
A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology

by

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ABSTRACT
The Eastern Collared Lizard (Crotaphytus collaris) has experienced extensive population declines over the past half century in the Ozark Mountains. Previous research suggests that glade habitat degradation through woody vegetation encroachment is an important factor resulting in population declines. In this dissertation I used information on time-energy budgets to investigate mechanisms leading to population declines of C. collaris the Ozarks. In chapter one, I addressed the influence of dense woody vegetation encroachment on age-specific growth, body size, body condition and reproduction of C. collaris in northern Arkansas. Results suggested that populations in degraded glade habitats experienced reduced body growth rates, delayed age of maturity, reduced clutch sizes and a nearly 50% decline in annual population fecundity rates. In chapter two, I investigated the importance of postural adjustment in C. collaris. Results suggested that the use of posture had significant effects on multiple indices of the thermal environment. I used findings from chapter two to account for C. collaris posture and to develop refinements in methodologies to compare the thermal environment among glade sites. In chapter three, I investigated the thermal sensitivity of digestion in C. collaris across four temperature treatments (28, 31, 34 & 37°C). Results in chapter three suggested that C. collaris digestion performance is affected by temperature, primarily through effects on passage times and voluntary feeding rates. In chapter four, I used results from the previous three chapters to erect several mechanistic hypotheses aimed at identifying the link between glade encroachment and reduced reproductive rates in C. collaris. Data in chapter four suggest C. collaris in encroached glades experience reduced environmental heat loads, shorter activity-times and less time-at-temperature suitable for digestive processing. The reduction in time-at-temperature for digestive processing resulted in lower energy available for growth and reproduction (~41%), which led to
a near 50% decline in energy devoted to reproduction (# of eggs). Results from this dissertation provide a compelling explanation to the mechanisms causing a shift in life history traits that appear to play an important role in population declines of C. collaris in the Ozarks.
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I. DISSE RTATION INTRODUCTION

Preface

Like many biologists, when I first began graduate school I was attracted to the idea of conservation of species. Although most biological research relates in some shape or form to conservation, it is not always possible to work on a biological question or study system with direct implications for conservation of biodiversity. Thus, in a lot of ways, I consider myself one of the “lucky ones” by having the opportunity to work in a study system that relates directly to conservation issues. I also consider myself lucky by being exposed to physiological-ecology, as this integrated subdiscipline of biology has completely shaped my research interests and presented me with some great opportunities in conservation. My general research interests are to understand the mechanisms by which species respond to environmental change. A powerful yet underutilized approach in conservation derives from an understanding of the complex interactions between the environment and time-energy allocations of organisms. An ideal model system to investigate mechanistic factors is one with a clear environmental shift, and a detectable species response. Here, I discuss an example of one such model system, the Eastern Collared Lizard (*Crotaphytus collaris*) in the Ozark glade habitats of Arkansas and Missouri.

INTRODUCTION

Ozark Glades and *Crotaphytus collaris*

*Crotaphytus collaris* is a medium sized (30–50g), sexually dimorphic lizard with conspicuous color patterns (McCoy et al. 1997; Macedonia et al. 2004). This species occupies xeric microhabitats and is adapted to rocky landscapes common in the desert southwest of North America (Sloan and Baird 1999; Hutchison 2003). Arkansas and Missouri represent the eastern
range limit of *C. collaris* (Templeton et al. 1990; McGuire et al. 2007) where it is restricted to glades habitats (xeric limestone prairies and red cedar glades; Baskin and Baskin 2000) and rocky outcrops within the Ozark and Ouachita mountains (Trauth et al. 2004). Historically, Ozark landscapes were maintained by natural and anthropogenic fire regimes (Strausberg and Hough 1997; Brisson et al. 2003). However, wildfires were excluded almost completely from the Ozarks throughout the 20th century (Templeton et al. 2001; Verble, 2012) allowing invasion of fire intolerant species into glade habitats (e.g. eastern red cedar, *Juniperus virginiana*) and providing conditions necessary for succession of other woody plants (Van Zandt et al. 2005; Neuwald and Templeton 2013). Over the last half century, *C. collaris* has experienced continued population declines and local extinctions through much of the Ozark Mountains (Templeton et al. 2011; Grimsley 2012; Neuwald & Templeton 2013; Arkansas Wildlife Action Plan 2016), prompting state conservation listing in Arkansas (S2) and Missouri (S4). For example, historically *C. collaris* in Arkansas inhabited all of the Interior Highlands ecoregion (including the Ozark Highlands, Arkansas Valley and Ouachita Mountains), but is now considered rare or extirpated throughout the Ouachita Mountains ecoregion (AWAP 2016).

Glade encroachment, characterized by invasion of dense woody vegetation (Laughlin 2004), has been associated with reductions in population size, gene flow (Hutchison and Templeton 1999; Brisson et al. 2003; Templeton et al. 2011), body size, and delayed age of maturity in *C. collaris* (Sexton et al. 1992; Brisson et al. 2003). Furthermore, studies in Missouri have recorded increased population sizes and gene flow as a result of tree removal and controlled fires (Brisson et al. 2003; Templeton et al. 2011; Neuwald and Templeton 2013; Table 1). Thus, it is clear that increased glade encroachment has contributed significantly to observed population declines of *C. collaris* in the Ozarks. However, what is not clear are the causal factors that link
environmental change (glade encroachment) to local extinctions in this study system. The focus of my dissertation research is to investigate the mechanisms leading to population declines of *C. collaris* in northern Arkansas.

**Operational Environments and Life Histories**

There are several potential mechanisms associated with glade encroachment that could lead to reduced population declines observed in the Ozarks. Causal factor in biological systems are seldom univariate (Quinn and Dunham 1983), and often include multiple interacting and complex variables (Dunham and Beaupre 1998). Thus, it is likely that several factors play a collective and interacting role in population declines of *C. collaris*. To determine the most effective research approach to investigate this complex ecological question requires consideration of a few fundamental principles. First, if a population is declining it must do so by way of population vital rates: survival, reproduction and migration (Cole 1954; Dunham and Overall 1994). There are no specific data on survival or reproductive rates of *C. collaris* in the Ozarks, and migration appears to cease as a function of glade encroachment (Brisson et al. 2003; Templeton et al. 2001). However, data from Sexton et al. (1992) suggested that growth rates declined with glade encroachment. Since clutch size is positively correlated with female body size in *C. collaris* (Ballinger and Hipp 1985), it is possible that populations in encroached glades have reduced fecundity rates as a result of reduced age-specific body size. Thus, at the outset, a decline in growth and reproduction would appear to be important variables to investigate.

Second, if *C. collaris* in encroached glades experience reduced growth, reproduction or survival rates, this necessarily means they are experiencing some factor that is resulting in a shift in their life histories. Here, I define a life history borrowing from Dunham et al. (1989): a set of
heritable rules that dictate allocation of time, resources, and reproductive effort. Vital rates (birth, mortality and migration rates; Cole 1954) are the emergent property of a life history. Thus, a life history phenotype can be viewed as the result of interactions between the environment and time-energy allocations that ultimately determine vital rates (Dunham et al. 1989). In short, an investigation into the factors that link environmental change to population declines inherently becomes an investigation into the ecological variables affecting life history characteristics of a population. Which leads to the third fundamental principal: woody vegetation encroachment could result in important changes to the operational environment available to C. collaris. Here, operational environment refers to the suite of biotic and abiotic environmental factors that an organism must contend with and that dictates its allocation of time, energy and reproductive effort (Beaupre 2002). Operational environments include (but are not limited to) the resource (i.e. food or water), biophysical (i.e. environmental temperature, moisture or pH), predation and social/demographic environments (Dunham et al. 1989).

At the outset, I anticipated that encroached glades would have reduced daytime environmental heat loads as a result of increased shade. Similarly, substantial changes in floral density and composition imply that arthropod (primary food source of C. collaris; McAllister 1985) densities could be affected by woody vegetation encroachment. The thermal and resource environments both play an important role in ectotherm mass-energy budgets (Andrews 1982; Congdon et al. 1982; Adolph and Porter 1993) and have been implicated as the primary source of variation in life history traits of several ectotherm study systems (Grant and Dunham 1990; Beaupre 1993; Angilletta 2001; Niewiarowski 2001; Dunham and Beaupre 1998). Since previous research in Missouri indicated dense woody vegetation is associated with reduced body sizes in C. collaris (Sexton et al. 1992), my research investigation of operational environments primarily
focused on the thermal and resource environments. Thus, the general framework of my dissertation research was to investigate potential differences associated with operational environments (thermal and food-resource) and life history traits that could be used to identify explanatory mechanisms linking habitat degradation to population declines of *C. collaris* in the Ozarks.

**Intact versus Encroached Glade Comparisons**

For the majority of my research, my general study design included population level comparisons between intact glades (with observably low levels of woody vegetation density) and encroached glades (with observably high levels of woody vegetation density). Although it is easy to find historic *C. collaris* sites that are now encroached, only a small fraction of them still have remnant populations (Grimsley 2012). Thus, in my first field season (summer 2013), I visited 22 historic *C. collaris* sites. At each site I spent several hours to multiple days searching for and capturing lizards – in an attempt to determine the best populations to use for comparisons. I designated three sites as intact glade populations, two of which are the largest natural populations of *C. collaris* that I am aware of in Arkansas. For the encroached sites, I attempted to choose the largest populations I could find, but where the site could still be defined as encroached. By the end of the summer, I had found a total of six populations in encroached sites. Two of the six encroached sites included populations of three-to-four adults, so I excluded those population from the study (although I continued to monitor those populations). In 2016, one of the four encroached glade populations that I had designated for comparisons reached a population size of zero (extirpated), so some of the comparisons in my dissertation include only three encroached glades. To make comparisons of habitat characteristics among glades extant *versus* extirpated of
C. collaris, I also included three extirpated sites. Information about each of the glade sites and their general locations are included in the following chapters. However, I excluded detailed location information because of concerns about C. collaris exploitation.

Research Approach: Specific Chapters

Chapter 1 – In chapter one, I addressed two primary questions in my dissertation research. First, what are the physical differences between intact and encroached glades. I address this first question by quantifying ten habitat variables assumed to be ecologically important for C. collaris. I also used habitat comparisons to look for differences and similarities between recently (within the past 10 years) extirpated sites, to that of intact and encroached sites. The habitat comparisons helped to define encroached and intact glades, and also identified physical differences that could be linked to variations in operational environments. I also addressed the question of whether there are any important life history trait differences between populations in intact and encroached glades. Here, I was primarily testing for differences in age-specific growth (mass and body length) or reproduction (age of maturity, clutches per year, number of eggs per clutch and population fecundity rates). I also tested for differences in body condition between populations. Results from chapter one provided the primary observations (variation in habitat characteristics associated with population level shifts in life history traits) that the remaining chapters are focused on understanding.

Chapter 2 – In chapter two, I focused on a few important details about the thermal ecology of C. collaris, and how best to quantify the effects of environmental temperature on basking lizard species. At the start of my dissertation study, I had envisioned that my second dissertation
Chapter would be a general thermal ecology study of *C. collaris*, with a focus on important thermal differences between intact and encroached glades. However, thermal ecology investigations require consideration of several important biophysical details (Porter and Gates 1969; Bakken et al. 1985), as well as dealing with multiple key assumptions (Bakken 1992; Bakken and Angilletta 2011; Wills and Beaupre 2000). Indeed, there is still substantial debate over the appropriate methodologies and applications in thermal ecology research (Heath 1964; Blouin-Demers and Nadeau 1999; Currin and Alexander 1999; Wills and Beaupre 2000; Bakken and Angilletta 2011). During the process of identifying the most suitable approach to investigate the thermal ecology of *C. collaris* in northern Arkansas, I noticed some behaviors (adjustment in body posture) that I was uncertain how best to account for. Before I could establish a suitable thermal ecology protocol for my study, I had to determine if body posture would have any important effects on my inferences - and if it did affect inferences, how best to account for it. Thus, chapter two demonstrates the importance of, and how to account for, postural adjustment in thermal ecology studies. Equally important, it represents a critical component of my dissertation by establishing the methodologies I used to investigate differences in the thermal environment between intact and encroached glades in chapter four.

**Chapter 3** – In chapter three, I investigated the thermal sensitivity of digestive performance in *C. collaris*. The focus of chapter three was spawned from two general observations provided from chapter one. First, data in chapter one implied that *C. collaris* in encroached glades appeared to experience a reduction in the assimilated energy allocated to growth and reproduction. One important way that ectotherms could experience a decline in their energy budgets is through a decline in digestive processing. Second, chapter one demonstrated the substantial difference in
shade cover produced by dense woody vegetation in encroached glades. The increase in shade cover means encroached glades experience a decline in available solar radiation, which could mean lizards in encroached glades experience reduced body temperatures. Thus, chapter three is focused on understanding the effects of temperature on digestive performance in *C. collaris*, to determine the potential role that it could have on the energy budgets of lizards in encroached glades.

*Chapter 4* – My final chapter is focused on combining information from the previous three chapters to investigate the link between environmental change (encroached glades) and life history traits (reduced annual fecundity rates) of *C. collaris* in northern Arkansas. My approach in chapter four draws on several seminal mechanism based squamate studies that used and defined the time-energy allocation framework (Dunham 1981; Congdon et al. 1982; Grant and Dunham 1990; Tinkle et al. 1993; Beaupre 1995; Dunham et al. 1998; Niewiarowski 2001). First, I addressed variation in the thermal and resource (prey availability) operational environments between glade types. Next, I tested for differences in activity-time, surface-active body temperatures, meal frequencies and movement rates. Finally, I used data from my previous chapters and from the literature to model the effect of the thermal environments, activity-time budgets, digestive processing rates, and net assimilated energy available for growth and reproduction in *C. collaris*. 
REFERENCES


II. Chapter 1: Habitat loss and local extinction: Linking population declines of Eastern Collared Lizards (Crotaphytus collaris) to habitat degradation in Ozark glades

Casey L. Brewster, Steven J. Beaupre, and John D. Willson

ABSTRACT

Habitat loss and degradation are the leading causes of local extinctions, making preservation and restoration of remaining habitat increasingly critical to conservation of biodiversity. However, mechanisms driving species extinction often begin with habitat loss and are seldom well-understood, which greatly limits our ability to mitigate their impacts. The first step in understanding mechanisms that drive local extinction is to identify vital rates that are affected by habitat degradation. Here we provide a case study of the impact of habitat degradation on individual growth and reproductive rates of Eastern Collared Lizards (Crotaphytus collaris), a species of special concern in the Ozark Mountains, USA. Our data suggest that C. collaris in habitats encroached by dense woody vegetation have reduced age-specific body size, primarily as a result of depressed individual growth rates in their first 2 yr of life. In turn, female C. collaris in habitats with high woody vegetation density have delayed age of maturity (by one year in 70% of females), smaller age-specific clutch size, and reduced clutch frequency (up to a 50% decrease in population annual fecundity). We conclude that depressed reproductive rates of C. collaris in degraded habitats likely contribute to population declines in Ozark glades. Our study provides the basis for understanding mechanisms driving population declines of C. collaris in the Ozarks and highlights an under-utilized perspective that can be used to link casual factors to local extinction.
INTRODUCTION

Effective conservation strategies aimed at ameliorating the negative impacts of habitat loss on biodiversity require explicit information on cause-and-effect and consequence of species declines (Cooke and O’Conner, 2010). Mechanism based inquiry not only allows investigators to better understand the root cause of population declines (Dunham and Beaupre, 1998; Carey, 2005; Tuff et al., 2016), but also allows greater predictive power under future scenarios (Kearney and Porter, 2009; Huey et al., 2012). Unfortunately, causal relationships driving population declines are seldom well understood, limiting our ability to mitigate impacts of environmental change. However, the most direct mechanisms associated with any change in population size (including extinction) are vital rates (birth, death, emigration, and immigration rates; Dunham et al., 1989; DeAngelis et al., 1991; Beaupre, 2002). Thus, studies that examine causal relationships among habitat loss and factors that influence the four fundamental rates provide invaluable information for determining effective conservation strategies (Cooke and O’Conner, 2010). Here we provide a case study that investigates the causal factors driving population declines in Eastern Collared Lizards (*Crotaphytus collaris*), a species of special concern in Arkansas and Missouri, USA (S2 and S4, respectively), as a result of habitat degradation in Ozark glade habitats.

Ozark glades are insular habitats consisting of xeric limestone prairies with exposed bedrock and thin soils within a savannah-woodland matrix (Baskin and Baskin, 2000). Historically, Ozark glades were maintained by natural and anthropogenic fire regimes (Strausberg and Hough, 1997). However, wildfires were excluded almost completely from the Ozarks throughout the 20th century (Jenkins et al., 1995; Verble, 2012) allowing invasion of fire intolerant species into glade habitats (e.g., Eastern Red Cedar, *Juniperus virginiana*), and providing conditions suitable for succession of other woody plants (Laughlin, 2004).
Encroachment of woody plants has resulted in habitat degradation of many Ozark glades (Jenkins et al., 1995; Laughlin, 2004), and in turn, substantial population declines in a glade-dependent predator, *C. collaris*.

Previous studies on declining *C. collaris* populations in the Ozarks have provided a compelling argument that habitat degradation through anthropogenic fire suppression is an important driver of population declines (Templeton et al., 1990; Grimsley, 2012; Neuwald and Templeton, 2013). However, no studies have investigated mechanisms that link habitat degradation to population declines. One study (Sexton et al., 1992) on a central Missouri population inhabiting a glade encroached by woody vegetation (encroached glade) reported reduced age-specific growth rates, compared to *C. collaris* in glade habitat with low woody vegetation density (intact glade). However, the central Missouri population represents the only reported case of reduced body size in *C. collaris*, so the generality of this phenomenon is unknown. Furthermore, Sexton et al., (1992) did not provide data quantifying vegetation encroachment, how habitat degradation affected reproductive rates, or any effects on population size. Therefore, the effect of woody vegetation encroachment and the mechanisms by which it might cause a decrease in age-specific body size of *C. collaris* remain unclear. Additionally, the long-term consequences of reduced body size and its effect on life history traits (i.e. reproductive potential) have not been examined.

Examining complex mechanisms driving population declines such as that of *C. collaris* in Ozark glades can be a daunting task. However, investigating impacts to vital rates provides a relatively straightforward starting point to address the most probable explanatory factors driving local extinctions. Our approach in this study was to test for an association between woody vegetation encroachment and reproductive rates in female *C. collaris* across multiple
populations. If *C. collaris* in encroached glades do not have reduced reproductive rates, then this implies that population declines in these habitats are driven primarily by a reduction in survival rates (or increased emigration rates); and further investigation should follow this line of questioning. Conversely, if reproductive rates are reduced, the study then turns to investigating casual mechanisms that could explain a decline in reproduction as a result of habitat degradation. Thus, our line of questioning was three-fold. First, we compared habitat characteristics among three Ozark glade types (intact, encroached, and glades recently extirpated of *C. collaris*) to quantify any important structural differences. Next, we compared age-specific body size, growth, and female reproduction rates between *C. collaris* in intact versus encroached glades. Lastly, we compared body condition (males) and the clutch-size to body-size relationship (females) of *C. collaris* from both glade types (intact and encroached) to uncover potential explanatory factors driving population declines in Ozark glades.

**METHODS**

**Study Sites**

We sampled 10 glade sites, and designated each site into one of three categories: 1) intact glades: extant population of *C. collaris*, with observably low levels of woody vegetation (*n* = 3); 2) encroached glades: extant population of *C. collaris*, with observably high levels of woody vegetation encroachment (*n* = 4) and; 3) extirpated glades: recently extirpated of *C. collaris*, with observably high levels of woody vegetation encroachment (*n* = 3). Sites were deemed extirpated after we made a minimum of four visits to each of the sites over a 2 yr span where no *C. collaris* were observed, and animals were known to have been captured in the previous 10 yr. All sites were naturally occurring glades located in the Ozark St. Francis National Forest (along the White
River and Buffalo River), within a radius of 80 km in north-central Arkansas. We have excluded the exact locality of populations in this study to reduce the possibility of human exploitation of *C. collaris* at these sites.

**Habitat Variables**

All habitat variables were measured in late May and early June in 2014 and 2015, to reduce potential differences in plant biomass associated with seasonality. We measured 10 total habitat variables including: density of large (+15 cm d. b. h.) and small (2.5 cm–15 cm d. b. h.) trees; percent cover of canopy, grass, shrubs, exposed rock, litter, shade on the ground surface at solar noon, and rocks ≥ 30 cm above the surface (the microhabitat used most frequently by *C. collaris* in this study); and area of total continuous glade habitat. We chose these 10 habitat variables with the goal of: 1) capturing the most important habitat variables in Ozark glade habitats, and 2) providing an approximately even representation of variables assumed to be related to woody vegetation density (tree densities, canopy, shrub, and shade cover), and variables not necessarily related to woody vegetation density (total area, and percent cover of rock, litter/moss, grass, and 30 cm rock). We utilized randomly designated (randomizing compass direction and distance) 100 m² blocks, with a minimum of eight blocks/site, to estimate density and percent cover variables (except for percent shade), and used the mean of these estimates per site to quantify habitat variables. Each 100 m² block was partitioned into four even quadrants and sub-sampled for each of the cover variables. Total habitat area was estimated using aerial imagery. For percent canopy cover, we scored the number of times canopy was observed through a densitometer along five 10 m transects (~2.5 m resolution) within the 100m² sampling blocks (25 measurements/block). Percent cover of shade was estimated as the fraction of 200 randomly
designated points (~2 m resolution) along a linear transect where a 4 cm object (a painted rock) was either covered or not covered with shade, at solar noon (± 30 min). All other percent cover estimates are the mean estimated percentage of each variable that covered the ground surface (or the sky, for canopy cover) in a block. Density estimates are the mean number of trees per 10 m$^2$ block and converted to density/hectare.

**Data Analysis: Habitat Variables**

We used a correlation matrix to assess collinearity among habitat variables. We used $r < 0.75$ as the maximum threshold for variables used to test for differences among glade types (intact, encroached, and extirpated) in a MANOVA. In the case where multiple variables were correlated with one another ($r \geq 0.75$), we used a single variable to represent correlated variables. We also used linear discriminant analysis (LDA; Cottenie, 2005), which is a form of discriminant-function analysis (Williams, 1983) in the R statistical software package, to assess priority of habitat variables in distinguishing among the three glade-types.

**Lizard Capture and Age Classification**

Although some *C. collaris* were originally captured and marked in 2013, the majority of data used here are from captures spanning 2014–2016. Animals were captured using a noose, toe-clipped to give a unique permanent mark, and released at the capture location. Upon capture we recorded SVL (snout-vent length) to the nearest mm using a metric ruler and mass to the nearest 0.5 g using a spring scale (60 g Pesola$^{TM}$). For each initial capture, we classified individuals as young-of-the-year (YOY), one-year-olds (1YO) or at least two years-old and older (adult) using conspicuous coloration patterns unique to the three age groups (McCoy et al., 1997). To classify
adult age classes (2YO–4YO), we only used individuals that were originally captured as YOY or 1YO’s, or when minimum age was known to be at least 4YO (e.g., classified as adult in 2014, recaptured in 2016). Although we did find individuals known to be at least 5YO, these instances were rare. Furthermore, most *C. collaris* tend to asymptote in size by 4YO (Sexton et al., 1992), so we grouped animals known to be 4YO and animals known to be at least 4YO into one age class (4YO+). *Crotaphytus collaris* is sexually dimorphic at maturity, and sex of juveniles was determined by the presence of (males) or absence of (females) enlarged post-anal scales (Telemeco and Baird, 2011).

**Age/Sex-Specific Body Size and Growth**

For comparisons of age- and sex-specific body size of 1YO and adults, we only used body size estimates obtained 1–30 May of each year. We used a narrow range of capture dates to reduce confounding variation in age-specific body size associated with time of year. As YOY typically do not start hatching until mid to late July in Arkansas (Brewster et al., 2013), age-specific body size of the YOY age class was estimated from captures dated between 20 August–20 September. We also compared age- and sex-specific differences of within-season SVL growth rate (mm/day). In all but the YOY age class, we used a minimum of 30 d between recaptures to reduce the influence of investigator measurement error in growth rate estimates. We observed our lowest recapture rate in the YOY age class. Thus, to increase sample size in growth rate estimates in the YOY age class, we used a minimum 14 d between recaptures. The YOY age class has the fastest growth rates of any of the age classes in *C. collaris* (Brewster et al., 2014), reducing potential impacts of measurement error over the shorter recapture interval.
Age-Specific Female Reproduction

We attempted to recapture females once every 2 wk through the peak of the reproductive season (early May–early July) to track reproductive condition (e.g. non-detectable, small follicles, large follicles, shelled oviductal eggs, post-oviposition, and clutch size; Sloan and Baird, 1999). Females typically reach maturity at 1YO, but smaller females may not reach maturity until 2YO (Trauth, 1978; Ballinger and Hipp, 1985). Reproductive condition was assessed by physical palpation or when knowledge of a clutch had been previously identified through palpation, the presence of dried mud on the dorsum, and recent significant weight loss, which are both indicative of recent oviposition (Baird et al., 2001). Although physical palpation has been used to estimate clutch size in lizards (Cueller, 1971; Turner et al., 1982; Olsson et al., 2002), in our experience with *C. collaris*, the only palpation estimates in which we are confident are those made once eggs have been shelled (large, turgid, and distinct). Thus, we only use clutch size estimates made when females had shelled oviductal eggs. However, we did use all reproductive condition categories to assess clutch frequency (zero, one, two, or three clutches/season), as long as a female had been captured a minimum of three times in a reproductive season.

Data Analysis: Body Size, Growth, and Reproduction

In all age-specific comparisons of body size, growth, and reproduction (clutch size/clutch frequency), we used linear mixed models (nlme package in R; Bates et al., 2015) to test for a difference between glade-types (intact and encroached). *Crotaphytus collaris* is sexually size dimorphic as adults (Fitch, 1956; McCoy et al., 1997; Brewster et. al., 2014), so to reduce model complexity, models were fit separately for males, and females. All response variables (SVL, mass and change in SVL) were log transformed, except for clutch frequency and clutch size,
where we used raw data. To estimate the predicted annual age-specific fecundity ($F_x$) between glade-types, we multiplied the annual mean clutch frequency for each age class times the corresponding mean clutch size of each age-class (Cole, 1954). To estimate the predicted cumulative age-specific fecundities ($\Sigma F_x$) we summed the estimated $F_x$ over each age-class from 1YO–4YO animals. Annual and cumulative age-specific fecundities ($F_x$ and $\Sigma F_x$) are derived estimates, so we do not use these two estimates for statistical comparison.

We tested for interactions of fixed effects among glade-type, site, and age ($\log_{10} \text{age}+1$; for growth rate, we used initial SVL as a covariate). Data on individual *C. collaris* were repeated over multiple years in many instances, so animal “ID” was included as a random factor. Model selection for fixed effects was based on AICs derived from maximum likelihood estimation (Bozdogan, 1987), and random effects based on restricted maximum likelihood estimates (Pinheiro et al., 2017). In all mixed model comparisons, positive general-definite covariance structure provided the best fit to our models.

**Body Condition and Clutch-Size to Body-Size**

To test for differences in body condition between glade-types, and the clutch-size to body-size relationship, we used linear mixed models with ID as a random factor. For body condition, we used the log$_{10}$ of mass as the response variable and log$_{10}$ of SVL as the predictor (testing for interactions among age, glade-type, and SVL). We used only male *C. collaris* for body condition comparisons because body mass of females can be confounded by variation in timing of gravidity. For the clutch-size to body-size comparison, we used the log$_{10}$ of clutch-size (number of eggs) as the response variable, and log$_{10}$ of SVL as the predictor variable (testing for interactions among age, glade-type, and SVL).
RESULTS

Habitat Variables

All five woody vegetation density variables (large tree density, small tree density, canopy, shrub, and shade cover) were highly correlated with one another \((r > 0.80)\), whereas none of the other habitat variables (total area, and rock, litter/moss, grass, and 30 cm rock cover) were highly correlated with one another \((r < 0.71)\). We chose to use shade cover in our analysis over the other variables because shade cover is a result of all of the other woody vegetation variables. We combined shade cover with the other five non-woody vegetation variables in the MANOVA and LDA (Table 1). The MANOVA suggested a significant difference among glade-types \((df = 1,5, P_{\text{Pillai}} = 1.86, F = 6.645, P = 0.0147)\), although the only statistically different variable among types was shade cover \((P< 0.001)\). Similarly, the LDA indicated shade cover as the leading discriminant variable (coefficient of linear discriminant, Table 1). A post-hoc Tukey test of shade cover among glade-types suggested a statistical difference between the intact and either encroached or extirpated glade-types \((P<0.001\) in both comparisons), but no statistical difference between encroached and extirpated glade-types \((P = 0.49; \text{Table 1})\). We provide a full summary of all habitat variables among sites in Appendix 1.

Age-Specific Female Reproduction

In all of our mixed models, REML indicated models that used ID alone as random effects were the most appropriate models. Low replication of individuals in adult age classes (3 and 4 YOs) in some of the encroached sites \((n \leq 5\) in multiple age classes in two of the glade sites) precluded models that included both site and glade-type as fixed effects. We found no statistically significant age x glade-type interactions on clutch size or clutch frequency. Age-specific clutch
frequency (number clutches/female) was significantly lower in females from encroached glades compared to intact glades (intercept = −0.34, SE = 0.174; slope = 0.71, SE = 0.218; df = 1,82, $F = 21.63, P<0.001$; Fig. 1A). Similarly, age-specific clutch size (number eggs/clutch) was lower in encroached glades (intercept = 3.85, SE = 0.603; slope = 1.17, SE = 0.810; df = 1,40, $F = 20.53, P<0.0001$; Fig. 1B).

**Age- and Sex-Specific Body Size and Growth**

We found no statistically significant age x glade-type interactions in the four age-specific body size comparisons (male or female SVL, and male or female mass). Both male and female age-specific SVL was significantly greater in *C. collaris* from intact glades than in encroached glades (males, intercept = 1.75 ± 0.011 SE; slope = 0.059 ± 0.014 SE; df = 1,90, $F = 25.99, P<0.0001$ and females, intercept = 1.77 ± 0.009 SE; slope = 0.050 ± 0.012 SE; df = 1,103, $F = 24.5, P<0.0001$; Fig. 2A, B). The average difference in mean SVL across age classes was 7.7 mm for females, and 8.2 mm for males. Similarly, age-specific mass for both sexes was significantly greater for *C. collaris* in intact glades (males, intercept = 0.86 ± 0.031 SE; slope = 0.158 ± 0.040 SE; df = 1,90, $F = 32.95, P<0.0001$ and females, intercept = 0.89 ± 0.033 SE; slope = 0.144 ± 0.043 SE; df = 1,103, $F = 28.13, P<0.0001$; Fig. 2C, D). The average difference in mean mass across age classes was 7.8 g for females, and 7.9 g for males. We observed a significant age x glade-type interaction on both male and female within-season growth rates (age x glade-type interaction for males, df = 1,27, $F = 9.81, P = 0.0041$ and females df = 1,26, $F = 17.58, P = 0.0003$; Fig. 3). We provide raw summary data of age-specific body size and growth of all animals used in the study in Appendix 2.
Body Condition and Clutch-Size to Body-Size

We observed no interactions among fixed effects (SVL, age, and glade-type) as they related to body condition or clutch-size to body-size. Male body condition was not significantly different between glade types (df = 1, 50, $F = 2.6$, $P = 0.115$; Fig. 4A). Similarly, the clutch-size to body-size relationship was not significantly different between glade types (df = 1, 39, $F = 0.43$, $P = 0.516$; Fig. 4B).

DISCUSSION

The goal of this study was to investigate factors that link habitat degradation in Ozark glades to population declines in *C. collaris*. Our first line of questioning was designed to quantify important habitat differences between encroached and intact sites. We recognize that the 10 habitat variables used in this study are not exhaustive, and one could hypothesize other potentially important habitat variables associated with Ozark glades (e.g., soil depth, plant composition, geological characteristics). However, our habitat variable assessments did allow us to provide a quantifiable justification of our a priori delineation of glade-types (intact and encroached). Not surprisingly, all five woody vegetation variables were highly correlated and substantially different between intact and non-intact (encroached and extirpated) sites. The LDA (coeff. LD1; Table 1) indicated shade as the best discriminant habitat variable among glade-types, and MANOVA suggested a significant difference among glade-types in the six habitat variables. However, the only variable that was significantly different was shade, and this variable was statistically indistinguishable between encroached and extirpated glade-types (Table 1). Similarly, we found no statistical difference in non-woody vegetation variables among sites. Therefore, other non-woody vegetation variables that should be important to *C. collaris* (e.g.,
size of the habitat, availability of basking rocks or amount of grasses which should provide necessary densities of Orthoptera, a primary prey source) were similar among all sites (Table 1, Appendix 1). We interpret our habitat assessment results to suggest that the most important difference between intact and non-intact glades was density of woody vegetation encroachment and resulting shade cover.

Our second line of questioning was designed to determine if *C. collaris* in encroached glades had reduced age-specific growth, body size, or reproductive rates. Both age-specific clutch size and clutch frequency were significantly lower in *C. collaris* from encroached sites. In fact, 70% of females in encroached sites did not produce their first clutch until 2YO; a full year later than 98% of females in intact sites. Similarly, females in encroached sites had smaller clutch sizes than females in intact sites. The potential impacts of these differences are apparent in the predicted cumulative number of eggs ($\Sigma F_x$) of a 4YO female in an intact versus an encroached glade (72.0 and 42.5 eggs, respectively; Fig. 1D). Taking the approximate mean age-structure of all sites (55% 1YO, 30% 2YO, 20% 3YO, 5% 4YO) multiplied by the estimated number of eggs per season for each age class ($F_x$; Fig. 1C), yields an approximate 50% decrease in annual population fecundity in an encroached glade compared to an intact glade (assuming the same initial population size). Depending on age-specific survival rates in encroached populations, the estimated deficits in reproduction would be consistent with population declines in encroached sites.

Age- and sex-specific body sizes were smaller in encroached glades compared to intact glades (Fig. 2; Appendix 2). However, in our within-season growth rate comparisons, we observed a significant interaction between glade-type and age. Thus, the effect of age on growth rates was different between glade types (intersecting slopes; Fig. 3A, B). The age x glade-type
interaction is likely because of the large difference between glade-types in growth rates of
younger age classes (YOY and 1YO), compared to the similar growth rates of older age classes
(3YO and 4YO; Fig. 3; Appendix 2). Superficially, it may be surprising that we did not observe
an age x glade-type interaction with body size, but did with within-season growth rates.
However, it is important to note that body sizes of *C. collaris* in intact glades were still
substantially larger than *C. collaris* in encroached glades at the older age classes (3YO and 4YO;
Fig. 2; Appendix 2), even though within-season growth rates were not. Thus, our data suggest
that animals in intact glades approach asymptotic body size (where growth rates would naturally
decline) at a younger age than animals in encroached glades, by experiencing greater growth
rates at younger age classes.

Unfortunately, we currently do not have data that could explain how or why *C. collaris*
would exhibit this difference in growth patterns between glade-types. One explanation could be
that *C. collaris* in encroached sites experience some factor (e.g., reduced energy acquisition or
digestive processing) that leads to lower total assimilated energy per unit time than *C. collaris* in
intact glades. If we assume that all *C. collaris*, regardless of glade-type, allocate proportionally
less energy to growth with increasing body size, then this would lead to an earlier decline in
growth of *C. collaris* (i.e., declines at younger ages) in intact glades compared to *C. collaris* in
encroached glades. This explanation is consistent with our observation of an age x glade-type
interaction in growth rates of *C. collaris* (i.e., intersecting slopes for age-specific growth), and a
significant difference of body size between glade-types (with no age x glade-type interaction).
However, without a better mechanistic understanding of variables driving differences in growth
between glade-types, the causal link between these patterns remains unclear. What is clear
however, is that reduced growth rates, primarily in their first 2 yr of age, result in reduced age-
specific body sizes in encroached glades compared to intact glades, and ultimately lead to reduced age-specific reproductive rates.

Our third line of questioning was designed to investigate potential factors, other than differences in age-specific body size, that could explain population declines of *C. collaris* in encroached sites. First, we found no statistical difference in body condition of males between glade-types (Fig. 4A). Thus, factors potentially linked to poor body condition (e.g. starvation) would appear to be similar between *C. collaris* in intact and encroached glades. Next, we tested for a difference in the SVL to clutch size relationship between glade-types (Fig. 4B) to determine if factors other than reduced age-specific body size contribute to reduced reproductive rates. Had we found a significant interaction (i.e. among SVL, age, or glade-type), or a significant difference in intercepts (clutch size as a function of SVL), this would have suggested that some factor other than age-specific body size contributes to clutch size variation. For example, if females in degraded glades had significantly lower clutch sizes as a function of SVL than females in intact glades, this would have indicated that animals differed in their proportions of allocation between growth and reproduction, and that differences in reproductive rates were not necessarily explained solely by age-specific body size differences. Because we found no statistical interaction, and no difference in clutch size as a function of SVL, we conclude that reduced age-specific body size is the primary factor driving reduced reproductive rates in *C. collaris* from degraded sites.

Our data suggest that *C. collaris* restricted to habitats with dense woody vegetation exhibit a shift in life history traits (body size, growth, and reproduction) that is consistent with explaining observed population declines. Specifically, we conclude that depressed growth rates within their first 2 yr of life leads to smaller age-specific body sizes and reduced reproductive
rates. Our findings provide a compelling argument that anthropogenic fire suppression in Ozark glades is driving extensive population declines in an important glade predator. Our results also underscore the importance of habitat restoration through prescribed fire and mechanical removal of dense woody vegetation in Ozark glade habitats. Indeed, the only reported cases of *C. collaris* population recoveries have been projects that included glade restoration through prescribed fire (Brisson et al., 2003; Neuwald and Templeton, 2013).

We recognize that multiple interacting factors likely drive variation in life-histories among *C. collaris* populations in Ozark glades, and some of these factors may be linked to variables other than woody vegetation density or shade cover. Future research will need to focus on the causal link between dense woody vegetation, and reduced growth. For example, one of the authors (C.L.B.) is currently investigating the impact of increased shade in encroached glades on available operative temperatures (Bakken, 1992), surface activity-time budgets (Grant and Dunham, 1988), and animal body temperatures; as well as any differences in prey densities between glade-types. We also note that dense woody vegetation may cause a decline in survival rates, as well as reproductive rates. Because understanding population level impacts requires data on all four fundamental rates, we cannot infer the entire impact of habitat degradation on *C. collaris* populations from the data presented here. However, because migration is extremely limited for this species through most of the Ozarks (Templeton et al., 1990; pers. obs.), unless dense woody vegetation increases survival rates substantially, the observed decline in reproduction of *C. collaris* in encroached sites will likely lead to population declines and extirpations of this species throughout the Ozarks.

We have provided a case study aimed at understanding the link between habitat degradation and local extinctions of an at-risk species. Our study shows the effectiveness of
using mechanism-based inquiry to investigate complex conservation issues and highlights the utility of investigating vital rates to uncover casual factors contributing to local extinctions. Projects such as ours are important not only to understanding how habitat loss and degradation are influencing at-risk species, but also to identifying effective strategies that can slow or even reverse current declines. For example, data from this project have helped prompt state level funding for conservation of *C. collaris* and will be used to guide habitat restoration efforts on five of the seven non-intact sites in this study.

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REFERENCES


Fig. 1. Adjusted means and ± 95% CI of age-specific A) clutch frequency, B) clutch size, C) predicted number of eggs per season ($F_x$), and D) the cumulative sum of $F_x$ ($\Sigma F_x$). $F_x$ and $\Sigma F_x$ are estimates derived from clutch frequency and clutch size, and not used to assess statistical differences.
Fig. 2. Adjusted means and ± 95% CI for age-specific body size (log transformed) between glade-types (encroached and intact) for: A) male SVL, B) female SVL, C) male mass, and D) female mass. \( \log \text{Age} = \log_{10}(\text{no. yr} + 1). \)
Fig. 3. Within season growth rate (log of mm/day) of male (A) and female (B) lizards from intact and encroached glades. \( \log \text{Age} = \log_{10}(\text{no. yr} + 1) \).

Fig. 4. Comparison of A) male body condition and B) female SVL to clutch size between lizards from intact and encroached glades.
Table 1. Group means of six habitat variables among three glade-types and coefficients of linear discriminant one (LD1) showing leading discriminant variable (% cover-shade). Four highly correlated variables not shown (see Appendix 1). Density (number of lizards/hectare) of 1YO and above C. collaris. Percent cover of shade at solar noon (shade), exposed bedrock (rock), rocks ≥ 30 cm above surface (bask. rock), grass, and leaf litter or moss (litter). Total glade habitat area (habitat) in hectares. Percent cover-shade used in post-hoc Tukey test: intact vs. encroached, $P<0.001$; intact vs. extirpated, $P<0.001$; encroached vs. extirpated, $P = 0.49$.

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<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
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LD1: 6.64 -1.43 -1.40 -0.81 -0.68 -0.09
Appendix 1. Summary data of habitat variables used to characterize 3 glade-types (intact, encroached, and extirpated). Density (number/hectare) of 1-year-old and above *C. collaris* (lizards), trees with d. b. h. (diameter at breast height) of 2.5 cm–15.0 cm (Sm.tree), and d. b. h. of 15 cm+ (Lg.tree). Percent cover of mid and over-story (canopy), under-story (shrubs), shade on ground surface at solar noon (shade), exposed bedrock (rock), rocks ≥ 30 cm above surface (bsk. rock), leaf litter or moss (litter), and grass and forbs (grass). Total continuous glade habitat area (habitat) in hectares. Predicted values from linear discriminant analysis (predicted LD1 values; proportion of trace, LD1 = 0.927, LD2 0.0783).

<table>
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<th>Lg. tree</th>
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Appendix 2. Summary table of mean, 95% CI, and n of age- (yr) and sex-specific SVL (mm), mass (g), and within season growth rate (mm/day) of *C. collaris* from encroached and intact glades.

<table>
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Chapter 2: The effect of body posture on available habitat and activity-time in a lizard: Implications for thermal ecology studies

Casey L. Brewster and Steven J. Beaupre

ABSTRACT

Ectothermic animals contend with variable environmental temperature through behavioral thermoregulation, including selection of activity-times and microhabitat spaces with suitable operative temperatures. Thus, an important component to understanding the influence of temperature on animals is through the assessment of thermal constraints on time and space usage. Thermal ecologists have recognized that postural adjustments are an important part of behavioral thermoregulation. However, the impact of postural adjustments on available space and time has received little attention. We hypothesized that postural adjustments would significantly affect the thermal availability of space and time for surface activity. To test our hypothesis, we used data collected over a four-year study of the thermal ecology of Eastern Collared Lizards (Crotaphytus collaris) in Arkansas. We used a novel approach to model three distinct postures used by C. collaris, and to assess the impact of posture on available space and time. For our study species and habitat, posture had a significant impact on several indices of available space and time including: a) a 13% increase in length of the reproductive activity season, b) a 35% increase in the frequency distribution of habitat within active body temperature range and c) a 42% increase in average thermal quality index. We conclude that posture can significantly impact space and time available for surface activity in species that employ it for thermoregulation. Thus, a clearer understanding of the thermal constraints on time-space usage
in ectotherms requires consideration of the impact of posture on the spatiotemporal distribution of thermally suitable microhabitats.

INTRODUCTION
Temperature affects organisms on multiple scales, from molecular to whole-animal processes (Hochachka and Somero 1973; Huey 1982), and from species distributions to ecosystem interactions (Porter and Gates 1969; Gilman et al. 2010). Understanding how rising air temperatures will influence organisms is critical to predicting ecological impacts of climate change. The ability of many animals to adjust behaviorally to changing environmental temperatures is often underappreciated in climate change studies (Huey and Tewksbury 2009). Recent work suggests that behavioral thermoregulation will play a key role in buffering the negative impacts of climate warming on ectotherms (Kearney et al. 2009; Huey et al. 2012). For many animals, behavioral thermoregulation includes selection of activity-times and microhabitat spaces with suitable environmental temperatures (Porter and Tracy 1983). Thus, an important approach for predicting the impact of changing environmental temperatures on animals is through estimation of available time and space for activity (Sinervo et al. 2010; Sears et al. 2010). However, one important component of behavioral thermoregulation, adjustment of body posture, has received little attention in terms of its impact on estimation of available space and time.

The effect of posture on the biophysical properties of animals is well known (DeWitt 1971; Muth 1977a; Stevenson 1985). Postural adjustments can influence several biophysical properties such as radiative, conductive, convective and evaporative heat flux rates (Porter and Gates 1969; Bakken and Gates 1975). Thus, posture can play an important role in determining $T_b$.
(body temperature; see Box 1 for a glossary of thermal-biology terms) and heating/cooling rates that animals experience in a given micro-environment. For example, Porter et al. (1994) showed that adjustments in posture can influence the metabolic rate of birds and mammals as a result of variation in heat transfer properties. One of the earliest observations of the potential importance of posture in the thermal biology of animals was by Cowles and Bogert (1944), in their discussion of “thermal control through behavior” in desert lizards. Since 1944, posture has been shown to influence the thermal ecology of several ectotherms including: grasshoppers (Chappell 1983), dragonflies (Anderson et al. 1979), snakes (Dmi’el and Borut 1972; Ayers and Shine 1997), marine iguanas (Bartholomew 1966; White 1973), crocodiles (Seebacher 1999), turtles (Grayson and Dorcas 2004) and several surface-active lizard species (i.e. Heatwole 1970; Pearson 1954; Losos 1985; Martin et al. 1995; McConnachie et al. 2009).

In terrestrial ectotherms, postural adjustments impact two physical characteristics that influence heat flux rates. First, posture influences the animal’s shape, and in turn, surface-area to volume ratios (Porter and Tracy 1983), the amount of surface-area in contact with the substrate and the amount of surface-area exposed to the sun/wind/radiating substrate. Second, posture can impact position and orientation, which influences the distance of the animal from the substrate and the orientation to the sun/wind/radiating substrate (Stevenson 1985; DeWitt 1971). In turn, variation in these two physical characteristics influences heat flux rates including: conduction (i.e. amount of heat flux to the body surface through the substrate), convection (i.e. fluid dynamics and amount of surface within the wind profile), radiation (i.e. surface area exposed to long and short-wave radiation) and evaporation (i.e. amount of surface exposed to the air) rates (Porter and Gates 1969).
Several studies have provided evidence that ectotherms use posture as a means to behaviorally thermoregulate (i.e. White 1973; Tracy et al. 1979; Waldschmidt and Tracy 1983; Stanton-Jones et al. 2018). For example, *Callisaurus dracanoides* uses an elevated posture when $T_b$ is high, a prostrate posture when $T_b$ is low, and a tail-down posture when $T_b$ is intermediate (Muth 1977b). Adopting the prostrate posture when air temperatures are colder than substrate temperatures increases the rate of heat gained through conduction, relative to the tail-down or elevated postures (DeWitt 1971; Muth 1977a). Alternatively, adopting the elevated posture reduces the rate of heat gained through conduction while increasing heat loss to the air (convection) compared to the tail-down posture (DeWitt 1971). Therefore, adjusting posture based on the current heat load can allow an ectotherm to behaviorally thermoregulate in a single microhabitat. However, standard procedures for investigating the thermal ecology of ectotherms involve modeling $T_e$ (Box 1; Bakken 1976) of target species using a single posture. Therefore, studies of thermal ecology typically lack information on postural adjustments in the study species.

We postulated that if posture is an important component of thermal ecology then it should impact estimates of the available space and time for surface activity. For example, posture could allow an ectotherm to extend surface activity for a longer period (e.g. by adopting the prostrate posture at sunset when environmental heat loads are low) before it experiences *restrictive $T_b$s* (Box 1), thus increasing available activity-time. Similarly, if posture allows an organism the opportunity to occupy a greater proportion of the habitat at a given time (e.g. by adopting an elevated posture in microhabitats with high heat loads), then this would be an example where posture increases the available activity-space for that organism.
The goal of this study was to investigate the impact of posture on the estimated available space and time for surface activity in a diurnal lizard; the Eastern Collared Lizard (*Crotaphytus collaris*). We evaluated the impact of posture using data collected during a four-year thermal ecology study on a *C. collaris* population in Northern Arkansas. First, we tested for an association between *C. collaris* posture and \( T_b \) or environmental heat load. Next, we characterized the impact of postural adjustments on steady state \( T_b \) of *C. collaris*. Lastly, we addressed the influence of posture on available space and time by asking: 1) does postural adjustment allow *C. collaris* the opportunity to occupy a greater proportion of habitat during typical activity times, and 2) does postural adjustment provide *C. collaris* the opportunity to extend their estimated daily surface activity time?

**METHODS**

**Study system and *C. collaris* posture**

Our study was conducted on a single *C. collaris* population in Northern Arkansas at a site along the White River in the Ozark National Forest. The habitat at this site consists of xeric-limestone prairie, with large expanses of exposed bedrock within a savannah-woodland matrix. We made weekly visits from late April through late August from 2013–2016. Posture used by *C. collaris* are similar to those reported by Muth (1977b) and DeWitt (1971) of other surface-active lizards (Fig. 1a–c). Postures can be categorized based on several characteristics including: body shape, area of contact with the substrate, distance of body from substrate, head and tail position, and geometry of the body plane to the substrate (DeWitt 1971; McConnachie et al. 2009). However, we chose to classify postures into three categories similar to that reported by Muth 1977b: 1) Elevated; forelimbs and hind-limbs extended and the body completely off of the substrate (Fig.
1a), 2) Intermediate; forelimbs extended (or partially so) and posterior of ventral surface in contact with substrate (Fig. 1b), and 3) Prostrate; all of ventral surface in contact with substrate (Fig. 1c). Frequently, lizards adopted postures not included in these three categories for short periods of time (i.e. during courtship displays, mating, patrolling territories and while pursuing prey items). However, in instances where posture could not be immediately categorized for an individual, the observer could typically wait 3–5min and the lizard would adjust into one of the three categories. In the majority of observations, lizard posture could be confidently placed into one of the three categories immediately.

OTM construction

We used three types of operative temperature models (OTM; Box 1; Bakken et al. 1985) for this study, all of which consisted of a 12cm length of copper pipe (~2.0cm outside diameter). We used a preserved *C. collaris* specimen (9.8cm SVL; University of Arkansas zoological collection) to estimate surface area variables of a mean adult sized lizard in the study population (~ 9.6cm SVL; Brewster et al. 2018) to compare to OTMs. The pipe dimensions were chosen as a compromise between the mean adult SVL of *C. collaris* in the population and a cylinder with a similar surface area (~ 100cm²) as an adult *C. collaris* (~105cm²). Models were painted using spray paint (Valspar™, Labrador Brown, #85046) with a similar mean percent reflectance value (11.7%) as reported for *C. collaris* (10.6% Porter 1967). We inserted a single iButton™ temperature data logger (Maxim Integrated Products) wrapped with a piece of aluminum screening material (to keep the iButton suspended in the center of the OTM) inside each OTM, and used a plastic plug (Mocap™, T-series tapered plug) to cover the ends of the pipe. We used an unmodified OTM (painted copper pipe, described above) to mimic the biophysical properties
of *C. collaris* in the intermediate posture (unmodified OTM; Fig 1d). The unmodified OTM had approximately 3cm² of ventral surface in contact with substrate (on a flat surface), compared to approximately 4cm² of an actual lizard in the intermediate posture. To mimic the biophysical properties of a lizard in the elevated posture (stilted OTM; Fig. 1d), we modified OTMs to match the mean distance above the surface of an elevated adult *C. collaris* (~3.5cm at the coronal and radial center of the body). We fastened two 4.5cm bolts (2.5mm diameter, drilled and fastened using appropriate size nuts) at one end of the OTM, and a single 2.5cm bolt at the opposite end of the OTM. To mimic the biophysical properties of a lizard in the prostrate posture (compressed OTM), we dorsoventrally compressed the copper pipe of an OTM (using a bench vise) to an approximately 1.0cm dorsoventral height and an approximately 2.5cm lateral width (Fig. 1d). The compressed OTM had approximately 18cm² of ventral surface in contact with the substrate (on a flat surface), compared to approximately 20cm² of an actual adult lizard in the prostrate posture.

**OTM validation**

To test for a linear relationship between *C. collaris* *Tb* and the *Te* estimate of the three OTM types used, we made comparisons of animals in a given posture to the *Te* of an OTM with a corresponding posture. We captured one-year-old and above *C. collaris* opportunistically, and only on days with low cloud and wind conditions. Upon observing a stationary lizard, we approached the animal to within 5m and waited a minimum of 15min before attempting to capture the lizard by noose. The minimum time before capture was chosen to allow at least two thermal time-constants (Box 1; ~6.5min for the mean *C. collaris* body size in our population; based on the regression from Grigg et al. 1979) to elapse, thus ensuring the animal was at or near
steady state $T_b$. If at any point the animal’s posture changed categories, or if it moved from the microhabitat location, the time to attempt capture was reinitiated. Immediately after capturing an individual, its cloacal $T_b$ was recorded using a quick read digital thermometer, and the appropriate OTM type (corresponding with posture adopted by the lizard) was placed in its exact location. Once the OTM had reached equilibrium (OTM time constant ~3.5min), we recorded $T_c$ using a quick read digital thermometer. If weather conditions changed (cloud cover or wind), the procedure was started over or excluded. This model validation procedure is similar to that reported by Grant and Dunham (1988) and allowed us to compare the steady state $T_b$ of lizards in each of the three posture categories, to that of the corresponding OTM type. We used major-axis regression to assess the linear relationship between $T_c$ and $T_b$.

**$T_c$ sampling**

We used a total of one hundred OTMs, placed at fifty microsites across the habitat. We arranged OTMs along five 10m transects and designated transects randomly (randomizing the distance and direction of each transect from the center of the site) across the habitat. Models were placed 1m apart, along each transect. At each microsite, we randomly assigned orientation of OTMs (East–West or North–South). We programmed iButtons™ to record $T_c$ estimates every 20 min, for ten-day time blocks, at two-time periods (May and late June to early July). We removed days with heavy cloud cover or rain from our dataset, leaving us with seven days early and seven days late season for comparisons. Microsites at the study site included exposed bedrock, loose sand/gravel or sparsely covered native grass substrates with intermittently distributed sandstone boulders and boulder piles. The study site has a relatively low tree density, so the majority of microsites were in full sun throughout the day.
**Crotaphytus collaris use of posture**

We used two different methods to quantify *C. collaris* use of posture with respect to heat loads experienced by surface active individuals. First, we recorded postures adopted by *C. collaris* simultaneously while recording the mean $T_e$ of the habitat (using the unmodified OTMs only). Here, we used mean $T_e$ as an index of the average environmental heat load impinging on surface active animals. We then compared the frequency of animals in a given posture to the range of mean $T_e$s. Second, we opportunistically captured surface-active *C. collaris* (from 2013–16), and recorded $T_b$s and the posture adopted by those animals. We then compared the frequency of animals in a given posture to $T_b$. For both frequency of posture comparisons, residuals fit normality assumptions and we used ANOVA for statistical comparisons.

**Posture and steady state $T_b$**

We evaluate the impact of posture on *C. collaris* steady state $T_b$ if they were to remain in the intermediate posture instead of adopting the prostrate posture when heat loads were low or the elevated posture when heat loads were high. We compared $T_b$s of individuals to an unmodified OTM opportunistically and using similar capture procedures as discussed above (see 2.3 OTM validation). Comparisons were made between a validated unmodified OTM and the $T_b$ of individuals in either the prostrate or elevated postures. We made comparisons at times when air temperatures (measured with a quick read thermometer, in the shade at 1m above the surface) were near the observed threshold of restricting surface activity in our population and all remaining individuals on the surface were in the prostrate (air temperatures below 27°C) or elevated (air temperatures above 37°C) postures. Thus, by using the unmodified OTMs validated to *C. collaris* in the intermediate posture (see 2.3 OTM validation), we were able to predict the $T_b$
of a lizard if it were to adopt the intermediate posture instead of the elevated or prostrate posture, near the air temperature thresholds for surface activity. Data on the predicted difference in $T_b$ among postures fit normality assumptions and were analyzed using ANOVA.

**Modeling $T_e$**

Our objective was to model $T_e$ under two scenarios: 1) assuming *C. collaris* postural adjustments have no impact on available space and time estimation, and all microsites can be modeled with a single (intermediate) posture (OTM\textsubscript{UNMODIFIED}; Box 1), and 2) assuming *C. collaris* adjusts posture to one of the three categories (prostrate, intermediate and elevated) based on mean $T_e$ at that time (OTM\textsubscript{IDEAL}; Box 1). To model OTM\textsubscript{UNMODIFIED}, we placed an unmodified OTM at every-other microsite (25 microsites). To model OTM\textsubscript{IDEAL}, we placed three OTMs (one of each type placed side-by-side oriented in the same direction) at the remaining microsites (25 microsites). In all replicates (time points), we used data on only one of the three OTM types to estimate $T_e$ for OTM\textsubscript{IDEAL}. To determine which OTM type to use at each time point, we used the mean $T_e$ thresholds where *C. collaris* adjusted from the intermediate to prostate posture, and the intermediate to elevated posture. To estimate these $T_e$ thresholds, we recorded the mean $T_e$ when the frequency of postures used by *C. collaris* intersected between postures (prostrate-intermediate posture = 33.5°C; intermediate-elevated posture = 39.5°C; Fig. 2a). Thus, to model $T_e$ based on OTM\textsubscript{IDEAL} we used the compressed OTMs when mean $T_e$ was below 33.5°C, and the stilted OTMs when mean $T_e$ was above 39.5°C. When mean $T_e$ was within 33.5–39.5°C, we used the unmodified OTMs. Therefore, our comparisons were between twenty-five microsites modeled solely with unmodified OTMs (OTM\textsubscript{UNMODIFIED}), and twenty-five microsites modeled
by one of three OTM types (compressed, unmodified and stilted), based on mean $T_e$ at that time point (OTM$^{\text{IDEAL}}$).

We used $T_b$ data collected over the four-year study to estimate the range of $T_{bs}$ for voluntary surface activity ($T_{ACT}$; Box 1). We used the central 99% of all $T_{bs}$ recorded from surface active lizards (31.2–42.6$^\circ$C) as our metric of $T_{ACT}$ (Grant and Dunham 1990). We used the percent of OTMs within $T_{ACT}$ to quantify the spatiotemporal frequency distribution of available $T_e$ (Grant 1990). A second metric for quantifying the availability of suitable $T_e$ is the estimate of the “thermal quality” of habitat (Hertz et al. 1993). Thermal quality ($d_e$; Box 1) is estimated by calculating the absolute difference between $T_{SET}$ (Box 1) and $T_e$. Although the concept of $T_{SET}$ has been criticized in the literature (Currin and Alexander 1999; and references therein), its use in thermal ecology studies is still common. Thus, to make our estimates comparable to other thermal ecology studies that are based on $T_{SET}$, we utilized the $d_e$ estimation. We used the central 60% of all $T_{bs}$ selected by $C.\ collaris$ in a laboratory gradient (34.8–38.1$^\circ$C; Firth et al. 1989) as our metric for $T_{SET}$. We note that $d_e$ does not directly estimate available time or space (instead, it measures absolute differences between $T_e$ and $T_{SET}$) for surface activity. However, it can be used as a metric to compare thermal quality of habitats (Diaz 1997; Blouin-Demers and Weatherhead 2001; Bakken and Angilletta 2014), and thus represents an additional method to test the impact of posture on available habitat for surface activity.

**Hypothesis testing**

**Hypothesis #1: Available Habitat**

We tested the null hypothesis that posture does not increase the proportion of available habitat that is in the thermally suitable range for $C.\ collaris$ surface activity. To test this hypothesis, we
compared the percentage of suitable habitat (% OTMs within T\textsubscript{ACT}) over a range of typical days and conditions throughout the activity season. We compared twenty-five microsites with unmodified OTMs only (OTM\textsubscript{UNMODIFIED}), to twenty-five microsites with OTMs in the ideal posture at that time (OTM\textsubscript{IDEAL}). Daily comparisons were made from 7:00am to 9:00pm, at 20min intervals, on four separate days (sunny day in May, June and July, and a cloudy day in June). We chose these four days (out of the 14 total days we had data for) to represent relatively typical daily climatic conditions across the C. collaris reproductive season in Arkansas (mid-April to mid-July; Brewster et al. 2018). The frequency distribution of T\textsubscript{e} data did not fit normality assumptions. Thus, we used Wilcoxon signed rank test to assess differences in available habitat between OTM\textsubscript{UNMODIFIED} and OTM\textsubscript{IDEAL}. If posture does not increase the space available for C. collaris surface-activity, then we did not expect a statistically significant increase in the proportion of thermally suitable habitat modeled with OTM\textsubscript{IDEAL} compared to OTM\textsubscript{UNMODIFIED}.

Hypothesis#2: Available Activity-Time

Here we tested the null hypothesis that posture does not increase the time available that is in the thermally suitable range for surface activity in C. collaris. To test this hypothesis, we compared the number of hours where the proportion of T\textsubscript{e} within T\textsubscript{ACT} is at or above the minimum threshold for surface activity (MIN\textsubscript{ACT}; Box 1). To determine the MIN\textsubscript{ACT}, we conducted a pilot study using thirty unmodified OTMs, and estimated the average percentage of OTMs within T\textsubscript{ACT} when lizards initiated or ceased surface-activity. We recorded eleven observations of MIN\textsubscript{ACT} in 2014 on the study population, with a mean of 10.3% (SE ± 1.95) of OTMs within T\textsubscript{ACT}. Thus, we used 10% as our MIN\textsubscript{ACT} threshold to compare available time within T\textsubscript{ACT}.
between microsites modeled with OTM\textsubscript{UNMODIFIED} to microsites modeled with OTM\textsubscript{IDEAL} (see 2.7 Modeling $T_e$). Comparisons were made by summing the total hours per day (from 7:00am to 9:00pm, at 20min intervals) where the habitat was greater than MIN\textsubscript{ACT} (10%). Daily time estimates were made across seven days in mid-May (early reproductive season) and seven days in late June (late reproductive season). Available activity-time data fit normality assumptions and were analyzed using ANOVA with model-type and day as a fixed effect. If posture does not increase the available time for surface-activity of \textit{C. collaris}, then we did not expect a statistically significant increase in the available time for surface activity for habitat modeled with OTM\textsubscript{IDEAL}, compared to OTM\textsubscript{UNMODIFIED}.

\textit{Hypothesis \#3: Thermal quality ($d_e$)}
We tested the null hypothesis that posture does not improve the thermal quality index ($d_e$) of available habitat for \textit{C. collaris}. Using the same model parameters as our previous two hypotheses (modeling 25 microsites with OTM\textsubscript{IDEAL} and 25 microsites with OTM\textsubscript{UNMODIFIED}) we estimated $d_e$ over seven days early and seven days late season (from 7:00am to 9:00pm). Daily mean $d_e$ fit normality assumptions and were analyzed using ANOVA with model-type and day as a fixed effect. If posture does not improve the thermal suitability of habitat for \textit{C. collaris}, then we should find no statistically significant decrease in mean $d_e$ of habitat modeled with OTM\textsubscript{IDEAL} compared to OTM\textsubscript{UNMODIFIED}.
RESULTS

OTM validation

In all three OTM to lizard $T_b$ comparisons, relationships were isometric with slopes indistinguishable from 1.0 and intercepts indistinguishable from 0.0 (Fig. 3). For the compressed OTM to lizards in the prostrate posture, the intercept was 0.485 (95% CI from -4.890 to 5.082) and slope was 1.019 (95% CI from 0.875 to 1.189). For the unmodified OTM to lizards in the intermediate posture, the intercept was -1.847 (95% CI from -7.755 to 3.27) and the slope was 1.009 (95% CI from 0.875 to 1.165). For the stilted OTM to lizards in the elevated posture, the intercept was -1.589 (95% CI from -11.31 to 6.322) and the slope was 0.989 (95% CI from 0.802 to 1.218; Fig. 3).

*Crotaphytus collaris* use of posture

Body temperature ($T_b$) of *C. collaris* was significantly different among the three postures (mean prostrate = 33.8°C, mean intermediate = 37.2°C, mean elevated = 39.7°C; $F_{1,182} = 566.8$, $P < 0.001$; Fig. 2b). Similarly, mean $T_e$ was significantly different among the three postures adopted by lizards (grand means for prostrate = 31.6°C, intermediate = 36.3°C and elevated = 43.2°C; $F_{1,101} = 238.6$, $P < 0.001$; Fig. 2a). We used the mean $T_e$ where the frequency of prostrate and intermediate postures intersected (33.5°C); and the frequency of intermediate and elevated postures intersected (39.5°C) to parameterize OTM$_{ideal}$ across time-points (Fig. 2a; see Methods, 2.7 *Modeling* $T_e$).
**Posture and steady state T\(_b\):**

We made a total of 10 OTM to \(T_b\) comparisons when air temperatures were near the threshold for surface activity. When air temperatures were near the minimum for surface-activity, lizard \(T_b\)s would have been an estimated 2.4°C colder (range of 2.3–2.8°C) if they were to adopt the intermediate posture instead of the prostrate posture (\(F_{1,8} = 12.3, P = 0.008\)). When air temperatures were near the maximum for surface-activity, lizard \(T_b\)s would have been an estimated 3.8°C hotter (range of 3.3–4.4°C) if they were to adopt the intermediate posture instead of the elevated posture (\(F_{1,8} = 29.5, P = 0.0006\)).

**Hypothesis testing**

*Hypothesis #1: Available habitat*

Null: Posture does not increase the proportion of available habitat that is in the thermally suitable range for *C. collaris* surface activity. On all four days, the proportion of suitable habitat was statistically greater for OTM\(_{IDEAL}\) compared to OTM\(_{UNMODIFIED}\) (Wilcoxon signed rank, \(P < 0.001\) all four days; Fig. 4a–d). Differences in the mean proportion of OTMs within \(T_{ACT}\) were greatest on the warmest days (Sunny day July, OTM\(_{IDEAL}\) = 54.7% vs. OTM\(_{UNMODIFIED}\) = 31.2%; Sunny day June, OTM\(_{IDEAL}\) = 49.5% vs. OTM\(_{UNMODIFIED}\) = 28.5%), compared to cooler days (Cloudy day June, OTM\(_{IDEAL}\) = 60.5% vs. OTM\(_{UNMODIFIED}\) 42.8%; Sunny day May, OTM\(_{IDEAL}\) = 60.2% vs. OTM\(_{UNMODIFIED}\) 45.5%; Fig. 4a–d).

*Hypothesis #2- Available activity-time*

Null: Posture does not increase the time available that is in the thermally suitable range for surface activity in *C. collaris*. In both early and late season, OTM\(_{IDEAL}\) had statistically greater
daily hours for activity (hr/day where \( T_e \) was within \( T_{ACT} \)) than OTM\textsubscript{UNMODIFIED} (early season: \( F_{1,6} = 8.7, P = 0.026; \) late season: \( F_{1,6} = 17.98, P = 0.005; \) Fig. 5). The mean hours available for activity were greater for both OTM\textsubscript{UNMODIFIED} and OTM\textsubscript{IDEAL} in the late season compared to early season (Fig. 5).

**Hypothesis #3: Thermal quality (\( d_e \))**

Null: Posture does not improve the thermal quality index (\( d_e \)) of available habitat for *C. collaris*. The mean daily \( d_e \) of available habitat across the activity season was significantly lower for OTM\textsubscript{IDEAL} (early season, \( 5.7^\circ C, SE = 0.55; \) late season, \( 3.6^\circ C, SE = 0.62, \)) than for OTM\textsubscript{UNMODIFIED} (early season, \( 7.9^\circ C, SE = 0.39, F_{1,11} = 11.1, P < 0.01; \) late season, \( 8.1^\circ C, SE = 4.3, F_{1,11} = 48.5, P < 0.001 \)).

**DISCUSSION**

The goal of this study was to assess the influence of posture on the available time and space for surface activity in *C. collaris*. We found an association between *C. collaris* posture and both \( T_b \) and mean \( T_e \) (Fig.3a–b). We recognize that *C. collaris* do not adjust posture as a function of mean \( T_e \) per se, but rather by the \( T_e \) they (individually) experience in the microhabitat they occupy. However, our data suggest that mean \( T_e \) provides a reasonable proxy for the heat load that most lizards experience at a given time (at least at this study site), as indicated by the timing of postures adopted by *C. collaris*. Our data also suggested that posture can have a marked effect on the heat flux rates experienced by *C. collaris*, depending on the environmental heat load at that time. Taken together, these two results (*C. collaris* use of posture and the impact of posture
on steady state $T_b$) implicate posture as an important means of behavioral thermoregulation in *C. collaris*.

Our data also suggest that altering posture affords *C. collaris* increased available space and time for surface activity. By adjusting posture favorably with environmental heat loads (mean $T_e$) throughout the day, *C. collaris* is able to occupy a greater spatial portion of the habitat during normal activity times without experiencing restrictive $T_b$s (Fig. 4a–d). The difference in the proportion of available habitat within $T_{ACT}$ for *C. collaris* that remain in the intermediate posture (OTM$_{UNMODIFIED}$) compared to animals that adjust posture (OTM$_{IDEAL}$) was greatest on the days with higher heat loads (sunny June and July days; Fig. 4b–c). The difference in magnitude of usable habitat among days is partially explained by the greater buffering effect that the elevated posture provides *C. collaris* when heat loads are high, compared to the effect of prostrate posture when heat loads are low (see Results; 3.3 Posture and steady state $T_b$). Nonetheless, our data suggest that even on colder days, *C. collaris* can greatly improve the usable proportion of habitat by adjusting posture (Fig. 4a, d).

Second, our results suggest that posture can significantly increase the activity-time available for *C. collaris* before experiencing restrictive $T_b$s. If *C. collaris* used only the intermediate posture (OTM$_{UNMODIFIED}$), they would have an average of 11 to 14% fewer hours per day (early or late season, respectively) for surface activity, compared to lizards that adjust posture (OTM$_{IDEAL}$: Fig. 5). Our results also indicate that by adjusting posture, *C. collaris* experience an approximately 42% improvement in thermal quality of the habitat ($d_e$) than if they ignored posture. The improvement in thermal quality implies *C. collaris* would have an easier time maintaining $T_b$ within $T_{SET}$, potentially facilitating optimal physiological performances (e.g. digestion and sprint speed), by adjusting posture.
OTMs used in this study

The benefits of using electroformed OTMs (Box 1; Bakken and Gates 1975) over cylinder shaped OTMs have been discussed in the literature (Bakken and Angilletta 2014). In short, electroformed OTMs are designed to mimic the surface area and shape of the actual organism (Walsberg and Wolf 1996), thus improving the odds that they will match the biophysical properties of the study animal. Nonetheless, several studies have successfully approximated $T_e$ of the target organism using cylinder shaped models (i.e. Peterson et al. 1993; Diaz 1997; Wills and Beaupre 2000; Row and Blouin-Demers 2006; Stanton-Jones et al. 2018). In the present study, we also found cylinder OTMs to provide a good approximation of the study organisms, indicated by our validation data (Fig. 3). We note that modeling $T_e$ in a species that uses three postures requires three times the number of OTMs to reach the same sample size. With the significant costs associated with electroformed OTMs (time and money; Bakken 1992; Watson and Francis 2015), we believe our methodology provides an accurate and cost-effective approach to modeling $T_e$ of ectotherms that use multiple postures.

The importance of posture in *C. collaris*

Our data show the importance of space and time characterization to understanding ecological patterns in the study population. For example, $T_e$ estimates based on OTM$_{UNMODIFIED}$ suggest that *C. collaris* surface activity should be bimodal and cease at mid-day (Fig. 4c). Yet even on days hotter than the “typical” sunny July day modeled here, *C. collaris* at this site remained surface active through the entire day by adopting elevated postures and restricting most of their activity to elevated (i.e. on top of a large rock) or shaded microhabitats. Alternatively, estimates based on OTM$_{IDEAL}$ correctly predicted that lizards should have the opportunity to remain surface active
through the hottest part of the day by adopting the elevated posture and restricting activity to microhabitats with the lowest heat loads (i.e. large rocks and shaded microhabitats).

Similarly, our predictions about total number of hours available for surface activity across the reproductive season were also impacted by posture. Our results suggested fewer hours available for daily activity (1.0–1.6hr; early and late season, respectively) for OTM\textsubscript{UNMODIFIED} compared to OTM\textsubscript{IDEAL}. With an approximately 90-day reproductive season length (Brewster et al. 2018), this would be an estimated 112 fewer hr (~13% shorter reproductive season) for surface activity for OTM\textsubscript{UNMODIFIED}, compared to OTM\textsubscript{IDEAL}. Thus, our study suggests posture has important implications for \textit{C. collaris} space and time availability that may have implications for the species ecology and life history as well.

\textbf{Is \textit{C. collaris} the exception or the rule?}

The target species in this study is a surface-active, diurnal lizard. The estimated difference in steady state $T_b$ among postures in this study (up to ~4.3°C), is consistent with estimates in another surface-active lizard (\textit{Callisaurus dracanoides}, ~6°C, Bakken 1989; ~4°C, Muth 1977b). Indeed, the magnitude of variation in $T_b$ associated with posture will vary among species, depending on factors such as body size, morphology and the range of climatic conditions that occur for that species (Stevenson 1985; Muth 1977a). Thus, it is difficult to know what impact posture has on time and space utilization in other species. We suspect that for some terrestrial ectotherms, posture may be a minor or even negligible influence on their thermal ecology. For example, posture will likely be of minor importance for nocturnal species or species that spend all of their time in full shade (e.g. in full canopy, or leaf litter). However, with the breadth of taxa reported in the literature that use posture for thermoregulatory purposes, we
suspect that *C. collaris* may represent more of the “rule” than the “exception” in sun-basking, terrestrial ectotherms.

**Implications for studies of thermal ecology**

Current methods for modeling $T_e$ (Bakken and Gates 1975; Hertz et al. 1993; Bakken and Angilletta 2014) typically lack information on postural adjustments. Our results suggest that in species that can and do use posture to buffer restrictive $T_{bs}$, omitting this information could lead to underestimation of available space and time for activity. The potential impact of errors in $T_e$ estimations on inferences about thermal ecology have been addressed in the literature. Bakken and Angilletta (2014) showed that habitat modeled with a $\pm 2^\circ$C systematic error would result in large effects on thermal indices (i.e. $d_e$, thermoregulatory effectiveness, and available surface activity-time), ranging from 8–32%. In our study, the difference in $T_{bs}$ among postures (up to $4.3^\circ$C) exceeds the $\pm 2^\circ$C modeled by Bakken and Angilletta (2014). Similarly, variation in estimates of available time and $d_e$ modeled with OTM$_{UNMODIFIED}$ versus OTM$_{IDEAL}$ mirrored (13% difference in activity-time) or exceeded (42% difference in $d_e$) those reported by Bakken and Angilletta (2014).

A relatively recent practice in thermal ecology studies is to model extinction risk of lizards to climate change by estimating hours of restriction ($h_r$; Box 1; Sinervo et al 2010; Lara-Reséndiz et al. 2015). The $h_r$ methodology assumes that when climate change causes activity-time to reach some minimum threshold for a particular species, that species will go extinct. Since ectotherms must balance competing functions of time and mass-energy budgets, all while contending with the available thermal environment (Dunham et al. 1989), the $h_r$ methodology is based on a critical component of ectotherm life histories: activity-time budgets (Adolph and
Porter 1993). However, current studies using the \( h_t \) methodology do not incorporate the potential variance in available activity-time associated with posture. Our findings suggest that in studies that make inferences based on the interaction between temperature and the availability of space and time, an assessment of the target species use of posture is warranted.

CONCLUSIONS

Our study presents a novel approach to investigating and quantifying the influence of postural adjustments on the thermal ecology of a terrestrial ectotherm. Our data show the importance of postural adjustments to the available space and time for \( C. \) collaris. Based on the frequency of ectotherm species in the literature that use posture as a means of behavioral thermoregulation, we conclude that posture can play a significant role in the available space and time for surface activity in species that use this behavior. A key component to predicting the biological impacts of climate change includes understanding the effects of rising air temperatures on the space and time budgets of ectotherms. Thus, our findings imply that posture can be an important component to accurately predict the impacts of changing temperatures on ectotherms that use it for behavioral thermoregulation. We urge investigators to consider the potential effects of posture in their target species, and when appropriate, account for variation in this behavior when making inferences associated with space and time utilization.

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REFERENCES


Box 1. A glossary of thermal-biology terms

\( d_e \) – habitat thermal quality; absolute difference between \( T_{SET} \) and \( T_e \)

**electroformed OTMs** – hollow copper OTM replicas of the target species

\( h_r \) – hours of restriction: the time where temperatures are predicted to be too hot or cold for surface activity

**MIN\text{ACT}** – minimum activity threshold; average percentage of OTMs within \( T_{\text{ACT}} \) when \( C. \text{collaris} \) initiated, or ceased surface activity

**OTM** – operative temperature model; physical model that estimates the operative temperature (\( T_e \)) of the study organism

**OTM\text{IDEAL}** – modeling method that uses one of three OTM types (compressed, unmodified or stilted) based on the mean \( T_e \) of the habitat at that time (parameterized from observations on \( C. \text{collaris} \) use of posture with respect to mean \( T_e \); Fig. 3a) to estimate \( T_e \) distribution

**OTM\text{UNMODIFIED}** – modeling method that uses a single OTM type (unmodified OTM) to estimate \( T_e \) distribution

**restrictive \( T_b \)** – \( T_b \)s too hot or cold for voluntary surface activity

**\( T_{\text{ACT}} \)** – voluntary surface-active \( T_b \) range

\( T_b \) – body temperature

\( T_e \) – operative environmental temperature; a single temperature index of the equilibrium temperature of an object with zero heat capacity and the same thermodynamic properties as the study organism in the same microclimate

**thermal time constant** – time for the temperature of an object to change by \(~ 63\%\) of the differential between the initial temperature and the ambient temperature

\( T_{SET} \) – the range of \( T_b \)s selected by the study species in a laboratory setting
Figure 1a–d: Images of C. collaris postures: a) elevated, b) intermediate, and c) prostrate. d) Image of the three OTM types used in study. Stilted OTM (top OTM; corresponding to an elevated lizard, Fig. 1a), unmodified OTM (middle OTM; corresponding to a lizard in intermediated posture, Fig. 1b) and compressed OTM (bottom OTM; corresponding to prostrate lizard, Fig. 1c.).
Fig. 2a–b. Frequency of *C. collaris* in prostrate, intermediate and elevated postures as a function of: a) mean $T_e$ of the habitat and b) individual lizard $T_b$. * = intersection points between intermediate-prostrate postures (Min = 33.5°C); and intermediate-elevated postures (Max = 39.5°C). Intersection points used to parametrize minimum and maximum mean $T_e$ thresholds for OTM_{IDEAL}. 
Figure 3. Linear relationships between $T_e$ and $T_b$ of *C. collaris* in three postures. Stilted OTM to lizards in an elevated posture ($r^2 = 0.87$), unmodified OTM to lizards in an intermediate posture ($r^2 = 0.91$) and compressed OTM to lizards in a prostrate posture ($r^2 = 0.93$).
Fig. 4a–d. Comparison of $T_c$ (% of OTMs within $T_{ACT}$) modeled with OTM_{UNMODIFIED} vs OTM_{IDEAL} over a typical sunny day in a) May, b) June and c) July; and d) a typical cloudy day in June. Shaded area represents the average minimum threshold for surface activity ($MIN_{ACT}$ =10%) observed by lizards in the study population.
Fig 5. Mean hr/day and SE of early (n = 7d) and late (n = 7d) seasons for habitat modeled: 1) exclusively by OTMs in the intermediate posture (OTM\textsubscript{UNMODIFIED}) and, 2) by OTMs in the optimal posture at that time (prostrate, intermediate or elevated; OTM\textsubscript{IDEAL}). hr/day measured as number of hours where a minimum of 10% of OTM's are within voluntary active $T_b$ ($T_{\text{ACT}} = 32.1$–$42.6^\circ C$).
IV Chapter 3: **Integrating bioenergetics and conservation biology: Thermal sensitivity of digestive performance in Eastern Collared Lizards (*Crotaphytus collaris*) may affect population persistence**

Casey L. Brewster, Jason Ortega, and Steven J. Beaupre

ABSTRACT

Information on bioenergetics can provide valuable insight into the ecology, life history and population dynamics of organisms. For ectothermic animals, thermal sensitivity of digestion is an important determinant of net assimilated energy budgets. A recent study in the Ozark Mountains indicated that Eastern Collared Lizards (*Crotaphytus collaris*) restricted to encroached glades (characterized by woody vegetation encroachment) experience reduced environmental heat loads and have reduced age-specific growth and reproductive rates compared to populations in intact glades. To assess the potential impact of reduced body temperatures on assimilation rates of *C. collaris* in encroached glades, we conducted feeding trials across four temperature treatments (28, 31, 34, & 37°C). We tested for temperature effects on voluntary feeding rates, passage times, apparent assimilated energy (AE), and metabolizable energy (ME). Passage times decreased and voluntary feeding rates increased significantly with increasing temperature. Consumption explained the majority of variance in AE and ME, followed by the effect of temperature treatments. Using data on voluntary feeding rates, passage times and ME as a function of temperature, we estimated over a 10-fold increase in predicted daily assimilated energy across temperature treatments (28°C = 0.58 kJ/d, 31°C = 1.20 kJ/d, 34°C = 4.30 kJ/d, 37°C = 7.95 kJ/d) . Thus, lower heat loads in encroached glades may cause reduced body temperature and result in restricted energy assimilation rates. Our study provides a novel
approach to the integration of bioenergetics and conservation, and shows the efficacy of using information on digestive performance to investigate causation in a conservation context.

INTRODUCTION

The integration of physiology and conservation is an emerging field (Wikelski and Cooke 2006) that has provided valuable perspective in addressing negative impacts on biodiversity (Madliger et al. 2016). Recently, researchers have expanded the “conservation physiologists toolbox” (Madliger et al. 2018), greatly improving our ability to address causation associated with environmental change. The importance of bioenergetics to conservation physiology has been recently emphasized (Tracy et al. 2006; Mochnacz et al. 2017; Leo et al. 2018; Madliger et al. 2018). Information from bioenergetics can provide valuable insight into the ecology, life history and population dynamics of species (Porter and Tracy 1983; Dunham et al. 1989; Adolph and Porter 1993; Dunham and Overall 1994; Beaupre 2002), which in turn, can be used to address conservation-based questions (Tracy et al. 2006). From a physiological standpoint, the thermal dependence of digestive performance strongly influences bioenergetics of free-ranging animals (Congdon et al. 1982; Angilletta 2001; Beaupre 2002; Beaupre and Zaidan 2013). Digestive physiology determines the net assimilated energy available for competing functions of growth, reproduction, maintenance, and storage (Congdon et al. 1982; Niewiarowsk 2001) – which directly affects fitness (Dunham and Overall 1994). Thus, information on digestive performance represents a valuable physiological tool that can be integrated into a conservation context.

In the Ozark Mountains of Arkansas and Missouri, the Eastern Collared Lizard (Crotaphytus collaris) has been listed as a species of special concern (S2 and S4 respectively) as a result of extensive population declines (Templeton et al. 2001; Grimsley 2012). Previous
research on *C. collaris* in northern Arkansas has documented reduced age-specific growth and reproductive rates in populations inhabiting encroached glades (e.g. glade habitats characterized by dense woody vegetation cover; Brewster et al. 2018). Encroached glades had increased shade cover compared to intact glades (e.g. glade habitats characterized by low woody vegetation density), reducing daily environmental heat loads in those habitats (Porter and Gates 1969; Huang et al. 2014). A reduction in environmental heat loads implies that *C. collaris* in degraded habitats may experience lower body temperatures (*Tbs*). Since most physiological functions are thermally dependent (Huey 1982; Peterson et al. 1993) a reduction in *Tb* could lead to a decline in digestive processing rates (van Marken Lichtenbelt 1997; Grant and Dunham 1990), ultimately causing reduced growth and reproduction of *C. collaris* in encroached glades. Thus, understanding the effect of *Tb* on the digestive performance of *C. collaris* is an important component to identifying potential explanatory mechanisms resulting in reduced reproductive rates and population declines of this species in Arkansas.

The objective of this study was to determine the thermal sensitivity of digestive performance in *C. collaris*. Specifically, we tested for differences in: 1) passage time (defined as time to first and last presence of a marked meal in feces), 2) apparent assimilated energy (AE; energy consumed – energy lost in feces), 3) metabolizable energy (ME; energy consumed – energy lost in feces – energy lost in uric acid) and 4) voluntary feeding rates of *C. collaris* from two populations across four temperature trials (28, 31, 34, and 37° C). We used these data to estimate the potential effect of reduced *Tbs* on energy assimilation of *C. collaris*. 
METHODS

Captive maintenance

We captured fifty wild *C. collaris* individuals from two rock quarries in northern Arkansas and transported them to the University of Arkansas. Individuals ranged in body mass from 11.2 – 34.0g and included 22 males and 28 females. Animals were housed individually in clear plastic terraria (38L) with butcher paper subflooring and a single plastic hide box (~0.5L). Terraria were placed on metal shelving inside of a temperature controlled walk-in environmental chamber with 12:12 photoperiod. The inside of terraria were spritzed daily with fresh water, and animals were fed crickets (*Acheta domestica*) every other day throughout the course of the study. Including acclimatization (3 days) and experimental trails (14 days), all animals were captured, studied and returned healthy to their point of capture in less than 20 days.

Experimental design: Voluntary feeding rates

Individuals were randomly assigned to one of six temperature treatments: 21, 25, 28, 31, 34 and 37°C, but subject to the constraint that sex and population of origin were equally represented in each treatment. Thermal treatments were chosen that fall within the range of *Tbs* observed in *C. collaris* during the activity season (20–42.6°C; CLB unpublished dissertation). For the 28–37°C treatments, we assigned ten individuals (4 males: 6 females at 31 & 37°C; 5:5 at 28 & 34°C) to each treatment group, with an even ratio of population of origin. We used five individuals per treatment group in the 21 and 25°C (2 males: 3 females and 2:3 ratio of population of origin). We placed 6 live adult crickets in each individuals’ terrarium and recorded the number of crickets consumed after two hours. For our metric of voluntary feeding rates, we used the number of crickets consumed in two hours as a function of temperature. Crickets used for
feeding trials were obtained from a commercial dealer (Fluker’s Cricket Farms, Baton Rouge, Louisiana).

**Experimental design: Energy extraction and passage time**

Temperature treatments for energy extraction and passage time (28–37°C) were chosen based on results from the voluntary feeding trials, which suggested *C. collaris* refused to eat at *Tbs* below 28°C. Thus, after voluntary feeding trials, individuals from the 21 and 25°C treatment groups were released healthy to their population of origin within five days of capture. The remaining 40 individuals from the 28–37°C trials were used, in their respective temperature treatments, for the digestion and passage time experiments.

Animals were fed a live cricket meal (2-8 crickets) equal to 5.5% (±0.25%) of their body mass every other day, starting on the day after capture. We weighed crickets and lizards to the nearest 0.001g using an analytical scale. Beginning with the second feeding event, we fed lizards a marked meal at every other feeding event. Previous research suggests that absorptive state and meal frequency can affect digestive processing estimates in lizards (Windell and Sarokon 1976; Skoczylas 1978; Waldschmidt et al. 1986). Thus, to reduce the potential effect of variation in meal frequency in our estimates, we did not use any feces corresponding to the first fed meal. Meals were marked by injecting ~ 0.02mL of inert UV fluorescent powder slurry (Slice of The Moon, Toronto, Canada) at a concentration of 0.015g mL⁻¹ and an estimated energy density of 23.6 kJ g⁻¹ into the body of each cricket used to make up a single meal. We corrected for the energy content attributed to marker powder (~0.76%) by subtracting the caloric value of marker from the caloric value of marked feces. We alternated the color powder used (green, pink, blue or orange) at every marked feeding event to improve delineation of specific meals for quantifying passage times.
Lizards were fed by holding the individual in one hand and presenting a cricket (using a pair of plastic forceps) in front of their face with the opposite hand. Although biting responses by *C. collaris* appeared to be more defensive than a true feeding response, in the majority of trials lizards willingly ate all of their cricket meals using this technique. In cases that animals refused to bite the cricket (this was more common in the 28°C temperature treatment), another researcher would gently tap the lizard on the snout (thus invoking a defensive biting response) while the other researcher quickly placed the cricket into the animal’s mouth. Using this feeding strategy, we were able to feed every subject their allotted meal on every feeding event (8 total feeding events). Previous research suggests that force-feeding *C. collaris* intact meals directly into the esophagus (precluding normal mastication) can have negative impacts on digestive estimates (Ruppert 1980). The feeding protocol we used in this study allowed all lizards (regardless of temperature treatment) to fully masticate every cricket fed to them.

During the experiment, terraria were checked for feces and uric acid every three hours from 7:00am – 7:00pm. At each check, we removed all waste material, recorded the date and time of the waste event, and checked feces for the presence of fluorescent marker using a black light. Feces and uric acid were then bagged separately in an individually marked Whirl-Pak® (Nasco; with animal ID, date, time, and wet mass of sample). We also collected five cricket samples (4-6 individual crickets per sample) representing a random subsample of crickets fed to *C. collaris* in the study to estimate the average water content and energy density of food consumed by individuals. We stored samples in a freezer until they could be processed at a later date. All samples were later lyophilized for 24hrs. We recorded the dry mass of all samples (to the nearest 1mg), and then combined samples (2-12 separate fecal samples per individual) to meet the minimum sample mass for calorimetry equipment at the University of Arkansas.
Samples were homogenized prior to analyses and energy densities were estimated using bomb calorimetry by an automated calorimeter at the Central Analytical Lab at the University of Arkansas.

We obtained several response variables from feeding trials including passage time (time in hours to first and last observed presence of a marked meal, TFM and TLM, respectively), total energy consumed (C in kJ), total energy in feces (F in kJ) and total energy in uric acid (U in kJ). We did not standardize the number of feeding events used to estimate energy lost in F and U. Instead, we used the maximum number of feeding events for each individual that we could confidently assign known feeding events to, based on the presence/absence of fluorescent markers in their feces.

**Statistical analyses**

We used mixed linear ANCOVA implemented with the nlme package (Bates et al. 2015) in R (v3.1.3, R Core Team, 2015), with temperature as the main effect in all of our comparisons. We designated population (2 populations) as a random factor, with sex as a fixed effect and body size (initial body mass) as a covariate. For AE, ME, F and U comparisons, we included consumption as a second covariate (Raubenheimer and Simpson 1994; Beaupre and Dunham 1995; Raubenheimer 1995). For passage time, we obtained 1-3 estimates of TFM and TLM for each individual, and we used all obtained estimates for statistical comparisons. Thus, for passage time, we included individual as a random factor in the ANCOVA models. We included individual meal size (dry mass of crickets) as a second covariate for passage time. We designated model covariance structure based on minimum AICc score (Bozdogan 1987). In all analyses, assumptions of normality and homogeneity of slopes were met.
RESULTS

Voluntary feeding rates

All individuals in the 21 and 25°C voluntary feeding trials ate zero crickets (Fig. 1). Thus, we only made statistical comparisons on the remaining 28–37°C temperature trials. We found a strong effect of temperature on voluntary feeding rates in *C. collaris* across the 28-37°C temperature trials ($F_{3/32} = 21.85$, $P < .0001$; Fig. 1). Mixed model ANCOVA found no significant effect of sex or body mass (covariate). Random effects (population) accounted for 4.5% of the total variance.

Passage times

The best model (based on AICc) for passage time comparisons included individual (without population) as a random effect. We converted wet mass of crickets consumed to dry mass using the equation: dry mass = 0.404*wet mass – 0.303 ($n = 21$, $r^2 = 0.95$, $P < 0.001$). Temperature had a strong effect on passage time for both TFM ($F_{3/34} = 17.48$, $P < .0001$) and TLM ($F_{3/34} = 17.80$, $P < .0001$; Fig. 2). Mixed model ANCOVA found no significant effect of sex, meal size (covariate) or individual body mass (covariate) on TFM and TLM. Random effects (individual) accounted for 1.5% (TFM) and 1.1% (TLM) of total variance.

Apparent assimilated energy

For all individuals, we were able to assign a minimum of three (two marked and one unmarked) and a maximum of five (three marked and two unmarked) feeding events in which to estimate C, F and U (grand mean of 4.1 meals, SE = 0.080). The number of meals used for analyses did not differ significantly among temperature treatments ($F_{3,36} = 1.333$, $P = 0.279$). The average energy
density of crickets was 22.6 kJ g\(^{-1}\) dry mass (SE = 0.60; \(n = 5\) samples), and the average energy density of feces was 18.41 kJ g\(^{-1}\) dry mass (SE = 0.35; \(n = 40\) samples). The average total energy consumed was 49.64 kJ (SE = 2.48) per individual and did not differ significantly across treatments (standard ANCOVA with sex and temperature as fixed effects, and body mass as a covariate; \(F_{3/34} = 1.895, P = 0.149\)). Energy lost in F was unaffected by body mass (covariate) or sex but was strongly affected by consumption (\(P < 0.0001\); Fig. 3a). The effect of temperature on F was also significant \((F_{3/33} = 6.926, P < 0.001\); Fig. 4a). Random effect of population accounted for less than 1% of the total variance.

Apparent assimilated energy was unaffected by body mass (covariate) or sex but strongly affected by consumption (\(P < 0.0001\); Fig. 3b). The effect of temperature on AE was also significant \((F_{3/33} = 4.71, P = 0.0076\); Fig. 4b). Population (random effect) accounted for less than 1% of the total variance. Linear regression of the effect of C on AE across all temperature treatments was: \(AE = 0.874C + 0.481\) (Adj. \(r^2 = 0.98, F_{1,38} = 3443, P < 0.0001, SE\) slope = 0.015, SE intercept = 0.767). Thus, apparent assimilation efficiency was approximately 87% across temperature treatments. We provide a summary table of linear-regression estimates of temperature-specific AE and F in Appendix 1.

**Metabolizable energy**

The average energy density of U was 10.80 kJ g\(^{-1}\) dry mass (SE = 0.127, \(n = 20\) samples). Energy lost in U was unaffected by body mass (covariate) or sex but was strongly affected by consumption (covariate; \(P < 0.0001\); Fig. 3c). The effect of temperature on U was significant \((F_{3/33} = 3.89, P = 0.0173\); Fig. 4a). Random effect of population accounted for 6.3% of the total variance.
For ME, we found no significant effect of sex or body mass. Mixed model ANCOVA revealed a significant positive effect of consumption ($P < 0.0001$; Fig. 3d). Temperature had a statistically significant effect ($F_{3/33} = 2.933, P = 0.0478$; Fig. 4b) on ME. Population accounted for 4.9% of the total variance in ME. The relationship between C and ME across all temperature treatments was: ME = 0.793C – 1.4621 (Adj. $r^2 = 0.96, F_{1/38} = 1072, P < 0.0001$; SE slope = 0.024, SE intercept = 0.124). Thus, metabolizable energy efficiency was approximately 79% across temperature treatments. We provide a summary table of linear-regression estimates of temperature-specific ME and U in Appendix 1.

**DISCUSSION**

The goal of this study was to investigate the potential impact that increased shade and reduced environmental heat loads (characteristics of encroached glades; Brewster et al. 2018) may have on energy assimilation rates of *C. collaris*. Specifically, our objective was to assess the thermal sensitivity of digestive performance by testing for a temperature effect on voluntary feeding rates, passage time, apparent assimilated energy and metabolizable energy in *C. collaris*. First, we found a strong effect of temperature on the voluntary feeding rates of *C. collaris* (Fig. 1). Individuals consumed no crickets at $T_b$s below 28°C, few crickets between 28–31°C, but consumed most or all crickets offered to them at 34 and 37°C. A recent study (Brewster and Beaupre 2019) suggests minimum voluntary active $T_b$ of *C. collaris* in northern Arkansas is 31.2°C (central 99% of surface-active $T_b = 31.2 – 42.6°C$). Thus, it is not overly surprising that *C. collaris* in this study consumed few crickets at $T_b$s below 31°C. Next, we found a strong effect of $T_b$ on passage rates of *C. collaris*. Passage times of animals at 28°C (TFM = 84.9h, SE
were approximately twice as long as animals at 37°C (TFM = 33.2h, SE = 5.26; TLM = 59.3h, SE = 5.12, n = 29; Fig. 2).

In all of our comparisons of energy extraction (F, U, AE, & ME), we found a significant effect of consumption, but no effect of body mass. Although each meal size fed to subjects was based on the body mass of individuals (5.5%, ±0.25%), the number of meals used to estimate energy extraction varied among individuals (3–5 meals). Thus, consumption was decoupled from body mass in our analyses and allowed differentiation between the effect of consumption (statistically significant) versus the effect of body mass (statistically nonsignificant) on energy extraction estimates. Additionally, all of our models suggested no effect of sex and that population explained a relatively small fraction of total variance (≤ 6.3%). Thus, data on energy extraction were explained primarily by consumption (Fig. 3) and, to a lesser extent, temperature (Fig. 4). We found a statistically significant effect of Tb on AE (C – F), as energy lost to F appeared to decrease nonlinearly across temperature (Fig. 4a–b). Conversely, energy lost to U appeared to increase nonlinearly across temperature (Fig. 4a), presumably as a result of increased metabolic rate with temperature (Coulson and Hernandez 1964; Beaupre et al. 1993; Hume 2005). Thus, our results imply that although AE appears to increase slightly across temperature treatments, this trend is lost by the increasing of uric acid production, which partially explains the relatively flat response in ME across Tb treatments (Fig. 4). We interpret these finding to suggest that consumption explains the majority of variance in C. collaris energy extraction indices, followed by the effect of temperature treatments over the 28–37°C Tb range used in the study.

All of our findings indicate that over the range of 28–37°C, digestive performance of C. collaris is sensitive to temperature from a statistical standpoint. The thermal dependence of
passage time and feeding rate appears to be ubiquitous in lizards (Van Damme et al. 1991; Beaufre et al. 1993; Chen et al. 2003; McConnachie and Alexander 2004). On the other hand, the thermal dependence of energy extraction indices in lizards appears to be species dependent, with some showing no effect of temperature (Christian 1986; van Marken Lichtenbelt 1992; Du et al. 2000; Chen et al. 2003), and others showing significant thermal dependence (Waldschmidt et al. 1986; Troyer 1987; McKinnon and Alexander 1999; Pafili et al. 2006). However, the more important question here is; are the observed differences in digestive performances biologically meaningful for *C. collaris*? Indeed, the ultimate goal of this study was to determine the potential effects of reduced *Tbs* in *C. collaris* on energy assimilation rates in northern Arkansas. Thus, to address the biological implications of our findings we estimated two indices for daily assimilation rates as a function of temperature: “standardized” ME rates and “realized” ME rates. We define standardized ME rates as the predicted metabolizable energy assimilated per day assuming a standard consumption rate (grand mean of daily consumption rate across energy extraction treatments) and the measured effect of temperature on ME and passage times (ME/TFM). Table 1 provides the results of the standardized ME rate comparisons and shows the substantial effect of temperature on daily metabolizable energy rates. Results in Table 1 also show the strong impact that passage rates (TFM as a function of *Tb*) have on energy assimilation rates, relative to ME (as a function of *Tb*). However, our comparisons of standardized ME rates assume that *C. collaris* consumption is insensitive to *Tb* – and our data suggest consumption is highly sensitive to *Tb* (Fig. 1). To account for *Tb* effects on consumptions rates, we define a second index – realized ME rates, which is the average metabolizable energy assimilated per day using the mean consumption rates from the voluntary feeding-temperature trials (ME*VF/TFM). Estimates of the realized ME rates (Table 1; ME*VF/TFM) show the strong effect that *Tb* would
have on daily assimilation rates in *C. collaris*, with a more than ten-fold increase in kJ/day across the 28°C to 37°C trials. Furthermore, the influence of *Tb* on ME appears to have little-to-no biological significance compared to the effect of *Tb* on feeding rates and passage times in *C. collaris*.

Our results indicate that predicted energy assimilation rates were greatest at 37°C. The peak in energy assimilation rates at 37°C in our study is consistent with the mean *Tb* of active *C. collaris* in intact glades (37.2°C; Brewster and Beaupre 2019). The maintenance of field-active *Tbs* that coincide with *Tbs* that maximize digestive processing rates is not uncommon in lizards (Angilletta 2001, Angilletta 2009; Beaupre et al. 1993; van Marken Lichtenbelt 1997). However, it is important to note that since *C. collaris* voluntary active *Tb* range is 31.2–42.6°C, our study cannot rule-out the possibility that digestive performance would continue to increase with *Tbs* above 37°C (Huey 1982). Because of animal care concerns, we chose to use the species mean field-active *Tb* as the high-temperature treatment. Regardless of the “optimal” digestive performance *Tb* of *C. collaris*, our study suggests that assimilation rates will decline when individuals experience *Tbs* below 37°C.

Results from this study have important implications for the conservation of *C. collaris* in the Ozarks. Encroached glades with greater shade cover and lower daily environmental heat loads (compared to intact glades) could force individuals to tolerate lower *Tbs* compared to individuals in intact glades. In turn, reduced *Tbs* in *C. collaris* can result in major declines in their energy assimilation rates (Table 1). Since *C. collaris* in encroached glades have reduced growth and reproduction, and energy available for growth and reproduction is a function of net assimilation rates (Congdon et al. 1982; Niewiarowski 2001; Beaupre and Zaidan 2013), our study provides a critical component to understanding factors that may drive population declines.
in the Ozarks. We note that a more complete understanding of the linkages among encroached glades, temperature, net assimilation rates, growth/reproduction and population declines in *C. collaris* will require data beyond those presented here. However, this study provides a robust mechanistic pathway to identifying the link between encroached glades and reduced growth/reproduction in *C. collaris*. Furthermore, this study highlights the critical data needed (e.g. *Tb* estimates and food availability) to understanding the complex patterns associated with *C. collaris* in the Ozark Mountains.

CONCLUSIONS

Our results indicate that digestive performance is sensitive to *Tb* in *C. collaris*. Using estimates from our data, we show that predicted energy assimilation rates (Table 1) can be influenced strongly by temperature through two primary variables (feeding rates and passage time), with the greatest assimilation rates occurring at the mean surface-active *Tb* of this species (37°C). We conclude that depending on the extent that *C. collaris* experiences reduced *Tbs* in encroached glades, we would expect decreases in energy assimilation rates, and subsequent declines in growth and reproduction. Herein, we describe a novel approach to the integration of bioenergetics and conservation physiology, and demonstrate the utility of using information on digestive performance to investigate conservation-based questions. We urge conservation biologists to consider bioenergetics when developing strategies to address cause-and-effect in their study systems.
ACKNOWLEDGEMENTS

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REFERENCES


**Figure 1**: Adjusted (mixed model ANCOVA) means (28–37°C) and raw means (21 & 25°C) of the number of crickets consumed voluntarily in 2 hours across temperature treatments. All individuals were offered six live crickets. Error bars = 95% CI. 28–37°C, $P < 0.0001$. 

![Voluntary Feeding Rates Graph](image)
Figure 2: Adjusted (mixed model ANCOVA) means of passage time (hr) across four temperature treatments. Passage time defined as time to first mark observed (TFM) and time to last mark observed (TLM). Error bars = ±95% CI. P < 0.001 for TFM & TLM.
Figure 3a–d: Linear relationship between consumption and energy lost in feces (F), assimilated energy (AE), energy lost in uric acid (U), and metabolizable energy (ME) across four temperature treatments. Raw data points of individuals, and linear regression lines by temperature treatment. Linear regression analysis for each line provided in Appendix 1.
Figure 4a–b: Covariate (consumption) adjusted means (mixed model ANCOVA) of energy utilization in *C. collaris* across four temperature treatments. a) Energy lost in F (feces) and U (uric acid). F, *P* < 0.001; U, *P* = 0.018. C (covariate) *P* < 0.001 for F and U. b) C (Consumption; raw means), AE (C – F) and ME (C – F – U). C, *P* = 0.15; AE, *P* < 0.001; ME, *P* = 0.057. C (covariate) *P* < 0.001 for AE and ME. Error bars = 2 SE.
Table 1: Predicted daily assimilation rates (kJ/day) of *C. collaris* across temperature treatments. Standardized metabolizable energy rates (ME/TFM) = the predicted daily assimilation rates using mean C across all trials and *Tb*-specific ME and passage rates (TFM). Realized metabolizable energy rates (ME*VF/TFM) = the predicted daily assimilation rates using *Tb*-specific voluntary feeding rates (VF) for C and *Tb*-specific ME and passage rates. TFM = adjusted (mixed model ANCOVA) mean number of days to the time of first observed marked feces.

<table>
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<tr>
<th>Tb (°C)</th>
<th>C (kJ)</th>
<th>ME (kJ)</th>
<th>TFM (d)</th>
<th>kJ/day</th>
<th>C (kJ)</th>
<th>ME (kJ)</th>
<th>TFM (d)</th>
<th>kJ/day</th>
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<td>7.95</td>
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Equations used for ME from mixed model ANCOVA fixed effects: 28°C = (0.775C + 0.399 kJ)/3.54d; 31°C = (0.775C – 0.262kJ)/2.43d; 34°C = (0.775C – 0.042kJ)/ 1.70d; 37°C = (0.775C + .332kJ)/1.38d. For ME/TFM, “C” = mean kJ consumed/day across all temperature treatments. For ME*VF/TFM, “C” = mean kJ voluntarily consumed (VF) for each temperature treatment.
### APPENDICES

**Appendix 1**: Linear regression analyses for the effect of C (consumption) and temperature on apparent assimilated energy (AE), metabolizable energy (ME), feces (F) and uric acid (U) in *C. collaris*. All estimates in kJ.

<table>
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<th>SE</th>
<th>y-int. SE</th>
<th>ME slope (C)</th>
<th>SE</th>
<th>y-int. SE</th>
<th>F slope (C)</th>
<th>SE</th>
<th>y-int. SE</th>
<th>U slope (C)</th>
<th>SE</th>
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Chapter 4: Using information on time-energy allocations to identify causal links of local extinctions: A case study of *Crotaphytus collaris* in Arkansas

Casey L. Brewster, Steve Beaupre and Jason Ortega

ABSTRACT

Accelerated global extinction rates have prompted an increased focus on the interplay between environmental change and species response. One well-established framework for identifying mechanisms that link environmental change to population demographics uses information on time-energy allocations of organisms. In this case study of Eastern Collared Lizards (*Crotaphytus collaris*) in northern Arkansas we use the time-energy allocation framework to identify variables that have resulted in reduced age-specific growth and reproduction.

Populations in encroached glades (defined by increased woody vegetation density) experienced similar prey availabilities, meal frequencies, surface active body temperatures (*Tbs*) and movement distances as in intact glades (defined by low woody vegetation density). In contrast, *C. collaris* in encroached glades did experience a decline in operative environmental temperatures suitable for surface-activity, reduced surface activity-time, and less time-at-*Tbs* suitable for digestive processing. We used data from our results to predict the effect of reduced time-at-*Tb* on the net assimilated energy available for growth and reproduction in *C. collaris* inhabiting degraded habitats. Our model predicts a ca. 50% decline in annual assimilated energy and a corresponding decline in annual fecundity – which is nearly identical to empirical observations of reproduction of *C. collaris* in northern Arkansas. We conclude that *C. collaris* in encroached glades experience reduced growth and reproduction primarily as a result of time-at-
Our study highlights the utility of using a time-energy allocation framework to investigate mechanisms driving population declines in at-risk species and integrating this information to address conservation-based questions.

INTRODUCTION

In the face of unprecedented extinction rates (Ceballos et al. 2015), the need for effective strategies to ameliorate the negative impacts of environmental change has become paramount to conservation biology. Conservation strategies often require information on cause and effect to successfully slow or reverse population declines and to predict future outcomes associated with environmental change (Carey 2005; Cooke et al. 2013). However, causal links to local extinction are typically complex, making identification of leading variables elusive (Tracy et al. 2006; Cooke and O’Conner 2010). A powerful, yet relatively underutilized tool in conservation derives from information on the interactions among the environment and time-energy allocations of individual organisms. Information on such interactions can be used to address questions associated with life histories and population vital rates (Dunham et al. 1989): thus, establishing a mechanistic basis from which to inform conservation strategies (Wikelski and Cooke 2006).

Eastern Collared Lizards (*Crotaphytus collaris*), which inhabit savanna-prairie-glade habitats in the Ozark Highlands ecoregion (Templeton et al. 1990), present a system where causal links between environmental change and recent population extinctions are unknown. Over a century of anthropogenic fire suppression has resulted in extensive woody plant encroachment of Ozark glade habitats (Neuwald and Templeton 2013). In turn, *C. collaris* populations have declined considerably in recent years throughout the Ozarks (Templeton et al. 2001; Grimsley 2012; Brewster et al. 2018), prompting conservation listing in Arkansas and Missouri (S2 and
S4, respectively). Previous research on *C. collaris* in Arkansas suggests that populations in habitats with greater woody vegetation density (encroached glades) have reduced age-specific growth and reproductive rates compared to populations in habitats with lower woody vegetation density (intact glades; Brewster et al. 2018). The decline in reproductive rates of *C. collaris* in encroached glades resulted in an estimated 50% decrease in annual population fecundity compared to populations in intact glades. Thus, the observed shifts in life history traits of *C. collaris* in encroached glades likely play an important role in population declines of this species throughout the Ozarks. The focus of our paper is to apply the previously established framework of time-energy allocations in ectotherms (Grant and Dunham 1988 & 1990; Dunham et al. 1989; Grant and Porter 1992; Dunham and Beaupre 1998) to investigate the links between environmental change (e.g. glade encroachment of woody vegetation) and a shift in life history traits (e.g. reduced age-specific growth and reproduction) in *C. collaris* (Fig. 1).

A life history can be defined as the heritable set of rules that determine environment dependent allocation decisions among competing functions. All animals must balance tradeoffs among functions that compete for time (e.g. foraging, social interaction, and behavioral thermoregulation) and functions that compete for mass-energy (e.g. growth, reproduction, storage, maintenance, and activity), within the constraints of the operational environment (biophysical, resource, social/demographic, and predation; Beaupre 2002). Furthermore, vital rates (birth, death and migration; Dunham and Overall 1994) are the emergent properties of a life history. For the present study, the life histories of interest are those that most-directly influence age-specific growth and reproduction. Following the above definition of a life history, our investigative focus is to determine the impacts of dense woody vegetation encroachment on the
operational environment of *C. collaris*, and how those impacts to the operational environment influence time-energy allocations in *C. collaris* (Fig. 1).

Typically, the operational environments that most directly influence time-energy allocation in lizards are the food-energy and thermal environments (Andrews 1982; Adolph and Porter 1993; Niewiarowski 2001; Sears 2005). Indeed, energy acquisition rates are highly dependent on food availability (Congdon et al. 1982). Additionally, environmental heat loads associated with the biophysical environment have a strong influence on ectotherm mass-energy budgets (Dunham et al. 1989; Grant and Dunham 1990). In the Ozark Mountains-*C. collaris* system, encroached glades are characterized by increased amounts of shade cover (Brewster et al. 2018), likely resulting in reduced daytime environmental heat loads compared to intact glades. Furthermore, recent data suggest that digestive processing time in *C. collaris* is strongly affected by temperature, with colder body temperatures (*Tb*) resulting in substantial decrements in the rate of energy assimilation (Brewster et al. 2019). Thus, it is possible that reduced environmental heat loads available to *C. collaris* in encroached glades ultimately result in reduced rates of energy assimilation. In the present study, we tested for differences in the biophysical environment (operative environmental temperatures; *Te*) and the resource environment (prey availability) that could force tradeoffs in time-energy allocations in *C. collaris* populations (Fig. 1).

Growth and reproductive rates result from net assimilated energy (NAE) allocated among competing functions of maintenance, storage, growth, reproduction and activity (Congdon et al. 1982). Therefore, the decline of growth and reproduction of *C. collaris* in encroached glades may ultimately result from: 1) a decrease in NAE (i.e. reduced consumption or assimilation rates) with no change in proportions of energy allocated among functions, 2) a change in
proportions of energy allocated among functions (i.e. more energy allocated to maintenance or activity), or 3) a combination of the two (Niewiarowski 2001). Thus, to determine the potential effects of differences in operational environments between glade types, we looked for population level differences in time-energy budgets that could result in decreased NAE or changes in proportions of NAE allocation (or both).

Specifically, we compared surface-active body temperatures ($T_b$s), daily activity-time, meal frequency, and individual movement rates between $C. collaris$ in encroached and intact glades (Fig 1). If $C. collaris$ in encroached glades have greater movement rates from increased thermoregulatory effort (i.e. shuttling among basking sites; Brewster et al. 2013) greater proportions of energy may be allocated to activity, leaving less energy available for growth and reproduction (A, Fig. 1). A decline in surface-active $T_b$ could suggest that $C. collaris$ in encroached glades suffer reduced physiological performance leading to reduced digestive performance (B1, Fig. 1; Huey 1982; Congdon 1989; Brewster et al. 2019) and/or reduced prey capture rates (C2, Fig. 1; Avery et al. 1982; Huey and Stevenson 1979; Smith and Ballinger 2001). A decline in time available for surface-activity could leave less time-at-$T_b$s where digestion is optimal (Dunham et al. 1989), resulting in reduced processing rates (B2, Fig. 1). Similarly, reduced activity-time could leave less time available for foraging (Adolph and Porter 1993; Grant and Dunham 1988), resulting in a reduction of energy consumed (C4, Fig. 1). Lastly, if $C. collaris$ in encroached glades spend more time with an empty gut as a result of low prey availability (C1, Fig. 1) and/or reduced capture performance, this could result in reduced energy consumed (C3, Fig. 1). We address potential differences in operational environments and time-energy allocations between populations using data collected from a five-year comparative $C. collaris$ study in northern Arkansas. We then combine data from our results with data on the
thermal sensitivity of digestive performance in *C. collaris* (Brewster et al. 2019) to model the effect of time-energy constraints on NAE available for growth and reproduction of individuals in intact and degraded habitats.

METHODS

Study Sites

We sampled *C. collaris* populations from six sites and designated each glade-site as either intact, with low levels of woody vegetation density and shade cover (*n* = 3), or encroached, with high levels of woody vegetation density and shade cover (*n* = 3; see Brewster et al. 2018 for summary data describing glade types). All glade-sites occurred within an 80 km radius along the White and Buffalo Rivers, in the Ozark-St. Francis National Forest in northern Arkansas.

**Intact versus Encroached: Statistical Comparisons**

*Prey Availability*

To compare prey abundances between glade types, we used linear observational transects to estimate arthropod densities per square meter sampled. All estimates included only numbers of arthropods within seven suborders known to represent 95% of *C. collaris* diet: Orthoptera, Coleoptera, Lepidoptera, Hymenoptera, Araneida, Hemiptera and Diptera (McAllister 1985). Sampling was conducted by walking along ten, 20m transects per site, and counting the number of arthropods observed from the ground to waist height, and within 0.5m on either side of the transect. Sampling was standardized by time of day (between 11:00am and 3:00pm), on sunny days, and only when *C. collaris* were active. We conducted linear transect sampling in May, June and July (10 replicates/month/site) for three consecutive summers (2014-2016). Data
analyses of prey densities between glade types was conducted using a mixed model (nlme package in R; Fox and Weisberg 2011), with glade-type (intact vs. encroached), month (May, June or July) and year (2014–2016) as fixed effects, and glade-site (6 total sites repeatedly sampled) as random effects.

*Operative Environmental Temperature*

We used two metrics to compare thermal environments between encroached and intact glades: 1) percent frequency distribution of operative environmental temperature ($Te$; Bakken 1992) within voluntary active $Tb$ range of *C. collaris*, and 2) thermal quality index ($de$; Hertz et al. 1993). A recent study suggested that *C. collaris* uses postural adjustments as a means to behaviorally thermoregulate, and this behavior can have a major influence on thermal ecology inferences in this species (Brewster and Beaupre 2019). Thus, we use similar $Te$ modeling procedures as Brewster and Beaupre (2019; “OTMIDEAL”) that account for postural adjustments by *C. collaris* to estimate $de$ and frequency distribution of $Te$ in this study. Briefly, we used three different operative temperature model (OTM) types modified from a 12cm (2.5cm diameter) length of copper pipe, and painted to match the reflective properties of *C. collaris*: 1) stilted OTM that mimics the biophysical properties of *C. collaris* in an elevated posture, 2) unmodified OTM that mimics the biophysical properties of *C. collaris* in an intermediate posture, and 3) compressed OTM that mimics the biophysical properties of *C. collaris* in a prostrate posture. All three OTM types were validated against live *C. collaris* individuals in the corresponding posture in the field (see Fig. 4b, Brewster and Beaupre 2019). For each glade-site, we used a total of 75 OTMs to estimate the $Te$ distribution of 25 randomly selected microsites. At each microsite we placed three OTMs (one of each type; silted, unmodified or compressed) side-by-side, oriented in the
same direction. We randomly designated the orientation direction of OTMs at each microsite (North–South or East–West). Using data on the frequency of postures used by *C. collaris* as a function of mean *Te* of the habitat, we estimated the minimum (33.5°C) and maximum (39.5°C) mean *Te* threshold that lizards remained in the intermediate posture. We used these two thresholds (33.5 and 39.5°C) to model the habitat with unmodified OTMs (OTM\textsubscript{IDEAL}, Brewster and Beaupre 2019). When mean *Te* was less than 33.5°C, we modeled the 25 microsites with compressed OTMs. When mean *Te* was greater than 39.5°C, we modeled the 25 microsites with stilted OTMs, and when mean *Te* was within 33.5–39.5°C, we modeled the habitat with unmodified OTMs. We suspended a single iButton\textsuperscript{TM} temperature data logger (Maxim Integrated Products) in the center of each OTM using a piece of aluminum screening and set loggers to record temperature every 20 min (OTM time constant ~3.5min). All thermal environment comparisons were made simultaneously between one encroached and one intact glade for a minimum of 5 days and alternated among glade sites across the activity-season. Each glade to glade comparison included 3 sunny days in May, June and July (9 days total for each glade-site).

Our first metric for comparing thermal environments between encroached and intact glades was to estimate the percentage of microhabitats (n = 25 for each site) within the voluntary active *Tb* range (*T\textsubscript{ACT}* of *C. collaris*). We used the central 99 % of all *Tbs* recorded on surface-active *C. collaris* in our study populations to estimate *T\textsubscript{ACT}* (31.2 - 42.6°C). Using OTM\textsubscript{IDEAL}, we estimated the percent of microhabitats at a given time point within *T\textsubscript{ACT}* . Percentages were calculated every 20 min from 7:00am to 9:00pm, and comparisons were made across days (n = 3 per glade-site) for May, June and July (9 days total per glade-site).
Our second metric for comparing thermal environments was to estimate the thermal quality index ($de$) between glade-types. The thermal quality index provides the average absolute deviation between $Te$ and the set-point $Tb$ range ($T_{SET}$; Hertz et al. 1993). We used the central 60% of all $Tbs$ selected by $C.\ collaris$ in a laboratory gradient (38.1-34.8; Firth, Turner and Ralph 1989) as our metric for $T_{SET}$. Using the OTM_{IDEAL}, we estimated the average absolute deviation between $Te$ and $T_{SET}$ at a given time point. Average absolute deviations were calculated every 20min from 7:00am to 9:00pm, and comparisons were made across days ($n = 3$ per glade-site) for May, June and July (9 days total per glade-site). We used a linear mixed model (nlme in R) to compare $de$ and percent $Te$ within $T_{ACT}$ by glade-type. We designated glade-type and season as fixed effects, and glade-site (sampled repeatedly) as a random effect.

**Surface Active Tb**

To determine if $C.\ collaris$ in encroached glades experience lower $Tbs$ during surface activity, we used $Tb$ data collected from 2013-2017. Animals were captured with a pole-and-noose from late April through late July, and cloacal $Tbs$ were recorded using a quick-read digital thermometer (Model HH800, Omega Engineering). We only used $Tb$ data on animals known to be surface-active for a minimum of two time-constants ($C.\ collaris$ time constant ~6.5mins; Grigg et al. 1979) to ensure they were near equilibrium $Tb$, and where $Tb$ was recorded within 2min after capture. Comparisons of $Tbs$ between glade-types were made using a mixed model (nlme package in R). We designated glade-type, sex and month as fixed effects, and subject ID (individuals were sampled repeatedly and at variable time-points) as a random effect.
Time Surface Active

To compare total daily surface-activity times between glade types, we made focal observations on *C. collaris* surface activity. A pilot study in 2014 suggested that surface-activity (percentage of animals in the population on the surface) was stable (>50% of animals) during mid-day (11:00am-3:00pm), assuming typical climatic conditions during the activity season. We estimated total activity-hours per day by recording the onset of surface activity in the morning, and end of surface activity in the evening. We conducted observational transects on 10–20min intervals starting at local sunrise until the onset of activity was observed, and then conducted hourly observational transects through the midday. Within two hours of local sunset, we again returned to 10-20min intervals between observational transects until lizards were no longer observed surface active. Activity-time estimates were made on three days for each glade-site, in May, June and July (9 days total per glade-site) in 2015–2016. Estimates were made only on days with typical climatic conditions for a given month. We used a mixed-linear model (nlme package in R) for statistical analyses of hours active between glade-types. We designated glade-type and month as fixed effects, and glade-site (repeatedly sampled) as a random effect.

Movement Rates

To determine if *C. collaris* in encroached glades expend more energy through increased locomotory rates, we estimated movement rates of individuals. Because of time constraints (all field sampling in this study was conducted by a single investigator, C.L.B.), we were only able to estimate movement rates on a sub-sample of individuals from one encroached and one intact glade. We opportunistically chose 5 males and 3 females from both glades to estimate movement rates. Subjects were observed through binoculars from a distance of 30–70m (so as to not impact
C. collaris behavior), for 10min time blocks, and only on days and times when multiple individuals were surface-active. All subjects had been previously captured within the previous 1–4 days and had been temporarily marked on their dorsum with a white paint pen (i.e. a single letter) to allow visual identification of the individual by the observer. We sampled movement rates of individuals over a ten-day period in May 2015, with a total of 5 observations (50min) per subject. We estimated the total distance moved (when animals moved greater than 10m at a time, we used a laser rangefinder; Nikon™ model AL-11, estimated to the nearest 1m) per 10min time block. Data analysis of movement rates were conducted using mixed models (nlme package in R). We designated glade-type and sex as fixed effects, and subject ID (individuals sampled repeatedly) as a random effect.

**Frequency of Recent Meal**

To test for differences between glade-types in the frequency of a recent meal, we used palpation data collected on C. collaris captured from 2013-2017. Upon capture, we physically palpated the abdomen of individuals to look for the presence of a recent meal. In our experience, females in late gravidity (those with shelled oviductal eggs) typically had an empty gut, suggesting that they stopped foraging until after oviposition. Thus, we excluded females from our palpation dataset if they contained large-soft follicles or oviductal eggs (reasonably distinct in shape and texture from food items). To analyze presence/absence data we used a general linear mixed model (glmer in R; Bates et al. 2015), fit with the binomial (logit) function. We designated glade-type and sex as fixed effects, and subject ID (individuals sampled repeatedly) as a random effect.
NAE Model

Time-at-Tb

We used the results from our statistical comparisons to predict their effects on the NAE available for growth and reproduction of *C. collaris* in our study populations. The NAE model specifically uses data on variation in time (hours active and inactive per day; see Results) and *Tbs* (mean active versus mean inactive *Tbs*; see Results) of individuals in encroached and intact glades to predict its effects (time-at-*Tb*) on digestive processing rates - based on *Tb* specific digestive processing data of *C. collaris* from Brewster et al. 2019.

To estimate *Tbs* of *C. collaris* during inactivity periods, we assumed that while inactive, the mean and variance in *Tbs* of animals would approximate the mean and variance of under-rock refugia (based on biophysical principals; Porter and Gates 1969). We estimated the mean and variance in *Te* of 5–8 deep crevice microsites used by *C. collaris*, at each of the six glades during our mid-season (June) estimates of *Te* distributions (see *Operative Environmental Temperature*). We wrapped a single iButton™ temperature data logger (Maxim Integrated Products) with aluminum foil and attached a length (~1.5m) of surveyor marking tape to the unit. We used a 1m dowel (2cm diameter) to guide the iButton™ unit into the refugia (~15-30cm deep), leaving the remaining length of surveyor tape on the surface for ease of retrieval. iButtons logged temperature on 20-minute intervals, over the same time periods as the surface *Te* estimates at a given site. Refugia sites were chosen opportunistically, based on previous observations of individuals using these sites. We did not make any formal statistical analyses on refugia temperatures. Instead, we use estimates of refugia temperatures to provide a 24hr *Tb* profile of *C. collaris* in our populations and to parameterize the NAE model.
To model NAE we estimated the daily energy budget of *C. collaris* assuming:

\[ NAE = ME - M \]

Where NAE is net assimilated energy, ME is metabolizable energy (energy consumed minus energy lost in feces minus energy lost in uric acid; C – F – U) and M is metabolic maintenance cost; all in kJ d\(^{-1}\). For consumption, we assumed that individuals would only ingest in a day the amount of energy that they had processed over the preceding 24 hrs (Avery 1972). To estimate the daily realized consumption (C), we first estimated the maximum energy a *C. collaris* would ingest in a day on an empty gut (max-C). We then estimated the fraction of energy processed (based on time and *Tb* effects on passage rate – see “Processing Rates” below) 24hrs later and assumed lizards would only consume (replace) that same fraction of energy the following day. To estimate max-C, we converted voluntary feeding rate estimates from Brewster et al. (2019; kJ of crickets consumed in a 2hr time block with lizards on empty gut at 37°C) to mass-specific rates (kJ g\(^{-1}\)d\(^{-1}\)). We bound the max-C input values into low and high using the mean mass-specific rate (0.86 kJ g\(^{-1}\)d\(^{-1}\)) as the low C value and the greatest observed mass-specific rate (1.02kJ g\(^{-1}\)d\(^{-1}\)) as the high C value (Brewster et al. 2019).

*Processing Rates and ME*\text{RATE}\*

To calculate the effect of temperature on daily digestive processing rates, we converted temperature-specific passage time (PT) estimates (Brewster et al. 2019) from total hours to % Processing Rates (% of ingested ME processed in 24hr). In carnivores, the bulk of digestive processing (i.e. breakdown, absorption, catabolism and biosynthesis; Secor 2009) of a meal is completed over the course of SDA (specific dynamic action; Dorcas et al. 1997; Beaupre 2002; Cox and Secor 2010), which consists of 60 – 80% of PT in Sceloporus lizards (70-80% in *S*.}
occidentalis, Harwood 1979 and Roe et al. 2005; ~60% in S. merriami, Beaupre et al. 1993a&b). Thus, we assumed that energy consumed was fully processed at 75% of PT in our calculations. We then converted PT estimates of C. collaris (Brewster et al. 2019) into time-at-\(T_b\) specific rates (% Processing Rate) using:

\[\text{% Processing Rate} = \left(\frac{\# \text{ hours Active}}{\text{PT @ mean active } T_b \times 0.75 \text{ SDA}}\right) + \left(\frac{\# \text{ hours Inactive}}{\text{PT @ mean inactive } T_b \times 0.75 \text{ SDA}}\right)\]

We then estimated the daily metabolizable energy rate (ME\(^{\text{RATE}}\) kJ d\(^{-1}\)) as:

\[\text{ME}^{\text{RATE}} = \text{ME} \times \text{% Processing Rate}\]

**Metabolic Maintenance Costs**

To estimate metabolic maintenance costs (\(M\) in kJ d\(^{-1}\)), we used mass-specific resting metabolic rates (RMR) of C. collaris from Dawson and Templeton (1963). Daily metabolic maintenance costs (\(M\)) in lizards typically ranges from 2.0 – 2.5* total daily RMR (Bennett and Nagy 1977; Anderson and Karasov 1981; Congdon et al. 1982; Nagy et al. 1999). Thus, we bound our estimates of \(M\) using 2.0*RMR (low \(M\)), 2.25*RMR (medium \(M\)) and 2.50*RMR (high \(M\)). To calculate \(M\), we used the equation:

\[M = 2.0 \text{ or } 2.25 \text{ or } 2.5 \left(\frac{\# \text{ hours active x RMR h}^{-1} \text{ @ mean active } T_b}{\# \text{ hours inactive x RMR h}^{-1} \text{ @mean inactive } T_b}\right)\]

We subtracted total \(M\) from the ME\(^{\text{RATE}}\) to estimate daily NAE. We multiplied total NAE (kJ d\(^{-1}\)) times the number of days in the activity-season. Activity season length in northern Arkansas is dependent on weather patterns especially in the early part of the activity-season (early April –
mid May; CLB personal observation). Thus, we bound our activity-season length to low (90d), medium (120d), and high (150d) to account for seasonal variation.

**Growth and Reproduction Potential**

We used estimates of available NAE from the time-at-$Tb$ model to predict the NAE available for reproduction (NAEr) in a one-year-old (1YO; 15g) and two-year-old (2YO; 25g) female *C. collaris*. We calculated the mean annual NAE allocated to mass growth and storage (NAEg) for a 1YO and a 2YO female *C. collaris* based on mean body mass data from Brewster et al. 2018. Body condition of *C. collaris* in intact and encroached glades does not differ (Brewster et al. 2018). Thus, we assumed *C. collaris* in intact and encroached glades allocate similar proportions of NAE to lean tissue growth and lipid deposition. To estimate NAER, we subtracted NAEg from total NAE. We used 8.6 kJ g$^{-1}$ dry mass (Peterson et al. 1999) as our estimate of the metabolic cost of growth (MCG). We used energy density estimates of *C. collaris* carcasses and *C. collaris* eggs from Vitt (1978) for our calculations of the energy density of lizards (EDL = 19.2 kJ g$^{-1}$ dry mass; water content, WCL = 74.7%) and lizard eggs (EDE, 25.6 kJ g$^{-1}$ dry mass; water content eggs, WCE = 54.6%). We estimated NAEg as:

$$\text{NAEg} = (\text{annual change in mass (g)}) \times (\text{MCG} + \text{EDL}) \times (1 – \text{WCL})$$

We used unpublished data provided by ME Gifford and CL Brewster to estimate the average egg wet mass of *C. collaris* in northern Arkansas (1.63g, range = 0.933–1.994g). We converted NAER to the predicted # of eggs for *C. collaris* using the equation:

$$\text{#Eggs} = \frac{\text{NAEr}}{(\text{MCG} + \text{EDE}) \times (1 – \text{WCE})} / 1.63g$$
RESULTS

**Intact versus Encroached: Statistical Comparisons**

*Prey Availability & Environmental Temperature*

Arthropod densities were significantly greater in encroached glades than intact ($F_{1,5} = 12.2, P = 0.018$) and declined each month across the activity season (May–July; $F_{2,387} = 3.8, P < 0.001$; Fig 2). The percent of microsites within T<sub>ACT</sub> increased each month ($F_{2,44} = 4.5, P = 0.016$), and were significantly greater in intact glades (across season daily mean = 47.31% ± 3.75 SE) compared to encroached glades (28.15% ± 3.75 SE; $F_{1,4} = 33.6, P = 0.004$; Fig. 3a-c). Similarly, $de$ declined over the activity season ($F_{2,44} = 19.6, P < 0.0001$), and was significantly lower in intact sites (across season daily mean = 4.43 °C ± 0.51 SE) than in encroached sites (6.51 °C ± 0.51 SE; $F_{1,4} = 24.9, P = 0.0075$; Fig 3d). We found no significant “glade-type x month” interactions on prey availability, percent T<sub>ACT</sub> or $de$.

*Surface-Active Tb & Time Surface Active*

Surface active $Tbs$ increased significantly over the activity season (May–July; $F_{2,168} = 16.21; P < 0.0001$), but were not different between glade types ($F_{1,168} = 2.82, P = 0.0947$; Fig. 4a). We found no significant “month x glade-type” interactions on surface-active $Tbs$. The number of hours per day *C. collaris* was observed surface active increased throughout the activity season ($F_{2,8} = 45.77, P<0.0001$) and was statistically greater in intact sites compared to encroached sites ($F_{1,4} = 108.14, P<0.0005$; Fig. 4b). We found no significant “month x glade-type” interactions on surface-activity times.
Movement Rates & Frequency of Recent Meal

Total distances moved did not differ significantly between glade types ($F_{1,13} = 0.64$, $P = 0.436$) or sex ($F_{1,13} = 2.50$, $P = 0.138$). Male lizards moved an average of $0.629 \text{m/min}$ in intact sites ($\pm 0.182 \text{ SE}$), and $0.471 \text{m/min}$ in encroached sites ($\pm 0.189 \text{ SE}$). In intact sites females moved an average of $0.367 \text{m/min}$ ($\pm 0.089 \text{ SE}$) and an average of $0.209 \text{m/min}$ ($\pm 0.102 \text{ SE}$) in encroached sites. We found no significant “sex x glade-type” interaction on distances moved. The frequency of a recent meal was not significantly different between glade types (intact = 90.1%, encroached = 85.4%; $X^2 = 0.914$, df = 1, $P =0.339$). We observed no significant “glade-type x sex” interaction on meal frequency.

NAE Model

To parametrize time-budgets in the NAE model, we used the grand mean of hours surface-active and inactive across the season (May–July) for individuals in intact sites (12:12, active: inactive) and encroached sites (9:15, active: inactive; Fig 4b). To parametrize active $Tb$ in the NAE model, we used the grand mean of surface-active $Tbs$ of $37^\circ \text{C}$ during activity time periods (Fig. 4a). To parameterize inactive $Tbs$, we used the mean overnight refugia temperatures ($20^\circ \text{C};$ Fig. 5). Figure 5 provides the 24hr $Tb$ profiles of $C. \text{collaris}$ in intact and encroached glades and illustrates the time-at-$Tb$ estimates used to parametrize the NAE model.

For PT calculations when $C. \text{collaris}$ was active, we used the $37^\circ \text{C}$ specific rates provided by Brewster et al. 2019. The minimum $Tb$ used to estimate PT in that study was $28^\circ \text{C}$. Thus, to predict PT at $20^\circ \text{C}$ during inactive times, we used a non-linear regression (PT data were nonlinear; Brewster et al. 2019) estimated from mean PT values across 28, 31, 34 & $37^\circ \text{C}$: $PT = –187.4(\ln^* Tb) +705.8$
A full summary of parameters, calculations and predictions of the NAE time-at-$Tb$ model are provided in Appendix 1 & 2. Depending on values used to calculate $M$ (low = $2.0 \times \text{RMR}$, medium = $2.25 \times \text{RMR}$, high = $2.5 \times \text{RMR}$), activity-season length (90, 120 or 150d), and max-C (low or high C; see METHODS), C. collaris in encroached glades would have a 37–51% (1YO, 15g female) or a 38–54% (2YO, 25g female) decline in annual NAE compared to intact sites based on model predictions (Fig. 7). The NAE model predicted 0–4.1 eggs for 1YO females in encroached glades and 1.7–8.4 eggs for 1YO females in intact glades, depending on variation in max-C, $M$ and activity-season length (90d, 120d or 150d; Fig. 8). Similarly, variation in 2YO max-C, $M$ and activity-season length would result in a predicted 3.0–10.6 eggs for females in encroached glades and 6.7–18.3 eggs for females in intact glades (Fig. 8).

DISCUSSION

The goal of our study was to determine the leading factors causing a reduction in growth and reproductive rates of C. collaris in northern Arkansas. Our first set of comparisons examined differences in operational environments (environmental temperatures or prey availability; Fig. 1) that could lead to constraints on the time-energy budgets of C. collaris in encroached habitats. Arthropod densities were statistically greater in encroached glades than in intact glades (Fig 2). Thus, our data suggest that C. collaris in encroached glades are not limited by prey availability. Alternatively, encroached glades had significantly lower percent $Te$ within $T_{\text{ACT}}$ than intact glades (Fig. 3a-c), and significantly greater $de$ indices than intact glades (Fig 3d). Taken together, our $Te$ comparisons imply that C. collaris in encroached glades could be experiencing a constraint on time-energy allocation associated with reduced availability of suitable $Te$. 
Our second line of questions examined differences in time-energy phenotypes that might explain a link between differences in operational environments and a shift in life history traits. First, we tested for differences in surface active $T_b$ between glade types. As our results suggested that increased shade leads to reduced proportion of microsites within surface-active $T_b$ range (Fig 3), it would be reasonable to anticipate that the $T_b$s of $C.\ collaris$ in encroached glades may be reduced. However, we found no significant difference in surface-active $T_b$ between glade-types (Fig. 5a). Thus, we concluded that $C.\ collaris$ in encroached glades do not experience a decline in physiological performance during surface-activity (i.e. digestion or prey capture rates) as a result of reduced surface-active $T_b$s (Fig. 1).

Next, we tested for a difference in surface-activity time between glade-types. Data from a previous study on one of the intact glades suggested that the minimum threshold for surface-activity was 10% (average minimum % of $T_e$ within $T_{ACT}$; Brewster and Beaupre 2019). Using 10% of $T_e$ within $T_{ACT}$ as a proxy for the minimum threshold for surface-activity, Figure 3a-c suggests that $C.\ collaris$ should have 2-4hrs fewer per day for surface-activity in encroached glades compared to intact glades. Our estimates of surface-activity time between glade-types (Fig. 4b) align with our $T_e$ assessment of available hours for activity (Fig. 3a-c). Thus, we conclude that $C.\ collaris$ in encroached glades do experience a reduction in the number of hours per day where the $T_e$ frequency distribution is suitable for surface-activity, which results in reduced daily-activity time.

Next, our results indicated that movement rates of $C.\ collaris$ in the encroached glade did not differ significantly from lizards in the intact glade. Thus, our data do not support the explanatory hypothesis that increased movement rates of $C.\ collaris$ in encroached glades leads to a proportional shift in NAE allocated to reproduction and growth. Lastly, we tested for a
difference in the presence of a recent meal of *C. collaris* in intact and encroached glades and found no significant difference. Our results suggested that *C. collaris* in both glade types experience an empty stomach relatively infrequently (9.9–14.6% of the time for intact and encroached respectively; see “Results”), which is consistent with our findings that prey densities are not lower in encroached glades (and are actually greater; Fig 2) and that animals experience similar active-*Tbs* (Fig 4a) between glade types. Thus, we conclude that if *C. collaris* in encroached glades do experience lower consumption rates, it is not because of reduced prey availability or reduced *Tb* associated capture performance.

In all of our statistical comparisons, the only variables that differed significantly in a way that could explain time-energy constraints of *C. collaris* in encroached glades were the availability of suitable *Te* (Fig. 3a–d), and the time animals were surface active (Fig. 4b). Thus, our results indicated that reduced activity-time budgets represented the most probable source of constraint in time-energy allocation experienced by *C. collaris* in encroached glades. Since our data suggested that the remaining variables (e.g. prey abundance, meal frequencies, active-*Tbs* and movement rates) did not explain time-energy constraints of *C. collaris*, we focused our modeling efforts on the potential effects of reduced activity-times on NAE.

Lizards in encroached glades spent less time surface-active (3–4 hr/day; Fig. 4b), which means they spent 3–4 hours per day longer in their under-rock refugia (Fig. 5). Assuming *C. collaris* *Tbs* approximate refugia *Tes* when they are inactive (based on fundamental biophysical principals; Porter and Gates 1969) would suggest that *C. collaris* in encroached glades spend 3-4 hrs a day longer at *Tbs* substantially lower (15–25°C) than their active-*Tbs* (34–39°C; Fig. 5). Digestive performance in *C. collaris* is substantially reduced at *Tbs* 28°C and below, primarily due to increased gut passage times and reduced feeding rates (Brewster et al. 2019). Thus, we
designed the NAE model to focus on the effects that variation in time-at-$Tb$ may have on NAE available for growth and reproduction (Fig. 6).

Results from the NAE model (Fig. 7; Appendix 1 & 2) suggested that female *C. collaris* in encroached glades experienced a substantial deficit in their available NAE by approximately 44% (range of 38–54% across both age-classes and all bounded input values; Appendix 1 & 2). By converting age-specific growth rates and total number of eggs produced by female *C. collaris* in intact and encroached glades (Brewster et al. 2018) into annual NAE (Appendix 1 & 2), we provided comparison of the observed annual NAE to the predicted annual NAE (Fig. 7). Predictions in annual NAE for both age-classes and between glade types are consistent with the observed annual NAE estimated from actual lizards (Fig. 7). Next, we converted the NAE available for reproduction (by subtracting NAEg estimated from observed annual growth in mass) into the predicted number of eggs (Fig. 8). Model predictions suggested females in encroached glades would produce far fewer eggs annually than in intact glades (average = 52% fewer, range = 84–39% fewer, across both age-classes and all input variables; Fig.8). Model predictions of a 52% decline in the annual number of eggs produced is consistent with observed declines in annual fecundity (~50%) of populations in encroached glades (Brewster et al. 2018). Furthermore, within age-class predictions from the NAE model bounded the observed number of eggs produced by females in intact and encroached glades (Fig. 8). Thus, the NAE model appears to provide a reasonable approximation of the actual observed differences in NAE allocated to reproduction of *C. collaris* in intact and encroached sites, all as a function of variation in time-at-$Tb$. In short, the NAE model implies that constraints on daily (24hr) digestive processing rates, as a function of time-at-$Tb$, may explain the majority of variation in growth and reproduction between *C. collaris* in intact and encroached glades.
We note that our study cannot rule out other potential factors contributing to observed shifts in life histories. Indeed, explanatory mechanisms in ecology are typically complex (Quinn and Dunham 1983; Dunham and Beaupre 1998). For example, we currently cannot rule out that some type of genotypic difference between *C. collaris* in intact and encroached glades is contributing to shifts in life histories. Similarly, there could be some other important differences not accounted for in our study [i.e. immunologically induced maintenance costs (Agugliaro et al. 2019) or predator effects on activity (Downes 2001) or predator induced shifts on food preferences (Hawlena and Pérez-Mellado 2009)] that contribute to constraints on time-energy budgets. However, given the large estimated differences in available NAE based on the time-at-*Tb* model, as well as the models consistency in predictions to actual estimates of growth and reproduction of *C. collaris* in northern Arkansas - we conclude that time-at-*Tb* effects play an important role in observed life history trait differences between intact and encroached glades.

**CONCLUSIONS**

Our study uses principals of time-energy allocations to investigate and identify important links between environmental change (e.g. glade encroachment of woody vegetation) and a shift in life history traits (e.g. reduced age-specific growth and reproduction) that are likely resulting in population declines of *C. collaris* in northern Arkansas (Brewster et al. 2018). Our findings suggest that woody vegetation encroachment reduces the availability of suitable *Te* and restricts surface activity-time in *C. collaris*. Shorter activity-times leave *C. collaris* with less time-at-*Tbs* suitable for digestive processing. We modeled the effect of time-at-*Tb* on the digestive processing rates of *C. collaris*, and the model predictions provide a compelling case to explain reduced NAE allocated to growth and reproduction of individuals in degraded habitats. As *C.
*collaris* have experienced extensive population declines and local extinctions through much of their historic range in the Ozark Mountains, our study provides a robust framework to identify explanatory mechanisms driving population declines as a result of habitat degradation. Our study reinforces the utility of using information of time-energy allocations to develop a mechanistic framework to address complex questions in conservation biology. We encourage conservation biologists to consider the importance of time-energy allocations in understanding patterns associated with at-risk species, and when appropriate, use this well-established framework to investigate complex conservation issues.

ACKNOWLEDGEMENTS

We thank M. Gifford for providing *C. collaris* egg mass data. We thank the many government agency staff members (AGFC, USFS, NPS, and TNC) including K. Irwin, K. Goodwin, J. Hawkins, C. Bitting, and D. Zollner for supporting our field efforts over the past 6 years. We thank M. Gifford and T. Baird for the valuable insights on working with *C. collaris*. All authors contributed critically to the experimental design, analyses and writing of this manuscript, and gave final approval for publication. The project was funded by a state wildlife grant from the Arkansas Game and Fish Commission [grant number AR-T-056], and through a scholarship to CLB from the Pat Tillman Foundation. This study was approved through the University of Arkansas Institutional Animal Care and Use Committee (protocol # 16057; AGFC permit # 030320171).
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FIGURES

**Operational Environment** | **Metric for environmental comparison** | **Quantifiable effects on C. collaris** | **Effects on energy budget**
--- | --- | --- | ---
Biophysical (temperature) | Habitat thermal quality index \((de)\) | Movement rates \(A\) | Fraction of \(NAE\) devoted to \(R \& G\)
Biophysical (temperature) | Frequency distribution of \(Te\) w/in \(T_{Act}\) | Surface active \(Tb\) \(B1\) | Processing rates
Resource (prey availability) | Arthropod density \(C1\) | Time surface active \(B2\) | Consumption rates
Resource (prey availability) | Frequency of recent meal \(C3\) | Consumption rates

**Fig. 1:** Conceptual framework for investigating and testing of factors that potentially link operational environments to reduced net assimilated energy (NAE) allocated to age-specific growth and reproduction of *C. collaris* in northern Arkansas. Diagram shows the primary variables to be tested in the current study, and how variables are related (directly or indirectly) to key potential differences between encroached and intact glades. \(A = NAE\) devoted to locomotion/activity; \(B1 = \) thermal sensitivity of digestion performance; \(B2 = \) time on the surface maintaining physiologically optimal \(Tbs\); \(C1 = \) prey capture opportunity; \(C2 = \) thermal sensitivity of prey capture performance; \(C3 = \) meal frequency; \(C4 = \) time on the surface available to capture prey; \(NAE = \) net assimilated energy; \(R = \) reproduction; \(G = \) growth
Fig 2: Mean and SD by month (May-July covers the reproductive season of *C. collaris* in northern Arkansas) of arthropod densities (includes only arthropods within seven suborders known to represent 95% of *C. collaris* diet; see Methods) in intact and encroached glades.
Fig 3a–d: Percentage of $Te$ within active $Tb$ range ($T_{ACT}$ a-c) and mean thermal quality index ($de$; d) between intact and encroached glades. $Te$ comparisons include three typical days for each site, for each month (9 total days for each of 6 sites). Error bars indicate SD. Shaded area (a-c) indicates the average minimum percentage of $Te$ models within $T_{ACT}$ (10%) where $C. collaris$ was observed surface active. Thermal indices were recorded from May-July, which covers $C. collaris$ reproductive season in northern Arkansas.
Fig. 4a-b: Mean and SD of surface-active Tb (a) and number of hours observed surface-active (b) between *C. collaris* in intact and encroached glades.
**Fig 5**: Comparison of the spatiotemporal $Tb$ profile of *C. collaris* in intact and encroached glades over a 24hr period. Data combines mean and SD of $Tb$ during surface-activity (Fig. 4a) with under-rock (refugia) $Tes$, which are assumed to match *C. collaris* $Tb$ while they are inactive and in refugia. $Tb^*$ = measured $Tbs$ (during surface activity) and assumed $Tbs$ (during surface inactivity).
Figure 6: Diagram of the effects of time-at-$T_b$ on processing rates (% of ingested ME processed in 24hr) and daily $M$ (maintenance metabolic costs) of *C. collaris* in encroached and intact glades.

$\%$ Processing Rates = \[\frac{\# \text{ hours Active}}{\text{PT} @ 37^\circ\text{C} \times 0.75 \text{ SDA}}\] + \[\frac{\# \text{ hours Inactive}}{\text{PT} @ 20^\circ\text{C} \times 0.75 \text{ SDA}}\]. 37°C PT (passage time) = 33.12hr, 20°C = 108.36hr (Brewster et al. 2019).

RMR @ 37°C (Active) = 4.51 J g$^{-1}$ hr$^{-1}$ RMR @ 20°C (Inactive) = 0.98 J g$^{-1}$ hr$^{-1}$ (Dawson and Templeton 1963).
Figure 7: Predictive model of the annual NAE (kJ yr$^{-1}$), assuming a 15g (1 YO; a & b) and 25g (2 YO; c & d) female C. collaris inhabiting an intact and encroached glade. Annual NAE calculated based on time-at-$T_b$ (Fig. 5) effects on digestive processing rates (Fig. 6) using digestive performance data from Brewster et al. (2019). Annual NAE estimates are bounded by assumed high and low max-C (High C, Low C), low (2.0*RMR), medium (2.25*RMR), high (2.5*RMR) assumed metabolic maintenance costs ($M$), and low (90d), medium (120d), high (150d) assumed activity-season length. Dotted line = the estimated NAE devoted to mass-growth (NAEg), calculated from observed age-specific changes in mass (annual) of female C. collaris in intact glades (Brewster et al. 2018). Dashed line = the estimated NAE devoted to NAEg, calculated from observed age-specific changes in mass of female C. collaris in encroached glades (Brewster et al. 2018). See Appendix 1 & 2 for summary of parameters, calculations and results of NAE model.
Figure 8: Predictive model of the annual number of eggs produced assuming a 15g (1 YO; a & b) and 25g (2 YO; c & d) female *C. collaris* inhabiting an intact and encroached glade. Model estimates based on the time-at-

"Tb" (Fig.5) effects on digestive processing rates of *C. collaris* (Fig. 6) using digestive performance data from Brewster et al. (2019). The NAE available for reproduction (NAEr) was calculated by subtracting the age-specific annual NAE devoted to mass-growth (NAEg) from the predicted annual NAE available to female *C. collaris* in intact and encroached glades (Fig. 7). NAEr was converted to the predicted number of eggs using: #Eggs = (NAEr/ (MCG + EDE)/ (1 – WCE))/1.63g

Predicted annual number of eggs are bounded by the assumed high and low max-C (High C, Low C), low (2.0*RMR), medium (2.25*RMR), high (2.5*RMR) assumed metabolic maintenance costs (M), and low (90d), medium (120d), high (150d) assumed activity-season length. Dotted line = the observed number of eggs of female *C. collaris* in intact glades (Brewster et al. 2018). Dashed line = the observed number of eggs of female *C. collaris* (Brewster et al. 2018). See Appendix 1 & 2 for summary of parameters, calculations and results of NAE model.
### Appendix 1: Summary table of NAE model calculations and results.

Table includes variation in low, medium and high $M$, assuming 120d activity season length. Act/Intact = hours/hours. Mass = grams. Eggs = number of eggs. All other variables in kJ. See methods for specific equations used and description of input assumptions.

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<th>Glade Type</th>
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Appendix 2: Summary table of NAE model calculations and results. Table includes variation in low, medium and high assumed activity-season length, and medium (2.25*RMR) M. Act/Intact = hours/hours. Mass = grams. Eggs = number of eggs. All other variables in kJ. See methods for specific equations used and description of input assumptions.

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VI. DISSERTATION CONCLUSIONS

The primary focus of my dissertation research was to determine the mechanisms linking glade encroachment to population declines of *C. collaris* in the Ozarks. My general approach was to test for variation in operational environments and life history traits that could be linked to population declines. Results from chapter one provided the general patterns in operational environments and life history traits which the remaining dissertation chapters were focused on explaining. Populations inhabiting encroached glades experienced higher levels of woody vegetation density, greater levels of shade cover, reduced age-specific body size, later age of maturity, lower clutch size/frequency and reduced annual population fecundities compared to animals in intact glades. I found no significant difference in body condition between glade-types. Taken together, results from chapter one suggested that *C. collaris* in encroached glades experienced decrements to their population fecundity rates that likely play an important role in population declines of *C. collaris* in the Ozarks.

In chapter two, I provided data that suggested postural adjustment can have a substantial effect on indices used in thermal ecology studies. Results in chapter two showed the functional significance of posture in *C. collaris* and presented a novel approach to account for this behavior in thermal ecology studies. I used information from chapter two to establish the thermal ecology methods for investigating variation in operative environmental temperature between intact and encroached glades in chapter four.

In chapter three, I investigated the thermal sensitivity of digestive performance of *C. collaris*. Passage times decreased and voluntary feeding rates increased significantly with increasing temperature. Consumption explained the majority of variance in AE and ME, followed by the effect of temperature treatments. Results from chapter three suggested that if *C.
collaris in encroached glades experience reduced Tbs, this could result in a decline in NAE rates. I used data from chapter three to model the potential impacts of time-at-Tb of C. collaris in encroached glades.

In chapter four, I found that C. collaris in encroached glades experienced a lower proportion of operative environmental temperatures suitable for surface-activity, reduced surface activity-time, and less time-at-Tbs suitable for digestive processing. I combined data from chapter four with digestion estimates from chapter three to model the effect of time-at-Tb on energy processing rates of C. collaris. Predictions from the NAE model suggest that individuals in encroached glades experience a substantial decrement in the available NAE for growth and reproduction as a result of reduced digestive processing rates. Results from chapter four appear to explain the link between reduced activity-times and observed declines in growth and reproduction of C. collaris in northern Arkansas.

Overall, this dissertation provides a compelling case to explain the link between degraded habitats and observed shifts in life history traits of C. collaris. One of the key shifts in life history traits observed in encroached glades was an ~50% decline in annual population fecundity rates. Thus, results from my dissertation may also explain the link between habitat degradation and population declines of C. collaris in the Ozarks. Indeed, such a substantial decline in fecundity could easily result in the population declines and local extinctions observed in the Ozarks. However, it is also possible that populations in encroached glades also experience a decline in survival rates. Thus, without population survival estimates, it is hard to know if reduced fecundity rates drive population declines or only contribute to them. Nonetheless, it is clear that an important link to population declines is reduced fecundity rates – and this dissertation
provides a compelling mechanistic pathway to explain shifts in life history traits that result in reduced fecundity rates of *C. collaris* in the Ozarks.
MEMORANDUM

TO: Steven Beaufre

FROM: Craig N. Coon, Chairman
       Institutional Animal Care
       And Use Committee

DATE: March 15, 2013

SUBJECT: IACUC Protocol APPROVAL
         Expiration date: March 15, 2016

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #13036 - “Conservation of eastern collared lizard (Crotaphytus collaris) populations in Arkansas”. You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 03-01-2016, you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian
MEMORANDUM

TO: Steven Beaupre
FROM: Craig N. Coon, Chairman
DATE: Apr 8, 2016
SUBJECT: IACUC Approval
Expiration Date: Apr 7, 2019

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # 16057 "Conservation of Eastern Collared Lizard (Crotaphytus collaris) populations in Arkansas"

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond Apr 7, 2019 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian
To: Steven Beaupre
Fr: Craig Coon
Date: May 8th, 2019
Subject: IACUC Approval
Expiration Date: May 2nd, 2022

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # 19082: Conservation of Eastern Collared Lizard (Crotaphytus collaris) populations in Arkansas.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond May 2nd, 2022 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Casey Brewster and Steven Beaupre. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp