | 0.358 | 0.0396 |
| Nest | Incident Radiation Intensity | Min | 0.134 _{1,8} | 0.11 | 0.7237 |
| Nest | Canopy Openness | Mean | 4.349 _{1,8} | 0.27 | 0.0705 |
| Nest | Canopy Openness | Max | 0.700 _{1,8} | 0.03 | 0.4275 |
| Nest | Canopy Openness | Min | 0.181 _{1,8} | 0.1 | 0.6816 |
| All | Incident Radiation Intensity | Mean | 154.1 _{1,33} | 0.82 | <0.0001 |
| All | Incident Radiation Intensity | Max | 53.12 _{1,33} | 0.61 | <0.0001 |
| All | Incident Radiation Intensity | Min | 0.041 _{1,33} | 0.03 | 0.8403 |

| | | | | | |
|-----|-----------------|------|-----------------------|------|---------|
| All | Canopy Openness | Mean | 64.88 _{1,33} | 0.65 | <0.0001 |
| All | Canopy Openness | Max | 33.03 _{1,33} | 0.49 | <0.0001 |
| All | Canopy Openness | Min | 0.131 _{1,33} | 0.03 | 0.7199 |

Figures

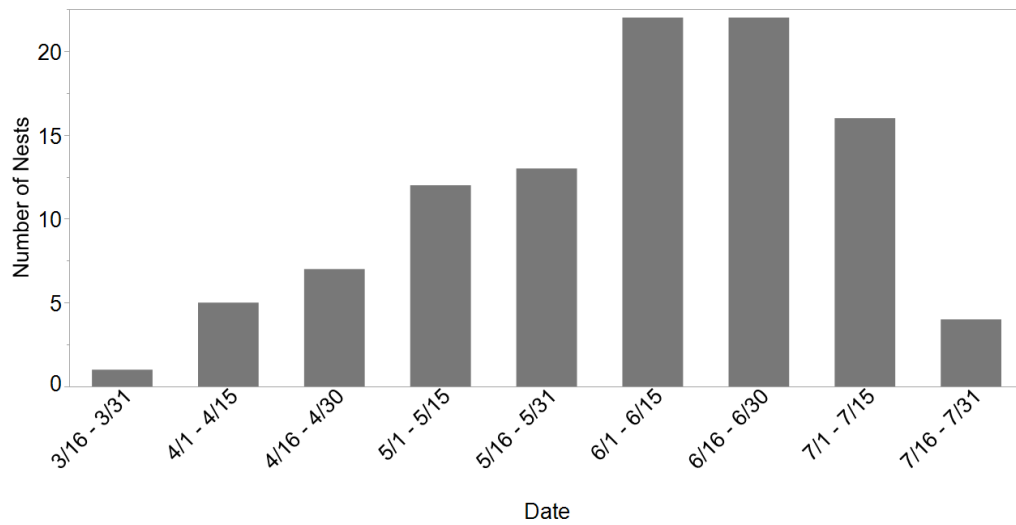


Figure 1: Seasonal timing of nesting in *A. ferox* at BHNP.

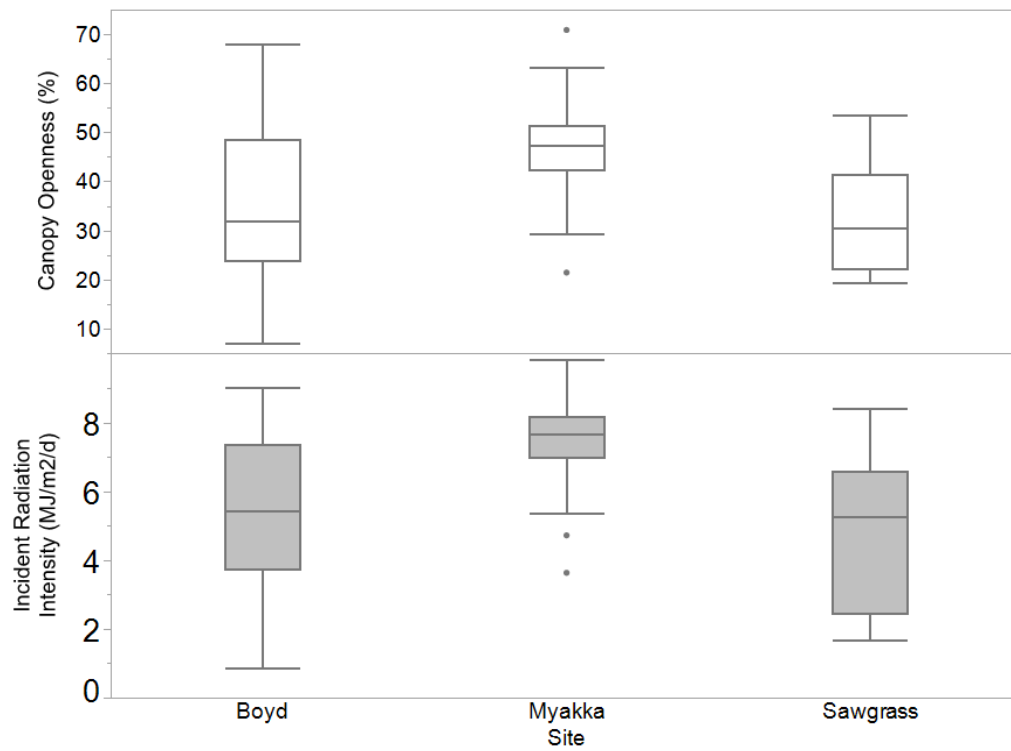


Figure 2: Comparison of canopy openness and incident radiation intensity among *A. ferox* nest sites.

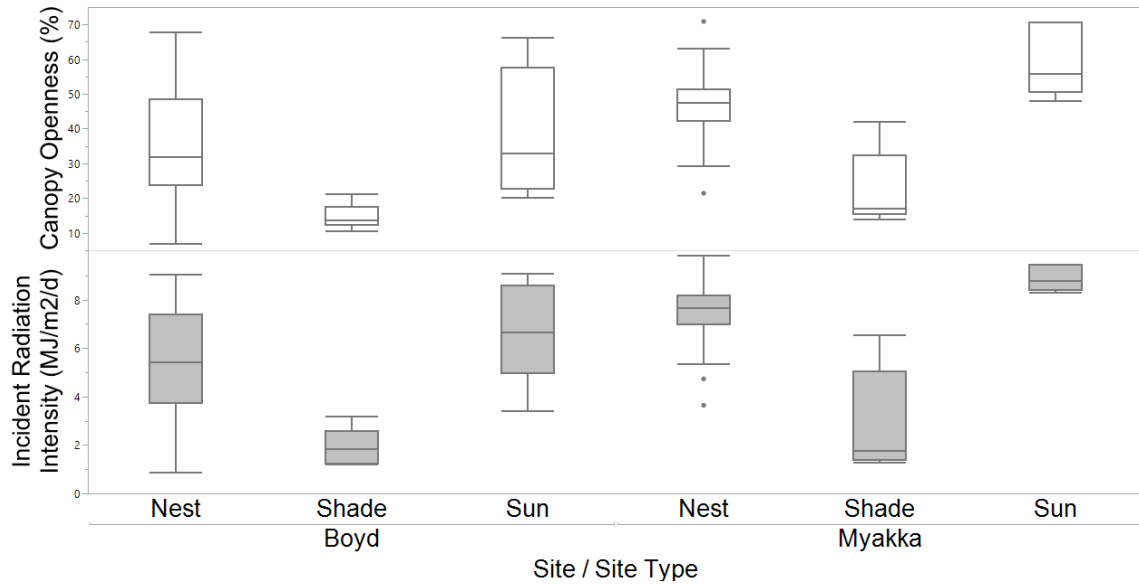


Figure 3: Canopy Openness and Incident radiation intensity at nest sites and potential nest sites.

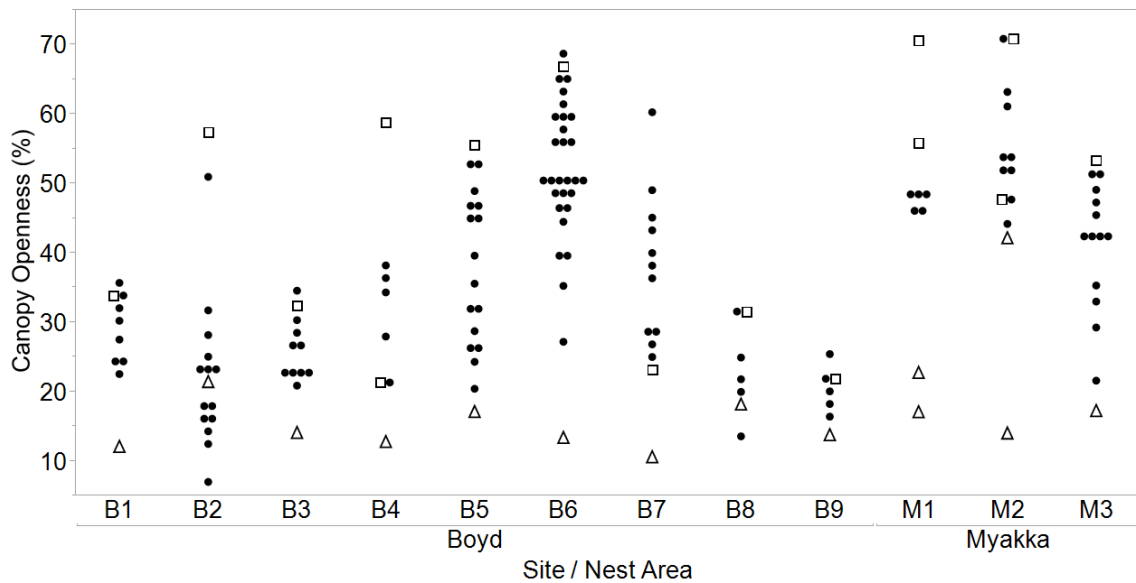


Figure 4: Comparison of canopy openness between nest sites and potential nest sites. Shaded sites are triangles, sunny sites are squares. Nest areas M1 and M2 include two of each potential nest site type; all other nesting areas include one of each potential nest site type.

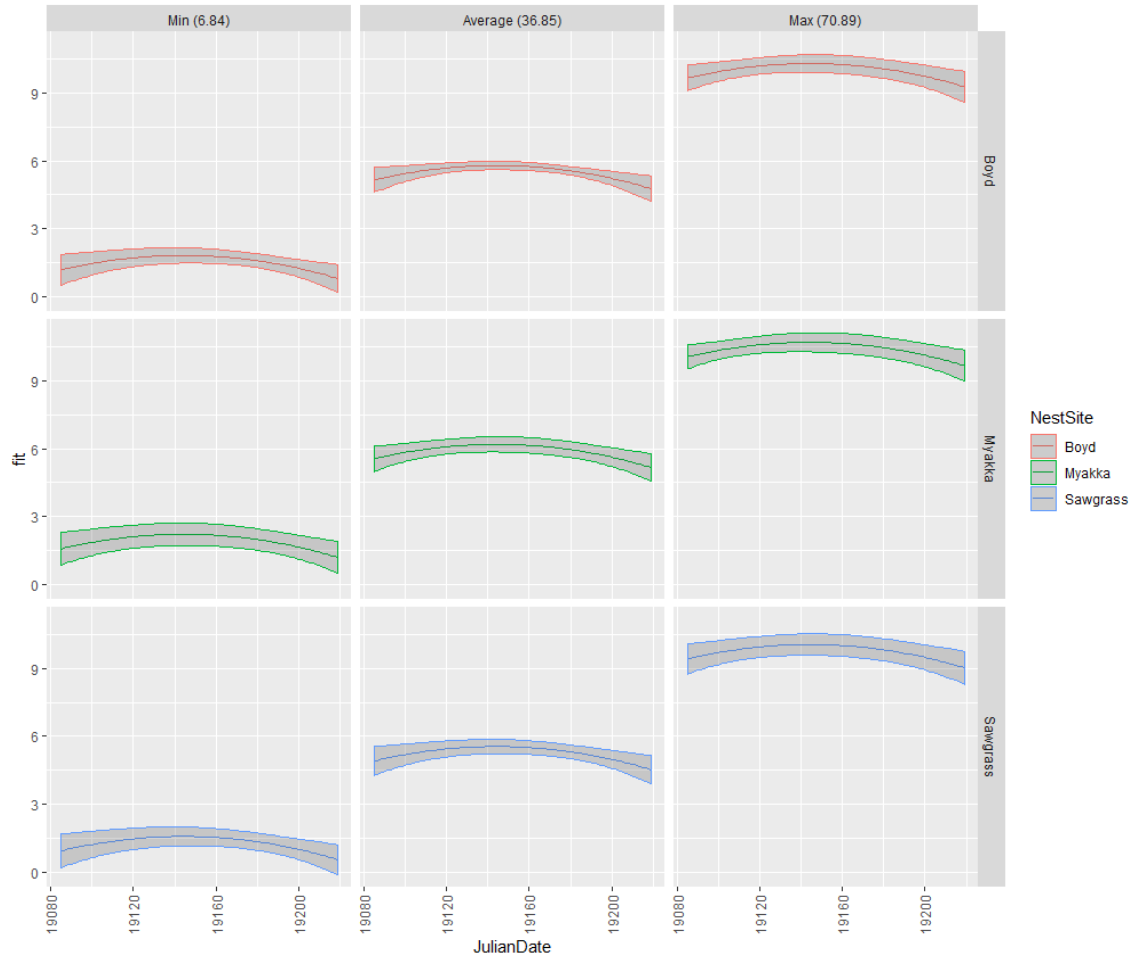


Figure 5: Model of best fit for incident radiation intensity (MJ/m²/d). Fit is the range of incident radiation intensity caused by the predicting values (julian date, nest site, and canopy openness). Canopy openness is represented by three values: the minimum, mean, and maximum.

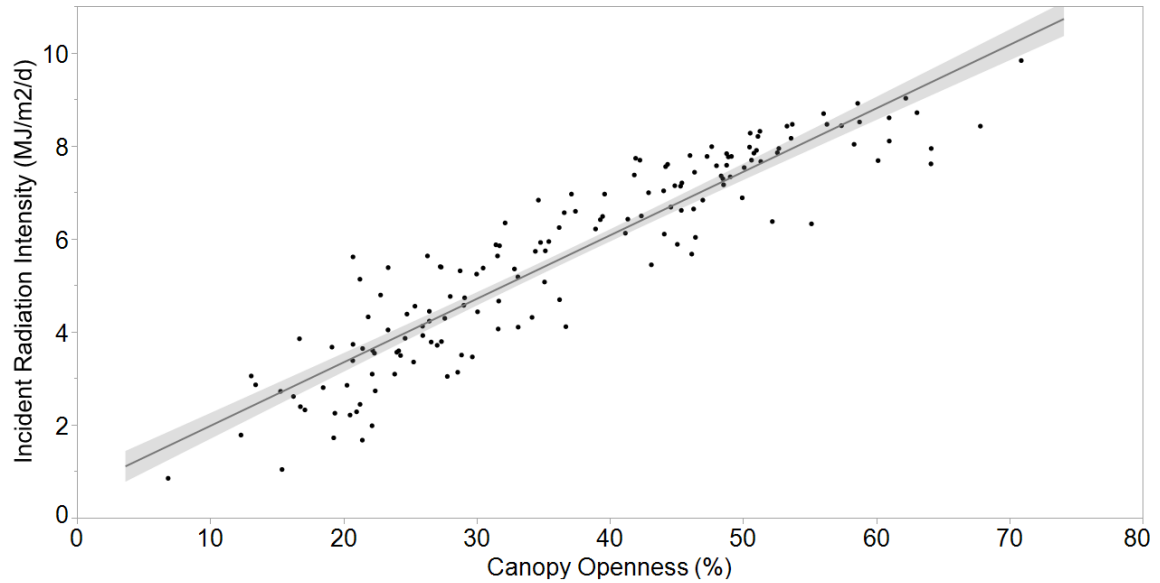


Figure 6: Relationship between canopy openness and incident radiation intensity of *A. ferox* nest sites.

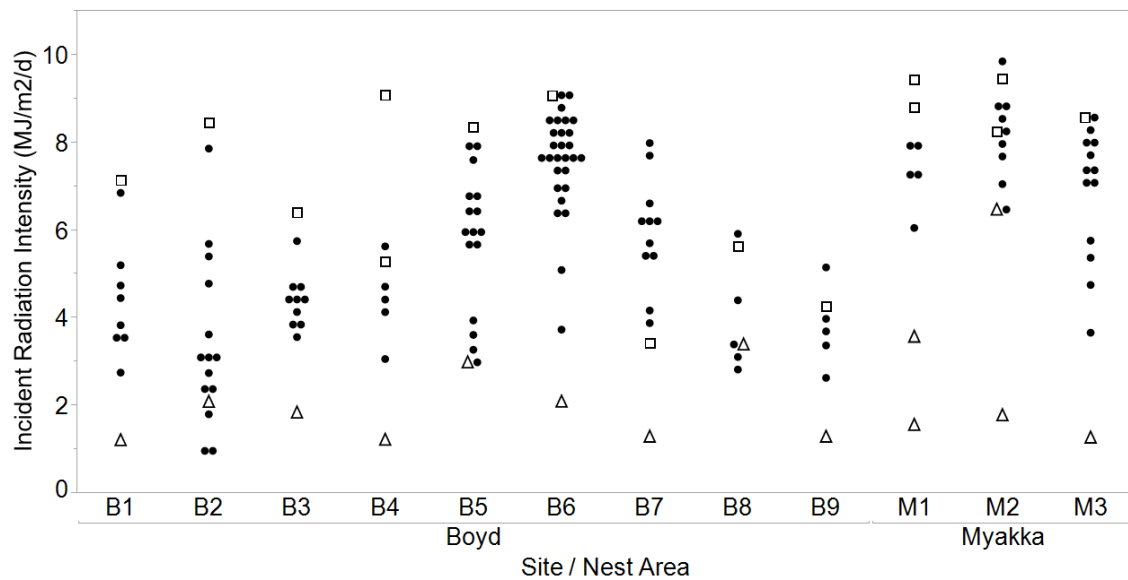


Figure 7: Comparison of incident radiation intensity between nest sites and potential nest sites. Shaded sites are triangles, sunny sites are squares. Nest areas M1 and M2 include two of each potential nest site type; all other nesting areas include one of each potential nest site type.

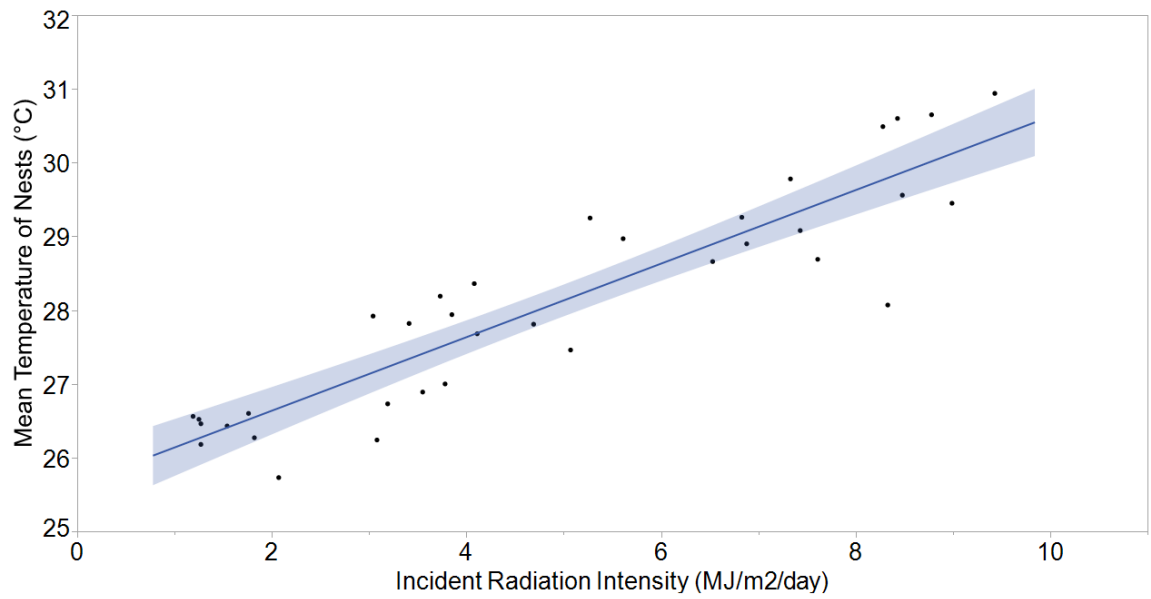


Figure 8: Relationship between incident radiation intensity and mean temperature across all nests.

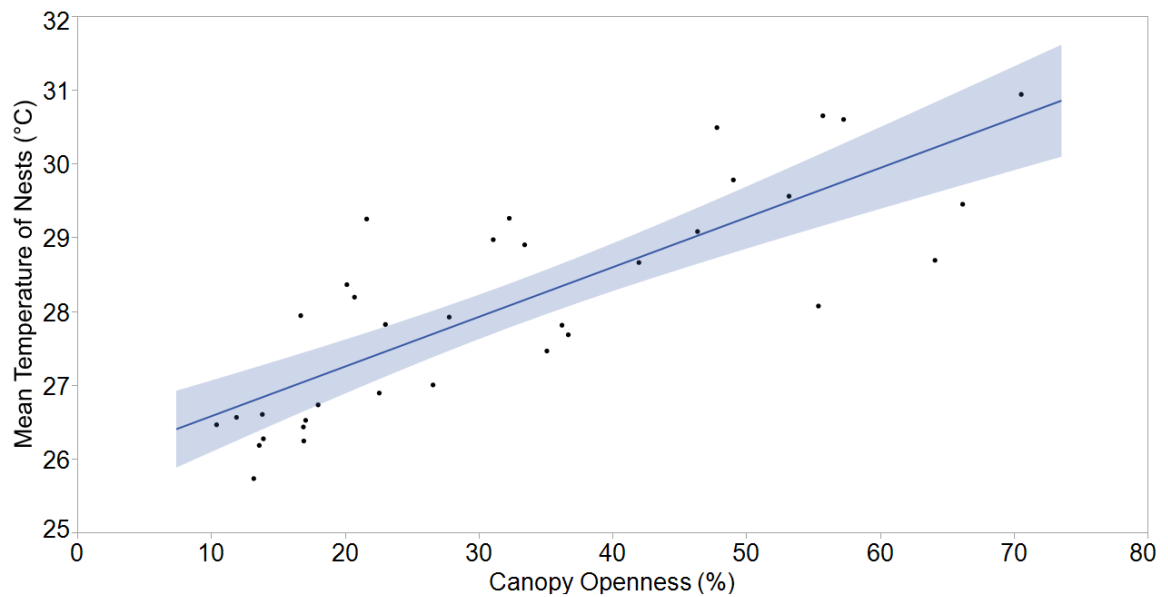


Figure 9: Relationship between canopy openness and mean temperature across all nests.

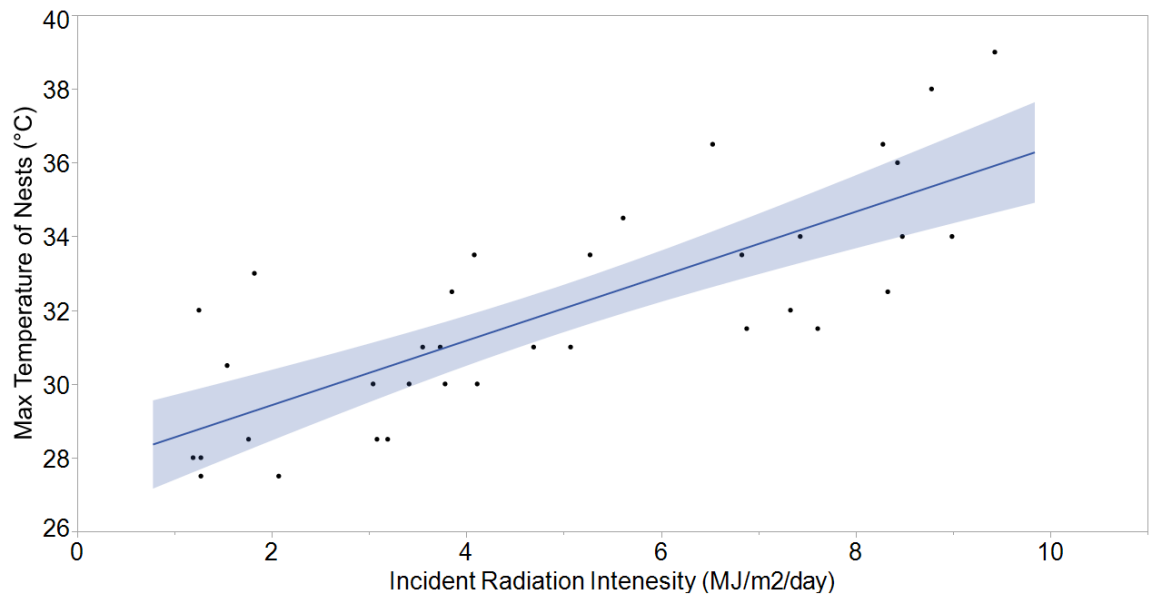


Figure 10: Relationship between incident radiation intensity and maximum temperature across all nests.

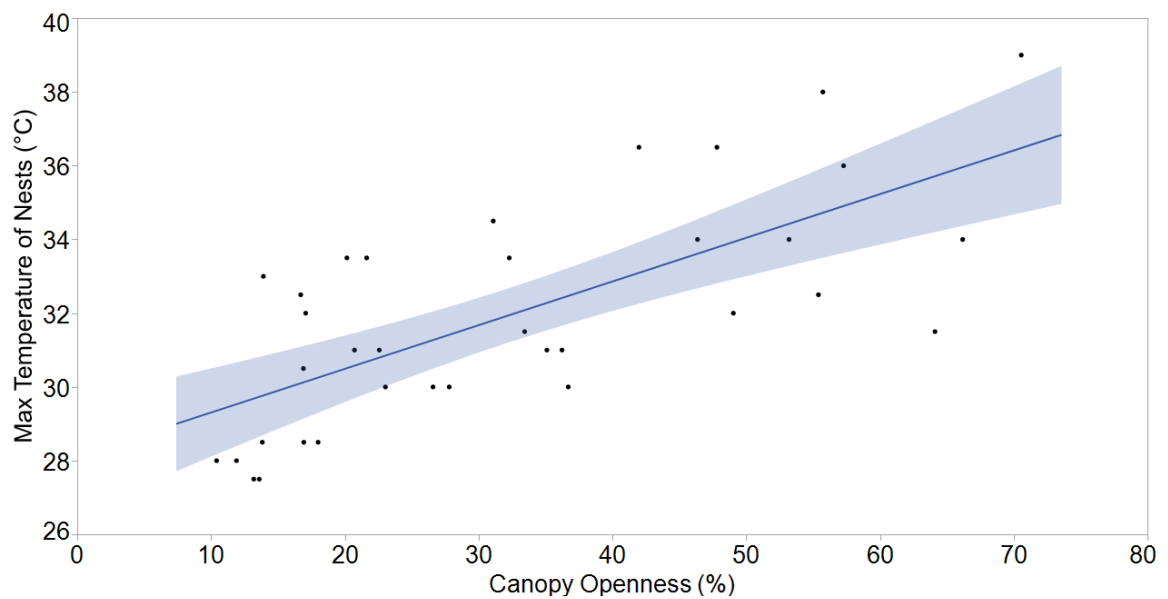


Figure 11: Relationship between canopy openness and maximum temperature across all nests.

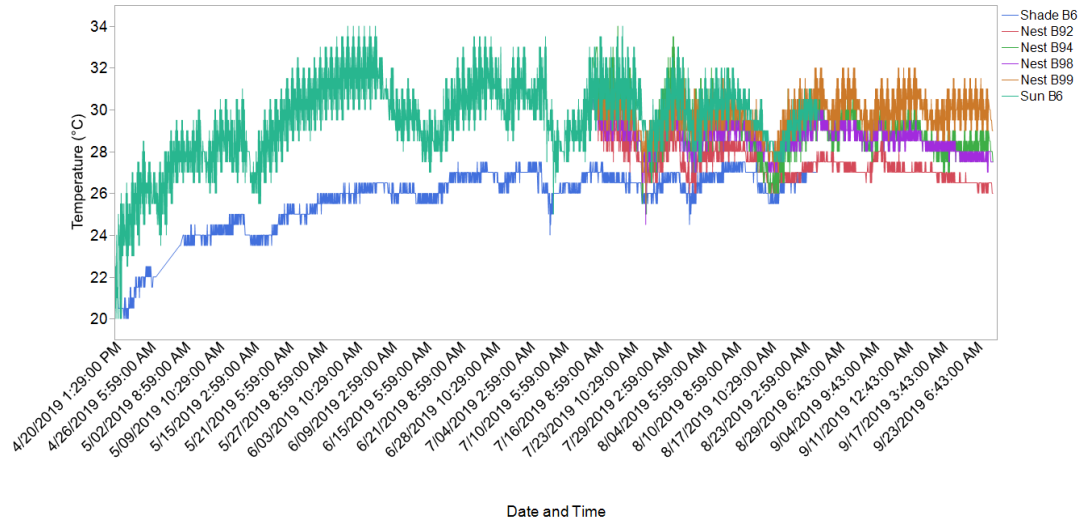


Figure 12: Temperature at four nest sites, the potential shaded nest site, and the potential sunny nest site in nesting area B6 (BHNP). Temperatures at potential nest sites run from 20 April to 25 August, temperatures at nest sites run from 16 July to 26 September. Temperature recorded every 90 minutes.

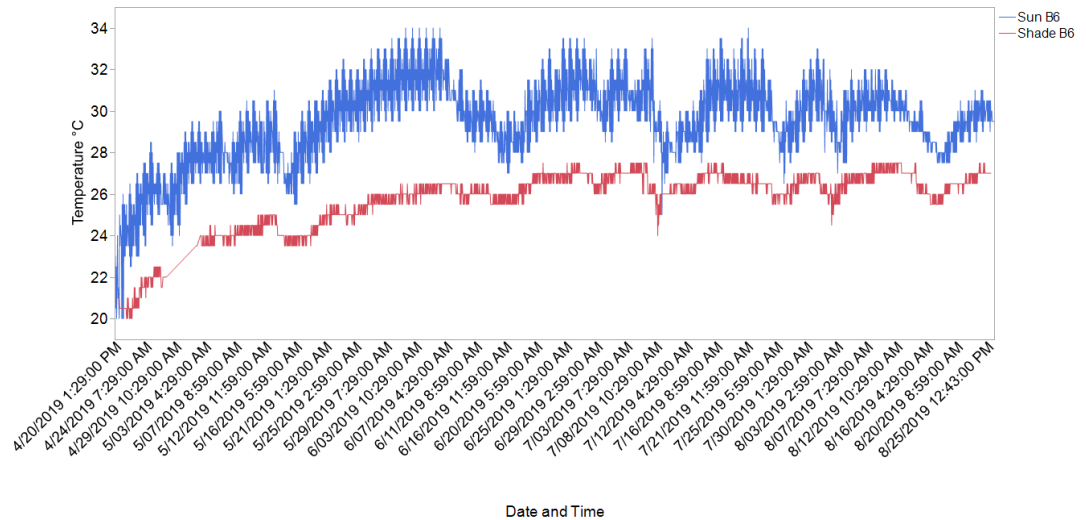


Figure 13: Temperature at the potential sun site and the potential shade site in nesting area B6 (BHNP). Temperature recorded every 90 minutes.

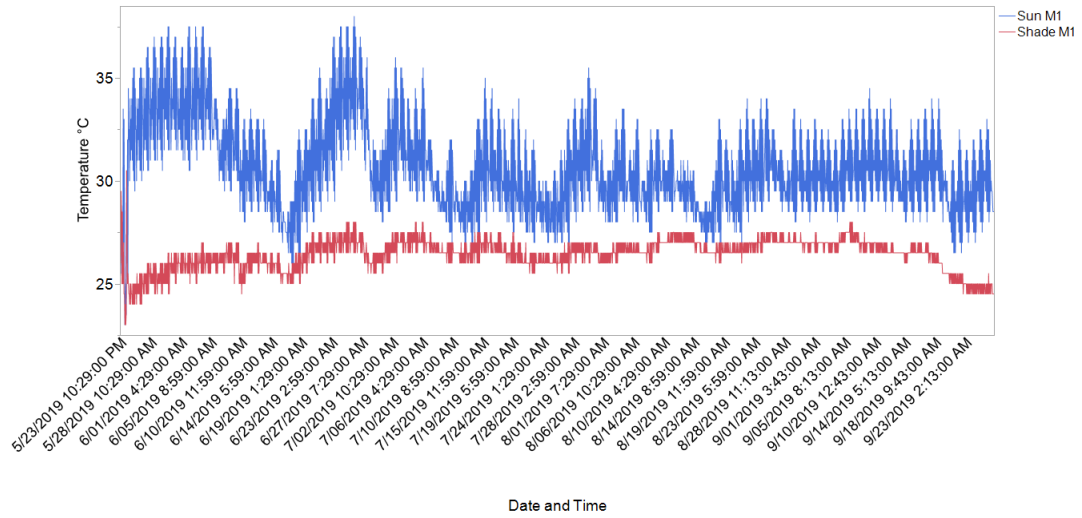


Figure 14: Temperature at the potential sun site and the potential shade site in nesting area M1 (MRSP). Temperature recorded every 90 minutes.

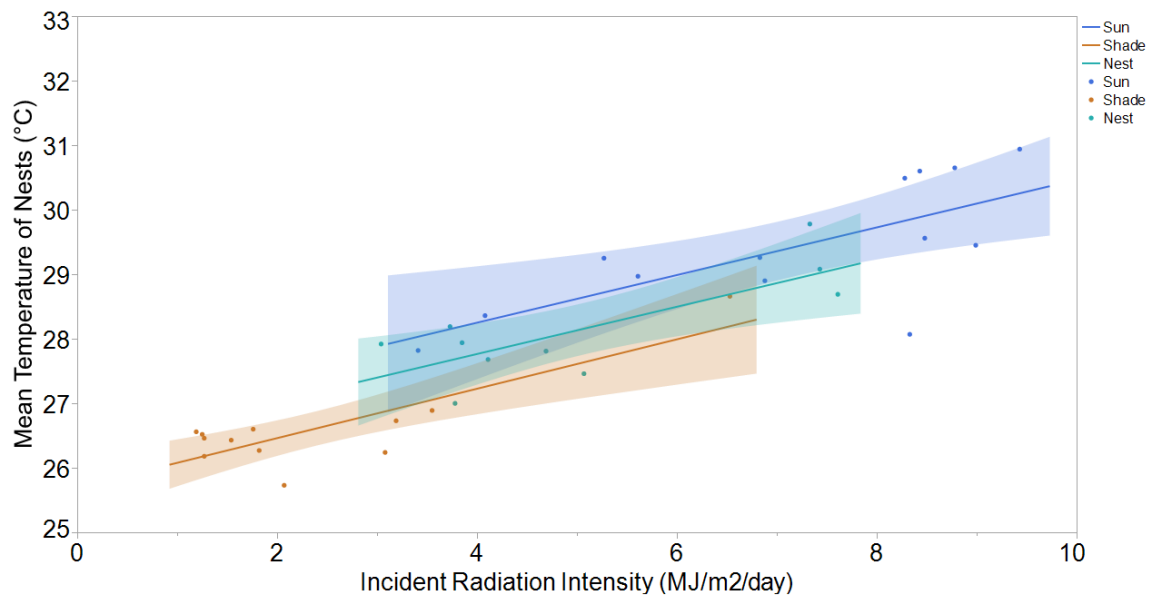


Figure 15: Relationship between incident radiation intensity and mean temperature separated by nest site type.

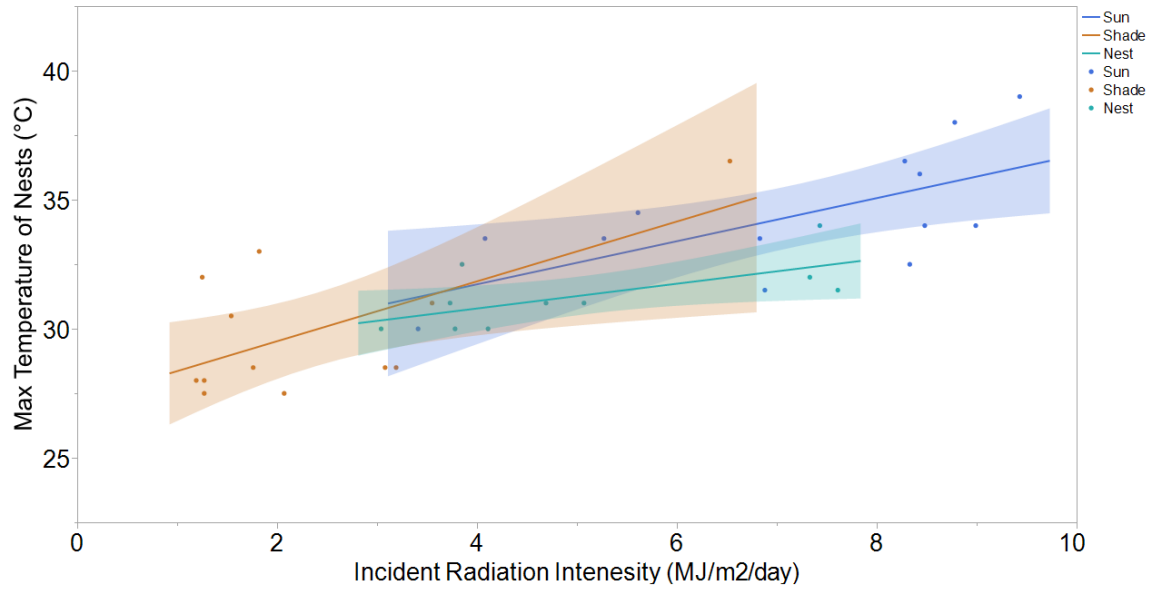


Figure 16: Relationship between incident radiation intensity and maximum temperature separated by nest site type.

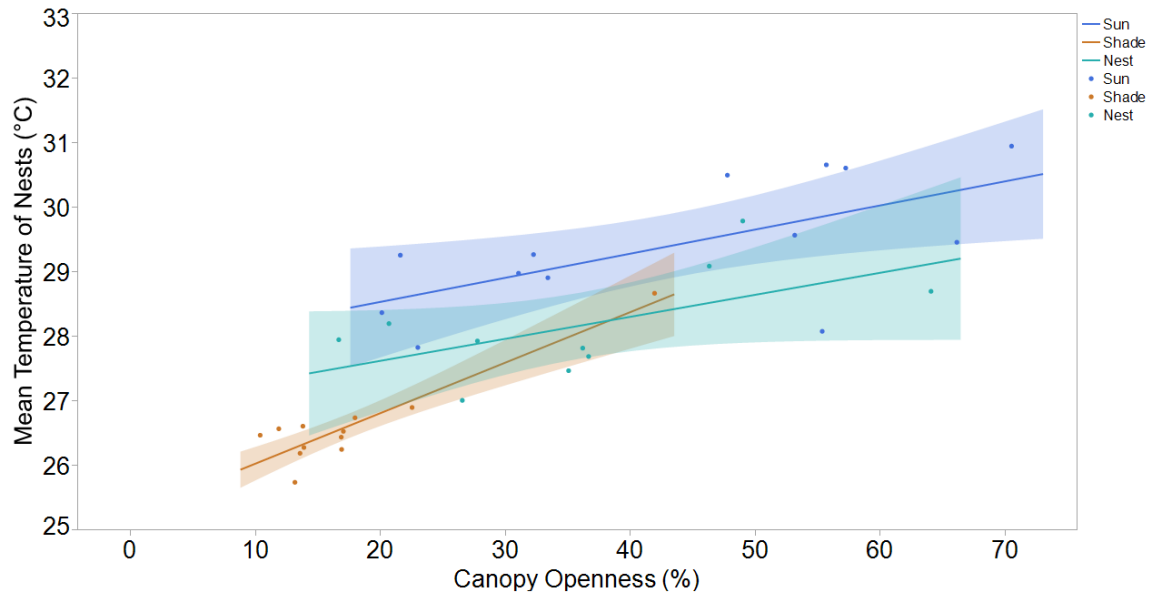


Figure 17: Relationship between canopy openness and mean temperature separated by nest site type.

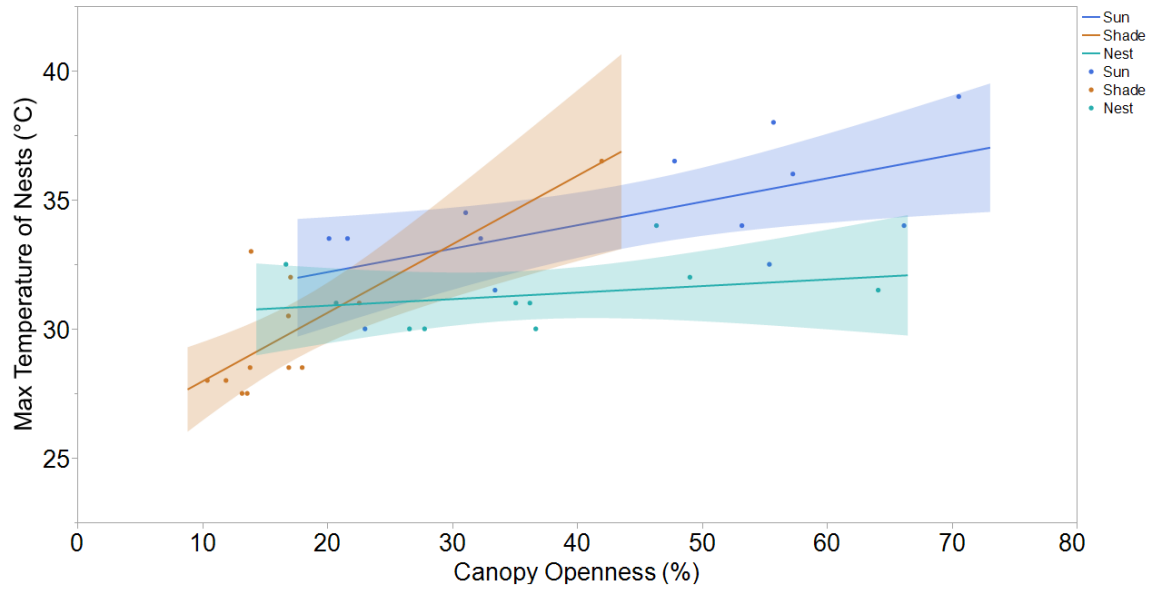


Figure 18: Relationship between canopy openness and maximum temperature separated by nest site type.

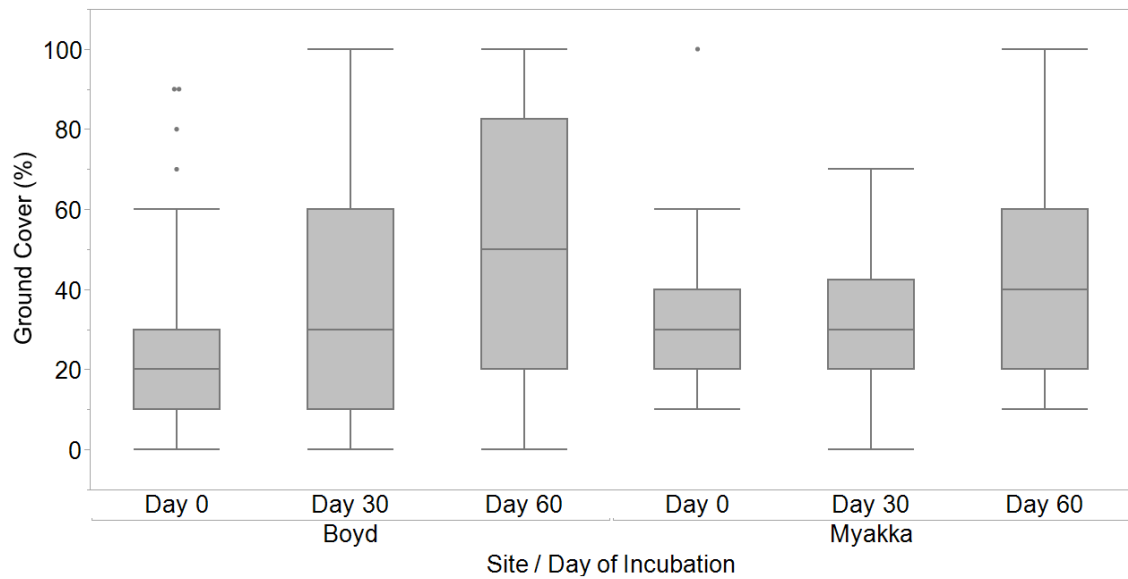


Figure 19: Comparison of ground cover throughout incubation. Day 0 is at or near the lay date and day 60 is near the predicted hatching date.

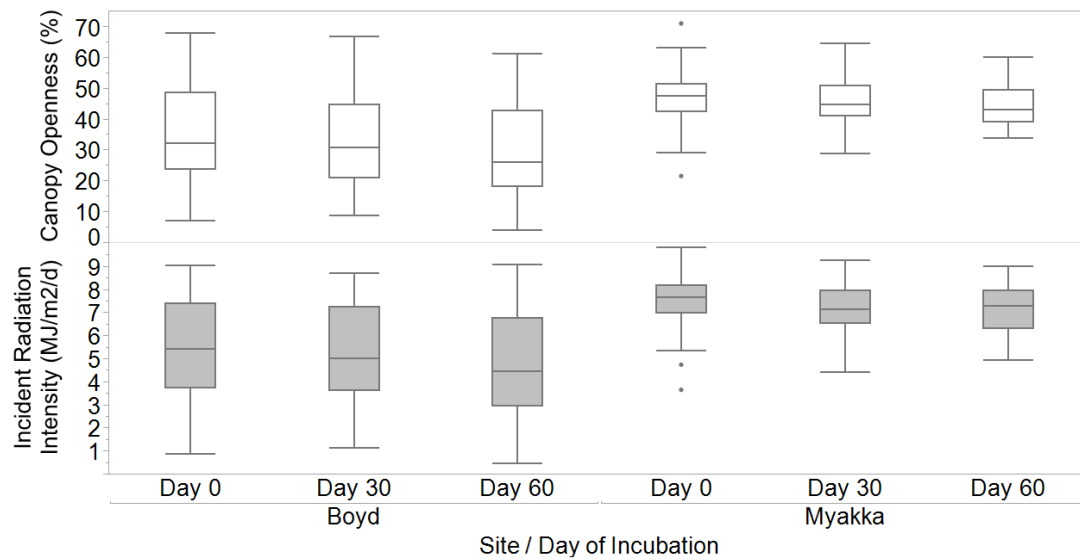


Figure 20: Comparison of incident radiation and canopy openness throughout incubation. Day 0 is at or near the lay date and day 60 is near the predicted hatching date.

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