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Here be dragons: Functional analyses of thermal adaptation and biogeography of reptiles in a changing world

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Here be dragons: Functional analyses of thermal adaptation and biogeography of reptiles in a changing world

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This dissertation is dedicated to my parents, Christopher and Deborah Telemeco, who have always encouraged my love and curiosity for the natural world.

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ABSTRACT

Environments around the world are changing rapidly and a major challenge for modern biologists is to understand how these changes affect organisms, communities, and ecosystems. Ideally, we would like to predict which taxa/populations are likely to remain stable, increase, or decline in response to predicted environmental perturbations. This information will allow us to create informed management plans and will provide insight into the ecological and evolutionary processes that shape biodiversity. For my Ph.D. dissertation, I examined factors that mediate the responses of reptile populations to rapid changes in the thermal environment, explored the ability of these factors to shift through phenotypic plasticity and evolution, and examined the power of a phenotypically plastic behavioral response to buffer populations from thermal environmental change.

In Chapters 2–4, I explored the evolutionary history and physiology of alligator lizards (*Elgaria coerulea*, *E. multicarinata*, and *E. panamintina*, family Anguidae) to identify mechanisms that mediate their responses to changing thermal environments. First, I integrated morphological data and species distribution modeling with prior molecular data to examine alligator lizard taxonomy. My results support the species status of *E. panamintina* and the existence of two cryptic taxa within *E. multicarinata*. Next, I examined the thermal physiology of confirmed alligator lizard taxa and explored the biogeographical implications of their thermal physiology. Adult alligator lizards are active at virtually identical body temperatures even though species occur in very different thermal environments, suggesting average differences in environmental temperature are not limiting. To examine whether extreme temperatures might be more limiting, I examined the effects of extreme temperatures

on the physiological stress response of alligator lizards. My results suggest that the thermal-stress response in alligator lizards is species specific and might be important for limiting alligator lizard biogeography. However, adults might not be the most thermally sensitive life-history stage. Thus, I also examined the effects of temperature during alligator lizard development and compared embryonic and adult thermal physiologies. My results suggest that the thermal physiology of alligator lizards changes across their ontogeny and embryonic thermal tolerances are more limiting than adult thermal tolerances. Together, my results suggest that relatively extreme thermal environments and the developmental thermal environment will have the greatest influence on how alligator lizards respond to changes in the thermal environment.

As environments change, many species may be able to respond adaptively through phenotypic plasticity, thereby countering any negative consequences of shifting environments. For example, the most common biotic response to ongoing global climate change is a plastic shift in spring phenology. While altered spring phenology is viewed as an adaptive response to changing thermal environments, this issue has not been examined directly. To test this hypothesis, in Chapter 6, I constructed a mechanistic model examining the power of shifting spring phenology to buffer populations from climate change, and examined this model using data on painted turtles (*Chrysemys pica*, family Emydidae), a species with temperature-dependent sex determination. Somewhat surprisingly, the model suggested that advancing phenology is a poor buffering mechanism, and only effectively counters the negative consequences of < 1.0 °C increase in environmental temperature.

In combination, my dissertation explores the diverse processes that mediate responses of reptiles to rapid changes in thermal environments such as those predicted to occur as a

result of global climate change. This information is necessary to better understand effects of major environmental changes on the ecology and evolution of species as well as for making accurate predictions for conservation/management.

CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

Environments around the world are changing rapidly. Numerous factors, many anthropogenic, are responsible for these changes including global climate change, habitat alteration/destruction, and the introduction of invasive species (e.g., Vitousek et al. 1997, Walther et al. 2009, Bloom 2010, Pyšek and Richardson 2010, IPCC 2013). To date, 40-50% of Earth's land surface is estimated to have been fundamentally altered by human activity (Vitousek et al. 1997, Sterling and Ducharne 2008). In fact, global environments have been altered so dramatically since the industrial revolution that many geologists argue that this period represents a new geological epoch, the Anthropocene, characterized by human alteration of the planet (Crutzen 2002, Zalasiewicz et al. 2010, Steffen et al. 2011, Zalasiewicz et al. 2011).

A major challenge for modern biologists is to understand how rapid environmental changes affect organisms, communities, and ecosystems. Most simplistically, populations can respond in one of three ways. If changes are tolerable, populations might remain stable. Alternatively, environmental changes might be beneficial, potentially releasing species from prior ecological constraints. Population sizes will increase if environmental changes increase carrying capacities or if additional regions become suitable for colonization. The successful introduction of invasive species is a prime example of how novel environments might be beneficial for some species (Morris and Heidinga 1997, Mika et al. 2008, Pyšek and Richardson 2010). Finally, environmental changes could induce population declines and

extinction. This last scenario is most worrisome, but is likely common. Even if some species benefit from changes to the environment, increases in their numbers will induce decreases in other species through competition or predation (Morris and Heidinga 1997). This process is predicted to result in biotic homogenization with relatively few "winners" dominating diverse landscapes (McKinney and Lockwood 1999, Baiser et al. 2012). Even if some species are able to track environmental changes through migration and dispersal, unless entire ecosystems move in concert, as species invade new regions they will induce novel community dynamics that may frequently induce local extinctions (Brooker et al. 2007, Gilman et al. 2010). Large numbers of taxa are already declining markedly as a result of various anthropogenic forcings; so much so that we might be in the midst of Earth's sixth great extinction event (McKinney and Lockwood 1999, Wake and Vredenburg 2008, Barnosky et al. 2011).

Multiple mechanisms interact to mediate population responses to environmental change (outlined in Fig 1). Most proximally, the physiological and behavioral tolerances of individuals will determine how populations respond to varying environments. In particular, these tolerances will set species' fundamental niches (Hutchinson 1957, Jackson et al. 2009, Kearney and Porter 2009, Wiens et al. 2009). Populations will remain stable if conditions stay within physiological tolerances, grow if conditions move closer to physiological optima, or decline if environments shift beyond individual tolerances. Importantly, physiological tolerances are unlikely to remain static, but will instead change through evolution or phenotypic plasticity (Parmesan 2006, Visser 2008, Fuller et al. 2010, Piersma and van Gils 2011, Urban et al. 2014). Phenotypic changes may be adaptive, allowing organisms to stably persist in novel environments, or maladaptive. Maladaptive phenotypes might evolve as a

result of genetic drift in small populations, or because of genetic constraints such as pleiotropy and linkage (Lande 1976, Crespi 2000). Plastic responses may be maladaptive when environmental cues become less informative, such as in environments that have not been previously experienced (Langerhans and DeWitt 2002, Ghalambor et al. 2007, Urban et al. 2014). Evolutionary changes and phenotypic plasticity will also interact in ways that are difficult to predict. For example, plasticity might drive evolutionary changes (i.e., behavioral drive/genetic assimilation, West-Eberhard 2003, Ghalambor et al. 2007, Aubret and Shine 2009, Lande 2009), stifle evolution (i.e., the Bogert effect, Huey et al. 2003, Ghalambor et al. 2007), or evolve itself (Garland and Kelly 2006, Lande 2009).

In response to changing environments, communities and ecosystems will also change as their constituent populations are altered (Fig 1). For example as populations of a focal species change, the competitive landscape, food availability, and/or predator density experienced by neighboring species will be altered, thereby inducing cascading changes throughout the community (Visser and Both 2005, Parmesan 2006, Pyšek and Richardson 2010, Walther 2010). Shifts in the community/ecosystem will, in turn, feedback on the focal population. These biotic interactions will set the species' realized niches (Hutchinson 1957). These community dynamics may eventually reach equilibrium, but equilibrium will be elusive as long as environments are in substantial flux.

Ideally, we would like to predict which taxa/populations are likely to remain stable, increase, or decline in response to predicted environmental perturbations. This information will allow us to create informed management plans and provide insight into the ecological and evolutionary processes that shape biodiversity. A common method for predicting how organisms will be affected by exposure to altered environments is correlative bioclimatic

envelope modeling (a.k.a. ecological niche modelling or species distribution modelling, Elith et al. 2006, Hijmans and Graham 2006, Wiens et al. 2009, Mbogga et al. 2010). Briefly, these models take known point locations for species (usually presence-only points) and statistically correlate these with measurements of environmental conditions (usually abiotic factors such as average precipitation and temperature, Wiens et al. 2009, Elith et al. 2011). Environmental conditions where individuals have been observed are assumed to be suitable whereas all other conditions are assumed to be unsuitable for species persistence (Wiens et al. 2009, Elith et al. 2011). The models produce maps displaying suitable and unsuitable regions, and thus show where species are predicted to occur (i.e., their bioclimatic envelope). Predictions for how environmental changes will affect species are made by projecting the predicted bioclimatic envelope into the future after accounting for predicted changes in environmental conditions (Hijmans and Graham 2006, Wiens et al. 2009, Araújo and Peterson 2012). While these models have been used extensively (e.g., Araújo et al. 2006, Mika et al. 2008, Lawler et al. 2009, Milanovich et al. 2010), their utility has been called into question (Davis et al. 1998, Pearson and Dawson 2003, Duncan et al. 2009, Lozier et al. 2009, Wiens et al. 2009). Correlative envelope models often have poor predictive power because their assumptions are routinely violated (Lozier et al. 2009, Wiens et al. 2009, Araújo and Peterson 2012). Perhaps most egregious, these models generally conflate the fundamental and realized niches (Davis et al. 1998, Pearson and Dawson 2003). Observed point locations describe the realized niche whereas the abiotic variables that are correlated with these point locations define the fundamental niche. If species are constrained by biotic interactions, which most species are, then these models will under-predict potential distributions (Davis et al. 1998, Wiens et al. 2009). The models will also under-predict

suitable ranges if point locations represent a biased sample, if tolerable conditions do not currently exist, or if taxa have not yet reached their equilibrium distribution (Pearson and Dawson 2003, Wiens et al. 2009, Araújo and Peterson 2012). Finally, these correlative models treat taxa as fixed units with uniform physiological and behavioral tolerances that are unable to adjust through either adaptive evolution or phenotypic plasticity (Pearson and Dawson 2003, Pearman et al. 2007, Wiens et al. 2009).

Mechanistic models that explicitly account for organismal biology are needed to predict accurately how rapid environmental changes will affect species (Pearson and Dawson 2003, Kearney and Porter 2009, Buckley et al. 2010). However, such models require substantial information describing the fundamental and/or realized niche, and sufficient data are unavailable for most species (Pearson and Dawson 2003, Pearman et al. 2007, Kearney and Porter 2009). Moreover, we do not know which factors are most important for delineating the fundamental and realized niches for most taxa. Thus, the most important factors to consider when constructing mechanistic models are generally uncertain. For example, we typically do not know whether average environmental conditions or environmental variability are more important for determining species distributions. In addition, we do not know which life-history stages are most limiting. If physiological and behavioral tolerances vary ontogenetically, a subset of life-history stages might determine the environments under which populations can persist. However, this idea has rarely been explored (but for examples in insects see, Zani et al. 2005, Chown and Terblanche 2007, Bowler and Terblanche 2008, Briscoe et al. 2012, Radchuk et al. 2013). Finally, we generally do not know which phenotypic traits best predict whether or not species can persist in various environments. For example, will running performance, foraging rate, stress

capacity, nest site choice/availability, or other traits be most important for determining population persistence? Given the challenges of describing fundamental and realized niches, it will not be possible to develop full mechanistic models for all taxa threatened by rapid environmental change. However, the factors that most limit where species can and cannot persist are likely conserved among related taxa and may be broadly generalizable (Wiens and Graham 2005, Wiens et al. 2009, Pearman et al. 2010). If so, information on these limiting factors will allow predictions for how broad taxonomic groups are likely to be affected by rapid environmental changes.

DISSERTATION ORGANIZATION

For my Ph.D. dissertation, I examined factors that mediate responses of reptile populations to rapid changes in the thermal environment, explored the ability of these factors to shift through phenotypic plasticity and evolution, and examined the power of a phenotypically plastic behavioral response to buffer populations from thermal environmental change. I examined effects of thermal environments on reptiles for multiple reasons. First, thermal environments are predicted to shift rapidly over the coming century as a result of global climate change (IPCC 2013). Current models predict average increases of 4.5–8.5 °C globally (Moss et al. 2008, IPCC 2013). In addition, other environmental perturbations, such as habitat alteration or the introduction of invasive competitors, may affect the available thermal landscape (Angilletta 2009). Thus, virtually all major environmental changes affect thermal environments. In most taxa, temperature defines a primary axis of the fundamental niche, and this is especially true for ectothermic organisms such as reptiles (Cowles and Bogert 1944, Hutchinson 1957, Angilletta 2009, Jackson et al. 2009). Temperature affects

most aspects of reptile biology including metabolic rate, foraging, energy assimilation, development, courtship, running/swimming performance, and, in some species, offspring sex (Cowles and Bogert 1944, Huey and Stevenson 1979, Huey 1982, Janzen and Paukstis 1991, Telemeco et al. 2010). Because of this tight link between thermal environments and biology, reptiles are hypothesized to be at high risk of climate-change induced decline, and are one of the most threatened groups of vertebrate animals, globally (Janzen 1994, Sinervo et al. 2010, Böhm et al. 2013). It is therefore imperative to understand factors that functionally mediate responses of reptile populations to changes in thermal environments. Moreover, because a single factor, temperature, has such dramatic biological effects, reptiles provide a relatively simple system for exploring the effects of environmental changes on populations of vertebrate animals.

In Chapters 2–4, I explored the evolutionary history and physiology of alligator lizards (*Elgaria coerulea, E. multicarinata,* and *E. panamintina,* family Anguidae, Fig 2) to identify mechanisms that mediate their responses to changing thermal environments. As taxa diverge, their ecological niche and physiology may also diverge. Therefore, before one can accurately predict how environmental perturbations will affect taxa, relevant operational taxonomic units (OTUs), such as species or subspecies, must be identified. In Chapter 2, I integrated morphological data and species distribution modeling with prior molecular data to examine the taxonomy of the *E. multicarinata–E. panamintina* species complex and delineate species/ OTUs. My results support the species status of *E. panamintina* and the existence of two additional cryptic taxa within *E. multicarinata*.

In Chapters 3 and 4, I examined the thermal physiology of confirmed alligator lizard taxa and explored the biogeographical implications of their thermal physiology. First, I

assessed the relative importance of extreme versus average thermal conditions on adult *E. multicarinata* and *E. coerulea*. These lizards are active at virtually identical body temperatures even though *E. coerulea* occurs in colder environments than *E. multicarinata* (Cunningham 1966, Stewart 1984, Kingsbury 1994, Sheen 2001, Beck 2009a, b), suggesting average differences in thermal environments are not limiting for adult alligator lizards. To examine whether extreme temperatures might be more limiting than average temperatures, I collaborated with Dr. Elizabeth Addis to examine the effects of extreme temperatures on the physiological stress response of adult *E. multicarinata* and *E. coerulea* (Chapter 3). My results suggest that the thermal-stress response in alligator lizards is species specific and might be important for limiting alligator lizard biogeography. However, this might not be the entire story. All previous work has focused on effects of temperature on adult alligator lizards. Yet, developing embryos might be more thermally sensitive than adults (Andrews and Schwarzkopf 2012, Briscoe et al. 2012, Miller et al. 2013, Radchuk et al. 2013). Therefore, in Chapter 4, I examined the effects of temperature during development on *E. multicarinata* and compared embryonic and adult thermal physiologies. My results suggest that the thermal physiology of alligator lizards changes across their ontogeny and embryonic thermal tolerances are more limiting than adult thermal tolerances. In addition, as an appendix to Chapter 4, I tested for temperature-dependent sex determination in *E. multicarinata*, but found no evidence for this sex-determining mechanism.

The most common biotic response to ongoing global climate change is a plastic shift in spring phenology (i.e., the onset of reproductive events, Visser and Both 2005, Parmesan 2006, Moser et al. 2009). While altered spring phenology is viewed as an adaptive response to changing thermal environments (Visser and Both 2005, Schwanz and Janzen 2008,

Telemeco et al. 2009), this issue has not been examined directly. To test this hypothesis, I collaborated with Drs. Karen Abbott and Fredric Janzen to construct a mechanistic model examining the power of shifting spring phenology to buffer populations from climate change (Chapter 5 and its appendices). Because alligator lizards and other anguid lizards are relatively poorly studied, sufficient data for accurate mechanistic modeling is unavailable. By contrast, painted turtles (*Chrysemys picta*, Fig 3) are well studied in both the field and laboratory. Moreover, *C. picta* display temperature-dependent sex determination (Janzen and Paukstis 1991), thus their basic demography is tightly linked to the thermal environment (Schwanz et al. 2010). We therefore estimated model parameters using data from a *C. picta* population that has been studied extensively for over 25 years. Our model suggests that nesting earlier in the year is a poor buffering mechanism, and only effectively counters the negative consequences of < 1.0 °C increase in environmental temperature on population demography.

In combination, my dissertation explores diverse processes that mediate responses of reptiles to rapid changes in thermal environments such as those predicted to occur as a result of global climate change. This information is necessary to better understand effects of major environmental changes on the ecology and evolution of species as well as for making accurate predictions for conservation/management.

FIGURES

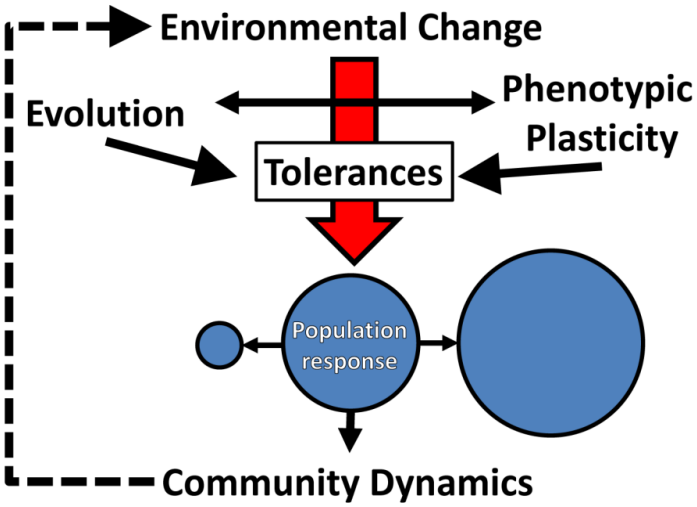

Figure 1—Schematic representation of the factors that mediate the effects of environmental change on populations. See the text for an explanation of the depicted interactions. Arrows represent the direction of effects. The size of the blue circles represents population size in numbers and/or space.

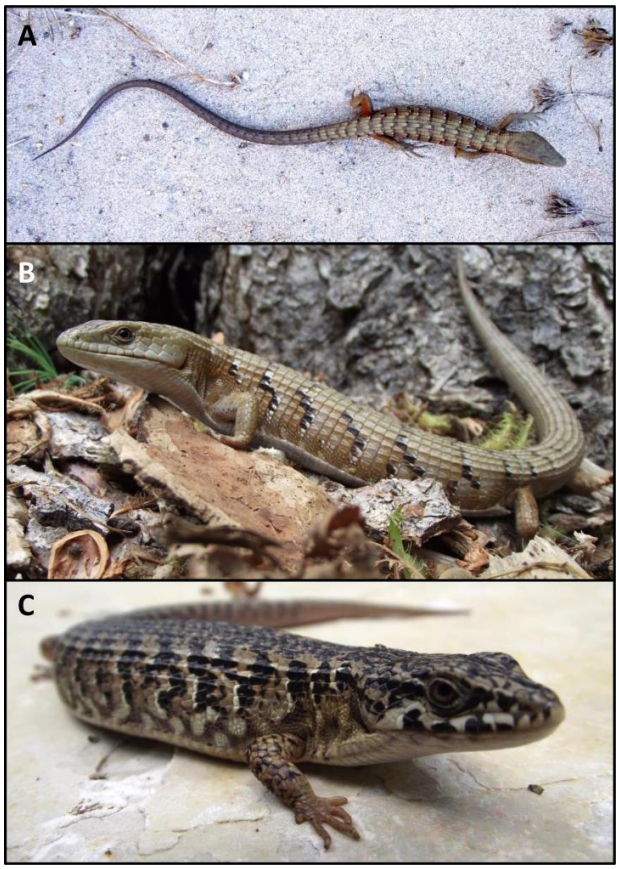

Figure 2—Photographs of southern alligator lizards (*Elgaria multicarinata*, A and B), and a northern alligator lizard (*Elgaria coerulea*, C). Alligator lizards were used as model systems for Chapters 2–4.

Figure 3—Nesting painted turtle (*Chrysemys picta*). Data from this species were used to estimate parameters for the mechanistic model presented in Chapter 5.

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CHAPTER 2

AN INTEGRATIVE TAXONOMIC ANALYSIS OF THE SOUTHERN AND PANAMINT ALLIGATOR LIZARD COMPLEX: COMBINING MORPHOLOGICAL, ECOLOGICAL, AND MOLECULAR EVIDENCE

A paper intended for publication in a scientific journal

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ABSTRACT

Describing standing biodiversity and its evolutionary origins are major goals of modern biology. While molecular genetic tools provide immense power to explore phylogenetic relationships among organisms, these tools are not without limitations. By employing an integrative approach that combines multiple datasets, we can better resolve the phylogenetic history of organisms, delineate species boundaries, and gain greater understanding of how lineages have ecologically diverged. I used such an integrative approach to test predictions from competing phylogenetic hypotheses for southern and Panamint alligator lizards (*Elgaria multicarinata* and *E. panamintina*, respectively). Recent mitochondrial DNA evidence contradicts the traditional taxonomy of these lizards, calling the species status of *E. panamintina* into doubt, and suggesting that *E. multicarinata* might be composed of 2–4 cryptic species. First, I examined male genital morphology to examine

potential reproductive isolation among putative clades. Next, I examined head morphology to assess potential ecological divergence among the clades. Finally, I further explored ecological divergence using species distribution modeling. My results support components of both the traditional and mitochondrial DNA phylogenies. All of my data support the species status of *E. panamintina*, and I suggest that the aberrant mtDNA phylogeny results from incomplete lineage sorting after peripatric speciation. My morphological data and species distribution modeling confirm the existence of two divergent cryptic clades within *E. multicarinata* (a Northern and Southern clade), as predicted by the mtDNA phylogeny. While these clades differ morphologically and ecologically, the extent to which they are reproductively isolated is unclear. These clades might represent incipient species or be reticulating into a single taxon. Even so, given their differences, I recommend considering these as separate operational taxonomic units for management purposes. By integrating morphometric analyses and species distribution modeling with prior molecular data, I obtained phylogenetic inferences that were impossible with any available dataset in isolation.

Keywords: *Elgaria multicarinata, Elgaria panamintina*, hemipene, geometric morphometrics, species distribution model

INTRODUCTION

Describing biological diversity and its evolutionary origins are major goals of modern biology. An accurate understanding of biodiversity is also necessary for informed conservation and management. The need to describe standing biodiversity is becoming ever more important as rapid changes to the world's environments are inducing marked reductions

in biodiversity around the globe (Pimm et al. 1995, Sala and Knowlton 2006, Rands et al. 2010, Böhm et al. 2013). While numerous models attempting to predict the effects of novel environments on species have been constructed (e.g., Buckley 2008, Mika et al. 2008, Carroll et al. 2010, Milanovich et al. 2010), the predictive power of these models is frequently poor (Davis et al. 1998, Pearson and Dawson 2003, Hijmans and Graham 2006, Duncan et al. 2009). One reason for poor predictive power is that these models frequently fail to account for local adaptation among populations or the presence of cryptic species (Wiens et al. 2009, Atkins and Travis 2010, Pearman et al. 2010). An accurate understanding of biodiversity and local adaptation among species is an important first step toward predicting how taxa will be affected by a changing world.

Molecular genetic techniques provide immense power to resolve the phylogenetic history of organisms; however, there are limitations to the inferences that can be drawn from such data. For example, phylogenies derived from few loci represent the evolutionary history of those loci, but not necessarily species (Pamilo and Nei 1988, Page and Charleston 1997, Rosenberg and Nordborg 2002). Differences between gene- and species-trees can result from processes such as incomplete lineage sorting and introgressive hybridization (Rosenberg and Nordborg 2002, Funk and Omland 2003, Leaché and McGuire 2006, McGuire et al. 2007). While the ability to examine extremely large numbers of loci simultaneously through next-generation sequencing can alleviate gene- and species-tree discordance (Emerson et al. 2010, Burleigh et al. 2011, Boussau et al. 2013), large-scale next-generation sequencing presents numerous bioinformatic challenges and such studies are still prohibitively expensive for many applications. Moreover, next-generation sequencing cannot alleviate all of the limitations of molecular data. For example, it is difficult to

delineate biological species boundaries using genetic data alone because these data provide little information about reproductive isolation (Coyne and Orr 2004, Sites and Marshall 2004, Frankham et al. 2012).

Because an organism's genotype and phenotype share a paired evolutionary history, phenotypic data can be used to augment our understanding of molecular phylogenetic relationships (Dayrat 2005, Padial et al. 2010, Schlick-Steiner et al. 2010). Analyses of the phenotype are particularly useful when molecular data are inconclusive (Bailey et al. 2010, Schlick-Steiner et al. 2010, Gauthier et al. 2012, Losos et al. 2012). For example, by examining phenotypic traits that lead to pre-zygotic reproductive isolation, we can delineate boundaries between hypothesized species/evolutionarily independent units (Böhme and Ziegler 2009, Bailey et al. 2010, Padial et al. 2010, Nunes et al. 2012). Analyses of ecologically important phenotypic traits can also provide information about local adaptation and differentiation among lineages (Will and Rubinoff 2004, Dayrat 2005). Thus, integrating phenotypic and molecular approaches should provide the best description of biodiversity and evolutionary history (Sites and Marshall 2004, Dayrat 2005, Padial et al. 2010, Schlick-Steiner et al. 2010).

Southern alligator lizards (*Elgaria multicarinata*, Blainville 1835) and Panamint alligator lizards (*E. panamintina*, Stebbins 1958) are anguid lizards native to the Pacific coast of North America (Stebbins 2003, Beck 2009, Mahrdt and Beaman 2009). Currently, *E. multicarinata* and *E. panamintina* are recognized as sister species that diverged ~1.5 MYA as a result of Pleistocene drying in western North America (Macey et al. 1999). However, a recent mitochondrial DNA (mtDNA) analysis contradicts this phylogenetic hypothesis (Feldman and Spicer 2006). *Elgaria multicarinata* (as currently recognized) are relatively

common, widespread lizards that occur from Baja California to Washington State (Stebbins 2003, Beck 2009). Three morphological subspecies are traditionally recognized, suggesting a high level of intraspecific diversity (Stebbins 2003, Beck 2009). By contrast, *E. panamintina* are uncommon and have a highly restricted geographic range, only occurring in isolated riparian/spring habitats in the Panamint Mountains of eastern California (Jennings and Hayes 1994, Stebbins 2003, Mahrdt and Beaman 2007). Few *E. panamintina* have been documented (25 museum specimens and 14 confirmed sightings, Mahrdt and Beaman 2009). *Elgaria panamintina* are classified as "vulnerable" by the IUCN and have been granted protected classifications from multiple government bodies (e.g., USFWS, USBLM, CA, Jennings and Hayes 1994, Mahrdt and Beaman 2007). *Elgaria panamintina* are easy to distinguish from *E. multicarinata* based on coloration and scalation, with *E. panamintina* having more defined banding and smoother dorsal scales than *E. multicarinata* (Stebbins 1958, 2003, Mahrdt and Beaman 2009). Hybridization between *E. multicarinata* and *E. panamintina* has not been documented; however, these species occur within 5km of each other (Mahrdt and Beaman 2009) and too little is known to rule out this possibility.

Feldman and Spicer (2006) examined mtDNA (900bp) from 45 *E. multicarinata* and 4 *E. panamintina* individuals, and their results largely contradict prior understanding of the phylogenetic history of these lizards. First, the mtDNA data implied that *E. multicarinata* is composed of four subclades, none of which corresponds to previously described subspecies. Feldman and Spicer (2006) named these the Northern California (NC), Southern Sierra Nevada (SSN), Coastal (C), and Southern California (SC) subclades based on their geographic locations (Fig 1). The NC and SSN subclades formed a larger "Northern" clade separate from the C and SC "Southern" clade (Fig 1). The Northern and Southern clades

apparently diverged from one another over 4 MYA and are so divergent that Feldman and Spicer (2006) suggested they might not be each other's closest relatives. Moreover, the mtDNA data did not support the species status of *E. panamintina*. The *E. panamintina* specimens formed a well-supported subclade nested within the Southern *E. multicarinata* clade and sister to the SC subclade (Feldman and Spicer 2006). The mtDNA data, therefore, suggested that *E. multicarinata* is composed of 2–4 cryptic species, one of which subsumes *E. panamintina* (Feldman and Spicer 2006).

Additional data from independent characters are needed to reconcile these conflicting phylogenetic hypotheses (Bailey et al. 2010, Padial et al. 2010, Schlick-Steiner et al. 2010). I examined the morphology and ecology of the clades proposed by Feldman and Spicer (2006) to explicitly test their phylogenetic hypothesis and develop a more integrative taxonomy for these alligator lizards. I first examined variation in hemipene shape (the paired intromittent organs of squamates). Phylogenies built using morphological variation in hemipenes correspond well with molecular phylogenies in the Anguidae and other diverse squamate families (Böhme and Ziegler 2009). Copulatory organs, such as hemipenes, should be phylogenetically informative for multiple reasons (Dowling and Savage 1960, Keogh 1999, Bailey et al. 2010, Padial et al. 2010). Because copulatory organ morphology is critical for successful reproduction, the shape of these structures should be under stabilizing selection within species (Dowling 1967, Eberhard et al. 1998, Keogh 1999, Hosken and Stockley 2004, Bailey et al. 2010). However, copulatory organs generally only function for copulation, and thus may be free to evolve divergent phenotypes between species. This is especially true for organs that are "ecologically hidden" such as hemipenes, which are stored within the tail base when not in use (Dowling and Savage 1960, Keogh 1999). Divergent

evolution of copulatory organs may occur through neutral drift, natural selection, or sexual selection (Coyne and Orr 2004, Hosken and Stockley 2004, Eberhard 2010, Masly 2012). When populations are sufficiently divergent that hybrid fitness is reduced, traits that act as pre-zygotic hybridization barriers are predicted to be under strong natural selection for reinforcement (Coyne and Orr 1998, Kirkpatrick and Ravigné 2002, Servedio and Noor 2003). Hemipenes and their corresponding female structures might be prime targets for such selection because their shapes strongly affect copulation without affecting other aspects of fitness (Dowling 1967, Keogh 1999, Böhme and Ziegler 2009). Hemipene shape might act to reduce hybridization through a mechanical "lock-and-key" mechanism (Dufour 1844, Sota and Kubota 1998, Masly 2012) or because tactile differences stimulate heterospecific rejection (Eberhard 1992, Coyne and Orr 2004, Masly 2012). Alternatively, hemipene shapes might deviate through divergent sexual selection in sister taxa, subsequently leading to reproductive isolation (Arnqvist 1998, Coyne and Orr 2004, Eberhard 2010, Masly 2012). Either way, the predicted outcome (documented by Böhme and Ziegler 2009) is little-to-no intraspecific variation in hemipenial morphology contrasting relatively great interspecific variation. Variation in hemipenial morphology between the clades proposed by Feldman and Spicer (2006) would therefore suggest that these taxa are reproductively isolated species.

I also examined the head morphology of individuals from each proposed clade. Unlike hemipene morphology, head morphology is intimately connected to the ecology of lizards and other vertebrate animals, affecting how they interact with predators, prey, and conspecifics (e.g., Adams and Rohlf 2000, Adams 2004, Kaliontzopoulou et al. 2012). Therefore, variation in head morphology among the alligator lizard clades would suggest ecological divergence. I further examined ecological divergence among the major *E.*

multicarinata clades using species distribution modeling. If the clades have diverged to occupy different niches, I predict that models considering each clade independently will better predict the species distribution than models that assume all *E. multicarinata* belong to a single clade with uniform tolerances (Pearman et al. 2010). Together, my analyses of hemipene and head morphology, along with my species distribution modeling, allow me to robustly test predictions of Feldman and Spicer's (2006) mtDNA phylogeny, and assess the degree of local adaptation that has occurred among the clades of *E. multicarinata* and *E. panamintina* alligator lizards.

MATERIALS AND METHODS

Morphometric Analyses

Collection of Specimens

I acquired specimens for the present study through field collection and from three museums (California Academy of Science [CAS], Museum of Vertebrate Zoology at the University of California, Berkeley [MVZ], and the Natural History Museum of Los Angeles County [LACM]). As part of a larger study of *E. multicarinata* ecology, I collected 51 adult lizards in California during May–July of 2010, 2011, and 2012 (RST specimens hereafter). I borrowed all other specimens (*E. multicarinata*: N = 238, *E. panamintina*: N = 18) from museum collections. A list of the specimens examined, including capture location and date, is given in Appendix A.

Prior to requesting museum specimens, I downloaded information on the capture location and date of every *E. multicarinata* and *E. panamintina* specimen in the CAS, MVZ, and LACM collections. Using DivaGIS software (version 7.5, Hijmans et al. 2012), I

selected 100 individuals (hoping to obtain ~50 males) from each mtDNA subclade (when available) as evenly distributed as possible throughout the hypothesized geographic range of each clade (from Feldman and Spicer 2006). When possible, I selected individuals that were analyzed by Feldman and Spicer (2006) and thus belonged to a known mtDNA clade (N = 37). I assigned all other individuals to a probable clade based on their geographic location of collection. *Elgaria multicarinata* are abundant in museum collections (> 3800 specimens in these three museums alone), so I limited my search to specimens collected since 1975. I requested all *E. panamintina* from each museum.

For each specimen, I assessed sex and snout-vent length (SVL), photographed the head, and collected a hemipene (from males). As part of my other studies, I euthanized all field collected *E. multicarinata* by decapitation and identified sex by examining the gonads during necropsy. I measured SVL in live animals immediately prior to euthanasia. I identified the sex of museum specimens by examining gonads through a small incision in the abdomen. Because many museum specimens were preserved in curved body positions, I measured SVL using a flexible sewer's measuring tape. I collected dorsal and lateral (left side) photographs of each specimen's head using a digital camera (Canon EOS Rebel T3i) mounted to a copy stand. In each photograph, I took care to minimize parallax distortion. For scale, I placed a thin ruler under each specimen. Because decapitation affects the shape of the posterior head, I photographed RST specimens live. I only collected photographs of the dorsal head surface from live animals because they would not remain in the necessary position for lateral photographs. For some museum specimens, I obtained only a dorsal or lateral photograph due to head damage affecting only one surface.

For all analyses, I only included individuals for which I could identify sex and SVL was > 8.5cm (N = 308, 28 of which were examined by Feldman and Spicer [2006], Appendix A). In total, I examined head and/or hemipene morphology of 89 C (female: N = 33, male: N = 56), 73 SC (female: N = 29, male: N = 44), 104 NC (female: N = 46, male: N = 58), and 22 SSN (female: N = 9, male: N = 13) *E. multicarinata* specimens, and 18 *E. panamintina* specimens (female: N = 6, male: N = 12, the 7 remaining known specimens were either unavailable or juveniles). The geographic distribution of these specimens is displayed in Fig 1.

Hemipene Preparation

For hemipene structures, I use the terminology of Dowling and Savage (1960), Keogh (1999), and Böhme and Ziegler (2009). I sampled the left hemipenis from male specimens (after euthanasia in field-collected specimens) using the methods of Myers and Cadle (2003) and Zaher and Prudente (2003). Briefly, I made a 1.5–2 cm lateral incision along the left ventral portion of the tail base to expose the hemipenis. I then cut the retractor muscle and the base of the hemipenis to allow removal of the organ. After excision, I soaked each hemipenis in a 3% KOH solution to soften the tissue prior to eversion (Pesantes 1994). I soaked formalin-fixed specimens in 3% KOH for ~3hrs, whereas specimens that were only preserved in ethanol (RST specimens) required ~10 min soaking. Once supple, I everted each hemipenis by gently rolling the base with forceps and pushing the remainder of the organ through the base. After eversion, I filled each hemipenis with petroleum jelly colored with red candle dye to visualize structures. I then tied off the hemipenis base with cotton thread and stored each everted/filled hemipenis in 70% ethanol for later examination. Once all hemipenes were prepared, I photographed their sulcal and asulcal surfaces using a digital

camera (Nikon DS-Vi1) attached to a dissection microscope (Nikon SMZ 745T). I used the camera's pre-set scale bar for the appropriate zoom of the microscope.

Quantification of Shape

Hemipene Shape

All hemipenes had the same basic structures (see Results) allowing morphometric comparison of hemipene shape. However, because hemipenes are soft and flexible, defining homologous landmarks for geometric morphometric analysis is problematic (Zelditch et al. 2004). I therefore quantified hemipene shape using traditional linear measurements, which should be more robust with flexible structures. Even so, I also quantified landmark-based shape data to aid visualization of shape differences. I only considered the shape of the distal, ornamented portion of each hemipenis to control for variation in hemipene preparation and removal.

I used the program tpsDig (version 2.17, Rohlf 2013) to make linear measurements of the hemipenes and to digitize hemipene landmarks. Because it was easiest to visualize structures on the asulcal surface (particularly the hemipene crotch), I used asulcal photographs for my analyses. I used four linear measurements to describe hemipene shape (Fig 1A): 1) flounce width (FW)–width of the hemipenis where the flounces first appear at the base of the organ, 2) crotch width (CW)–width of the hemipenis at the crotch, 3) crotch-flounce length (C-FL)–distance along the hemipenis' axis of symmetry from the FW line to the CW line, and 4) lobe length (LL)–length of the hemipenis' right lobe along its axis of symmetry.

Using the same photographs, I digitized 19 hemipene landmarks (Fig 1B). Three of these were fixed landmarks, defining the onset of the flounces and the crotch. The remaining 16 landmarks were sliding, semi-landmarks defining the curved sides of the hemipenes. Each landmark was placed inside the flaps of the flounces, and thus described the primary shape of the hemipenes rather than the shape of the flounce ornamentation.

Head Shape

Because lizard heads are solid structures with homologous features, I compared head shapes solely using geometric morphometric analysis (Zelditch et al. 2004). I examined the shape of the dorsal and lateral head surfaces separately. I used 26 landmarks to describe dorsal head shape (Fig 1C). Sixteen of these were fixed landmarks that together define the midline, nostrils, eye crests, and anterior and posterior extremes of the masseter muscles. The 10 remaining landmarks were sliding, semi-landmarks used to define the shape of the masseter muscle curves.

I used 11 fixed landmarks to define the shape of the lateral head surface (Fig 1D). To control for variable mouth positions, I only used landmarks associated with the upper jaw and skull. Together, these landmarks described the mouth, lower and upper maximum of the ear opening, tip of the snout, eye, and upper extreme of the masseter muscle. All landmarks were digitized using tpsDig software (version 2.17, Rohlf 2013)

Statistical Analyses

All analyses were performed using the program, R (version 3.0.2, R Core Team 2013). Prior to analyses, I extracted shape variables from the landmark-based data using

generalized Procrustes superimposition with sliding semi-landmarks (Gower 1975, Rohlf and Slice 1990, Bookstein 1997a) with the *gpagen* function in the *geomorph* package (version 1.1-5, Adams and Otarola-Castillo 2013). This function superimposes landmark data by minimizing Procrustes distances between each object. Shape variables are then projected into Kendall's tangent space such that they can be used for multivariate analyses (Rohlf 1999). I used principal components analyses (PCA) to ordinate each dataset (hemipene linear measurements, superimposed dorsal- and lateral-head shape variables) and examined PC plots to identify outliers. No outliers were apparent in the hemipene or dorsal-head data, but three outliers were present in the lateral-head data and were removed from further analyses.

I tested for effects of clade (NC, SSN, C, SC, and *E. panamintina*) on hemipene shape using Euclidean distance-based non-parametric multivariate analysis of variance (NP-MANOVA, also known as permutational MANOVA; see Anderson [2001] for details). My linear hemipene measurements were the dependent variable in this analysis and SVL was a covariate. The interaction between SVL and clade was not significant ($P > 0.1$), so it was removed from the final model. Statistical significance was determined using 1000 permutations. I used the *adonis* function in the *vegan* package for this analysis (version 2.0-10, Oksanen et al. 2013). I examined pairwise differences between clades using Euclidean distance-based pairwise tests (10,000 permutations).

I used Procrustes ANOVAs (NP-MANOVA with landmark-based shapes as dependent variables) to examine the effects of clade on dorsal and lateral head shape. I included SVL as a covariate and sex as an additional factor in these analyses. Three-way interactions were never significant ($P > 0.10$) and were removed from all final models. I

performed Procrustes ANOVAs using the function *procD.lm* in the *geomorph* package. When Procrustes ANOVA was significant, I used the *pairwiseD.test* function in the *geomorph* package to perform Euclidean distance-based pairwise tests (1000 permutations). Because SVL and sex had interactive effects on head shape, I conducted pairwise tests on males and females separately.

I also examined the effects of clade on the centroid size of the dorsal and lateral head surfaces (derived from Procrustes superimposition of landmarks). I used Euclidean-distance based NP-ANOVA for these analyses (function *adonis* in the *vegan* package, 1000 permutations) with SVL as a covariate and sex as an additional factor. When clade was significant in NP-ANOVA, I employed pairwise tests (10,000 permutations) to further explore the data. I performed separate pairwise tests for each sex because of interactions between sex and SVL on centroid size.

For many models, I found significant effects of clade on shape and/or centroid size (see Results). However, because I largely defined clade based on geographic location of the sample, I wanted to test whether or not collection locality better explained the variation than my clade assignments. To do this, I constructed models with the latitude and longitude of specimen collection added as covariates for analyses that exhibited significant effects of clade. I examined the influences of latitude and longitude on the head shapes of each sex separately.

I began all analyses with full models and removed non-significant terms by backwards selection (see Zuur et al. [2009] for details). Because I was primarily interested in differences among clades, I never removed clade from the models. I used a conservative threshold *P*-value of 0.10 for including factors in the final models, but a traditional value of *P*

< 0.05 for interpreting results. When non-significant results are presented, I derived values from the simplest models containing those factors. For pairwise tests, I controlled for multiple comparisons by interpreting *P*-values after accounting for false discovery (Benjamini and Hochberg 1995, Garcia 2004) using the *p.adjust* function with *method = "fdr"* in R.

To visualize hemipene and head shape variation, I constructed thin plate spline deformation grids from the landmark data for each clade (Zelditch et al. 2004). These plots were created using the *plotRefToTarget* function in the *geomorph* package. For each plot, I used the grand mean shape from all specimens as the reference. Therefore, these plots represent the transformation of the grand mean shape into each target clade's mean shape.

Species Distribution Modeling

If *E. multicarinata* is actually composed of two or more cryptic species, these cryptic taxa may have evolved different tolerances as a result of local adaptation. To test this hypothesis, I constructed species distribution models (also known as ecological niche models or correlative bioclimatic envelope models) for *E. multicarinata* assuming that all individuals represent either a single species (with uniform tolerances) or two species with potentially divergent tolerances. These models were constructed using maximum entropy species distribution modeling (MaxEnt version 3.3.3k, Phillips et al. 2006, Phillips and Dudík 2008) and visualized using Diva-GIS software (version 7.5, Hijmans et al. 2012). MaxEnt was chosen because it consistently performs as well as, or better than, other presence-only species distribution modeling methods (Elith et al. 2006, Hernandez et al. 2006, Hijmans and Graham 2006, Scheldeman and van Zonneveld 2010, Elith et al. 2011).

I downloaded *E. multicarinata* presence points from the HerpNet2 data portal (www.herpnet.org). Prior to analyses, I removed points occurring well outside the known range of *E. multicarinata* or in nonsensical locations (e.g., the Pacific Ocean). In addition, I removed all points on islands for which mtDNA was not analyzed by Feldman and Spicer (2006). This process left $N = 3970$ point locations for analysis. Because the mtDNA evidence and my shape data (see Results) best support considering *E. multicarinata* to be two taxa (the "Northern" and "Southern" clades of Feldman and Spicer [2006]), I constructed species distribution models for the Northern and Southern *E. multicarinata* clades (Northern and Southern models, respectively) and compared those to the predicted distribution of *E. multicarinata* taken as a whole (single-taxon model). I assigned presence points to either the Northern ($N = 1034$) or Southern ($N = 2936$) clades based on their geographic locations (Fig 1C) compared to the hypothesized distribution of each clade from Feldman and Spicer (2006).

To construct species distribution models, I correlated the *E. multicarinata* presence data with the Bioclim variable dataset at 30 arc-second resolution (grid cells are approximately $1km^2$, Hijmans et al. 2005). The Bioclim dataset is composed of 19 bioclimatic variables representing annual trends, seasonality, and extremes in temperature and precipitation (www.worldclim.org). For model construction, all duplicate point locations for each clade within single grid cells were removed to control for sampling biases. In addition, I set aside 20% of the presence points for subsequent model testing. I examined the area under the receiver operating characteristic curve (AUC) of the test data from each model to assess the overall quality of the models. To quantify the relative importance of each bioclimatic variable, I examined the percent contribution of each variable to model

construction. Finally, I qualitatively compared the predicted distributions of each model to the known distribution of *E. multicarinata*. If the Northern and Southern clades have diverged ecologically, I predicted that the models of the Northern and Southern clades would better represent the known distribution of *E. multicarinata* than the single-taxon model.

RESULTS

Hemipene Morphometrics

All hemipenes had a similar gross morphology (see Fig 3 for representative specimens). Hemipenes were bilobed and slightly clavate. The sulcus spermaticus was weakly bifurcated, extending slightly into each apical lobe. As in most squamate reptiles (Dowling 1967, Böhme and Ziegler 2009, Nunes et al. 2014), the basal region was naked. More distally, the hemipenes displayed undifferentiated ornamentation comprised of scalloped, transverse flounces. Similar to most lizards (Nunes et al. 2014), we did not observe calcified hemipene ornamentation.

Although gross morphology was similar, both clade ($F_{4,128}$ = 4.38, P = 0.001, Table 1, Figs 4 and 5) and SVL ($F_{1,128}$ = 7.81, P = 0.006) influenced hemipene shape, with no interaction ($F_{4,124}$ = 0.58, P = 0.72). The effect of clade remained significant ($F_{4,126}$ = 3.40, P = 0.006) after adding latitude and longitude of collection as covariates ($F_{1,126}$ = 1.87, P = 0.171 and $F_{1,126}$ = 2.90, P = 0.07, respectively). The hemipenes of *E. panamintina* differed from those of three of the four *E. multicarinata* clades (NC: D = 1.856, P = 0.0009, SSN: D = 2.436, P = 0.0019, C: D = 1.343, P = 0.0186), and marginally differed from the fourth clade (SC: D = 1.058, P = 0.0729) (Fig 4). In general, *E. panamintina* hemipenes had reduced apical lobes compared to those of the *E. multicarinata* clades, and *E. panamintina*

hemipenes narrowed distally whereas *E. multicarinata* hemipenes broadened distally (Figs 3 and 5).

Within *E. multicarinata*, differences in hemipene shape among clades generally matched predictions of the mtDNA phylogeny (Feldman and Spicer 2006), with SC and C forming a group separate from an NC and SSN group. The hemipene shapes of SC differed from those of NC (D = 0.814, P = 0.0107) and SSN (D = 1.391, P = 0.0235), but not C (D = 0.316, P = 0.3666) (Fig 4). Similarly, the hemipene shape of C marginally differed from that of NC (D = 0.526, P = 0.0786) and SSN (D = 1.113, P = 0.0706) (Fig 4). NC and SSN did not differ from one another (D = 0.598, P = 0.3635) (Fig 4). PC plots suggest that the differences among clades may be driven largely by variation in hemipene size, as PC1, which commonly represents size in multivariate morphometric data (Jolicoeur and Mosimann 1960, Bookstein 1997b), best separates the *E. multicarinata* clades (Fig 4). Even so, hemipenes from NC and SSN had relatively narrower apical lobes and had a slightly more pronounced point where the apical lobes connect to the hemipene base than the C and SC hemipenes (Fig 5).

Head Morphometrics

Dorsal Head Shape

Clade ($F_{4,283}$ = 3.37, P = 0.001, Figs 6A, 6B, and 7A-7J), SVL ($F_{1,283}$ = 48.86, P = 0.001), and sex ($F_{1,283}$ = 9.05, P = 0.001) influenced dorsal head shape. The interaction between SVL and sex approached significance ($F_{1,283}$ = 2.18, P = 0.061), but all other interactions were not significant (P > 0.10 for all). When males and females were analyzed separately, clade and SVL significantly affected both sexes (male: $F_{4,158}$ = 2.39, P = 0.004,

and $F_{1,158}$ = 36.78, P = 0.001, respectively; female: $F_{4,112}$ = 1.92, P = 0.003, and $F_{1,112}$ = 12.54, P = 0.001, respectively), with no interactions (male: $F_{4,158}$ = 1.03, P = 0.387, and female: $F_{4,112}$ = 0.90, P = 0.611). In males, including latitude and longitude in the models reduced the impact of clade ($F_{1,161}$ = 1.48, P = 0.071). Both latitude and longitude affected dorsal head shape ($F_{1,161}$ = 2.66, P = 0.025, and $F_{1,161}$ = 5.44, P = 0.001, respectively). There were no interactions between clade, latitude, longitude, and SVL (P > 0.10 for all). In females, only longitude affected head shape (latitude: $F_{1,114}$ = 0.95, P = 0.327, longitude: $F_{1,114}$ = 2.37, P = 0.007), but this did not subsume the effect of clade on head shape ($F_{4,114}$ = 1.47, P = 0.022). Again, no substantive interactions were apparent (P > 0.10 for all).

Results from pairwise tests for dorsal head shape are given in Table 2, the location of each clade in PC space is presented in Figs 6A and 6B, and the shapes of each clade are illustrated in Figs 7A–7J. In general, males had broader heads than females, particularly in the rear portion of the head defined by the masseter muscles, and females had relatively longer snouts than males (Fig 7).

In males, dorsal head shape of *E. panamintina* differed from all *E. multicarinata* clades except SSN (NC: D = 0.034, P = 0.003, SSN: D = 0.025, P = 0.112, C: D = 0.035, P = 0.005, SC: D = 0.034, P = 0.007, Fig 6A). Male *E. panamintina* had relatively narrow and long heads, reminiscent of *E. multicarinata* females (Fig 7). For *E. multicarinata* males, differences among clades did not match predictions from Feldman and Spicer's (2006) mtDNA phylogeny and no distinct groupings were apparent. The only significant differences were between C and SSN (D = 0.026, P = 0.014), and C and SC (D = 0.018, P = 0.016, but not apparent from PC1 and PC2) (Fig 6A). In both cases, males from the C clade had broader heads with shorter snouts than those from the SSN or SC clades (Fig 7). In addition,

SSN marginally differed from both NC (D = 0.021, P = 0.063) and SC (D = 0.024, P = 0.034) (Fig 6A), with SSN males displaying a generally narrower and longer head than males in the other clades (Fig 7).

In females, few differences in dorsal head shapes were apparent (Figs 6B and 7). Female *E. panamintina* did not differ significantly from the *E. multicarinata* clades (NC: D = 0.027, P = 0.052, SSN: D = 0.032, P = 0.065, C: D = 0.026, P = 0.096, SC: D = 0.031, P = 0.028, Fig 6B). However, *E. panamintina* females marginally differed from NC, SSN, and SC females, with the former displaying very narrow heads with poorly defined masseter muscles compared to the other females (Fig 7). Similar to males, differences in dorsal head shape of females from each *E. multicarinata* clade did not match predictions from Feldman and Spicer's (2006) mtDNA phylogeny. The dorsal head shape of SC significantly differed from C (D = 0.026, P = 0.002) and SSN (D = 0.032, P = 0.003) (Fig 6B). In both cases, SC females had relatively broader heads, particularly at the eye crests (Fig 7). In addition, there were marginal differences between NC and SSN (D = 0.024, P = 0.030), NC and SC (D = 0.016, P = 0.028), and C and SSN (D = 0.026, P = 0.069), while NC and C did not differ (D = 0.244, P = 0.244).

Dorsal Head Size

In general, males had larger heads than females (Figs 8A and 8B). However, interactions were evident between SVL and sex ($F_{1,276}$ = 55.66, P = 0.001), and sex and clade ($F_{4,276}$ = 2.57, P = 0.041) on the centroid size of the dorsal head surface, so we examined the effects of clade and SVL on the size of male and female heads separately. For males, both SVL ($F_{1,159}$ = 1279.14, P = 0.001) and clade ($F_{4,159}$ = 4.59, P = 0.001, Fig 8A) significantly

affected dorsal head size, with no interaction ($F_{4,159}$ = 1.31, P = 0.253). The effect of clade remained after adding latitude and longitude to the model ($F_{4,161}$ = 4.60, P = 0.006), neither of which significantly affected male dorsal head size (latitude: $F_{1,161}$ = 0.85, P = 0.353, longitude: $F_{1,161}$ = 0.05, P = 0.829). For females, only SVL substantially affected head size ($F_{1,113}$ = 778.51, P = 0.001), although the effects of clade and the interaction between SVL and clade approached significance ($F_{4,113}$ = 2.46, P = 0.056, Fig 8B, and $F_{4,113}$ = 2.09, P = 0.085, respectively).

Results from pairwise tests for dorsal head centroid size are given in Table 3. For males, the only significant difference was between *E. panamintina* and the NC clade of *E. multicarinata* (D = 8.201, P = 0.004), with NC males having the largest heads while *E. panamintina* males had the smallest heads (Fig 8A). For females, pairwise tests revealed no differences in head sizes between any of the groups examined (Table 3, Fig 8B).

Lateral Head Shape

Similar to the dorsal surface, the shape of the lateral head surface was affected by clade ($F_{4,228}$ = 4.29, P = 0.001, Figs 6C, 6D, and 7K-7T), SVL ($F_{1,228}$ = 28.41, P = 0.001), and sex ($F_{1,228}$ = 4.18, P = 0.002), with a marginally significant interaction between SVL and sex ($F_{1,228}$ = 2.01, P = 0.050). In general, the posterior portion of the head was longer in males than in females, and the eye ridge and masseter muscle were shifted farther back relative to the snout in males (Fig 7). When analyzed separately, clade and SVL affected the lateral head shape of males (clade: $F_{4,127}$ = 3.51, P = 0.001, Fig 6C, and SVL: $F_{1,127}$ = 22.14, P = 0.001) and females (clade: $F_{4,92}$ = 1.77, P = 0.004, Fig 6D, and SVL: $F_{1,92}$ = 6.45, P = 0.001), with a marginal interaction between SVL and clade in females only ($F_{4,92}$ = 1.42, P =

0.076). When latitude and longitude were added to the models, clade remained significant in males ($F_{4,126}$ = 2.72, P = 0.001), but not in females ($F_{4,94}$ = 1.23, P = 0.191). In males, latitude of collection affected head shape while there was a marginal effect of longitude (latitude: $F_{1,126}$ = 4.27, P = 0.003, and longitude: $F_{1,126}$ = 2.06, P = 0.052), whereas in females only longitude explained variation in lateral head shape (latitude: $F_{1,94}$ = 1.46, P = 0.152, and longitude: $F_{1,94}$ = 4.28, P = 0.001). There were no interactions between SVL, clade, latitude, or longitude (P > 0.10 for all).

Results from pairwise tests examining lateral head shape variation among clades are given in Table 4, the location of each clade in PC space is presented in Figs 6C and 6D, and lateral head shapes are illustrated in Figs 7K–7T. The lateral head shape of *E. panamintina* males differed from males of each *E. multicarinata* clade (NC: D = 0.031, P = 0.005, SSN: D = 0.031, P = 0.034, C: D = 0.032, P = 0.003, SC: D = 0.032, P = 0.007, Fig 6C), with *E. panamintina* males displaying heads that were more dorso-ventrally flattened than those of *E. multicarinata* males (Fig 7). Within *E. multicarinata* males, differences among clades generally match predictions from Feldman and Spicer's (2006) mtDNA phylogeny: C and SC form a group (D = 0.011, P = 0.256) that differs from both NC (C: D = 0.016, P = 0.003, SC: D = 0.018, P = 0.002) and SSN (C: D = 0.023, P = 0.010, SC: D = 0.025, P = 0.004), which together form a group (D = 0.017, P = 0.081, Fig 6C). C and SC males had deeper heads (particularly at the masseters) and more forward slanted heads than males from the NC and SSN clades (Fig 7). Among females, no differences in lateral head shape were apparent after accounting for the false discovery rate.

Lateral Head Size

Similar to the dorsal head surface, we found a significant interaction between SVL and sex on the centroid size of lateral head surfaces ($F_{1,221}$ = 31.55, P = 0.001). Analyzing the sexes separately, clade and SVL significantly influenced centroid size in both males ($F_{4,124}$ = 3.14, P = 0.019, Fig 8C, and $F_{1,124}$ = 755.89, P = 0.001, respectively) and females ($F_{4,93}$ = 4.02, P = 0.004 Fig 8D, and $F_{1,93}$ = 582.13, P = 0.001, respectively), with no interactions (P > 0.10). Clade continued to affect lateral head size after including latitude and longitude of collection as covariates in the models (males: $F_{4,126}$ = 3.36, P = 0.009, and females: $F_{4,94}$ = 3.11, P = 0.024), which had no effects on lateral head size (males: $F_{1,126}$ = 2.12, P = 0.122, and $F_{1,126}$ = 0.02, P = 0.884, respectively; females: $F_{1,94}$ = 2.32, P = 0.138, and $F_{1,94}$ = 0.77, P = 0.353, respectively).

Results from pairwise tests exploring the effects of clade on lateral head centroid size are presented in Table 5. Results match those for dorsal head centroid size. In males, the only significant difference was between *E. panamintina* and the NC clade of *E. multicarinata* (D = 4.930, P = 0.003), with NC males having the largest heads and *E. panamintina* having the smallest heads (Fig 8C). In females, pairwise tests were unable to detect significant differences in lateral head size among any of the clades (Fig 8D).

Species Distribution Modeling

All species distribution models performed well. The test-data AUC for the single-taxon model was 0.934, suggesting a high ability of the model to distinguish suitable versus unsuitable areas (AUC's range from 0.5 [random correspondence] to ~1 [perfect correspondence], Phillips et al. 2006, Elith et al. 2011). Even so, considering the Northern

and Southern clades separately improved the test-data AUCs (Northern model AUC = 0.978 and Southern model AUC = 0.956). For all three models, precipitation of the wettest and coldest quarters (BIO18 and BIO19) contributed most to model construction (single-taxon model: 44.0% and 27.1%, Northern model: 37.5% and 27.0%, and Southern model: 32.8% and 31.8%, respectively) followed by isothermality (BIO3 [(mean diurnal temperature range / annual temperature range) x 100]; single taxon model: 10.7%, Northern model: 9.9%) or temperature seasonality (BIO4; Southern model: 5.60%), and mean temperature of the wettest quarter (BIO8; single-taxon model: 9.40%, Northern model: 10.4%, Southern model: 18.6%). Together, these bioclimatic variables contributed to ≥ 85% of each model.

Maps of the predicted distributions of *E. multicarinata* as a single taxon, the Northern clade, and the Southern clade are displayed in Fig 9 along with the currently recognized range limit of *E. multicarinata* (Stebbins 2003, Beck 2009). Colored areas represent regions with predicted presence values above the 10 percentile training threshold, thus 90% of training points fall within this region (Scheldeman and van Zonneveld 2010). The single taxon model (Fig 9A) and a combination of the Northern and Southern models (Fig 9B) both largely accord with the known distribution of *E. multicarinata*. The greatest differences between the models occurred at the northern and southern extremes of the range (Oregon and Baja California, respectively). The single taxon model predicted that *E. multicarinata* does not occur throughout much of Oregon or southern Baja California (Fig 9A). By contrast, the Northern and Southern clade models correctly predicted that *E. multicarinata* occur through much of their known range in these regions (Fig 9B). Moreover, the Northern and Southern clade models predicted higher probabilities of occurrence than the single-taxon model throughout the known range (Fig 9). The only region where the two-taxon model deviates

from the recognized distribution is in southern Baja California. In general, the Northern and Southern models, when taken together, better match the known distribution of *E. multicarinata* than the single taxon model.

DISCUSSION

Accurate delineation of species boundaries and the phylogenetic relationships among those species are necessary for most essential endeavors within the biological sciences (Coyne and Orr 2004, Sites and Marshall 2004). As native biota increasingly suffer globally, it is imperative that remaining biodiversity is accurately described; both so that we can understand the evolutionary and ecological processes that gave rise to this diversity and to create informed management plans (Pimm et al. 1995, Sala and Knowlton 2006, Rands et al. 2010, Böhm et al. 2013). An integrative approach that incorporates molecular, phenotypic, and ecological data should provide the best test of phylogenetic/species hypotheses (Dayrat 2005, Bailey et al. 2010, Padial et al. 2010), because all three methods can have high error rates when considered alone (Schlick-Steiner et al. 2010). I employed such an integrative approach to examine phylogenetic relationships within the *E. multicarinata-E. panamintina* species complex of alligator lizards. Given that multiple hypothesized clades within this complex could be at high risk of climate change-induced decline (e.g., *E. panamintina* and the SSN clade of *E. multicarinata*, Jennings and Hayes 1994, Feldman and Spicer 2006, Mahrdt and Beaman 2007), an accurate phylogeny with species/operational taxonomic unit delineations is imperative. My results suggest that neither previous phylogenetic hypothesis is fully accurate, and instead supports a phylogeny for *E. multicarinata* and *E. panamintina* that combines features of both.

First, my morphological data support the traditional status of *E. panamintina* as a biological species (*á la* Stebbins 1958). The hemipenes of *E. panamintina* were substantially different from those of the *E. multicarinata* clades, both in shape and size. These differences in hemipene morphology are likely sufficient to induce at least partial pre-zygotic reproductive isolation. *Elgaria panamintina* males also differed in head shape from *E. multicarinata* males, whereas female head morphology did not differ. These differences in head morphology resulted from reduced sexual dimorphism in *E. panamintina* compared to *E. multicarinata*. Head sexual dimorphism might reflect ecological niche differentiation between the sexes or be sexually selected (Shine 1989, Andersson 1994). In alligator lizards, males use their mouths to hold females by the head during copulation (Svihla 1942, Langerwerf 1981), thus male head morphology is likely under sexual selection. Reduced head dimorphism in *E. panamintina* compared to *E. multicarinata* might indicate different reproductive behaviors, which would act to further reproductively isolate these species (Coyne and Orr 2004).

Similar to prior morphological evidence (Stebbins 1958), my data contradict the mtDNA hypothesis (Feldman and Spicer 2006) suggesting that *E. panamintina* is polyphyletic and indistinct from *E. multicarinata*. The mtDNA pattern could result from either mitochondrial introgression or incomplete lineage sorting (Rosenberg and Nordborg 2002, Funk and Omland 2003, Leaché and McGuire 2006, McGuire et al. 2007). If mitochondrial introgression elicited the observed mtDNA pattern, then hybridization between *E. panamintina* and the Southern *E. multicarinata* clade is likely rare. If hybridization and introgression are common, *E. panamintina* haplotypes should be scattered throughout the Southern *E. multicarinata* clade, rather than forming a monophyletic group within that clade

(Funk and Omland 2003). Thus, incomplete lineage sorting resulting from peripatric speciation may better explain the observed mtDNA pattern. When a peripheral population speciates peripatrically (i.e., breaks off from a population of a large, substructured species), the new species is predicted to be more genetically similar to its parent population than to other populations within the parent species (Funk and Omland 2003, Kruckenhauser et al. 2014). Such "budding speciation" results in the new species initially being genetically nested within the parent species, just as Feldman and Spicer (2006) observed. The aberrant genetic pattern will persist until genetic sorting restores monophyly to the parental species (Funk and Omland 2003). Given the relatively recent estimated divergence between the two species (Macey et al. 1999), the peripheral nature of known *E. panamintina* populations (Stebbins 2003, Mahrdt and Beaman 2009), and the mtDNA monophyly of *E. panamintina* within the *E. multicarinata* clade to which it is geographically closest (Feldman and Spicer 2006), I suggest that incomplete lineage sorting after peripatric speciation of *E. panamintina* is the most parsimonious explanation of the entire dataset.

Within *E. multicarinata*, my data support the existence of the Northern and Southern clades as proposed by Feldman and Spicer (2006), but I could not detect their proposed C, SC, NC, and SSN subclades. Both hemipene and lateral head morphology were divergent between the Northern and Southern *E. multicarinata* clades. I did not detect similar differences between the subclades, with the C and SC subclades and the NC and SSN subclades not significantly differing from one another, respectively. While insufficient to refute the existence of these subclades, my morphological data suggest that the subclades have not diverged ecologically and likely are not reproductively isolated. I therefore recommend that these subclades not be elevated to species status.

The species status of the Northern and Southern clades is less clear. Mean hemipene morphology differed among the Northern and Southern clades, but there was broad overlap in hemipene morphology. My estimates of hemipene differences between the clades are likely conservative because specimens were assigned to clades based on their collection locations, thus misclassification might explain some of the observed shape overlap. Even so, the variation in hemipene morphology that I observed was minimal and may be insufficient to induce strong reproductive isolation between the two clades. Regardless, clade differences in hemipene shape indicate reduced gene flow because stabilizing selection should homogenize hemipene morphology within taxa (Dowling 1967, Eberhard et al. 1998, Keogh 1999, Hosken and Stockley 2004, Bailey et al. 2010). The subtle differences in hemipene morphology could result from genetic drift, divergent sexual selection among the clades, or early selection for a reinforcement mechanism (Eberhard et al. 1998, Coyne and Orr 2004, Hosken and Stockley 2004, Eberhard 2010, Masly 2012). Regardless of the ultimate mechanism, the differences in hemipene shape that I observed between the Northern and Southern clades corroborate the mtDNA evidence, suggesting that these clades represent unique evolutionary lineages.

By contrast, patterns of sexual dimorphism in head shape did not differ significantly among the *E. multicarinata* clades. All *E. multicarinata* subclades displayed strong sexual dimorphism in both head shape and size (dorsal and lateral); however, the general pattern of head dimorphism was conserved (i.e., no significant interactions between sex and clade on head shape). Not surprisingly, the sexes differed in their head-shape allometry, with head shape changing with body size differently in males and females (inferred from the interactions between SVL and sex on head shape). Males also had larger heads than females,

with male heads growing more rapidly relative to body size than female heads. The general conservation of head sexual dimorphism among the *E. multicarinata* clades suggests that the heads of these lizards experience similar sex-based ecological niche differentiation and/or sexual selection. If true, all *E. multicarinata* clades may display similar reproductive behaviors (at least those involving the head). Thus, pre-copulatory behavioral isolation might not reproductively isolate the Northern and Southern *E. multicarinata* clades (Coyne and Orr 2004).

Even if sexually-selected differences in head morphology are relatively unimportant, my analyses of head morphology suggest that the Northern and Southern *E. multicarinata* clades have diverged ecologically. Variation in lateral head shape is characteristic of the clades, with Northern and Southern clades grouping as predicted by the mtDNA phylogeny. The clades generally differed in head depth and the relative lengths of their snouts and the rear portion of their jaws. Such shape variation affects gape size and bite force, and thus could influence interactions with prey, predators, and conspecifics (Adams and Rohlf 2000, Herrel et al. 2001, Kaliontzopoulou et al. 2012). Dorsal head shape, by contrast, did not correspond to the mtDNA phylogeny, and collection location explained much of the observed variation. Such geographic differences could result from local adaptation and/or phenotypic plasticity matching dorsal head shape to local environments (Aubret et al. 2004, Aubret and Shine 2009, Buckley et al. 2010). Thus, differences in dorsal head shape likely reflect local ecological differences among populations, whereas lateral head shape variation might reflect clade-specific ecological variation.

My species distribution models also suggest that the Northern and Southern clades are ecologically divergent. The two-taxon models for the Northern and Southern clades predict

limited overlap between the clades, and better predict the known *E. multicarinata* distribution (particularly at the latitudinal extremes) than the single-taxon model. Initially, the two-taxon model appears to over predict appropriate habitat in Baja California, at the southern extent of the *E. multicarinata* range. However, this discrepancy likely results from the recognized range limit in this region being incorrect. Figure 1C demonstrates that many *E. multicarinata* specimens have been collected from the southern portion of Baja California. Thus, the majority of the peninsula, particularly coastal regions, likely falls within *E. multicarinata*'s range. Even though the species distribution models suggest that the Southern and Northern clades have ecologically diverged, some niche conservatism is apparent (Wiens and Graham 2005). For example, all models suggest that precipitation, particularly in winter (the wettest season within their range), and intra-annual temperature variability correlate with the ecological niche of these lizards. Because these factors may also correlate with additional aspects of the environment (e.g., annual precipitation, annual temperature, presence of other species, etc.), they may or may not be causative. Even so, differences in the predicted distributions of the Northern and Southern clades suggest that suitable values for these factors, or their environmental correlates, have diverged. Still, the predicted distributions of the clades overlap somewhat and divergence likely is not complete. Regions where the clades overlap might represent hybrid zones or, if reproductive isolation is complete, areas of intense interspecific competition.

Together, the mtDNA evidence (Feldman and Spicer 2006) and hemipene morphology data suggest that the Northern and Southern *E. multicarinata* clades represent unique evolutionary lineages. Moreover, the head morphology data and species distribution models imply that these lineages have diverged ecologically and are adapted to different

environments. Most likely, the Northern and Southern clades began diverging approximately 4 MYA when they were forced into allopatry as the Pacific Ocean intruded through the Monterrey Bay, and by the formation of the Sacramento-San Joaquin Delta (Dupré 1990, Dupré et al. 1991, Feldman and Spicer 2006). Today, these two clades are parapatric, and may have come into secondary contact relatively recently (Feldman and Spicer 2006). The question is: Are the Northern and Southern *E. multicarinata* clades reproductively isolated species or are they reticulating into a single taxon? Current data are insufficient to distinguish between these hypotheses, thus I am unable presently to determine whether these clades should be considered subspecies or be elevated to species. Detailed analyses of gene flow at the boundaries between the Northern and Southern clades are needed. Still, given their evolutionary and ecological differences, I recommend that the Northern and Southern clades be considered separate operational taxonomic units for conservation management and planning.

By integrating phenotypic and ecological information with molecular analyses we can test phylogenetic and taxonomic hypotheses with increased power (Coyne and Orr 2004, Dayrat 2005, Padial et al. 2010, Schlick-Steiner et al. 2010). My morphometric analyses and species distribution modeling allowed me to test the competing phylogenetic hypotheses for *E. multicarinata* and *E. panamintina* and discover that a combination of prior hypotheses is best supported. My results suggest that *E. panamintina* is both reproductively isolated and ecologically divergent from *E. multicarinata*, and thus support the species status of *E. panamintina*. In addition, my results confirm the existence of the divergent Northern and Southern *E. multicarinata* clades as proposed by Feldman and Spicer (2006). My integrative approach allowed me to simultaneously test taxonomic/species boundaries and examine

differences in the ecology of the supported taxa. Such analyses comprise a necessary step in better understanding the recent evolutionary history of taxa and for preserving biota.

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TABLES

Table 1—Results from pairwise tests comparing multivariate hemipene shape in *Elgaria multicarinata* clades and *E. panamintina*. Values above the diagonal are exact *P*-values and those below the diagonal are observed Euclidean distances. Bold values indicate significant differences ($P < 0.05$) whereas italicized values indicate marginal differences ($0.05 \leq P < 0.10$) after correcting for the false discovery rate. Clade designations follow Feldman and Spicer (2006): NC is Northern California, SSN is Southern Sierra Nevada, C is Coastal, SC is Southern California (each of these are *E. multicarinata* clades), and *pan* is *E. panamintina*.

	NC	SSN	C	SC	*pan*
NC		0.3635	*0.0786*	**0.0107**	**0.0009**
SSN	0.598469		*0.0706*	**0.0235**	**0.0019**
C	0.526435	1.112888		0.3666	**0.0186**
SC	0.8138	1.391077	0.315894		*0.0729*
pan	1.856284	2.436184	1.342933	1.058196	

Table 2—Results from pairwise tests comparing dorsal head shape in *Elgaria multicarinata* clades and *E. panamintina*. Values above the diagonal are exact *P*-values and those below the diagonal are observed Euclidean distances. Bold values indicate significant differences ($P < 0.05$) whereas italicized values indicate marginal differences ($0.05 \leq P < 0.10$) after correcting for the false discovery rate. Clade designations follow Feldman and Spicer (2006): NC is Northern California, SSN is Southern Sierra Nevada, C is Coastal, SC is Southern California (each of these are *E. multicarinata* clades), and *pan* is *E. panamintina*.

	NC	SSN	C	SC	*pan*
Male					
NC		*0.063*	0.237	0.172	**0.003**
SSN	0.021867		**0.014**	*0.034*	0.112
C	0.010605	0.026239		**0.016**	**0.005**
SC	0.011738	0.02415	0.018321		**0.007**
pan	0.034221	0.025431	0.034995	0.033798	
Female					
NC		*0.03*	0.244	*0.028*	*0.052*
SSN	0.024107		*0.069*	**0.003**	*0.065*
C	0.011498	0.025851		**0.002**	0.096
SC	0.015571	0.031781	0.025851		*0.028*
pan	0.027369	0.032334	0.025851	0.031093	

Table 3—Results from pairwise tests comparing the centroid size of the dorsal head surface in *Elgaria multicarinata* clades and *E. panamintina*. Values above the diagonal are exact *P*-values and those below the diagonal are observed Euclidean distances. Bold values indicate significant differences ($P < 0.05$) whereas italicized values indicate marginal differences ($0.05 \leq P < 0.10$) after correcting for the false discovery rate. Clade designations follow Feldman and Spicer (2006): NC is Northern California, SSN is Southern Sierra Nevada, C is Coastal, SC is Southern California (each of these are *E. multicarinata* clades), and *pan* is *E. panamintina*.

	NC	SSN	C	SC	*pan*
Male					
NC		0.116	0.091	0.261	**0.004**
SSN	3.817363		0.704	0.446	0.198
C	2.83269	0.984673		0.584	0.05
SC	1.873625	1.943738	0.959065		0.021
pan	8.200747	4.383384	5.368057	6.327122	
Female					
NC		0.387	0.25	0.131	0.816
SSN	1.909843		0.124	0.932	0.413
C	1.545525	3.455368		0.02	0.738
SC	2.113562	0.20372	3.659087		0.313
pan	0.635964	2.545806	0.909562	2.749526	

Table 4—Results from pairwise tests comparing lateral head shape in *Elgaria multicarinata* clades and *E. panamintina*. Values above the diagonal are exact *P*-values and those below the diagonal are observed Euclidean distances. Bold values indicate significant differences ($P < 0.05$) whereas italicized values indicate marginal differences ($0.05 \leq P < 0.10$) after correcting for the false discovery rate. Clade designations follow Feldman and Spicer (2006): NC is Northern California, SSN is Southern Sierra Nevada, C is Coastal, SC is Southern California (each of these are *E. multicarinata* clades), and *pan* is *E. panamintina*.

		NC	SSN	C	SC	*pan*
Male						
	NC		0.09	*0.059*	**0.024**	**0.005**
	SSN	0.021445		**0.022**	**0.009**	**0.034**
	C	0.016941	0.027769		0.259	**0.003**
	SC	0.018549	0.03001	0.013653		**0.007**
	pan	0.031121	0.031026	0.032384	0.031883	
Female						
	NC		0.708	0.081	0.039	0.022
	SSN	0.015443		0.466	0.422	0.253
	C	0.01667	0.019051		0.557	0.111
	SC	0.018979	0.02014	0.012806		*0.063*
	pan	0.035647	0.030023	0.028676	0.032238	

Table 5—Results from pairwise tests comparing the centroid size of the lateral head surface in *Elgaria multicarinata* clades and *E. panamintina*. Values above the diagonal are exact *P*-values and those below the diagonal are observed Euclidean distances. Bold values indicate significant differences ($P < 0.05$) whereas italicized values indicate marginal differences ($0.05 \leq P < 0.10$) after correcting for the false discovery rate. Clade designations follow Feldman and Spicer (2006): NC is Northern California, SSN is Southern Sierra Nevada, C is Coastal, SC is Southern California (each of these are *E. multicarinata* clades), and *pan* is *E. panamintina*.

		NC	SSN	C	SC	*pan*
Male						
	NC		0.277	0.121	0.081	**0.003**
	SSN	1.89139		0.965	0.868	0.168
	C	1.955756	0.064366		0.867	0.108
	SC	2.169249	0.277859	0.213493		0.147
	pan	4.92979	3.0384	2.974034	2.760541	
Female						
	NC		0.359	0.137	0.53	0.747
	SSN	1.231724		0.061	0.649	0.725
	C	1.290234	2.521959		*0.071*	0.275
	SC	0.569569	0.662155	1.859803		0.995
	pan	0.550771	0.680953	1.290234	0.018797	

FIGURES

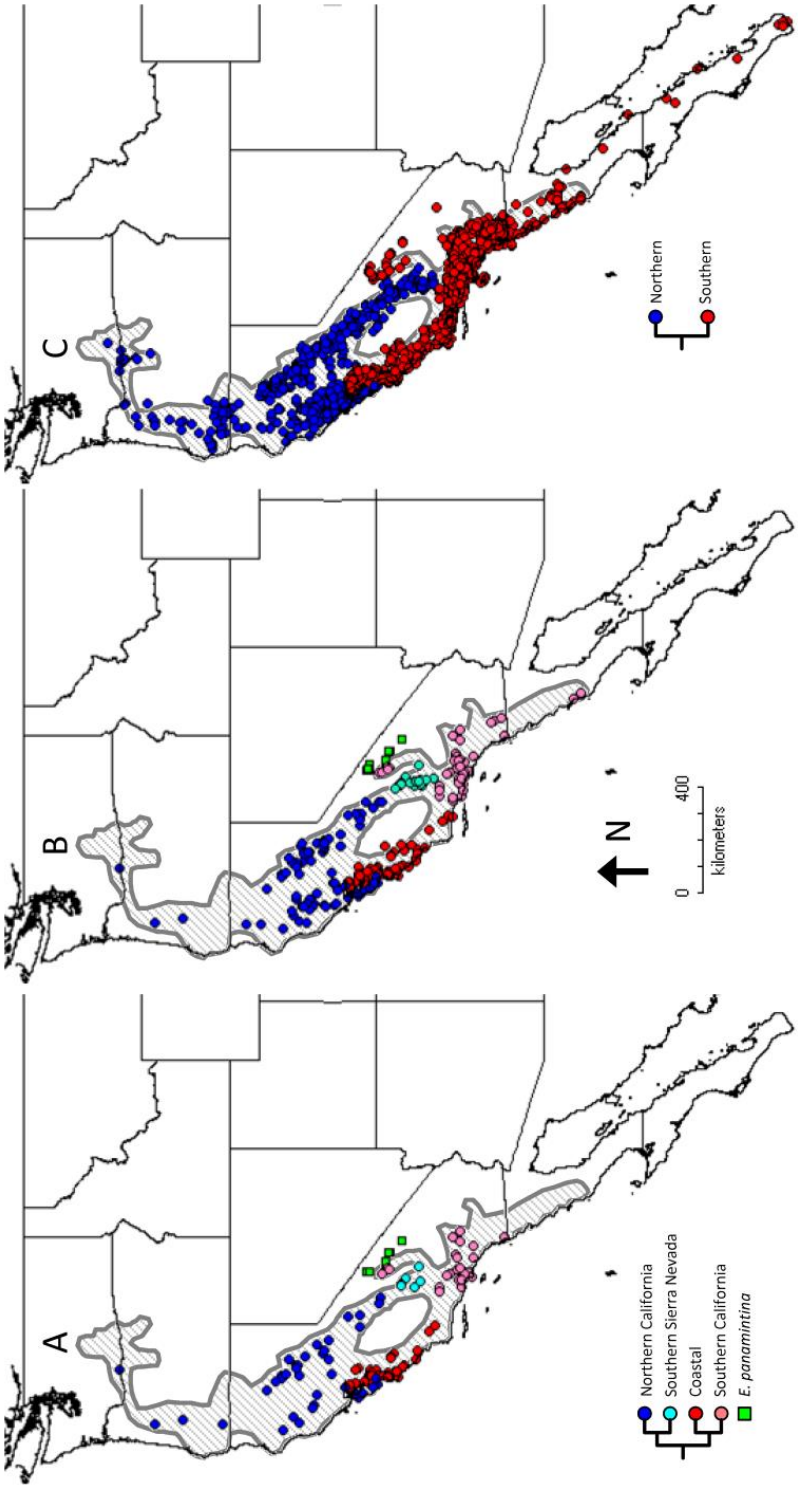

Figure 1—Collection locations of *Elgaria multicarinata* and *E. panamintina* specimens: A) specimens for hemipene shape analyses, B) specimens for head shape analyses, and C) specimens used to construct species distribution models. The hashed region in each map represents the current, accepted distribution of *E. multicarinata*. For the ecological niche models, the Northern California and Southern Sierra Nevada clades were analyzed together as the "Northern" clade, and the Southern California and Coastal clades were analyzed together as the "Southern" clade. *Elgaria panamintina* specimens were not included in the ecological niche models. The legend in (A) is also for (B). Phylogenetic clade relationships are from Feldman and Spicer (2006).

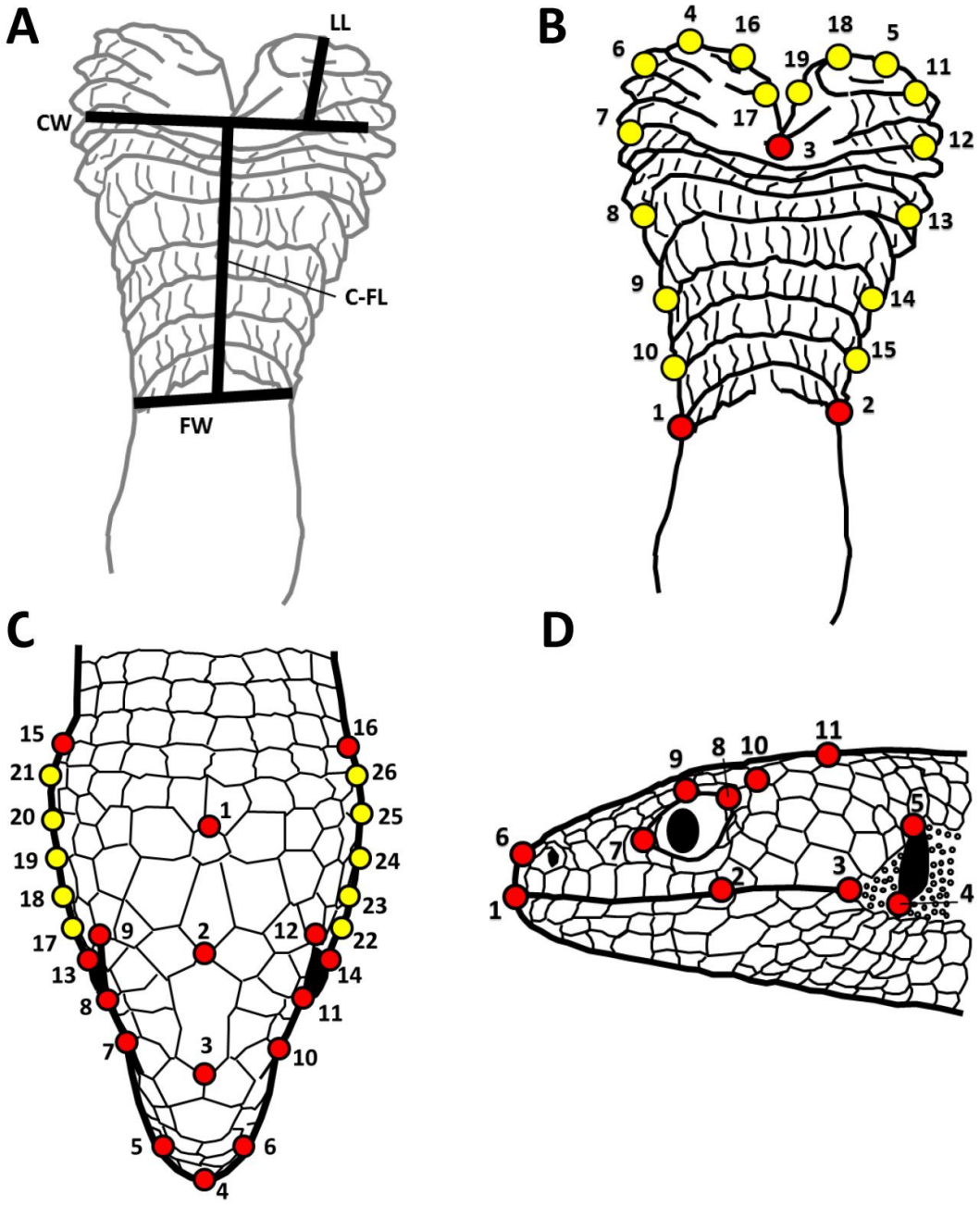

Figure 2—Linear measurements and landmarks used for morphometric analyses. Bold lines represent linear measurements, red dots represent fixed landmarks, and yellow dots represent sliding semi-landmarks. A) Hemipene length measurements for multivariate morphometric analyses. See text for descriptions of each measurement. B) Hemipene landmarks. These were only used for visualizing shape differences. C) Dorsal head landmarks. D) Lateral head landmarks. Head landmarks were used for full geometric morphometric analyses.

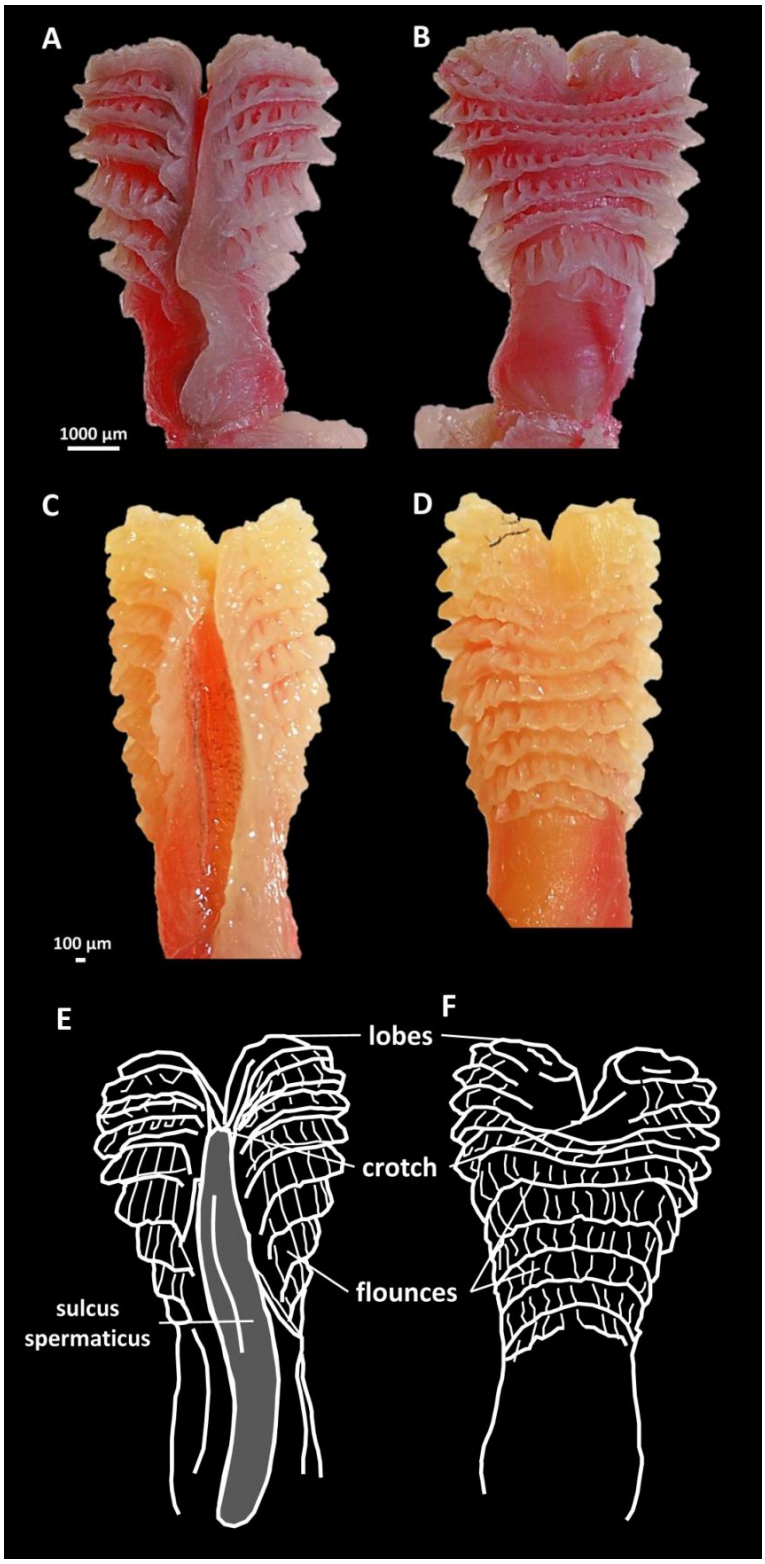

Figure 3—Photographs of representative hemipenes from *Elgaria multicarinata* (A, B) and *Elgaria panamintina* (C, D), with line drawings describing basic structures (E, F). Sulcal (A, C, E) and asulcal (B, D, F) surfaces of each hemipene are presented. The upper scale bar is for (A) and (B) whereas the lower scale bar is for (C) and (D).

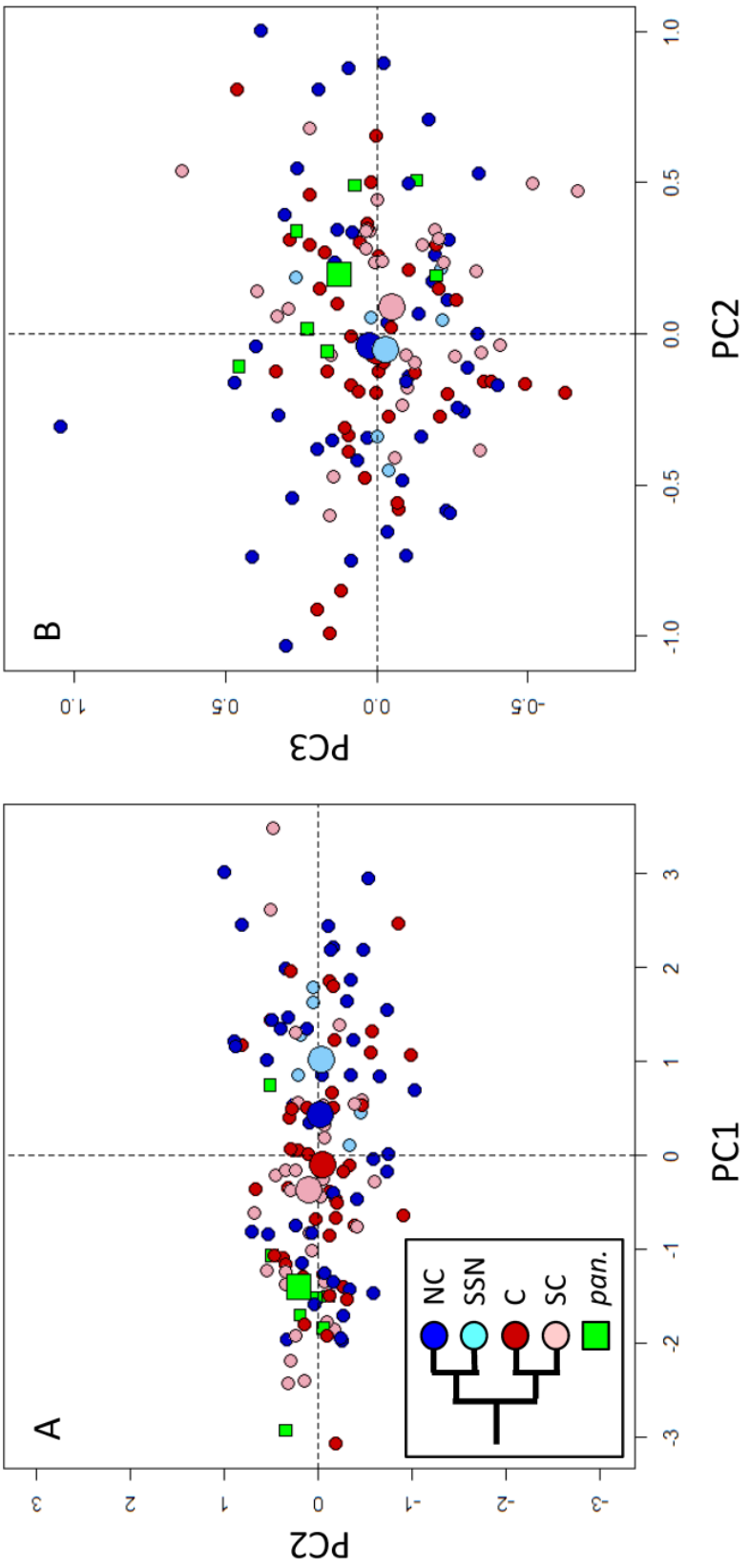

Figure 4—Principle component plots of hemipene shape of *Elgaria multicarinata* and *E. panamintina* by clade: A) plot of PC1 and PC2, and B) plot of PC2 and PC3. The data are from linear hemipene measurements. PC1 commonly represents size variation in multivariate morphometric data, thus (B) may better represent true "shape" variation. Smaller points represent the raw data whereas larger points represent mean values for each clade. Phylogenetic clade relationships are from Feldman and Spicer (2006): NC is Northern California, SSN is Southern Sierra Nevada, C is Coastal, SC is Southern California (each of these are *Elgaria multicarinata* clades), and *pan* is *E. panamintina*.

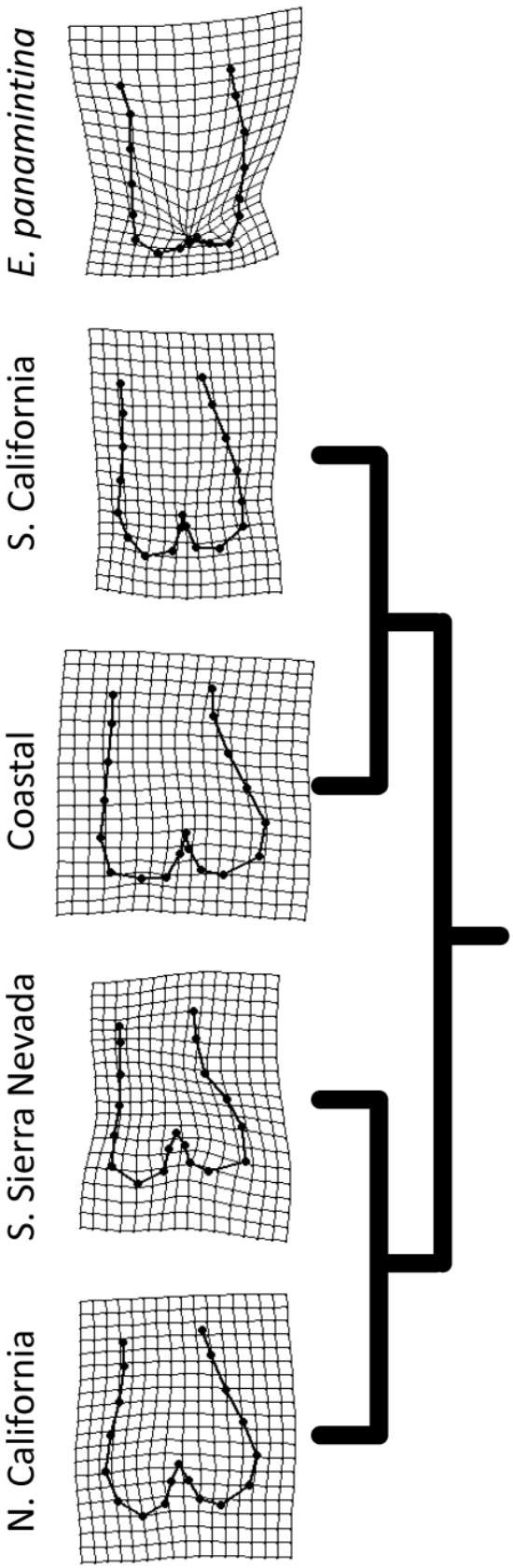

Figure 5—Deformation grid plots illustrating differences in the mean shape of hemipenes from the *Elgaria multicarinata* clades and *E. panamintina*. Deformations are from the grand-mean hemipene shape and are magnified by three to better illustrate differences. Phylogenetic relationships are from Feldman and Spicer (2006).

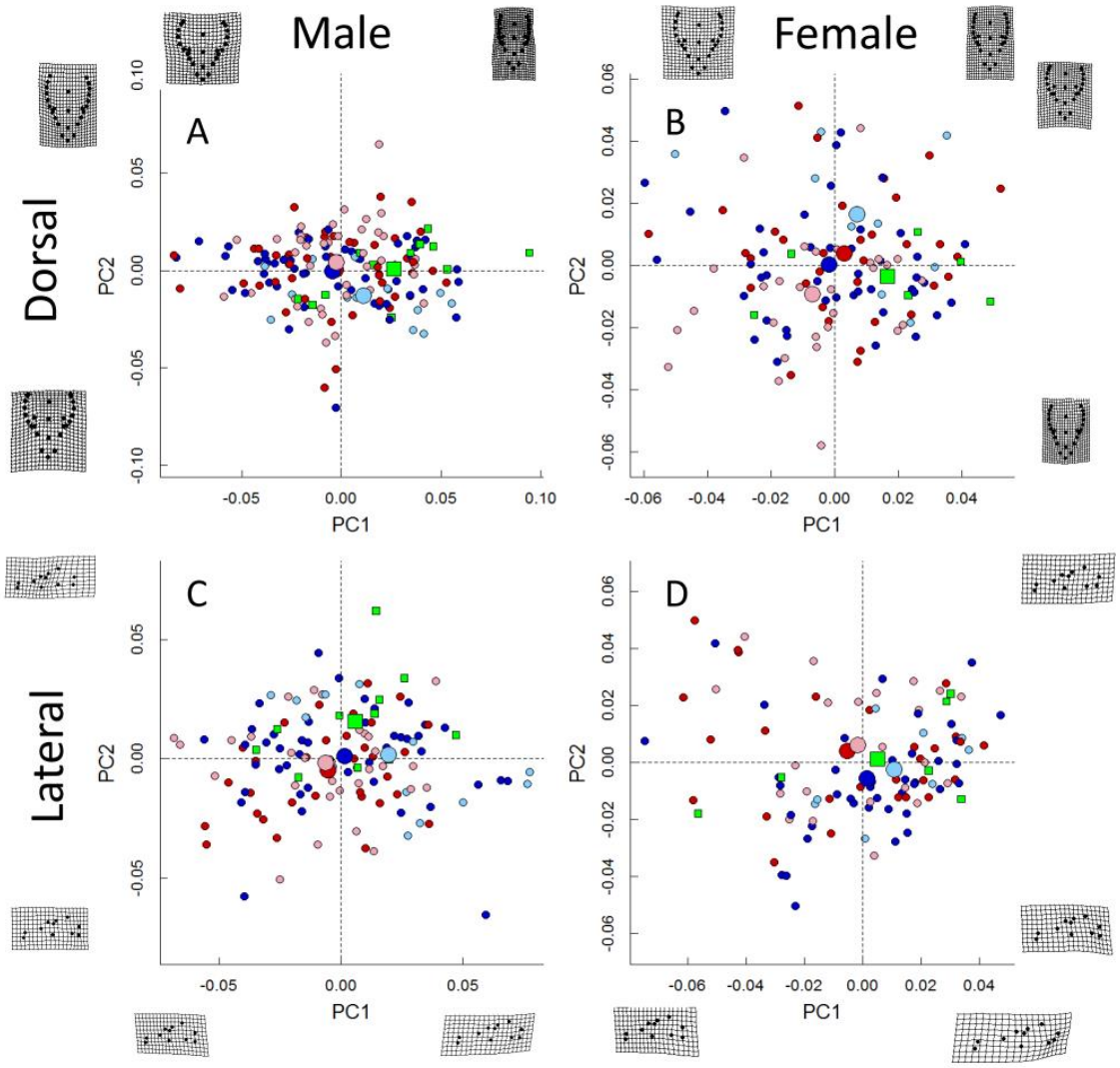

Figure 6—Principle component plots of dorsal (A, B) and lateral (C, D) head shapes of *Elgaria multicarinata* and *E. panamintina* by clade (PC1 versus PC2). Male (A, C) and female (B, D) data are displayed separately. The data are from landmark-based shape data with size removed. Small points represent the raw data whereas large points represent mean values for each clade. Color assignments and symbols are the same as for Figure 4. Deformation plots represent the shapes at the ends of each PC axis, and thus illustrate how shape changes along the PC axes. Deformations are from the grand mean dorsal or lateral head shape, as appropriate.

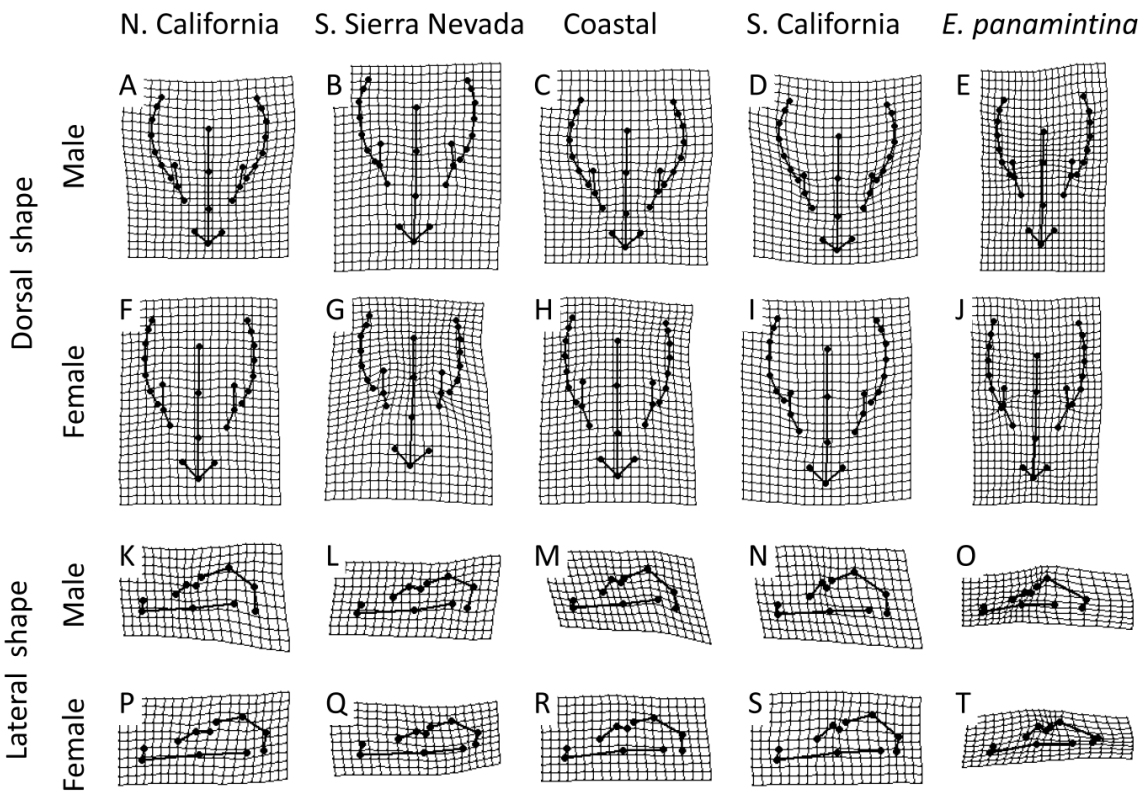

Figure 7—Deformation grid plots illustrating differences in mean dorsal (A–J) and lateral (K–T) head shapes from males (A–E, K–O) and females (F–J, P–T) of each *Elgaria multicarinata* clade and *E. panamintina*. Each deformation illustrates differences between the target clade and the grand mean (dorsal or lateral, as appropriate). Dorsal head deformations are magnified by three and lateral head deformations are magnified by five to better illustrate differences.

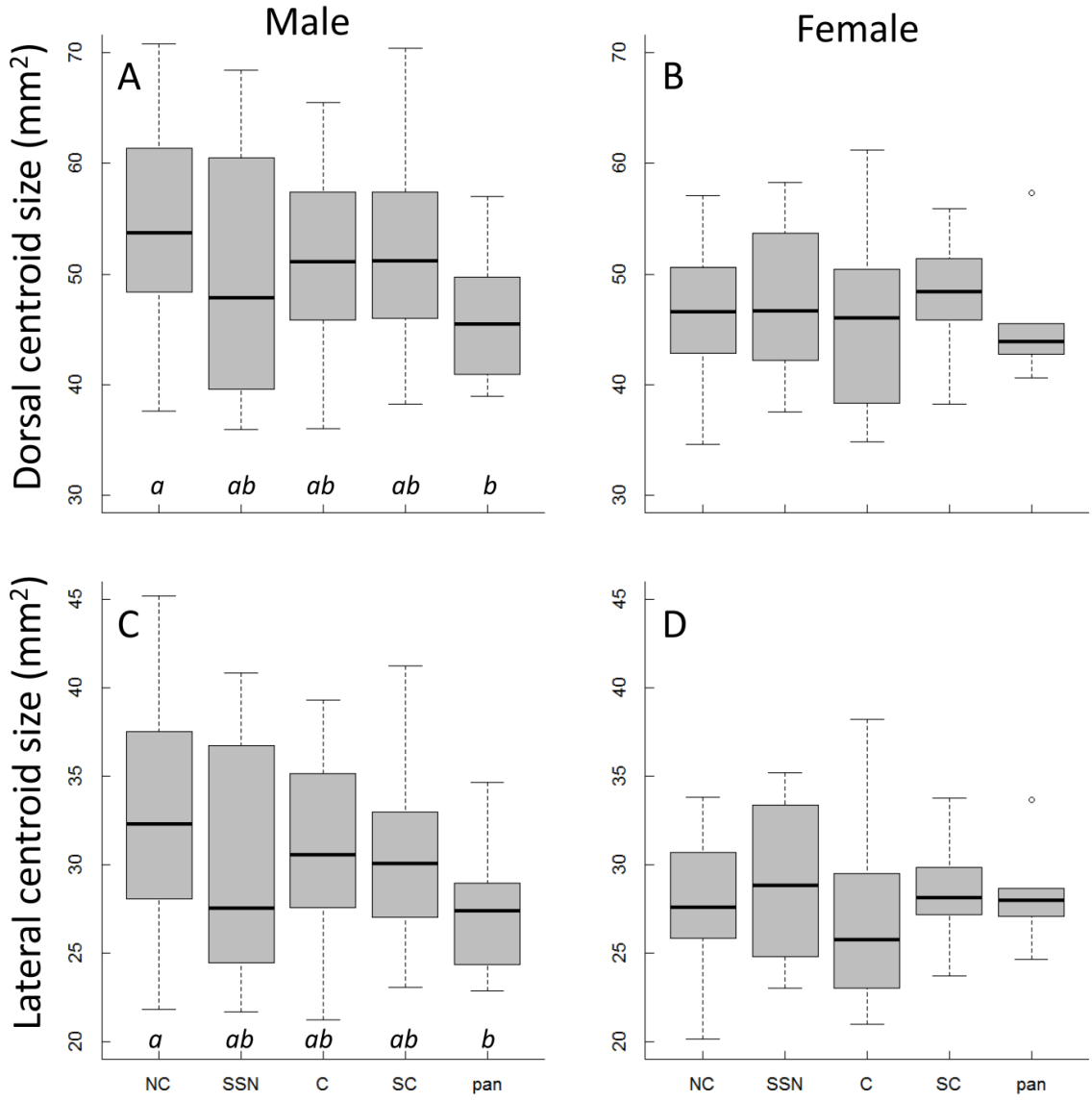

Figure 8—Box plots of the centroid sizes of dorsal (A, B) and lateral (C, D) head surfaces from male (A, C) and female (B, D) *Elgaria multicarinata* (split by clade) and *E. panamintina*. NC is Northern California, SSN is Southern Sierra Nevada, C is Coastal, SC is Southern California, and pan is *E. panamintina*. The lines within the boxes represent medians, box limits depict the first and third quartiles, and box whiskers are 1.5 times the interquartile range. Different letters below the boxes indicate significant differences from pairwise tests.

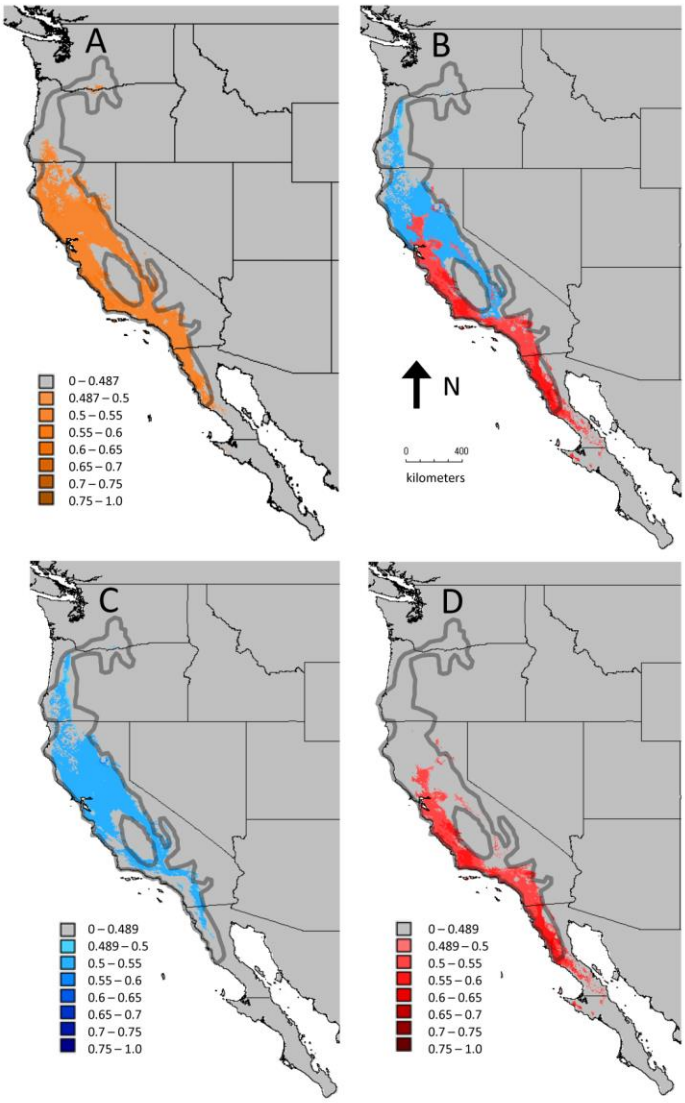

Figure 9—Ecological niche models for *Elgaria multicarinata* constructed using MaxEnt: A) predicted distribution assuming *E. multicarinata* is a single, uniform taxon, B) predicted distribution assuming *E. multicarinata* is composed of two, independent taxa with potentially different tolerances, a Northern (blue) and Southern (red) clade, C) predicted distribution of the Northern *E. multicarinata* clade alone, D) predicted distribution of the Southern *E. multicarinata* clade alone. Panel (B) is a composite overlay of panels (D) on top of (C), and their respective legends apply. Darker colors indicate higher predicted probabilities of occurrence. Dark gray lines represent the current, accepted distribution of *E. multicarinata*. The gray regions represent areas outside the 10 percentile training presence thresholds for each model, thus the colored regions contain 90% of the training points for each model. Values in the legends are logistic probabilities of occurrence from the MaxEnt models. For species of low prevalence sampled over a long period of time, such as *E. multicarinata*, suitable habitats are expected to have values around 0.5.

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CHAPTER 3

ARE EXTREME TEMPERATURES PHYSIOLOGICALLY STRESSFUL? THERMAL EFFECTS ON CORTICOSTERONE IN LIZARDS

A paper submitted for publication in *General and Comparative Endocrinology*

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ABSTRACT

In response to conditions that threaten homeostasis and/or life, vertebrates generally upregulate production of glucocorticoid hormones, such as corticosterone (CORT), which induces an emergency physiological state referred to as the stress response. Given that extreme temperatures pose a threat to performance and survival, glucocorticoid upregulation might be an important component of a vertebrate ectotherm's response to extreme thermal conditions. To address this hypothesis, we experimentally examined the effects of body temperature (10, 20, 28, and 35°C; 5-hr exposure) on CORT in two congeneric species of lizard naturally exposed to different thermal environments, northern and southern alligator lizards (*Elgaria coerulea* and *E. multicarinata*, respectively). While medium and high temperatures affected CORT in both species similarly, CORT was only elevated at low temperatures in southern alligator lizards. We also examined CORT before and after

adrenocorticotrophic hormone (ACTH) challenge. In both species, ACTH induced higher CORT levels than any temperature, suggesting that these animals maintained reactive scope and could respond to further stressors. Finally, we compared our laboratory results to measurements of CORT in field-active southern alligator lizards. CORT levels from our laboratory experiment had the same mean and less variance than the field lizards, suggesting that our laboratory lizards displayed CORT within natural levels. Our results demonstrate that body temperature directly affects CORT in alligator lizards. Moreover, the CORT response of these lizards appears to be adapted to their respective thermal environments. Species-specific differences in the thermal CORT response might be common in vertebrate ectotherms and have implications for species' biogeography and responses to climate change.

Keywords: alligator lizard, *Elgaria coerulea*, *Elgaria multicarinata*, glucocorticoid, GC, CORT

INTRODUCTION

Temperature broadly affects the performance and function of organisms (Cowles and Bogert 1944, Huey and Stevenson 1979, Angilletta 2009). In general, the relationship between body temperature (T_B) and performance can be described by a left-skewed, hump-shaped curve (thermal response curve): performance increases with T_B until an optimum is reached, then rapidly drops off (Huey and Stevenson 1979, Huey and Kingsolver 1989, Angilletta 2009). Within a range around the thermal optimum, organisms can maintain homeostasis and physiological performance is high (Huey and Kingsolver 1989, Angilletta et al. 2002). At extreme T_B (above and below the optimal range), organisms are unable to

maintain homeostasis and performance is compromised (Huey and Kingsolver 1989, Angilletta et al. 2002, Bradshaw 2003, Angilletta 2009). Endothermic animals expend large amounts of energy metabolically controlling T_B near their physiological optima (Randall et al. 2002, Angilletta 2009). Ectothermic animals, by contrast, have limited capacity to metabolically control their T_B, which is more dependent on environmental temperature (Cowles and Bogert 1944, Huey and Stevenson 1979, Chown and Terblanche 2007, Angilletta 2009). Ectotherms primarily thermoregulate behaviorally, moving between thermal microenvironments (Huey and Slatkin 1976, Avery 1982, Huey and Hertz 1982, Huey et al. 2003, Chown and Terblanche 2007). Exposure to non-optimal T_B can compromise multiple organismal processes including water balance, energetics, and gas-exchange (Dawson and Templeton 1966, Huey 1982, Pörtner 2002, Angilletta 2009). To avoid extreme T_B (particularly hot temperatures), many ectotherms seek thermal refugia and cease activity when environmental temperatures become too extreme for successful thermoregulation (Cowles and Bogert 1944, Grant and Dunham 1988, Bashey and Dunham 1997, Sinervo et al. 2010).

Exposure to extreme T_B is likely stressful for ectotherms (Cowles and Bogert 1944, Van Berkum et al. 1986, Bradshaw 2003). While the term "stress" has many connotations, we follow the convention of considering an organism physiologically "stressed" when an environmental perturbation (i.e., stressor) drives them out of homeostasis (Selye 1936, 1950, Greenberg and Wingfield 1987, Orchiinik 1998, Bradshaw 2003, Romero et al. 2009). "Stressful" situations/environments challenge homeostasis and must be countered by the individual to maintain homeostatic function and high performance (Greenberg and Wingfield 1987, Bradshaw 2003, Romero et al. 2009).

One mechanism whereby vertebrates perceive and respond to stressors is through the upregulation of glucocorticoid hormones (GCs, Greenberg and Wingfield 1987, Bradshaw 2003, Norris 2007, Romero et al. 2009). Glucocorticoid production is controlled by the hypothalamus-pituitary-adrenal (HPA) axis (Romero 2004, Norris 2007, Nelson 2011). In response to stressful environmental stimuli (either predictable or unpredictable), the hypothalamus induces the pituitary gland to release adrenocorticotrophic hormone (ACTH), which in turn induces the adrenal gland to intensely secrete GC hormones (Greenberg and Wingfield 1987, Romero 2004, Norris 2007, Nelson 2011). High GC levels induce an emergency physiological state: digestion, immunity, and reproduction are impaired, while energy is mobilized for emergency use (Greenberg and Wingfield 1987, McEwen and Wingfield 2003, Squires 2003, Norris 2007, Romero et al. 2009). In addition, behaviors such as territory defense, courtship, and foraging may be suppressed while escape behaviors are promoted (Greenberg and Wingfield 1987, Belthoff and Dufty 1998, Orchiinik 1998, Belliure et al. 2004). The primary action of GC hormones is in intermediary metabolism, inducing gluconeogenesis and inhibiting the use of glucose by peripheral tissues (Norris 2007, Nelson 2011). In this way, GCs increase energy availability for tissues of primary import, such as the brain (Norris 2007). These GC mediated changes are frequently adaptive, allowing organisms to escape stressful environments and maintain homeostasis (Bradshaw 2003, Nelson 2011). However, extreme elevations of GCs, either in duration or magnitude (i.e., chronic stress/ homeostatic overload/ allostatic overload), can reduce fitness and even induce pathology (Selye 1936, Sapolsky 1992, Broom and Johnson 1993, Bradshaw 2003, McEwen and Wingfield 2003, Norris 2007, Romero et al. 2009). Because GCs are an important component of an organism's response to environmental stressors, GC elevation is

often used to indicate whether or not organisms are exposed to stressful environments (Broom and Johnson 1993, Romero 2004, Busch and Hayward 2009, Nelson 2011).

Because extreme T_B can disrupt homeostasis and even cause death (Bradshaw 2003, Norris 2007), GC elevation might be an important component of a vertebrate ectotherm's response to non-optimal temperature (Bradshaw 2003, Cree et al. 2003, Dupoué et al. 2013). Many effects of GC elevation might induce organisms to seek thermal refugia and thus be adaptive in response to dangerous temperatures. Given that both temperature and GC production have direct effects on metabolism (Bradshaw 2003, Preest and Cree 2008, Sykes and Klukowski 2009), any relationship between GCs and T_B could have important biological implications. Moreover, if temperature affects GC secretion, this endocrine pathway might influence how vertebrate ectotherms respond to climate change. Increased frequency of extreme heat events is an important prediction of many climate change models (Bloom 2010, IPCC 2013). If these heat events are physiologically stressful, they might induce chronic GC elevation. Because chronic GC elevation has been linked to reduced fitness and pathology (Bradshaw 2003, Romero 2004), this endocrine response could contribute to population declines as a result of climate change.

To date, little is known about the relationship between GC hormones and T_B in reptiles and other ectothermic vertebrates. In reptiles, corticosterone (CORT) is the primary GC (Idler 1972, Greenberg and Wingfield 1987, Norris 2007). Multiple researchers have found correlations between CORT and T_B in reptiles in the field (Dunlap and Wingfeld 1995, Tyrrell and Cree 1998, Jessop et al. 2000, Cree et al. 2003, Woodley et al. 2003). However, such correlations could arise through three, non-mutually exclusive, pathways (outlined in Fig 1). First, CORT and T_B might be simultaneously affected by an outside factor. For

example, CORT can fluctuate with time of day and season (Dunlap and Wingfeld 1995, Kotrschal et al. 1998, Jessop et al. 2000, Romero 2002, Woodley et al. 2003, Dickmeis 2009, Nelson 2011, Eikenaar et al. 2012), but temperature also varies temporally. Second, CORT might directly induce changes in T_B. Experimental elevation of CORT affects thermoregulatory behavior in some lizards, and not necessarily in the same way (Belliure and Clobert 2004, Belliure et al. 2004, Preest and Cree 2008). Finally, T_B might directly affect CORT concentrations. Experimental exposure to non-optimal temperatures in two snake species induced elevated CORT levels (Dupoué et al. 2013, Schwartz and Bronikowski 2013) but had little effect on CORT in a third species (Sykes and Klukowski 2009). While these results are limited, they suggest that T_B and CORT might be functionally linked in reptiles.

We expand on this work by experimentally examining the effect of T_B on CORT in two congeneric species of alligator lizard (family Anguidae), northern alligator lizards and southern alligator lizards (*Elgaria coerulea* [Wiegmann, 1828] and *E. multicarinata* [Blainville, 1835], approximately 6.6 million years divergent [Macey et al., 1999]). These lizards occur in different thermal environments: *E. coerulea* occurs at higher elevation and latitude (i.e., colder environments) than *E. multicarinata* (Stebbins 2003, Beck 2009a, b). Alligator lizards are best described as facultative thermoregulators (Kingsbury 1994), and will remain active across a broad range of temperatures. *Elgaria coerulea* and *E. multicarinata* are naturally active at remarkably similar body temperatures (both species display a mean active temperature of ~24-25 °C with a range of ~10-35 °C, Cunningham 1966, Stewart 1984, Kingsbury 1994, Sheen 2001). This observation seems paradoxical because species' thermal tolerances are predicted to be locally adapted to their thermal environment (Huey and Kingsolver 1993, Gilchrist 1995, Angilletta et al. 2002, Kingsolver

and Gomulkiewicz 2003). Because *E. coerulea* persist in colder environments than *E. multicarinata*, their thermal optimum range is predicted to be colder than that of *E. multicarinata*. However, the response of these animals to extreme thermal events might be more important than their average active temperatures (Bradshaw 2003, Pörtner and Knust 2007, McKenchnie and Wolf 2009, Telemeco et al. 2013). We generally consider extreme temperatures to be those that are beyond an organism's optimal performance range. In practice, this could be measured as temperatures outside the 80% performance breadth (Huey and Stevenson 1979, Huey and Kingsolver 1989, Angilletta et al. 2002) or greater than two standard deviations from mean activity temperatures (Telemeco et al. 2013).

First, we tested the hypothesis that T_B affects CORT in *E. coerulea* and *E. multicarinata*. We quantified CORT after exposing lizards to four controlled temperature treatments (constant 10, 20, 28, and 35°C for 5 hrs each), as well as laboratory control conditions (at ~23.5°C) and an ACTH challenge. Next, we used these data to test the hypothesis that *E. coerulea* and *E. multicarinata* differ in their CORT response to temperature. If variation in the thermal stress response contributes to biogeographic variation in these species, we predict that *E. coerulea* will have elevated CORT levels at warm temperatures that do not affect *E. multicarinata*, and vice versa. Finally, we examined CORT in field sampled *E. multicarinata* for comparison to our experimental results.

MATERIALS AND METHODS

Laboratory Experiment

Lizard Collection and General Laboratory Maintenance

We collected adult *E. multicarinata* and *E. coerulea* during the active seasons of 2010 and 2011. Most lizards were collected from the central-coast region of California, which corresponds to the southern-most region where *E. multicarinata* and *E. coerulea* coexist. A few individuals, however, were collected in north-central California. Precise capture locations and body-size data are given in Table 1. Lizards were collected by hand via active searching in appropriate habitat, primarily from under rocks, logs, or anthropogenic debris. After collection, we transported the lizards to Iowa State University (ISU) and entered them into captive colonies. During active seasons (March–December), we housed the lizards in plastic containers with mesh tops. In 2010 and 2011, we housed the lizards either individually or in size-matched, male-female pairs whereas, in 2012, all lizards were housed individually. Enclosures housing individual lizards measured 33 cm long x 20 cm wide x 14.5 cm tall, whereas enclosures housing pairs measured 39 cm long x 26 cm wide x 29 cm tall. Each enclosure was outfitted with plastic hides (14 cm diameter x 2 cm tall, one per lizard) and water dishes with standing water provided *ad libitum.* Twice per week, we fed each lizard 3 crickets (*Acheta domesticus*) dusted with reptile vitamins (1:1 mixture Exo-Terra calcium and multi-vitamin powder supplements). We misted the cages with water daily. Enclosures were stored on a metal shelving system and illuminated with 40W ReptiSun 5.0 UVB bulbs (ZooMed Inc.) set on a 12 hr light cycle suspended above each enclosure. Additional room lights turned on 1 hr before the enclosure lights and turned off 1 hr after. Flex Watt heat tape (7.6 cm wide) on the shelves, under the rear portion of each

enclosure, maintained a surface heat gradient within the enclosures ranging from 26–32°C for 5 hrs/day (middle of the 12 hr light cycle) to allow behavioral thermoregulation.

Outside of the active season (mid-December to mid-February), we hibernated the lizards at 6°C in a dark room to mimic natural conditions. During hibernation, we housed the lizards individually in small containers with moist peat moss. Prior to the onset of hibernation, room temperature and light exposure was gradually reduced over 1 month. Similarly, temperature and lighting was gradually increased over 1 month at the end of hibernation.

We quantified body temperatures maintained by thermoregulating lizards in our captive colony in September 2011. We affixed iButton thermal data loggers (Maxim Integrated, San Jose, CA; diameter: 15 mm, height: 6 mm, mass: 3.3g) programmed to record temperature every 10 minutes to six post-reproductive *E. coerulea* (see Telemeco et al. 2010 for detailed methods) that were each housed individually. We affixed the data loggers to the dorsum of the lizards with superglue, above the pectoral girdle. The data loggers did not affect lizard movement, and body surface temperatures measured in this way correlate highly with internal body temperatures in lizards (Robert and Thompson 2003). We collected the data loggers after they naturally fell from the lizards as they sloughed. Grand-mean lizard body temperatures ± 1.0 s.e. over 10 days were 21.22 ± 0.13°C when both the heaters and lights were off, 23.56 ± 0.20°C when only the lights were on, and 27.70 ± 0.29°C when both the heaters and lights were on.

Experimental Protocol

We examined thirty adult lizards for the present study: 15 *E. coerulea* (6 male, 9 female) and 15 *E. multicarinata* (12 male, 3 female). When the lizards were brought out of hibernation in February 2012, we measured them (snout-vent length [SVL] and mass) and placed them individually in home enclosures for the season. These lizards received minimal human interaction until the onset of experiments in April 2012. During this period and throughout the experiments, lizard care was highly controlled, with lizards misted daily and fed each Tuesday and Saturday. We identified the sex of each lizard after the experiments by examining the gonads of euthanized individuals (data in Table 1). Because they were housed individually following hibernation, no females were pregnant/gravid.

On 12 April 2012, we collected blood samples (for details see Blood Collection, below) before (laboratory control) and after ACTH challenge. ACTH challenge induced the HPA axis to secrete CORT, and thus allowed estimation of the potential CORT-response capacity of the lizards (Romero and Wingfield 1999, Squires 2003, Romero and Wikelski 2006, Phillips and Klukowski 2008, Klukowski 2011). Beginning at 09:10 h, we removed individual lizards from their enclosures and bled them at approximately 5 minute intervals. When the laboratory control samples were collected, the enclosure lights, but not heaters, were switched on. We therefore estimate that lizard body temperatures were approximately 23.5°C (see Lizard Collection and General Laboratory Maintenance, above) at the time of blood collection. Immediately following blood collection, each lizard was injected with ACTH (Sigma A6303, fragments 1–39 porcine) in the anteroventral portion of the right hindlimb using a 30G disposable insulin syringe. Injections consisted of 0.1 IU ACTH/µL/g body mass (based on mass measured in Feb 2012, Table 1). In previous studies, this dosage

was effective but not supraphysiological in both squamate reptiles and birds (Romero and Wingfield 1999, Romero and Wikelski 2006, Phillips and Klukowski 2008, Klukowski 2011). After ACTH injection, we returned the lizards to their home enclosures and allowed them to rest for 60 min (± 1.5 min) before collecting a second blood sample. Previous studies have shown that lizards display a CORT response to ACTH 60 min after exposure (e.g., Klukowski 2011and citations therein). During the waiting period, the lizards were at room temperature (~ 22°C). After the second blood collection, we returned the lizards to their home enclosures on the shelving units.

We allowed the lizards to rest with minimal human contact until thermal experiments began in June 2012. For these experiments, we exposed each lizard, in random order, to four temperature treatments (10ºC, 20ºC, 28ºC, and 35ºC) for 5 hours each. These temperatures spanned the range of possible active T_B in *E. coerulea* and *E. multicarinata*. The thermal extremes are approximately the minimum and maximum active T_B recorded in both species (Brattstrom 1965, Cunningham 1966, Stewart 1984, Kingsbury 1994), whereas 28°C optimizes ATPase and skeletal muscle activity (Licht 1964, 1967) in *E. multicarinata* and is their preferred body temperature in the field and laboratory (Licht 1964, Dawson and Templeton 1966, Kingsbury 1994). This latter temperature therefore appears to correspond to the physiological optimum for *E. multicarinata*. Similar published data are not available for *E. coerulea*, but we found that *E. coerulea* in our colony maintained body temperatures near 28°C when allowed to thermoregulate (see Lizard Collection and General Laboratory Maintenance, above). Finally, 20°C approximates the average active temperature for these species (Brattstrom 1965, Cunningham 1966, Stewart 1984, Kingsbury 1994, Sheen 2001).

The temperature experiments were separated by one week, beginning 8 June 2012. For each experiment, we placed the lizards in one of four thermal chambers (7 or 8 lizards per chamber) illuminated with LED lights. We confirmed chamber temperatures using iButton thermal data loggers. The time when lizards were in each chamber was staggered to allow post-treatment blood collection: we placed lizards in the 10ºC chamber from 09:30–14:30, the 20ºC chamber from 09:45–14:45, the 28ºC chamber from 10:00–15:00, and the 35ºC chamber from 10:15–15:15 (± 1.0 min for each experiment). These treatment times approximately correspond to when under-cage heaters were on in the animal room as well as the warmest part of the day in nature. To reduce external stressors, we left the lizards in their original home enclosures while in the thermal chambers, and only handled the lizards to collect blood. In addition, at 17:30 the evening prior to each experiment, we moved the lizards (within their home enclosures) from their animal room to the room that housed our thermal chambers. The lizards then stayed overnight in the thermal-chamber room, which was maintained at ~21.5ºC and had the same light cycle as the animal room. Immediately following each 5-hr temperature treatment, we collected blood from each lizard (for details see Blood Collection, below) and returned the lizards to their shelves in the animal room. After each experiment, we allowed the lizards to rest one week, after which we repeated the entire process until each lizard was exposed to each thermal treatment. This protocol allowed us to have a complete, randomized, repeated-measures statistical design.

Assessment of Field Corticosterone and Body Temperature

To assess natural alligator lizard CORT levels, we collected plasma samples and T_B measurements from 10 field-active *E. multicarinata*. These lizards were located via active

searching in July 2013 in Sonoma County, California (precise capture locations and body-size data in Table 1). After capture, we immediately collected a blood sample and measured cloacal body temperature using a pre-calibrated thermocouple and digital thermometer. All blood samples and body temperatures were collected within 2.5 minutes of initially observing the lizards. We then measured lizard body mass and SVL, and released the lizards where they were originally observed.

Blood Collection

At each sampling time, we collected ~50 µL of blood from a lizard by piercing its post-orbital sinus with a 75 µL heparinized micro-hematocrit capillary tube (Fisher #22-362-566). After blood was drawn into the tubes by capillary action, we carefully removed the tube and applied pressure to the orbital region using a clean cotton pad until bleeding stopped (usually a few seconds). We then returned the lizards to their home enclosures (laboratory experiment) or released them (field study). Blood was usually collected within 1 minute of the onset of handling, and the entire process was usually complete within 2 minutes. We placed the blood samples on ice immediately following collection. Within 5 hours, we centrifuged the blood samples (7000 rpm for 10 minutes), then pippetted off the plasma and stored it at -80°C for later corticosterone quantification. Field-collected plasma samples were shipped to the laboratory at ISU on dry ice within 2 days of collection and stored at -80°C.

Collecting blood from the post-orbital sinus in lizards induces minimal stress (Langkilde and Shine 2006). As with other studies (e.g., Thaker et al. 2009, Calsbeek et al. 2010, Gowan et al. 2010), we observed resumption of normal behaviors (e.g., foraging,

exploring, etc.) within seconds of blood collection. For our laboratory experiments, we collected six blood samples from each lizard. To reduce the potential negative consequences of repeated blood collection, we alternated orbital sinuses at each bleeding (each sinus was bled three times in experimental lizards), leaving at least 2 weeks for recovery between consecutive blood draws from the same orbital sinus.

Quantification of Plasma Corticosterone

We quantified plasma CORT concentration using the ImmunChem Double Antibody Corticosterone I-125 RIA kit (Catalogue # 07-120103, MP Biomedical, Orangeburg, NY), as modified for squamate reptiles (Robert et al. 2009). To validate the use of this radioimmunoassay with alligator lizards, we tested for parallelism between the kit standards and serial dilutions of a pool derived from our plasma samples (hereafter "plasma pool," derived from 8 *E. multicarinata*, 2 samples from each temperature treatment). The serial dilutions of the standards and our plasma pool were parallel after logit transformation (alligator lizard: slope = -1.963, R^2 = 0.96; CORT standards: slope = -1.939, R^2 = 0.99; Fig 2), confirming the validity of quantifying plasma CORT in alligator lizards with this radioimmunoassay.

Following validation, we assayed two replicates of each sample at a 1:40 dilution. We re-assayed samples whenever the intra-assay coefficient of variation (CV) of replicate samples was > 10, or if CORT estimates were outside the bounds of the standard curve. For the former, samples were re-assayed until the intra-assay CV was ≤ 10, and for the latter, samples were further diluted until they fell within the standard curve (dilutions were accounted for when calculating final CORT concentration). During each assay, we also

quantified CORT from replicate samples of the plasma pool to calculate inter-assay variation, the CV of which was 25.96. To control for this variation, we transformed all CORT estimates prior to analyses such that the plasma-pool estimates were equal across assays.

Statistical Analyses

Laboratory Experiment

All analyses were performed using the program R (version 3.0.1, R Core Team 2013). Prior to analyses, we assessed normality graphically using boxplots, histograms, and q-q plots (Zuur et al. 2009). CORT concentration estimates were non-normally distributed, so we log-transformed these data to meet the assumptions of parametric statistics. After transformation, we used boxplots to identify outliers, which were removed (N = 5 out of 180) prior to analyses. We used generalized linear mixed-effects models (GLMM) to test for effects of treatment on CORT concentrations in *E. multicarinata* and *E. coerulea.* We examined two models: the first tested for effects of our experimental temperature treatments (10, 20, 28, and 35°C, "temperature model" hereafter) on CORT, whereas the second model compared CORT before and after ACTH challenge ("ACTH model" hereafter). Experimental treatment, species, and their interaction were included as fixed-effects in these models, and individual lizard was included as a random intercept. We selected random-intercept-only models because they modeled the data better than models that included random intercepts and slopes (ΔBIC = -20.27 and -7.90, respectively, and analyses of residuals showed better homogeneity of the random-intercept-only model in both cases). Because we found a significant interaction between species and treatment, we also tested for effects of treatment on CORT for each species separately. We used the "lme" function in the

nlme package for these analyses (Pinheiro et al. 2013). We validated the assumptions of the final models graphically by examining histograms of the residuals, and plots of the residuals vs. fitted values (Zuur et al. 2009). To assess pairwise differences in plasma CORT concentrations between the thermal treatments, the laboratory control (at ~ 23.5°C), and in response to ACTH challenge, we used *post hoc* Tukey tests (function "lsmeans" in the lsmeans package, Lenth 2013).

The *E. multicarinata* that we examined were collected from a broad geographic area and likely represent multiple populations (Table 1). While population boundaries are unclear, evidence from mitochondrial DNA suggest that *E. multicarinata* can be divided into two major clades (Feldman and Spicer 2006). Based on their geographic location at collection and the predicted distribution of the mitochondrial DNA clades from Feldman and Spicer (2006), we assigned each *E. multicarinata* to a clade. Early in our analyses, we included clade of origin as a fixed-effect factor in our models. Neither clade of origin nor its interactions significantly affected CORT in any model ($P > 0.10$ for all). We therefore removed clade of origin from the final models. In addition, models including the order that the lizards were exposed to each temperature treatment and/or sex showed that neither of these factors (nor their interactions) affected CORT concentration ($P > 0.10$ for all), so we also removed these factors from the final models. Time of day when blood-samples were collected could not be included in the models because we sampled each treatment at the same time during each experiment, thereby conflating thermal and temporal effects. Even so, all samples were collected within the same hour (although on different days), which should minimize any effect of the diel cycle on our results. Moreover, the lack of an effect of

experiment order on our results suggests that slight differences in timing of blood collection did not significantly affect our results.

While examining boxplots of the original, untransformed data, we noticed apparent variation in the dispersion of plasma CORT between the two species. We tested this hypothesis using F-tests comparing CORT variation between the two species within each treatment. For this analysis, we used our original, untransformed, CORT estimates and the "var.test" function in the base installation of R.

Field Study

As with the laboratory experiment, we log transformed the CORT estimates from our field-sampled *E. multicarinata* so that the data met the assumptions of parametric statistics. We examined concordance between our laboratory and field CORT estimates by comparing their mean and variance using a Welch's two-sample *t*-test and an *F*-test, respectively. We used log-transformed data for the *t*-test but untransformed data for the *F*-test. In addition, we tested for an effect of body temperature on plasma CORT using linear and quadratic regressions. For these analyses, we created a "laboratory" group by pooling our CORT estimates from *E. multicarinata* exposed to all four experimental temperature treatments.

RESULTS

Laboratory Experiment

Both the "temperature" and "ACTH" models found a significant interaction between species and treatment on plasma CORT concentration (temperature model: $F_{3,81} = 4.42$, $P = 0.0063$, ACTH model: $F_{1,26} = 9.30$, $P = 0.0052$, Fig 3). When analyzed separately,

experimental temperature affected CORT in both *E. multicarinata* ($F_{3,40}$ = 7.36, P = 0.0005, Fig 3A) and *E. coerulea* ($F_{3,41}$ = 8.62, P = 0.0001, Fig 3A). Generally, the relationship between temperature and CORT was similar between species: CORT displayed a parabolic, concave-up pattern in response to temperature (Fig 3). In both species, CORT was lowest at 20°C and increased with warmer temperatures, leveling off at 28°C (Fig 3A, Table 2). In *E. multicarinata*, CORT also increased as temperature cooled to 10°C, but CORT did not increase with colder temperatures in *E. coerulea* (Table 2, Fig 3A). In both species, ACTH challenge elevated CORT levels (*E. multicarinata*: $F_{1,14}$ = 150.75, P < 0.0001, *E. coerulea*: $F_{1,12}$ = 60.41, P < 0.0001) much higher than any temperature treatment, and this increase was greater for *E. multicarinata* than *E. coerulea* (Table 2, Fig 3B). We estimated, *a priori*, that the laboratory control samples represent samples from ~23.5°C (see Lizard Collection and General Laboratory Maintenance, and Experimental Protocol sections above for details). Concordant with this hypothesis, mean CORT levels in these samples fell between those from the 20°C and 28°C treatments in both species (Fig 3). When ACTH samples were collected, lizard T_B was ~22°C (see Experimental Protocol above), thus ACTH greatly elevated CORT beyond that expected from T_B alone. Whenever plasma CORT concentration differed between the species, *E. multicarinata* had higher levels than *E. coerulea* (Fig 3, Table 3). The dispersion of CORT estimates also differed between the two species (Table 4). For all treatments, untransformed CORT estimates for *E. multicarinata* were at least an order of magnitude more variable (s^2) than those for *E. coerulea* (Table 4).

Field Study

Mean T_B of the field-sampled lizards was 23.61°C (range = 19.4–29.5°C) and mean (± s.e.) plasma CORT concentration was 108.84 ± 47.84 ng/ml (Fig 4). Mean CORT concentration did not differ between *E. multicarinata* sampled in the field and laboratory (t = 0.75, df = 10.55, P = 0.4715). However, field CORT estimates were more variable than those from the laboratory (field s^2 = 22885.95, laboratory s^2 = 6716.097, $F_{9,57}$ = 3.41, P = 0.0041). Neither linear ($F_{1,8}$ = 0.169, P = 0.6918) nor quadratic (T_B: $F_{1,7}$ = 0.1479, P = 0.7119, T_B^2: $F_{1,7}$ = 0.0018, P = 0.9669) regression found a significant effect of T_B on plasma CORT in the field collected lizards (Fig 4). Even so, CORT from the field-sampled *E. multicarinata* appeared similar to, but more variable than, CORT from the laboratory lizards (Fig 4).

DISCUSSION

Temperature broadly affects the biology of ectotherms and exposure to extreme T_B can by highly detrimental, if not fatal (e.g., Cowles and Bogert 1944, Bradshaw 2003, Chown and Terblanche 2007, Angilletta 2009). An important component of the response of vertebrate ectotherms to dangerous situations is the upregulation of GC hormones such as CORT that act to initiate an emergency physiological state commonly referred to as the stress response (Selye 1950, Bradshaw 2003, Norris 2007). The stress response might be adaptive when vertebrate ectotherms are exposed to non-optimal T_B, potentially inducing animals to seek thermal shelter. Even so, the effects of temperature on CORT in vertebrate ectotherms, such as reptiles, are not well understood. Results from our experiment suggest that T_B

directly affects CORT in northern and southern alligator lizards. Temperature thus appears to be an important factor affecting the physiological stress response in these lizards.

Our measurements for the T_B of active *E. multicarinata* concord with previous observations (Brattstrom 1965, Cunningham 1966, Stewart 1984, Kingsbury 1994, Sheen 2001) confirming the biological relevance of our experimental temperature treatments. The 20°C and laboratory control [~23.5°C] treatments modeled average thermal conditions experienced by these lizards (Cunningham 1966, Stewart 1984, Kingsbury 1994, Sheen 2001), while the other treatments modelled progressively more extreme conditions. While both species have been observed with active T_B of approximately 10°C and 35°C (Cunningham 1966, Stewart 1984, Kingsbury 1994, Sheen 2001), we did not observe lizards with T_B this extreme. Even so, these treatments should represent biologically-relevant extreme temperatures that are occasionally encountered by active alligator lizards.

Elgaria multicarinata displayed generally higher CORT levels and a greater response to ACTH than *E. coerulea*, but the CORT response of each species to $T_B \geq 20$°C was similar. Because all animals were acclimated under common-garden laboratory conditions for at least 10 months prior to the onset of experiments, any species-specific differences in the CORT response likely reflect genetic or developmental differences rather than short-term/reversible phenotypic plasticity to their capture environments. In both *E. coerulea* and *E. multicarinata*, CORT levels were lowest when lizards were exposed to 20°C and increased with temperature (plateauing at 28°C). 20°C approximates, but is slightly below, the average activity temperature observed in both species (Brattstrom 1965, Cunningham 1966, Stewart 1984, Kingsbury 1994, and present study). Because prior work suggests that the optimum temperature in *E. multicarinata* is 28°C (Licht 1967, Kingsbury 1994), the result that 20°C

induced the lowest CORT levels is somewhat surprising. While 28°C maximizes ATPase activity (Licht 1967), it is possible that this is not the optimum temperature for the entire organism. However, both laboratory experiments (Licht 1964) and field observations (Kingsbury 1994) have shown that *E. multicarinata* thermoregulate to ~28°C when able. Moreover, we observed *E. coerulea* thermoregulating to approximately 28°C in the laboratory (27.7°C when the lights and heater were on). These results suggest that alligator lizards seek out T_B that induce CORT levels elevated above the minimum, but more frequently experience T_B that induce reduced CORT levels. It is also possible that lizards displayed lower CORT levels at 20°C than at 28°C simply because they had reduced metabolic rates at 20°C. At more extreme temperatures, activation of the HPA axis may override this basal metabolic effect, inducing elevated CORT at extreme cold and warm temperatures.

Because CORT was elevated, our results suggest that 5 hr exposure to temperatures ≠ 20°C in *E. multicarinata* and > 20°C in *E. coerulea* challenge homeostasis. However, no temperature treatments induced CORT levels as high as ACTH challenge in either species. Temperature treatment therefore failed to induce a maximal CORT response from the HPA axis (Romero and Wingfield 1999, Romero and Wikelski 2006, Phillips and Klukowski 2008, Klukowski 2011). Given that alligator lizards can be observed active at all of our experimental temperatures in nature (Brattstrom 1965, Cunningham 1966, Stewart 1984, Kingsbury 1994) and they had reactive scope for further CORT response to additional stressors, the CORT response that we observed likely allowed maintenance of reactive homeostasis (á la Romero et al. 2009). Even so, longer exposure to 10°C or 35°C, or

exposure to even more extreme temperatures, can be fatal and thus likely challenges homeostasis beyond the counteracting ability of CORT.

CORT levels increased with temperature above 20°C in both species, but only *E. multicarinata* displayed increased CORT levels at 10°C. This result supports the hypothesis that the thermal CORT response is adapted to the respective thermal environments of these species. Exposure for a longer period or to a colder temperature might significantly elevate CORT in *E. coerulea* as in *E. multicarinata.* Even so, variation in the thermal CORT response could partially explain the biogeographic differences between *E. coerulea* and *E. multicarinata*. Even if 5 hr. exposure to 10°C does not stress *E. multicarinata*, per se, maintaining a CORT response is energetically expensive and many features of this response (e.g., inhibition of foraging or courtship) incur additional costs (Bradshaw 2003, Norris 2007, Nelson 2011). Because *E. multicarinata* and *E. coerulea* are ecologically similar (Brattstrom 1965, Stebbins 2003), they presumably are under intense competition with each other. Competitive exclusion would explain why, even though their tolerances are similar, these species are rarely found at the same location (e.g., Hutchinson 1959, Armstrong and McGehee 1980). If *E. multicarinata* incur CORT-induced costs at cold temperatures and *E. coerulea* do not, presumably as a result of general adaptation to cooler environments, then *E. coerulea* will be competitively superior in these environments. The potential effect of the thermal CORT response on the competitive landscape also has implications for how species are affected by climate change. In species where temperature affects CORT, exposure to novel thermal environments will alter the physiological stress experienced by populations. Subtle species-specific differences in the stress response might affect the competitive

landscape sufficiently that species go locally extinct through displacement well before environmental temperatures exceed critical thermal limits.

In addition to having higher mean CORT levels in our laboratory experiment, *E. multicarinata* displayed more variation in CORT than *E. coerulea*. If this variation is heritable and additive, the CORT response of *E. multicarinata* to temperature will have greater evolutionary potential than that of *E. coerulea* (Falconer and Mackay 1996). This could have important implications for the ability of these animals to invade new thermal habitats or to respond to impending climate change. An alternate explanation for the difference in variance that we observed is that these variances reflect how the animals were sampled. Most of the *E. coerulea* examined were collected from a single site, whereas the *E. multicarinata* examined were collected over a broad geographic range, and thus might be expected to be more variable. However, *E. multicarinata* clade of origin (Feldman and Spicer 2006) did not affect CORT, suggesting that the CORT response has not diverged among *E. multicarinata* clades. Furthermore, the *E. multicarinata* that we assayed for our field study displayed greater CORT variation than those from our laboratory experiment, even though the field-study lizards were sampled from a single county. These observations suggest that natural populations of *E. multicarinata* display high variance in their CORT response.

While our experimental data demonstrate an effect of T_B on CORT, we did not detect a correlation between T_B and CORT in field-active *E. multicarinata.* This lack of correlation likely resulted from the small sample size of our field study and the high variance of CORT in *E. multicarinata*. Moreover, many additional factors can affect CORT in the field (e.g., time of day, nutritional status, previous predator encounters, etc, Bradshaw 2003, Romero

2004, Nelson 2011) but were controlled in the laboratory. It is therefore not surprising that we were unable to detect a correlation between T_B and CORT in the field. Even so, mean CORT of *E. multicarinata* in the field and laboratory did not differ and the variance that we observed in the laboratory was within the bounds observed in the field. These observations suggest that our laboratory CORT results likely apply to natural populations.

Our data provide evidence that CORT and T_B are causally related in reptiles and not simply correlated (e.g., Fig 1). In other reptile species, experimentally elevated CORT alters thermoregulatory behavior, either increasing heat seeking (Belliure et al. 2004, Preest and Cree 2008) or cooling behaviors (Belliure and Clobert 2004), depending on species. By contrast, we demonstrate that T_B directly affects CORT levels in *E. multicarinata* and *E. coerulea*. T_B also affects CORT in garter snakes (Schwartz and Bronikowski 2013) and Children's pythons (Dupoué et al. 2013). The causal pathway between CORT and T_B might thus function in both directions in reptiles. If so, this could represent an important regulatory feedback loop that could partially control thermoregulation. Exposure to extreme T_B could increase CORT thereby inducing thermoregulatory behaviors (heating or cooling) as appropriate. This might generally be a negative feedback loop, with high T_B inducing cooling behaviors through elevation in CORT. Alternatively, a positive feedback loop might help maintain high CORT levels during energetically demanding periods, such as the reproductive season. Given the effects of CORT and T_B on metabolism (Squires 2003, Norris 2007, Preest and Cree 2008, Nelson 2011), such a system might maximize the availability of free energy. Further work is necessary to understand how T_B, CORT, and thermoregulatory behavior interact, and the potential importance of this interaction as a regulatory mechanism.

To conclude, T_B and CORT are causally related in northern and southern alligator lizards. The exact relationship between T_B and CORT is species specific and the thermal CORT response appears to be adapted to the thermal environment of each species. Physiological stress likely plays an important role in the thermal ecology of these species and may affect the outcome of their competitive interactions in different thermal environments. Although poorly explored to date, causal relationships between T_B and CORT may be common in vertebrate ectotherms. If so, the thermal CORT response of species will affect how they are geographically distributed and how they respond to impending climate change.

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TABLES

Table 1—Capture and body size data for the lizards used in the current experiment. *Elgaria multicarinata* is denoted by *E.m.* and *Elgaria coerulea* is denoted by *E.c.* Coordinates are based on the WGS84 datum. All lizards were collected in the State of California and their County of collection is given. Snout-vent length (SVL) is in mm and mass is in g. For lizards used in the laboratory experiments, sex was determined by gonadal examination of euthanized individuals, and SVL and mass were recorded in February 2012 when lizards were removed from hibernation prior to experiments. For lizards used for field measurements, a hypothesized sex is provided based on external characteristics, and SVL and mass were recorded at the time of capture.

Species	Capture Date	Latitude	Longitude	County	Sex	SVL	Mass
Laboratory experiment specimens							
E.m.	July 2010	37º07'55"N	122^{0}10'19"W	Santa Cruz	M	127	31.46
E.m.	July 2010	37º07'55"N	122^{0}10'19"W	Santa Cruz	M	133	32.96
E.m.	July 2010	39º44'27"N	121^{0}28'45"W	Butte	F	130	32.78
E.m.	July 2010	38º46'01"N	120^{0}41'40"W	El Dorado	M	125	30.69
E.m.	July 2010	37º23'05"N	122^{0}11'24"W	Santa Clara	M	110	23.82
E.m.	June 2011	36º38'11"N	121^{0}47'02"W	Monterey	M	103	16.02
E.m.	June 2011	36º38'11"N	121^{0}47'02"W	Monterey	M	121	24.24
E.m.	June 2011	36º38'05"N	121^{0}47'13"W	Monterey	F	125	16.9
E.m.	June 2011	36º36'10"N	121^{0}47'14"W	Monterey	M	110	21.61
E.m.	June 2011	36º38'11"N	121^{0}47'11"W	Monterey	M	97	12.15
E.m.	June 2011	36º38'14"N	121^{0}46'56"W	Monterey	M	113	18.06
E.m.	June 2011	36º38'05"N	121^{0}47'12"W	Monterey	F	120	23.48

Table 1 Continued

Species	Capture Date	Latitude	Longitude	County	Sex	SVL	Mass
Laboratory experiment specimens							
E.m.	June 2011	36º38'14"N	121^{0}46'57"W	Monterey	M	115	18.65
E.m.	July 2011	37º04'04"N	122^{0}03'06"W	Santa Cruz	M	128	26.67
E.m.	May 2011	NA	NA	San Mateo	M	132	33.05
E.c.	July 2010	40º04'44"N	121^{0}33'33"W	Butte	F	114	23.34
E.c.	July 2010	36º59'46"N	121^{0}53'46"W	Santa Cruz	F	110	32.15
E.c.	June 2010	37º23'46"N	122^{0}17'37"W	San Mateo	F	112	26.36
E.c.	June 2010	37º23'15"N	122^{0}15'57"W	San Mateo	M	109	24.39
E.c.	July 2010	39º52'06"N	121^{0}10'24"W	Plumas	F	107	20.19
E.c.	June 2011	37º04'56"N	121^{0}51'08"W	Santa Cruz	F	108	17.67
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	F	106	17.26
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	F	115	21.02
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	M	88	11.5
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	M	98	14.23
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	F	105	15.36
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	F	100	14.02
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	M	106	17.09
E.c.	June 2011	37º05'21"N	121^{0}53'25"W	Santa Cruz	M	109	21.51
E.c.	June 2011	36º59'26"N	121^{0}48'17"W	Santa Cruz	F	108	19.37
Field specimens							
E.m.	July 2013	38º13'20"N	122º37'43"W	Sonoma	F	105	15.5
E.m.	July 2013	38º12'54"N	122º37'54"W	Sonoma	M	93	13
E.m.	July 2013	38º12'34"N	122º38'35"W	Sonoma	M	100	15
E.m.	July 2013	38º12'37"N	122º38'31"W	Sonoma	?	70	9.5
E.m.	July 2013	38º12'40"N	122º38'26"W	Sonoma	M	130	36
E.m.	July 2013	38º12'40"N	122º38'26"W	Sonoma	M	85	9
E.m.	July 2013	38º12'40"N	122º38'26"W	Sonoma	F	118	27.5
E.m.	July 2013	38º12'45"N	122º37'07"W	Sonoma	M	71	9

Table 1 Continued

Species	Capture Date	Latitude	Longitude	County	Sex	SVL	Mass
Field specimens							
E.m.	July 2013	38º12'54"N	122º37'55"W	Sonoma	M	127	41
E.m.	July 2013	38º12'54"N	122º37'55"W	Sonoma	M	60	5.5

Table 2—Matrix of results from between-treatment pairwise comparisons of plasma corticosterone levels in northern and southern alligator lizards (*Elgaria coerulea* and *E. multicarinata*). Untransformed mean (± s.e.) plasma corticosterone concentrations (ng/ml) for each treatment are presented on the diagonals (shaded regions). *Z*-scores and *P*-values from pairwise tests are displayed below and above the diagonals, respectively. While exact, uncorrected *P*-values are presented, bold-font indicates significant differences ($P < 0.05$) after a Tukey correction for multiple comparisons. Control indicates laboratory control samples and ACTH indicates samples after adrenocorticotrophic hormone challenge (lizard T_B's were approximately 23.5°C and 22°C, respectively).

Treatment	10C	20C	28 C	35 C	Control	ACTH
Elgaria coerulea						
10C	9.3 ± 1.3	0.41693	**0.00776**	0.10116	0.97191	**<0.00001**
20C	0.814	9.0 ± 2.0	**0.00039**	**0.01241**	0.44838	**<0.00001**
28C	-2.66	-3.54	21.1 ± 3.0	0.29652	**0.00829**	**0.00001**
35C	-1.641	-2.50	1.04	14.9 ± 1.9	0.10115	**<0.00001**
Control	0.04	-0.76	-2.64	-1.64	8.8 ± 1.0	**<0.00001**
ACTH	-7.10	-8.07	-4.53	-5.57	-6.98	85.3 ± 17.9
Elgaria multicarinata						
10C	89.0 ± 31.5	**0.00001**	0.65025	0.01005	0.18452	**<0.00001**
20C	4.49	14.1 ± 5.5	**0.00005**	0.04303	**0.00129**	**<0.00001**
28C	0.45	-4.06	68.71 ± 23.0	0.03393	0.38234	**<0.00001**
35C	2.57	-2.02	2.12	23.5 ± 3.9	0.21221	**<0.00001**
Control	1.33	-3.22	-0.87	1.25	54.2 ± 22.2	**<0.00001**
ACTH	-9.93	-14.01	-10.38	-12.50	-11.26	765.0 ± 156.7

Table 3—Results from pairwise comparisons of plasma corticosterone between northern and southern alligator lizards (*E. coerulea* and *E. multicarinata*) at each experimental treatment level. *Z*-values and *P*-values are shown. While exact, uncorrected *P*-values are presented, bold-font indicates significant differences ($P < 0.05$) after a Tukey correction for multiple comparisons. Control indicates laboratory control samples and ACTH indicates samples after adrenocorticotrophic hormone challenge (lizard T_B's were approximately 23.5°C and 22°C, respectively). Untransformed means (± s.e.) for each species and treatment level are given in Table 2.

Treatment	*Z*	*P*
10C	-4.36	**0.00001**
20C	-1.22	0.22294
28C	-1.82	0.06913
35C	-0.93	0.35007
Control	-3.26	**0.00110**
ACTH	-6.62	**< 0.00001**

Table 4—Dispersion of plasma corticosterone concentrations in southern and northern alligator lizards (*Elgaria multicarinata* [*E.m.*] and *E. coerulea* [*E.c.*]) exposed to each experimental treatment. Sample variance (s^2) and results from *F*-tests for equal variances with untransformed data are displayed. Control indicates laboratory control samples and ACTH indicates samples after adrenocorticotrophic hormone challenge (lizard T_B's were approximately 23.5°C and 22°C, respectively). Significant *P*-values (< 0.05) are in bold font.

Treatment	*E.m.* s^2	*E.c.* s^2	*F*-value	*d.f.*	*P*-value
10C	14873.9	23.7	627.82	14,13	**< 0.00001**
20C	386.5	60.5	6.39	12,14	**0.00161**
28C	7957.0	132.5	60.07	14,14	**< 0.00001**
35C	226.8	51.4	4.41	14,14	**0.00886**
Control	7368.0	12.5	587.92	14,12	**< 0.00001**
ACTH	368069.1	4777.6	77.04	14,14	**< 0.00001**

FIGURES

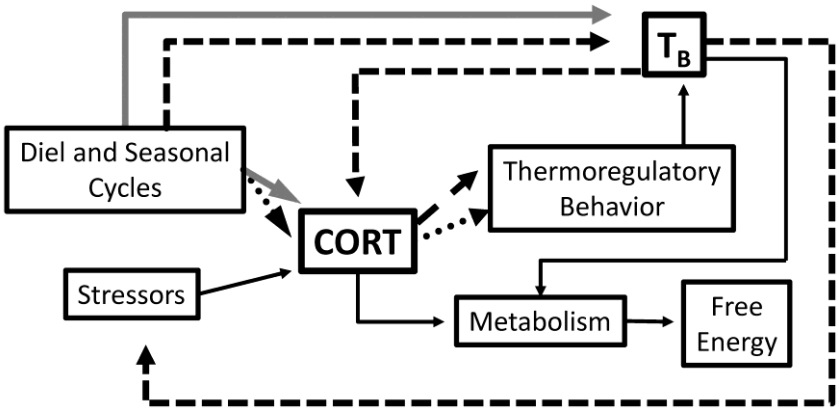

Figure 1—Concept map of the potential causal relationships explaining correlations between plasma corticosterone concentration (CORT) and body temperature (T_B) in vertebrate ectotherms. The thin solid lines represent well-supported relationships. The grey lines represent hypothesis 1: diel and seasonal cycles affect both CORT and T_B independently. The dotted lines represent hypothesis 2: CORT affects T_B through its effects on thermoregulatory behavior. The dashed lines represent hypothesis 3: T_B directly affects CORT (either baseline or stress levels), and this represents a pathway whereby temperature affects metabolic rate. Importantly, many of the connections proposed under these hypotheses are not mutually exclusive. Free energy refers to energy available for immediate use by the organism.

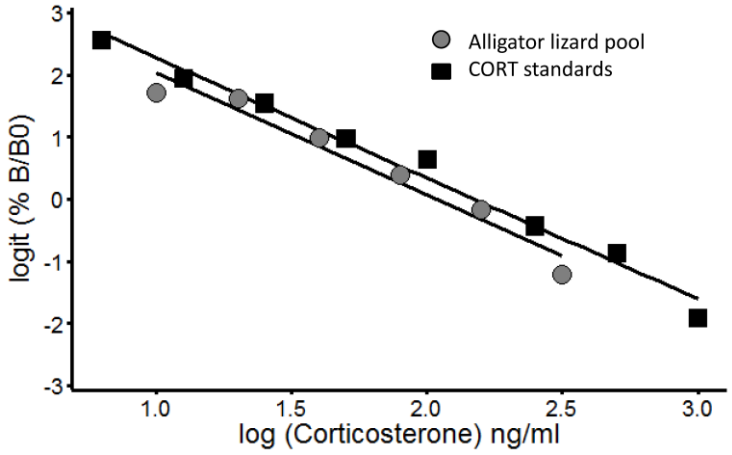

Figure 2—Parallelism plot comparing the sensitivity of the radioimmunoassay with corticosterone (CORT) standards and a serially diluted pool of southern alligator lizard (*Elgaria multicarinata*) samples. Samples were plotted on a logit-log scale to linearize them.

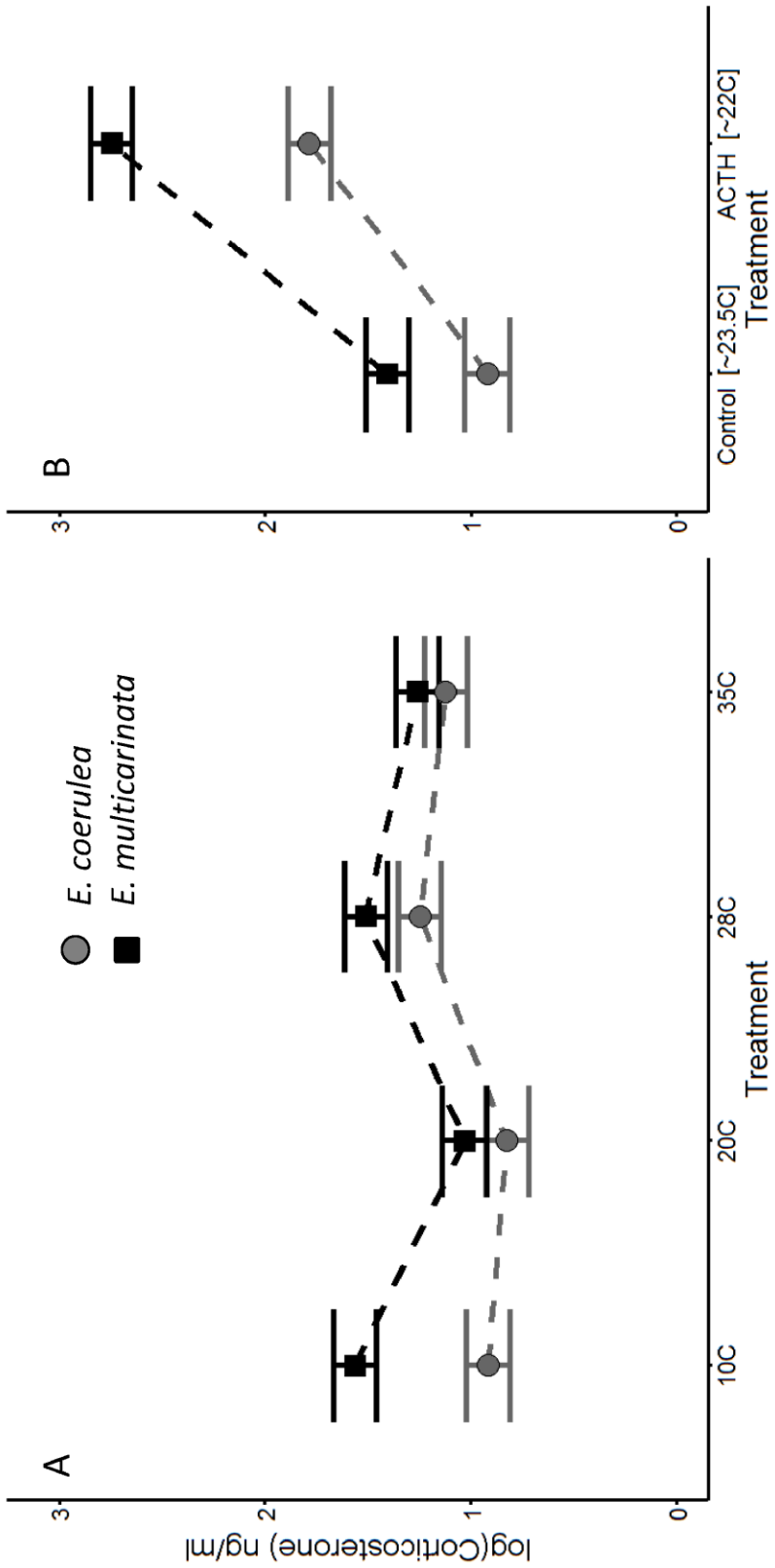

Figure 3—Treatment effects on plasma corticosterone (CORT) concentrations in northern and southern alligator lizards (*Elgaria coerulea* and *E. multicarinata*). Least-squares means ± standard errors are displayed from A) the experimental temperature treatments and B) the laboratory control and ACTH treatments (lizard T_B's were approximately 23.5°C and 22°C for these treatments, respectively). Each model also included species and the interaction between treatment and species as fixed effects, and individual as a random effect. Data are from log-transformed CORT estimates. Results from pairwise tests are given in Tables 2 and 3.

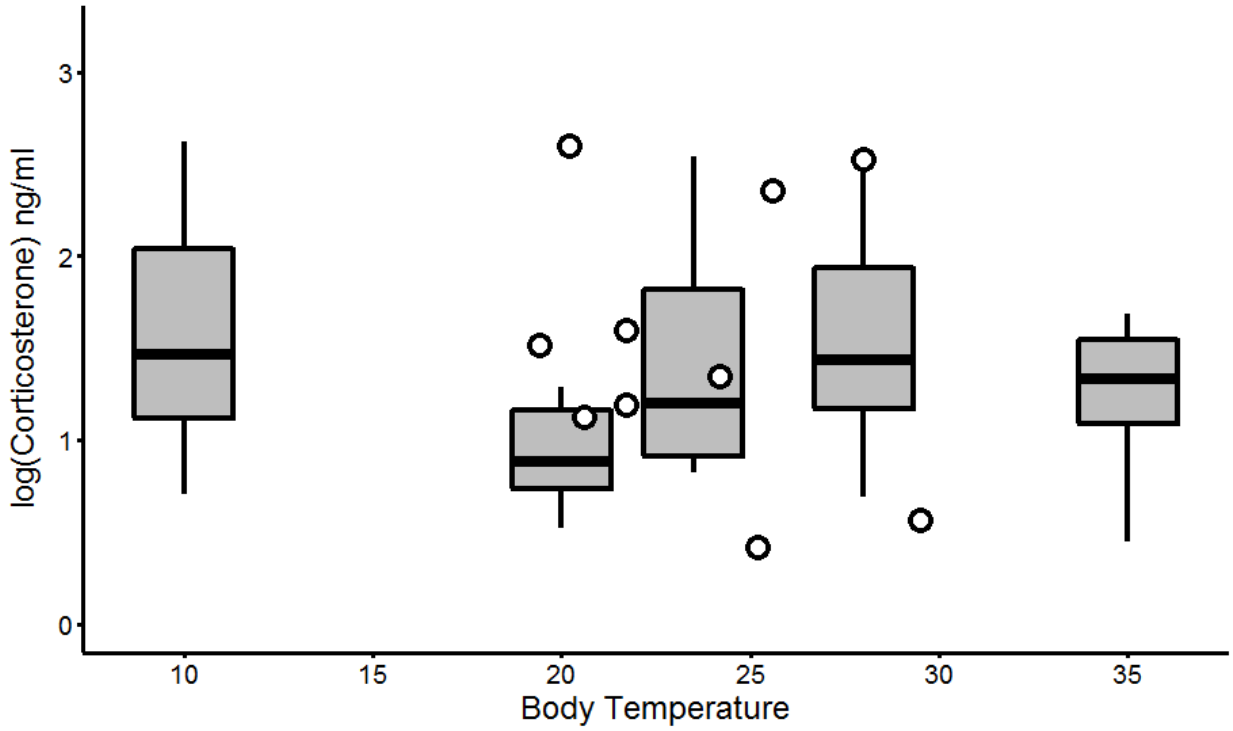

Figure 4—Effect of body temperature on CORT in southern alligator lizards (*Elgaria multicarinata*) from the laboratory and field. Boxplots represent CORT estimates from our laboratory experiment, including the ~23.5°C laboratory control. The lines within the boxes represent medians, box limits depict the first and third quartiles, and box whiskers are 1.5 times the interquartile range. The overlaid scatterplot represents the relationship between CORT and T_B of 10 active lizards in the field.

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CHAPTER 4

IMMOBILE AND MOBILE LIFE-HISTORY STAGES HAVE DIFFERENT THERMAL PHYSIOLOGIES IN A LIZARD

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ABSTRACT

Temperature affects multiple aspects of an organism's biology, and thus defines a major axis of the fundamental niche. For ectotherms, variation in the thermal environment is particularly important because most of these taxa have a limited capacity to thermoregulate via metabolic heat production. While temperature affects all life-history stages, stages can differ in their ability to respond to the thermal environment. For example, in oviparous organisms, free-living adults can behaviorally thermoregulate whereas developing embryos are at the mercy of the nest environment. These differences in the realized thermal environment should select for life-history stages to have different thermal tolerances, although this has been rarely examined. I tested the hypothesis that stage-specific thermal reaction norms can evolve independently using southern alligator lizards (*Elgaria multicarinata*, Anguidae). Using incubation experiments (five temperatures: 24, 26, 28, 30, and 32 ºC), I described the thermal reaction norm for embryonic development and compared

these results to previous studies on the thermal ecology of adults. Offspring survivorship and morphology were similarly affected by incubation temperature. While developing embryos had the same optimum temperature as adults (approximately 28 ºC), the breadth of their thermal reaction norms differed. My results suggest that developing embryos of *E. multicarinata* are more sensitive to variation in the average thermal environment than are adults. Variation in the thermal sensitivity of life-history stages might be common and has implications for how organisms respond to variation in the thermal environment. Identifying those life-history stages that are most sensitive/limiting will be important for developing models that best predict species' responses to impending environmental change.

*Keywords***:** alligator lizard, Anguidae, biogeography, developmental reaction norm, *Elgaria multicarinata,* incubation, temperature

INTRODUCTION

Temperature defines a major component of the fundamental niche for many species (Cowles and Bogert 1944, Hutchinson 1957, Angilletta 2009, Jackson et al. 2009). In ectotherms, virtually all biological processes are affected by the thermal environment (e.g., metabolism, physiology, and development, Cowles and Bogert 1944, Huey and Stevenson 1979, Huey 1982, Angilletta et al. 2002b, Chown and Terblanche 2007, Hoffmann 2010). Thus, environmental thermal variation has major ecological and evolutionary consequences (Angilletta 2009, Jackson et al. 2009, Clusella-Trullas et al. 2011). The relationship between temperature and organismal performance in ectotherms can generally be described by a left-skewed, hump-shaped reaction norm: performance increases with temperature until an

optimum temperature is reached, above which performance rapidly declines (Huey and Stevenson 1979, Huey and Kingsolver 1989, Angilletta 2009). Both the optimum temperature and the breadth of the thermal reaction norm might evolve (reviewed by Huey and Kingsolver 1989, Angilletta et al. 2002b), with selection predicted to drive the optimum performance temperature to mirror the average temperature in the environment, and the breadth of the performance curve to increase with environmental thermal variance (Huey and Kingsolver 1993, Gilchrist 1995, Angilletta et al. 2002b, Kingsolver and Gomulkiewicz 2003).

Ectotherms frequently experience different environmental conditions across their ontogeny. For example, in species with complex life histories (e.g., holometabolous insects and amphibians), the egg, larval, pupal (in insects), and adult stages may fundamentally differ in mobility, size, and habitat. Even in species with simpler life histories (e.g., hemimetabolous insects, fish, and reptiles), size and mobility generally change across ontogeny. Such variation in phenotype and habitat expose life-history stages to different thermal environments, and these differences are predicted to select for dissimilar thermal reaction norms at each life-history stage (Kingsolver and Gomulkiewicz 2003, Zani et al. 2005, Marais and Chown 2008, Miller et al. 2013). Mobile and immobile life-history stages, in particular, should experience distinct selective regimes due to the fundamental difference in how these stages interact with the thermal environment. Mobile stages can thermoregulate behaviorally by moving among microclimates (Vitt et al. 1996, Huey et al. 2003, Chown and Terblanche 2007), often enabling individuals to maintain body temperatures near their physiological optima during activity periods (Avery 1982, Huey 1982, Christian et al. 2006). By contrast, immobile stages, such as eggs, are generally at the mercy of the nest

environment (Bowler and Terblanche 2008, Refsnider and Janzen 2010, Woods 2013). Free-living and embryonic stages therefore differ greatly in their ability to maintain body temperatures near a thermal optimum. Because immobile stages should be exposed to a broader range of temperatures, they are predicted to be under selection for broader reaction norms (potentially with lower optima) than mobile stages (Huey and Kingsolver 1989, Angilletta et al. 2002b, Zani et al. 2005). However, thermal sensitivity might be genetically correlated across life-history stages and thus unable to evolve independently (Falconer and Mackay 1996, Kingsolver and Gomulkiewicz 2003). If the stages are not free to adapt to their specific thermal conditions, thermal tolerances at one stage could limit the habitats occupied by other stages (Chown and Terblanche 2007, Briscoe et al. 2012, Radchuk et al. 2013).

Most analyses of thermal sensitivity in ectotherms have focused on a single life-history stage, with ontogenetic changes in thermal sensitivity largely unexplored (e.g., Dawson and Templeton 1966, Nice and Fordyce 2006, Pörtner and Knust 2007, Buckley 2008). Variation in thermal sensitivity among life-history stages has been best examined in insects, which generally show large changes in thermal sensitivity across ontogeny (Zani et al. 2005, Chown and Terblanche 2007, Bowler and Terblanche 2008). Moreover, ontogenetic changes in thermal sensitivity have implications for insect biogeography, with the geographic distribution of species limited by a subset of life-history stages (Briscoe et al. 2012, Radchuk et al. 2013). The breadth of the thermal reaction norm generally decreases with age in insects such that earlier stages are more thermally tolerant than later stages, as predicted by theory (reviewed in Bowler and Terblanche 2008). However, this pattern is not

universal (e.g., Krebs and Loeschcke 1995) and its applicability to non-insect ectotherms (particularly vertebrates) is uncertain.

In general, oviparous reptiles are highly mobile after hatching and are able to behaviorally thermoregulate (Avery 1982, Huey 1982, Vitt et al. 1996, Christian et al. 2006). In contrast, developing embryos are confined to immobile eggs and largely at the mercy of the nest environment (Ackerman and Lott 2004, Refsnider and Janzen 2010), although limited behavioral thermoregulation within the egg has been suggested (Du et al. 2011, Zhao et al. 2013). Thus, the free-living and embryonic stages of oviparous reptiles likely differ greatly in their ability to maintain body temperatures near a thermal optimum. Such differences in response to the thermal environment have likely played an important role in reptile evolution and biogeography– for example, substantial evidence suggests that viviparous reptiles can exist in colder climates than oviparous reptiles because immobile eggs perish when exposed to cold conditions (reviewed in Shine and Bull 1979, Shine 2005, Blackburn 2006). Behavioral thermoregulation allows viviparous females to increase the temperatures to which their developing embryos are exposed and thus persist (e.g., Shine 1995, Shine 2002). Similar to insects (Briscoe et al. 2012, Radchuk et al. 2013), mismatches between the thermal tolerances of life-history stages might have major biogeographical implications for reptiles, with range limits being set by tolerances within a subset of stages.

I tested the hypothesis that vertebrate ectotherms exposed to different thermal conditions across their ontogeny evolve stage-specific thermal reaction norms using southern alligator lizards (*Elgaria multicarinata*; Anguidae, Blainville 1835). The thermal ecology of free-living/adult *E. multicarinata* has been well studied: adults are thermal generalists, best described as facultative thermoregulators, that are active at a broad range of body

temperatures in nature (Cunningham 1966, Dawson and Templeton 1966, Kingsbury 1994). While mean active body temperatures in the field are approximately 23–24 ºC ($\bar{x}$ ± 1.0 s.d. = 21.1 ± 6.22 [Cunningham 1966], 23.8 ± 1.90 [Kingsbury 1994], and 23.9 ± 6.5 [Sheen 2001]), these lizards have been observed with active field body temperatures ranging from 5–36 ºC (4.9–35.7 [Cunningham 1966], 10.0–34.0 ºC [Kingsbury 1994]). Adult *E. multicarinata* maintain activity at temperatures substantially cooler than those tolerated by most sympatric lizards (Cunningham 1966, Dawson and Templeton 1966). This broad activity range is unusual among squamate reptiles (snakes and lizards), which usually maintain narrow body temperatures around a thermal optimum during activity (Huey 1982, Christian et al. 2006). Alligator lizards are hypothesized to maintain such broad and cool active body temperatures as an adaptation to living in dense, shaded habitat, which has limited opportunity for behavioral thermoregulation (Kingsbury 1994). Even so, *E. multicarinata* bask in nature (Kingsbury 1994), and thermoregulate to ~28 ºC in the field and laboratory (Cunningham 1966, Dawson and Templeton 1966, Kingsbury 1994). A body temperature of 28ºC also optimizes ATPase activity in this species (Licht 1967). Together, these data suggest that ~28 ºC is the optimum temperature for performance in adult *E. multicarinata*.

Using constant-temperature incubation experiments, I described the thermal reaction norm for embryonic development in *E. multicarinata*. I assessed whole-organism performance in response to temperature during development by examining offspring survival, morphology, and running speed. I used these data to test the hypothesis that the thermal reaction norm (both optimum temperature and breadth) is conserved across the

embryonic and adult stages in *E. multicarinata*, and discuss the potential biogeographic implications of my results.

Materials and Methods

Eggs were obtained from wild-caught southern alligator lizards collected during the 2010 and 2011 activity seasons (3 collection trips: 1 May–8 May 2010 to southern California, 2 July–13 July 2010 to central California, and 14 June–4 July 2011 to central California; five additional lizards were collected in southern California by a volunteer in June 2010, see Table 1 for detailed collection location information). Lizards were collected by hand via active searching in appropriate habitat, primarily from under rocks, logs, or anthropogenic debris. *Elgaria multicarinata* do not display strong sexual dimorphism- the sexes are very similar in external character and females frequently possess enlarged hemiclitores that are easily confused with male hemipenes (personal observation). In addition, abdominal palpation cannot be effectively used to ascertain gravidity because the dermal ossicles on the ventrum of these lizards obstruct feeling the eggs. Therefore, it was not possible to determine sex or reproductive condition in the field, and all adult lizards collected ($N = 51$) were transported to the laboratory at Iowa State University (ISU).

In the laboratory, lizards were housed individually in conditions suitable for oviposition. Enclosures consisted of plastic containers with mesh tops (33 cm long x 20 cm wide x 14.5 cm tall) each outfitted with a plastic hide (14 cm diameter x 2 cm tall), water dish, and nest box (14.5 cm long x 14.5 cm wide x 4.5 cm tall). Each nest box was half filled with a 1:1 mixture of moist vermiculite and peat moss, and had a small opening in the top (~ 2cm square) for lizard access. The enclosures were illuminated with ReptiSun 5.0 UVB

bulbs (ZooMed Inc.) set on a 12hr light cycle. Additional room lights turned on 1hr before and 1hr after enclosure lights. Lizard enclosures were kept in rooms maintained at approximately 22 ºC. In addition, Flex Watt heat tape (7.6 cm wide) under the rear portion of each enclosure maintained a surface-heat gradient within the enclosures ranging from 26–32 ºC for 8 hrs/d (middle of the 12 hr light cycle) to allow behavioral thermoregulation. Lizards were fed crickets dusted with reptile vitamins (1:1 mixture Exo Terra calcium and multi-vitamin powder supplements) twice per week, standing water was provided *ad libitum*, and enclosures were misted with water daily.

Nest boxes were visually inspected for eggs twice per day. Eleven lizards oviposited, producing a total of 119 eggs (see Table 1 for maternal collection and reproductive data). Similar to previous captive studies, females appeared to stay with their eggs and potentially egg guarded (Langerwerf 1981, Greene et al. 2006). As in most squamate reptiles, *E. multicarinata* oviposit when their embryos have completed approximately the first third of development (using the criteria of Dufaure and Hubert [1961] embryos are between stages 29 and 31, Sheen 2001). As soon as eggs were discovered, they were removed from nest boxes, weighed, and placed individually in 140-ml glass jars approximately 2/3 filled with moist vermiculite (water potential = -150 kPa). Eggs were gently pressed into the vermiculite such that they were 1/2–2/3 buried. Throughout this process, care was taken to not roll the eggs. Each jar was then covered in clear plastic wrap and sealed with a rubber band to prevent evaporation (Warner et al. 2012). Eggs from each clutch were evenly distributed among temperature treatments in cabinet incubators. In 2010, eggs (N = 81) were placed in one of three constant temperature treatments: 26.0, 28.0, and 30.0 ºC. These temperatures were chosen because previous work suggested that development progresses well at 27–28 ºC

(Langerwerf 1984) and because the optimum temperature for adult *E. multicarinata* is ~28 ºC (Dawson and Templeton 1966, Licht 1967). Eggs produced in 2011 (N = 38) were divided among these temperature treatments and two new treatments, 24.0 and 32.0 ºC, to test the effects of extreme temperatures on development. Temperatures within each incubator were confirmed with iButton thermal data loggers (Maxim Integrated, San Jose, CA.) placed in the incubators throughout the duration of the experiment. To control for thermal gradients within the incubation chambers, egg position within each chamber was rotated three times per week. Eggs were checked daily for hatching.

At hatching, I removed juveniles from their incubation jars and measured their morphology (snout-vent length [SVL] and tail length [TL] to the nearest 0.5mm, and mass to the nearest 0.0001 g). Hatchlings were then permanently marked via toe clipping and housed in groups of five in conditions otherwise identical to those described above for adults. Initially, hatchlings were uniformly/haphazardly assigned to housing groups as they hatched (i.e., if three individuals hatched on the same day, each would be assigned a different housing group haphazardly). However, to minimize potential competition/aggression, I also attempted to keep lizards size-matched. Therefore, I began 2–4 housing groups at a time, uniformly filled those with hatchlings, after which I would initiate another set of housing groups. This allowed individuals of approximately the same age (and size) to be maintained together. Because incubation treatment affected hatching date (see Results for details), groups tended to consist of individuals from the same treatment. Generally, lizards stayed in their initial group throughout the experiment. However, in a few instances, size disparities became apparent as the animals grew or there were signs of aggression at feeding and the

offending lizards were reassigned to new housing groups such that similar-sized animals were housed together.

At 7- and 30-days of age, hatchling morphology was re-measured, and racing trials were used to quantify running performance. Measuring individuals at multiple time points allowed me to determine whether or not the effects of incubation temperature on hatchlings persisted over time, or displayed delayed onset. For racing trials, I raced hatchling lizards at each time point down a 1-m racetrack 3 times with a minimum 15-min rest between successive runs. Lizards were "chased" with a paint brush to stimulate flight, and the time that it took lizards to run the track was measured using infrared motion sensors positioned every 25-cm on the racetrack (Elphick and Shine 1998, Telemeco et al. 2011). For analyses, I calculated mean 1-m and mean fastest 25-cm speeds for each lizard (estimates of long-distance speed and sprint speed, respectively).

Hatchlings from the 2010 cohort were maintained in the laboratory until Dec. 2012 when any individuals that had not already died were euthanized by lethal injection of sodium pentobarbital. Hatchlings from the 2011 cohort were euthanized by decapitation for use in other analyses after measurement at 30 days of age.

Statistical Analyses

Because female lizards were collected from a broad geographic area (Table 1), I first examined the effects of maternal region of origin on offspring survivorship and phenotype. Females were assigned to groups based on whether they were derived from the "Northern" or "Southern" mitochondrial clade as described by Feldman and Spicer (2006). Models that included the region of maternal origin generally did not show a significant effect of this

factor and these models never outperformed models that instead incorporated maternal identity (assessed using p-values and AIC). Therefore, maternal identity was included in final models rather than region of origin.

To test the hypothesis that incubation temperature affected survivorship, I used mixed-effects general linear models (GLM) with a binomial distribution (i.e., logistic regression). I examined the effects of incubation temperature on survivorship from oviposition to hatching, hatching to 30-d post hatching, and the entire period from oviposition to 30-d post hatching. Temperature was included as a fixed effect while maternal identity was included as a random effect (intercept only, models including random intercept and slope failed to converge). Eggs belonging to clutches from which no eggs hatched were removed from analyses.

I tested the effects of incubation temperature on offspring morphology and speed at each measurement age (hatching, 7 days, and 30 days) using Euclidean-distance based, nonparametric multivariate analyses of variance (NP-MANOVA, also known as permutational MANOVA, see Anderson 2001 for details). NP-MANOVA was used because this analysis is robust to small sample sizes and because the data failed to meet the assumptions of parametric statistics. Both incubation temperature and maternal identity were included as fixed factors in these analyses, and SVL was included as a covariate in the analyses of speed. Offspring sex was not included in analyses because sex cannot be determined non-lethally in juvenile *E. multicarinata* and because previous work shows this species does not display temperature-dependent sex determination (see Appendix B). The dependent variable "morphology" was composed of SVL, TL, and mass, and "speed" was composed of mean fastest 25-cm speed and mean 1-m speed. Mass was cube-root

transformed to ensure that all aspects of "morphology" were in commensurate units. In addition, many measures within "morphology" required log-transformation to normalize their distribution. To ensure commensurate units and make the distributions as normal as possible, SVL, TL, and mass were each log-transformed. Statistical significance for NP-MANOVAs was determined using 10,000 permutations. The 24ºC level was dropped from analyses of "morphology" and "speed" at 30d of age because only one individual from this treatment survived to this measurement point. When NP-MANOVAs suggested a significant effect of temperature, I further examined the effects of temperature and maternal identity on each component of "morphology" or "speed" individually using NP-ANOVA (significance determined using 10,000 permutations). When NP-ANOVA suggested significant effects of temperature, I examined pairwise differences using permutational pairwise tests (10,000 permutations).

To further explore the effects of incubation temperature on morphology, I analyzed the effect of temperature on offspring body condition at hatching, 7, and 30 days of age. I used the residuals from regressions of body mass on SVL (each log-transformed) at each time point as proxies for body condition (larger values indicate a greater than predicted mass for a given length, Jakob et al. 1996, Cox et al. 2010). Histograms and q-q plots suggest that these residuals were normally distributed. I therefore analyzed the effects of temperature on body condition at each time point using mixed-effect model ANOVAs with mothers as random effects (random intercept only- models including random intercepts and slopes failed to converge, Zuur et al. 2009).

NP-ANOVA was used to examine the effects of incubation temperature and maternal identity on the length of the incubation period (incubation duration). To estimate the

developmental zero temperature for *E. multicarinata* (cold temperature at which developmental rate is effectively zero), I regressed incubation temperature against the inverse of incubation duration (Georges et al. 1994, Les et al. 2007). The x-intercept extrapolated from this regression is the approximate developmental-zero temperature: this method for estimating the developmental zero temperature assumes developmental rate increases linearly with temperature at all temperatures. While this assumption is not true exactly (e.g., Georges et al. 2005), the method provides a useful starting estimate.

For all statistical tests, I began with full models and used backwards selection to remove non-significant terms (see Zuur et al. 2009 for details). Because incubation temperature was my factor of primary interest, this main effect was never removed from models. I chose a conservative threshold p-value of < 0.10 for factor inclusion in final models, although I used the traditional alpha value of 0.05 for interpretation of results. When non-significant factors are discussed in the results, the degrees of freedom and p-values were derived from the simplest models that still contained those terms. In addition, I report exact p-values for all tests, rather than p-values corrected for multiple comparisons because there is no consensus for how or when to make such corrections (Perneger 1998, Cabin and Mitchell 2000). While sequential Bonferroni (Holm 1979, Rice 1989) is frequently used, numerous authors advocate against this (e.g., Perneger 1998, Cabin and Mitchell 2000, Moran 2003). I largely controlled for multiple comparisons by using NP-MANOVAs; still the potential exists for one or more of my reported p-values to represent a Type-1 error. Even so, most of my results suggest the same pattern (see below), which is extremely unlikely to occur by chance alone.

All analyses were performed using the program R version 2.15.2 (R Core Team 2013). NP-MANOVA/ANOVA were performed using the function *adonis* in the *vegan* package (Oksanen et al. 2013), mixed-model ANOVAs were performed using the function *lme* in the *nlme* package (Pinheiro et al. 2013), and mixed-model logistic regressions were performed using the function *glmer* in the *lme4* package.

RESULTS

Incubation temperature affected survivorship to hatching ($\chi^2 = 28.49$, d.f. = 4, $P < 0.0001$, Fig 1A) and marginally affected juvenile survivorship to 30-d of age ($\chi^2 = 7.59$, d.f. = 3, $P = 0.0553$, Fig 1B). When survivorship over the entire period from oviposition to 30-d post hatching was examined, temperature had a substantial effect ($\chi^2 = 26.38$, d.f. = 4, $P < 0.0001$, Fig 1C). Survivorship to hatching was relatively high when eggs were incubated from 24–28 ºC (~74%), but dropped dramatically at 30 ºC (~42%), with zero survivorship when eggs were incubated at 32 ºC (Fig 1A). By contrast, juvenile survivorship was highest at warmer temperatures, particularly 28 ºC (~ 95%, Fig 1B). When taken together, survivorship from oviposition to 30-d post hatching was "hump shaped," with maximum survivorship when eggs were incubated at 28 ºC (~74%) and reduced survivorship when eggs were incubated at both cooler and warmer temperatures (Fig 1C). Mortality dropped after lizards reached 30-d of age. Of those individuals from the 2010 cohort that survived to 30-d post hatching, 86% survived to 3-mo. of age (approximately equivalent to the onset of first hibernation) and 72% survived at least 6 mo.

As in other species, incubation duration was negatively correlated with temperature ($F_{3,44} = 416.51$, $P < 0.0001$, Table 2, Fig 2A). This relationship was roughly linear with

development taking 52.2 days at 24 ºC and 39.5 days at 30 ºC, on average. Maternal identity also affected incubation duration ($F_{8,44}$ = 195.13, P < 0.0001), but there was no interaction effect ($F_{14,30}$ = 1.02, P = 0.4703). The developmental-zero temperature for *E. multicarinata* was approximately 7.3 ºC (Fig 2B).

At each measurement age, both incubation temperature (hatching: $F_{3,32}$ = 4.52, P = 0.0043, 7d: $F_{3,39}$ = 4.84, P = 0.0025, 30d: $F_{2,22}$ = 4.69, P = 0.0177) and maternal identity (hatching: $F_{8,32}$ = 17.08, P = 0.0001, 7d: $F_{8,39}$ = 10.10, P = 0.0001, 30d: $F_{8,22}$ = 14.96, P = 0.0001) affected juvenile *E. multicarinata* morphology (Table 2, Fig 3). Generally, temperature had a "hump-shaped" effect on morphology, with larger individuals produced at intermediate incubation temperatures and smaller individuals produced at extreme temperatures (Fig 3). At hatching, temperature and maternal identity interacted to influence morphology ($F_{15,32}$ = 2.05, P = 0.0175), likely driven by an interactive effect of these factors on tail length ($F_{15,32}$ = 3.83, P = 0.0007, Table 2). This interaction was not present at 7- or 30-days of age ($F_{12,27}$ = 1.35, P = 0.2210 and $F_{9,22}$ = 2.15, P = 0.0579, respectively). NP-ANOVA revealed effects of temperature on SVL and TL at hatching ($F_{3,47}$ = 4.44, P = 0.0080 and $F_{3,32}$ = 26.08, P = 0.0001, respectively; Table 2, Figs 3A and 3B), as well as TL and mass at 7-days post hatching ($F_{3,27}$ = 25.42, P = 0.0001 and $F_{3,39}$ = 2.89, P = 0.0449, respectively; Table 2, Figs 3F and 3G) and 30-days post hatching ($F_{2,22}$ = 13.13, P < 0.0001 and $F_{2,31}$ = 3.25, P = 0.0493, respectively; Table 2, Figs 3J and 3K). Maternal identity affected every morphological trait measured at each measurement time ($P \leq 0.0001$ for all), and there were occasional interactions between maternal identity and temperature (see Table 2 for details). Body condition was not affected by incubation temperature at either hatching ($F_{3,47}$ = 2.49, P = 0.0717, Fig 3D) or 7-d of age ($F_{3,39}$ = 0.64, P = 0.5925, Fig 3H).

Incubation temperature did affect the body condition of 30-d old juveniles ($F_{2,31}$ = 7.50, P = 0.0022, Fig 3L), with those individuals incubated at 30 ºC being in higher condition than those incubated at 26 or 28 ºC. However, given the high mortality of individuals incubated at 30 ºC by 30-d of age (N = 8 surviving from the 30 ºC treatment versus N = 14 and N = 20 surviving from the 26 ºC and 28 ºC treatments, respectively), this result might be an artifact of differential survivorship.

Incubation temperature did not influence running speed (Table 2, Fig 4). However, at 7-days of age there was an interaction between temperature and SVL on running speed ($F_{3,39}$ = 4.30, P = 0.0155). This interaction resulted from SVL having a positive effect on running speed (both mean-fastest-25cm speed and mean-1m speed) at all incubation temperatures except for 28 ºC, where SVL had a negative effect (data not shown). By 30d, this interaction disappeared ($F_{3,29}$ = 0.55, P = 0.6580), and neither SVL nor temperature affected running speed ($F_{1,32}$ = 1.09, P = 0.3043 and $F_{3,33}$ = 1.82, P = 0.161, respectively, Table 2, Figs 4C and 4D). Maternal identity had no effect on running speed at 7d ($F_{7,32}$ = 0.73, P = 0.6639), but a strong effect on running speed by 30d ($F_{8,32}$ = 6.94, P = 0.0001, Table 2).

DISCUSSION

Developmental Thermal Reaction Norm of *Elgaria multicarinata*

The results of this study allow description of the developmental thermal reaction norm for *E. multicarinata*. The critical thermal maximum was ~31 ºC (between 30 ºC and 32 ºC) and the optimum temperature for development was ~27–28 ºC (more discussion below). I estimated the developmental-zero temperature (temperature below which no development occurs) to be ~7.3 ºC. However, this estimate requires substantial extrapolation (Fig 1B).

Offspring survivorship suggests that 24 ºC is near the critical thermal minimum temperature for constant temperature development. These results reveal that developing *E. multicarinata* display a narrow "hump-shaped" reaction norm/performance curve typical of other squamate reptiles (reviewed in Birchard 2004). Many of the effects of incubation temperature examined persisted for 30-d after hatching. As in other reptiles, incubation temperature strongly affects developmental success and offspring phenotype in *E. multicarinata*.

Survivorship to hatching was uniformly high at temperatures ≤ 28 ºC and rapidly dropped at warmer temperatures. By contrast, juvenile survivorship to 30-d of age was highest when embryos were incubated at 28 ºC and reduced when embryos were incubated at either cooler or warmer temperatures. Taken together, survivorship over the entire experimental period from oviposition to 30-d post hatching displayed a distinct "hump-shaped" performance curve. Because all hatchlings were maintained under identical conditions, differences in survivorship to 30-d post hatching result from embryos experiencing different incubation temperatures. These results suggest that cool temperature incubation has a delayed effect on offspring mortality, while the effects of warm temperatures are apparent at hatching. High mortality in juvenile reptiles incubated at constant cool temperatures is common (reviewed in Deeming 2004), and might result from depleted energy stores at hatching (e.g., Booth and Thompson 1991, Booth 1998, Angilletta et al. 2000, Warner et al. 2012). However, this mechanism does not explain the reduced survivorship that I observed in cold-incubated *E. multicarinata* because hatchlings from the cool treatments grew and maintained high body condition, suggesting that their assimilated energy exceeded maintenance costs. Further work is necessary to understand the proximate mechanisms responsible for the delayed mortality observed.

Offspring phenotype was affected by incubation temperature as well. Both SVL and TL through 7 days of age were highest when offspring were incubated at ~28 ºC, mirroring the effects of incubation temperature on survivorship over the entire experiment. By 30-d of age, the impacts of incubation temperature on SVL disappeared, but both mass and body condition were positively affected by incubation temperature. These results suggest that incubation temperature has lasting effects on offspring phenotype, at least through 30d of age. Those individuals that survived and were of the best condition by the end of the experiment likely were of the highest quality (i.e., most likely to have survived, acquired resources, and reproduced in nature). My results therefore suggest that ~28 ºC is the optimum temperature for constant-temperature development in *E. multicarinata* because it produced offspring that could maintain high body condition and were likely to survive. While 30 ºC produced individuals of higher condition than those from 28 ºC, 30 ºC also resulted in high mortality, making it suboptimal.

Organisms experience thermal variation at multiple scales. At the acute level, temperature may vary dramatically over a single day. More chronically, average daily temperatures may vary with season or year. My results suggest that differences in constant temperature during development (i.e., chronic thermal variation) affect the survivorship and phenotype of neonatal *E. multicarinata.* Natural *E. multicarinata* eggs likely experience acute thermal variation during development as well, which I was not able to examine. It is likely that short-term exposure of embryos to temperatures above 31ºC or below 24 ºC is not harmful and, in some circumstances, might be beneficial (e.g., Ashmore and Janzen 2003, Du and Ji 2006, Les et al. 2007, Warner and Shine 2011, Angilletta et al. 2013). So long as acute temperature variation remains sub-lethal, constant and fluctuating temperature

incubation regimes with the same mean generally affect developing reptiles similarly, although the mean may need weighted (e.g., the CTE, Ashmore and Janzen 2003, Georges et al. 2005, Du and Ji 2006, Warner and Shine 2011). My experiment models the effects of variation in the average thermal environment on *E. multicarinata* development. This allows me to describe the chronic thermal reaction norm for development. Further work is necessary to understand how acute thermal variation might impact the developmental trends that I observed.

Thermal Sensitivity of Immobile and Mobile Life-history Stages

Comparing my results to previous studies of adult *E. multicarinata* suggest that some, but not all, aspects of the reaction norm to chronic thermal variation are shared across life-history stages of this species. The optimum temperature is approximately 28 ºC in both the free-living (neonates-adults, Cunningham 1966, Dawson and Templeton 1966, Licht 1967, Kingsbury 1994) and immobile (eggs, present study) stages. However, the breadth of the reaction norm differs, with adult *E. multicarinata* frequently active at temperatures outside the range suitable for constant-temperature embryonic development. Active body temperatures in adults range from 5–36 ºC (Cunningham 1966, Kingsbury 1994), whereas successful development is only possible from ~24–31 ºC (present study). Notably, when embryos were incubated at the mean body temperature experienced by active adults in nature (24 ºC, Cunningham 1966, Kingsbury 1994) they had poor post-hatching survivorship. Laboratory experiments have also shown that adult *E. multicarinata* have high physiological functioning at constant temperatures outside the suitable range for embryonic development (Dawson and Templeton 1966). Therefore, as observed in non-vertebrate ectotherms (e.g.,

Bowler and Terblanche 2008, Vorhees and Bradley 2012, Miller et al. 2013), thermal sensitivity varied across the ontogeny of southern alligator lizards.

The narrow chronic thermal reaction norm that I observed in developing embryos relative to adults is counterintuitive given that, unlike adults, the embryos cannot maintain preferred body temperatures via behavioral thermoregulation (although see, Du et al. 2011, Zhao et al. 2013). If embryos are exposed to high environmental thermal variance, they should be under selection for broad thermal tolerance (Huey and Kingsolver 1989, Angilletta et al. 2002b, Kingsolver and Gomulkiewicz 2003, Angilletta et al. 2013). One explanation for embryos displaying a narrow thermal tolerance relative to adults might be that developing *E. multicarinata* are not exposed to variation in the average thermal environment in nature and thus are not under selection for broad thermal tolerance. To my knowledge, no natural *E. multicarinata* nests have been documented. However, in naturalistic enclosures, females constructed nests as shallow chambers under cover stones, similar to other lizards (Langerwerf 1981). This description matches the nest construction that I observed in the artificial nest boxes used for this study. These observations suggest that *E. multicarinata* nests are typical for lizards, which generally experience high thermal variance associated with diel and seasonal cycles, and may vary substantially from year to year (e.g., Shine et al. 2002, Ackerman and Lott 2004, Huang and Pike 2011). Therefore, *E. multicarinata* eggs/embryos most likely experience a variable thermal environment at multiple scales in nature, and a broad thermal tolerance should be adaptive. If so, my results suggest that the evolution of thermal reaction-norm breadth in developing *E. multicarinata* is constrained. Most reptiles display a narrow developmental sensitivity to constant-temperature regimes

similar to that observed for *E. multicarinata* (reviewed in Birchard 2004). The breadth of the thermal reaction norm of embryonic reptiles thus appears to have low evolutionary potential.

The relatively broad thermal tolerance of adult *E. multicarinata* observed in the field and laboratory (Cunningham 1966, Dawson and Templeton 1966, Kingsbury 1994) is also initially perplexing. Because they can thermoregulate to preferred temperatures, adult ectotherms are predicted to have narrow thermal performance curves, potentially with high optima (Huey and Kingsolver 1989, Angilletta et al. 2002b). Moreover, the thermal performance curves of these species are predicted to have low evolutionary potential because behavioral thermoregulation negates environmental variation and thus selection (the "Bogert effect," Bogert 1949, 1959, Huey et al. 2003). Matching prediction, most adult reptiles have narrow thermal reaction norms (e.g., Huey 1982, Van Berkum et al. 1986, Angilletta et al. 2002a). It is plausible that *E. multicarinata* escaped the "Bogert effect" and evolved a broader thermal reaction norm by first reducing their behavioral thermoregulation. Adult *E. multicarinata* only thermoregulate facultatively, when the costs are low (Kingsbury 1994). When the costs of behavioral thermoregulation are high or ideal temperatures are unavailable, *E. multicarinata* remain active at easily accessible body temperatures (Cunningham 1966, Kingsbury 1994) allowing for selection on the thermal reaction norm. Adult *E. multicarinata* thus appear to have evolved a broader thermal tolerance while the thermal tolerance of embryonic *E. multicarinata* has been constrained to the ancestral condition.

Implications for Alligator Lizard Biogeography

Differences in the evolutionary lability of each life-history stage's thermal reaction norm are likely common in ectotherms and might induce important trade-offs (e.g., Andrews and Schwarzkopf 2012, Briscoe et al. 2012, Miller et al. 2013, Mitchell et al. 2013). In alligator lizards, the apparent inability of embryos to evolve broader thermal tolerance likely constrains their geographic range. Alligator lizards are relatively poor at dispersing long distances (Kingsbury 1994, Rochester et al. 2010), similar to most reptiles (Gibbons et al. 1990, Doughty et al. 1994, Araújo et al. 2006, Warner and Shine 2008, Vitt and Caldwell 2009). Thus, *E. multicarinata* are only predicted to persist where all life-history stages are successful. Because adults can persist at temperatures unsuitable for embryos, the availability of suitable nesting sites likely limits the geographic range of *E. multicarinata.* The geographic range of some insects appears to be similarly constrained by the thermal sensitivity of a single life-history stage (Radchuk et al. 2013), and this might be a common phenomenon among ectotherms.

Constraints imposed by the thermal sensitivity of developing embryos might partially explain landscape-level biogeographic patterns in the genus *Elgaria.* *Elgaria multicarinata* and its congener, *E. coerulea* (northern alligator lizard, Wiegmann 1828, approximately 6.6 million years divergent, Macey et al. 1999) are largely sympatric but not syntopic; *E. coerulea* exists at higher elevation and higher latitude than *E. multicarinata* (i.e., in colder areas, Stebbins 2003, Beck 2009). Even so, these lizards occur in similar habitat, fill similar niches, and are active with virtually identical body temperatures (both mean and range, Stewart 1984, Kingsbury 1994, Stebbins 2003). Therefore, neither biotic interactions nor adult thermal tolerances likely are responsible for the biogeography of these lizards.

However, *E. coerulea* is viviparous and pregnant females tightly maintain body temperatures of ~25 ºC (Stewart 1984, Sheen 2001). This temperature is thus suitable for development in *E. coerulea* and is likely near the optimum. By contrast, *E. multicarinata* embryos maintained at 25 ºC are expected to experience high mortality within the first 30 d of life (present study). Thus, *E. multicarinata* is predicted to have low reproductive success in conditions as cold as those suitable for *E. coerulea*, thereby confining *E. multicarinata* to warmer regions.

Conclusions

My results suggest that thermal tolerances are not fixed across the ontogeny of *E. multicarinata*. Different life-history stages in ectotherms might frequently evolve different environmental tolerances, particularly when the stages differ in their mobility or habitat (e.g., Bowler and Terblanche 2008, Hoffmann 2010, Angilletta et al. 2013, Miller et al. 2013). However, the evolutionary potential of each stage may also differ depending on the quantitative genetic architecture present (Lande and Arnold 1983, Falconer and Mackay 1996, Delph et al. 2005). The extent to which stage-specific tolerances can evolve will have major biogeographic implications, particularly for poor dispersers, because these organisms can only persist where suitable habitats for all life-history stages are present. Understanding the differences in environmental sensitivity across the ontogeny of ectotherms will also have implications for our ability to more accurately predict organismal responses to changes in environment and climate (Andrews and Schwarzkopf 2012, Briscoe et al. 2012, Radchuk et al. 2013, Telemeco et al. 2013). Numerous authors advocate mechanistic models that account for physiology when generating predictions for how organisms will respond to our

changing world (e.g., Dunham et al. 1989, Pearson and Dawson 2003, Chown and Terblanche 2007, Angilletta 2009, Buckley et al. 2010). However, these models only provide usable predictions if the physiological tolerances used to estimate parameters are derived from the most sensitive/limiting life-history stages. Currently, little is known about which life-history stages or physiological processes will be most limiting for the vast majority of species. As in at least one insect species (Radchuk et al. 2013), my results suggest that the thermal sensitivity of a single life-history stage (developing embryos) limits the geographic range of southern alligator lizards. Moreover, because developing embryos were less tolerant of variation in average temperature than adults, this stage will likely play an important role in determining how southern alligator lizards respond to climate change. This result might be general to the Anguidae and numerous other reptiles; however, insufficient data are currently available to be confident of this conjecture. Further work is needed to understand how physiological tolerances differ among life-history stages, and which stages most limit the distributions of groups of organisms.

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TABLES

Table 1—Collection and reproductive data for gravid female southern alligator lizards (*Elgaria multicarinata*) used in the present study. Coordinates are based on the WGS84 datum , elevation (elev) is in m, snout-vent length (SVL) is in mm, mass is in g, Csize is the number of eggs in each clutch, % hatched is the percent of eggs in a clutch that successfully hatched. Individuals for which no eggs hatched were removed from analyses.

Collection date	Latitude	Longitude	Elev	SVL	Oviposition date	Csize	Egg mass mean ± se	% hatched
6 May 10	32°39'6"N	117°02'5"W	60	129	2 Jun 10	18	desiccated	0
6 May 10	32°39'6"N	117°02'5"W	60	119	8 Jun 10	12	0.80 ± 0.02	42
30 Jun 10	32°45'1"N	116°27'1"W	1170	129	13 Jun 10	13	1.07 ± 0.01	54
30 Jun 10	32°39'6"N	117°02'5"W	60	112	2 Jul 10	12	0.81 ± 0.01	0
3 Jul 10	37°25'2"N	122°10'4"W	40	113	10 Jul 10	12	1.16 ± 0.01	83
6 Jul 10	37°01'4"N	121°50'4"W	350	128	4 Aug 10	8	0.86 ± 0.01	100
7 Jul 10	39°44'3"N	121°28'5"W	385	115	27 Jul 10	6	1.01 ± 0.02	67
17 Jun 11	36°38'1"N	121°47'1"W	104	123	21 Jul 11	11	0.99 ± 0.01	55
22 Jun 11	36°38'1"N	121°47'1"W	95	128	20 Jul 11	11	0.71 ± 0.02	55
23 Jun 11	36°39'1"N	121°43'5"W	34	121	12 Jul 11	9	0.86 ± 0.02	67
1 Jul 11	37°04'1"N	122°03'1"W	132	135	25 Jul 11	7	1.16 ± 0.02	43

Table 2**:** Results from NP-MANOVA/ANOVA examining effects of incubation temperature, maternal identity, and snout-vent length (SVL, for tests of running speed only) on offspring phenotype in southern alligator lizards (*Elgaria multicarinata*) at hatching, 7-, and 30-days of age. Italics indicate multivariate phenotypes composed of the traits listed directly below them. Interactions included in final models ($P < 0.10$) are listed and those that were significant ($P < 0.05$) are in bold. All values are from the final/reduced models. *P*-values were obtained using permutation tests (see text for details). Significant *P*-values are in bold. SVL and TL were measured in mm, Mass in g, and Speeds in m/s. Mass was cube-rooted to ensure all measures were in similar units for MANOVA. In addition SVL, TL, and Mass were log-transformed for analyses.

		Temperature (T)			Mother (M)			SVL (S)			Interactions	Model R^2
Age	Phenotype	*d.f.*	*F*	*P*	*d.f.*	*F*	*P*	*d.f.*	*F*	*P*		
Incubation duration		3,44	416.51	**<0.0001**	8,44	195.13	**<0.0001**	–	–	–	–	0.985
Hatching												
	Morphology	3,32	4.52	**0.0043**	8, 32	17.08	**0.0001**	–	–	–	**T:M**	0.850
	SVL	3,47	4.44	**0.0080**	8, 47	18.13	**0.0001**	–	–	–	–	0.849
	TL	3,32	26.08	**0.0001**	8, 32	15.60	**0.0001**	–	–	–	**T:M**	0.891
	Mass	3, 47	0.46	0.7101	8, 47	13.71	**0.0001**	–	–	–	–	0.839
7 days												
	Morphology	3,39	4.84	**0.0025**	8,39	10.10	**0.0001**	–	–	–	–	0.710
	SVL	3,39	1.48	0.2429	8,39	10.02	**0.0001**	–	–	–	–	0.684
	TL	3,27	25.42	**0.0001**	8,27	10.13	**0.0001**	–	–	–	**T:M**	0.639
	Mass	3,39	2.89	**0.0449**	8,39	12.07	**0.0001**	–	–	–	–	0.730
	Speed	3,39	2.35	0.0785	–	–	–	1,39	0.23	0.6887	**T:S**	0.450

Table 2 Continued

		Temperature (T)			Mother (M)			SVL (S)			Interactions	Model R^2
Age	Phenotype	*d.f.*	*F*	*P*	*d.f.*	*F*	*P*	*d.f.*	*F*	*P*		
Mean fastest 25 cm		3,39	2.33	0.0884	–	–	–	1,39	0.21	0.6376	**T:S**	0.459
30 days												
	Morphology	2,22	4.69	**0.0177**	8,22	14.96	**0.0001**	–	–	–	T:M	0.871
	SVL	2,22	2.92	0.0705	8,22	36.19	**0.0001**	–	–	–	**T:M**	0.938
	TL	2,22	13.13	< **0.0001**	8,22	21.98	**0.0001**	–	–	–	**T:M**	0.920
	Mass	2,31	3.25	**0.0493**	8,31	11.29	**0.0001**	–	–	–	–	0.861
	Speed	3,32	1.82	0.161	8,32	6.94	**0.0001**	–	–	–	–	0.660
Mean fastest 25 cm		3,32	1.98	0.1386	8,32	6.71	**0.0001**	–	–	–	–	0.654
	Mean 1m	3,32	0.98	0.406	8,32	8.11	**0.0001**	–	–	–	–	0.686

FIGURES

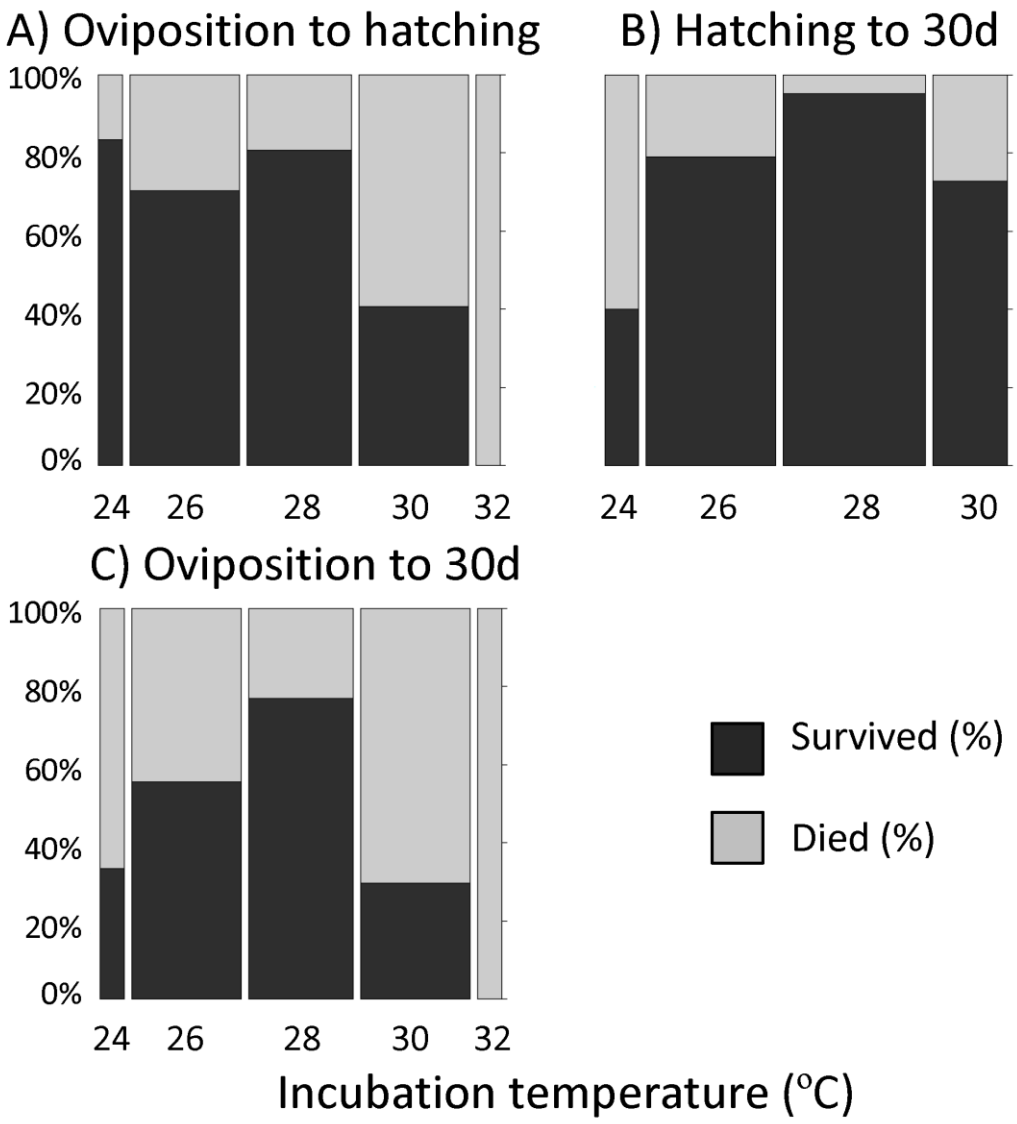

Figure 1—Percent of southern alligator lizard (*Elgaria multicarinata*) offspring incubated at five constant temperatures that survived from A) oviposition to hatching (i.e. hatching success), B) hatching until 30d post-hatching, and C) oviposition to 30d post hatching. For A) and C), incubation temperature affected survivorship ($P < 0.05$), whereas temperature marginally influenced survivorship for B) ($P = 0.058$). The 32 ºC treatment is not included in B) because no individuals incubated at this temperature hatched. Bar width is scaled to sample size.

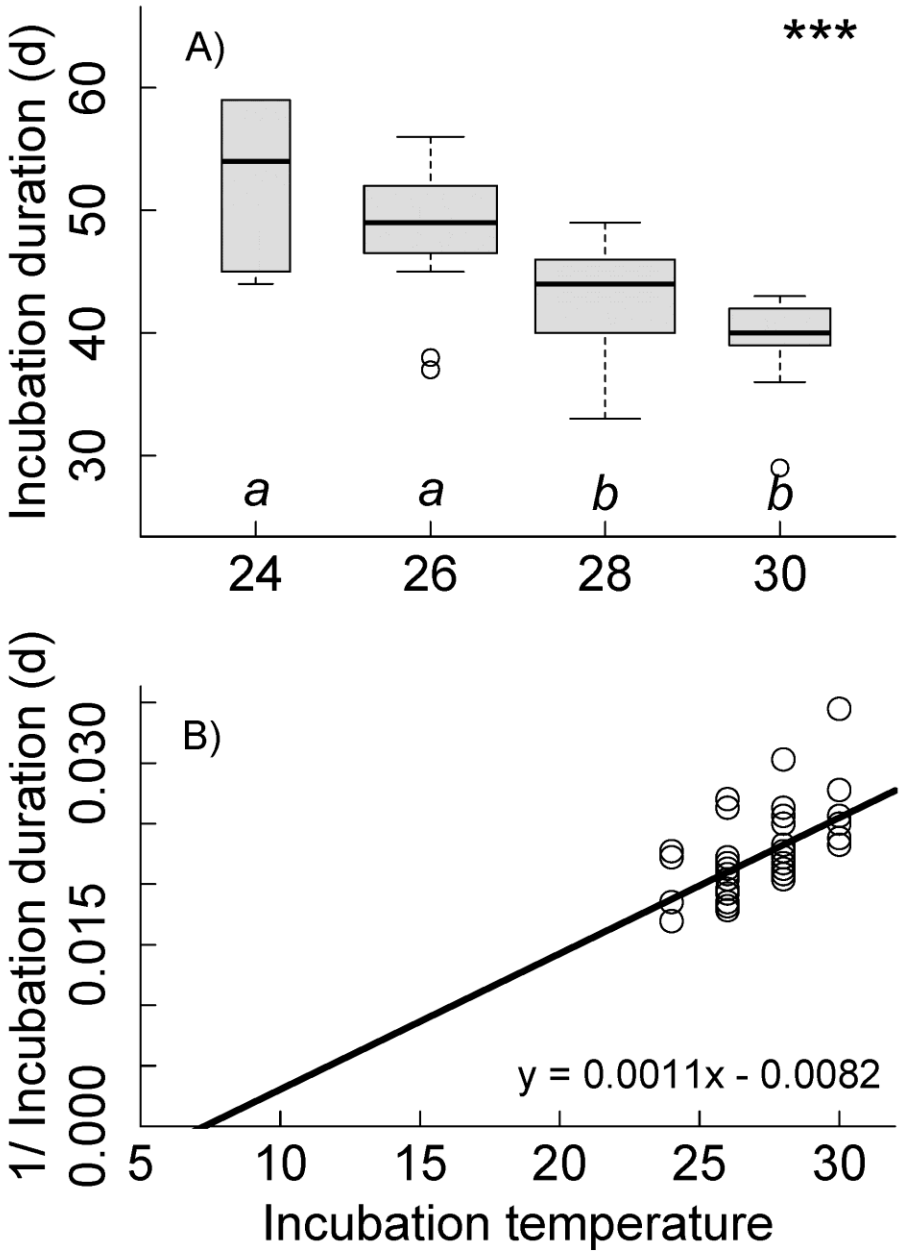

Figure 2—Effect of temperature on incubation duration in southern alligator lizards (*Elgaria multicarinata*). A) Box plots for temperature vs. duration with box width scaled to sample size. Different letters under boxes indicate significant differences from pair-wise tests ($P < 0.05$). B) Scatterplot of temperature vs. the inverse of incubation duration. The line is a least-squares regression line (equation shown) and was used to estimate the developmental-zero temperature.

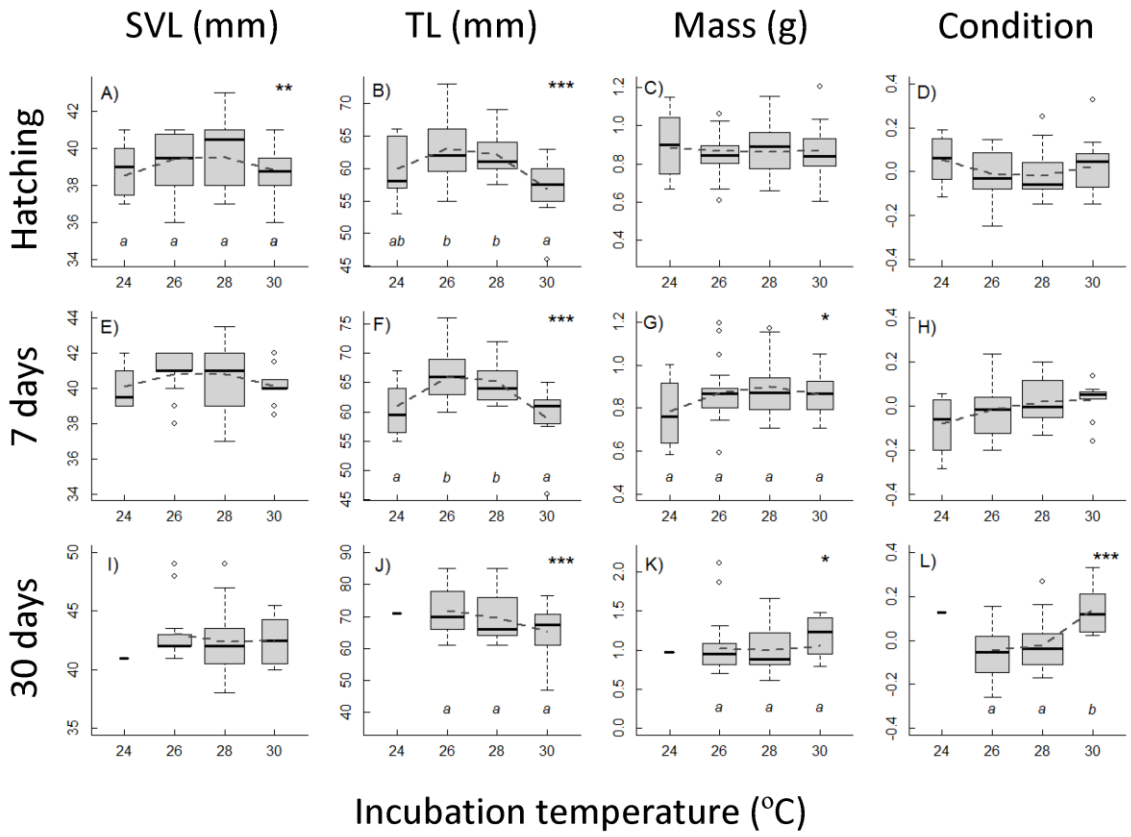

Figure 3—Boxplots displaying the effects of incubation temperature on morphology (snout-vent length [SVL]: A,E,I; tail length [TL]: B,F,J; mass: C,G,K, and body condition [Condition]: D,H,L) in offspring southern alligator lizards (*Elgaria multicarinata*) at three time points: hatching (A–D), 7-days of age (E–H), and 30-days of age (I–L). MANOVAs included SVL, TL, and mass at each time point and showed significant effects of incubation temperature ($P < 0.05$). Asterisks indicate significant ANOVAs (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Box width is scaled to sample size and different letters under the boxes indicate significant differences from pair-wise tests. Dashed lines represent quadratic least-squares regressions fitted to these data and are presented to illustrate the shape of estimated reaction norms. Only one individual incubated at 24 ºC survived to 30 days of age; this point was not included in statistical analyses. Body condition was estimated as the residuals from a regression of log-transformed mass on log-transformed SVL.

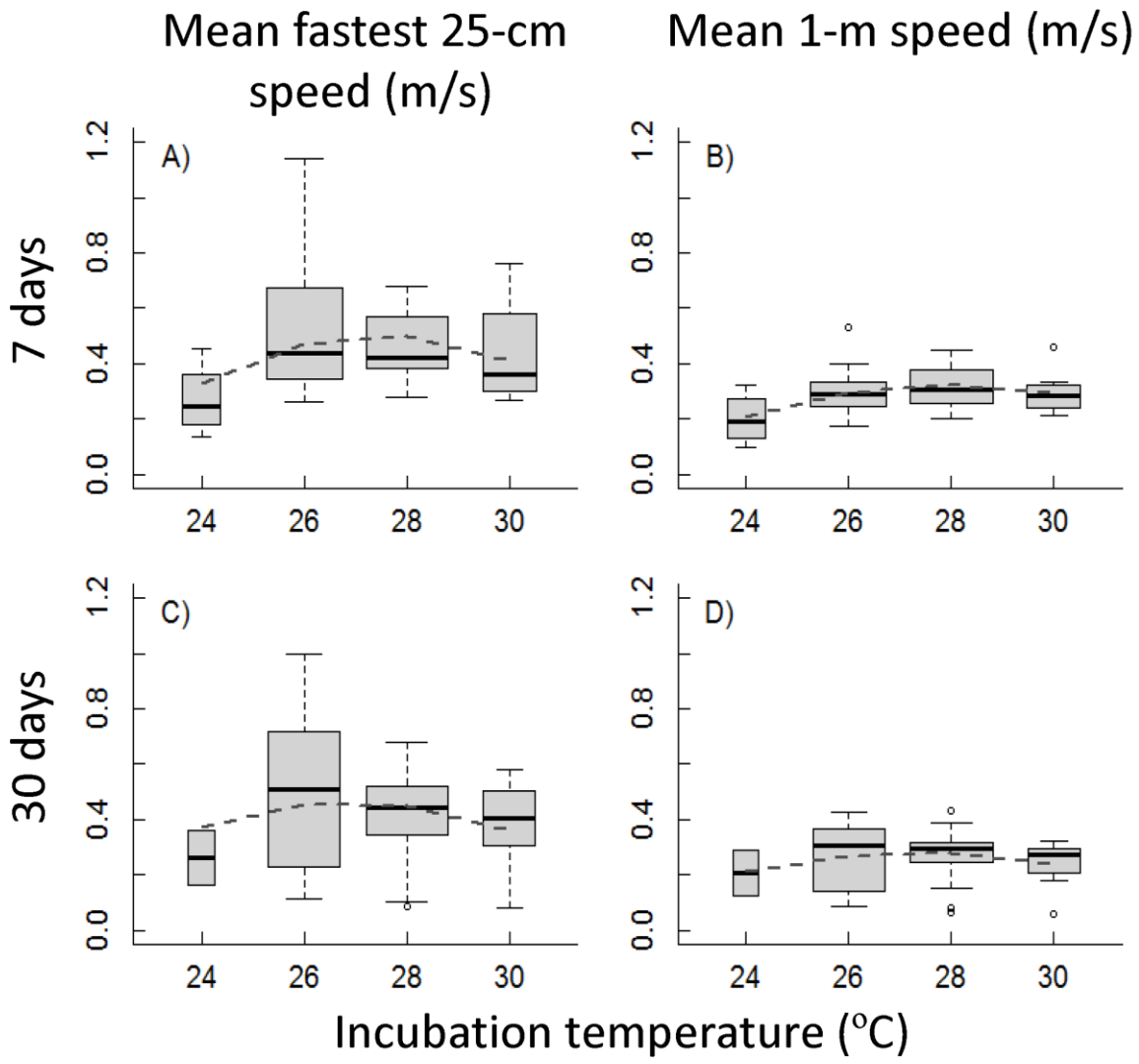

Figure 4—Boxplots displaying the effects of incubation temperature on running speed (mean fastest 25-cm speed: A, C, and mean 1-m speed: B, D) in offspring southern alligator lizards (*Elgaria multicarinata*) at two time points: 7-days of age (A, B) and 30-days of age (C, D). MANOVAs did not reveal an effect of temperature at either time point ($P > 0.05$), although the interaction between temperature and snout-vent length was significant at 7 days ($P < 0.05$). Box width is scaled to sample size. Dashed lines represent quadratic least-squares regressions fitted to these data and are presented to illustrate the shape of each estimated reaction norm.

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CHAPTER 5

MODELING THE EFFECTS OF CLIMATE-CHANGE INDUCED SHIFTS IN REPRODUCTIVE PHENOLOGY ON TEMPERATURE-DEPENDENT TRAITS

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ABSTRACT

By altering phenology, organisms have the potential to match life-history events with suitable environmental conditions. Because of this, phenological plasticity has been proposed as a mechanism whereby populations might buffer themselves from climate change. We examine the potential buffering power of advancing one aspect of phenology, nesting date, on sex ratio in painted turtles (*Chrysemys picta*), a species with temperature-dependent sex determination. We developed a modified constant-temperature equivalent model that accounts for the interaction between climate change, oviposition date, and seasonal thermal pattern on temperature during sexual differentiation and thus on offspring sex ratio. Our results suggest that females will not be able to buffer their progeny from the negative consequences of climate change by adjusting nesting date alone. Not only are offspring sex ratios predicted to become 100% female, but our model suggests that many

nests will fail. Because the seasonal thermal trends that we consider are experienced by most temperate species, our result that adjusting spring phenology alone will be insufficient to counter the effects of directional climate change may be broadly applicable.

Keywords: temperature-dependent sex determination, constant temperature equivalent (CTE), development, *Chrysemys picta*

INTRODUCTION

The most commonly observed biotic response to climate change is a shift in phenology (Visser and Both 2005, Parmesan 2006, Moser et al. 2009). Examples include advances in flowering date, tree bud burst, arrival of migrant birds and butterflies, frog breeding, and nesting in birds and reptiles (e.g., Beebee 1995, Menzel and Fabian 1999, Forister and Shapiro 2003, Visser et al. 2004, Both et al. 2006, Telemeco et al. 2009). These temporal shifts in response to climate change presumably result from individuals using thermal cues to time life-history events (Parmesan 2006, van Asch and Visser 2007), an important consequence of which is that temperatures at the onset of these events remain relatively stable from year-to-year. Plasticity in phenology therefore has been proposed as a mechanism whereby individuals might buffer themselves from the impacts of climate change (Visser and Both 2005, Schwanz and Janzen 2008, Telemeco et al. 2009).

Shifting phenology, however, only allows individuals control of conditions at the onset of life-history events. Because temperature trajectories during the growing season are roughly parabolic (increasing from spring to summer and then decreasing), if organisms respond to increasing average temperatures by starting their spring phenological cycles

earlier, they will face steeper seasonal temperature changes. Thus, even though temperatures at the onset of life-history events could be held constant by adjusting phenology, temperatures over the periods that follow might still increase (Fig 1). This shift could be particularly important for oviparous organisms that lack parental care, such as many ectotherms (Vitt and Caldwell 2009), because environmental temperatures during embryogenesis affect survivorship and phenotype (Du et al. 2003, Shine 2005, Booth 2006). Even if these species advance their phenology such that temperatures at nesting are constant, temperatures during embryogenesis might still increase. Thus, many species might be unable to compensate for the effects of warming climates on their developing offspring by advancing phenology.

We explored the power of advancing phenology to buffer populations from climate change using painted turtles (*Chrysemys picta*), a species with temperature-dependent sex determination (TSD, Bull 1980). Temperature during development directly determines the sex of individuals with TSD (Bull 1980, Godfrey et al. 2003, Warner and Shine 2008). In painted turtles, warm temperatures produce females and cool temperatures produce males (Janzen and Paukstis 1991). Species with TSD provide a unique opportunity for studying the biological impacts of shifting phenology in response to climate change because temperature directly affects a key trait without additional complicating factors. Moreover, species with TSD might be at risk of climate-change induced extinction because directional changes in temperature could skew sex ratios (Janzen 1994a, Telemeco et al. 2009, Wapstra et al. 2009). By advancing nesting date (observed in numerous TSD species, e.g., Doody et al. 2006, Schwanz and Janzen 2008, Telemeco et al. 2009), females might control the temperatures to which their developing offspring are exposed and counter these negative effects. However,

sex in species with TSD is generally determined during the middle third of development (termed the thermosensitive period, TSP, Janzen and Paukstis 1991, Georges et al. 2005, Shine et al. 2007) which, in turtles, begins a month or more after oviposition and continues for another month (Janzen and Paukstis 1991, Georges et al. 2005, Shine et al. 2007). Early nesting will buffer progeny from sex-ratio shifts induced by climate change only if temperature at oviposition predicts temperature during the TSP (Telemeco et al. 2009, Fig 1).

Generally, laboratory trials involving temperature assess the effects of constant-temperature treatments. While informative, such studies poorly replicate natural conditions where temperatures fluctuate daily and seasonally (Ackerman and Lott 2004, Angilletta 2009). Methods are therefore needed to translate results from constant-temperature experiments such that they make accurate predictions about organisms exposed to natural conditions. One such method is the constant-temperature equivalent model (CTE, Georges 1989), which condenses diel thermal variation from natural nests into a single number (the CTE) that can be used to predict the sex ratios of species with TSD (more below, Georges 1989, Georges et al. 1994). The CTE model accurately predicts sex ratios in many species (e.g., Les et al. 2007, Delmas et al. 2008, Mitchell et al. 2008), although it is not universally applicable (Warner and Shine 2011).

We developed a modified CTE model that accounts for the effects of nesting date and seasonal increases in temperature on offspring sex ratio. We then applied our modified CTE model to a population of painted turtles to examine whether or not advances in nesting date could buffer populations of species with TSD from biased sex ratios induced by climate change.

THE MODEL

The CTE model calculates the temperature above and below which half of development occurs, or the temperature associated with the median developmental rate (Georges 1989). We briefly present the original CTE model (for further details see Georges 1989, Georges et al. 2004), and then describe our modifications.

The original CTE model assumes that developmental rate (*ds/dt*) increases linearly with temperature (*T*):

$$\frac{ds}{dt} = \begin{cases} A(T - T_0) & T > T_0 \\ 0 & T < T_0 \end{cases} \qquad \text{(Equation 1)}$$

where $A > 0$ is the rate of increase and T_0 is the critical thermal minimum below which no significant development occurs. Temperature varies daily in a simple, periodic fashion around a constant mean (*M*) with a daily range equal to 2*R*:

$$T = R\cos t + M \qquad 0 \leq R < M \qquad \text{(Equation 2)}$$

Time (*t*) is scaled so that 1 day = 2π units. In natural nests, the daily range frequently varies, and an average *R* can be estimated using the statistical thermal variance (σ^2, L. Harmon unpublished): $R = \sqrt{2\sigma^2}$. The amount of development (*s*) that takes place from time 0 to time *t* is $\int_0^t \frac{ds}{dt}\,dt$. Because the cosine function is symmetrical, the entire period of development can be characterized by considering only the half-day interval (0, π). During this period, there is a time (*t'*) that divides the half-day cycle into two intervals in which total

development is exactly equal. The temperature associated with this time is the CTE. We can find *t'* by solving:

$$\int_0^{t'} \frac{ds}{dt} dt = \int_{t'}^{\pi} \frac{ds}{dt} dt \qquad \text{(Equation 3)}$$

and then the CTE (termed T' in Georges 1989) by solving:

$$CTE = R \cos t' + M \qquad \text{(Equation 4)}$$

Sex ratios can be predicted by comparing the CTE value from natural nests to the temperature that results in a 1:1 sex ratio under constant incubation conditions (pivotal temperature, T_{PIV}).

We elaborated Georges' CTE model by adding two features: 1) a maximal temperature above which development is zero, and 2) a seasonal increase in daily mean temperature. As others have pointed out (e.g., Georges et al. 2005, Delmas et al. 2008), the assumption in Eq. 1 that developmental rate increases with temperature linearly to infinity is flawed. Within an optimal thermal range, a linear relationship is appropriate (Sharpe and DeMichele 1977, Georges et al. 2005), but the rate rapidly approaches zero if temperatures rise above this range. Curvilinear approaches to modeling this relationship have been advanced (Georges et al. 2005, Neuwald and Valenzuela 2011), but each has many parameters that are difficult to estimate. In addition, the curvilinear models seem to perform similarly well, regardless of their complexity (Georges et al. 2005). We developed a simple

alternative by adding a critical thermal maximum (T_{MAX}), above which no development occurs, to Eq. 1 (Fig 1A from Appendix D).

$$\frac{ds}{dt} = \begin{cases} A(T - T_0) & \text{if } T_0 < T < T_{MAX} \\ 0 & \text{if } T < T_0 \text{ or if } T > T_{MAX} \end{cases} \quad \text{(Equation 5)}$$

While this simplified approach has less resolution than curvilinear models within the thermal range where developmental rate decreases, that range is narrow (Georges et al. 2005) and embryos experience little development here. A model that allows developmental rate to decrease more realistically at extreme high temperatures neither markedly outperforms this simple approach with our test data, nor affects our qualitative results (described in Appendix D). The primary advantage of our approach is that T_{MAX} is relatively easy to estimate.

An important assumption of the original CTE model is constant mean temperature throughout the TSP (Georges 1989). Our second elaboration relaxes this assumption, allowing temperature to change linearly to reflect seasonal warming:

$$T = R\cos t + M' + wt \qquad 0 \leq R < M' \quad \text{(Equation 6)}$$

where *M'* is the mean temperature on the first day of the TSP, and *w* is the rate of increase in mean daily temperature. By adjusting these two parameters, we can explicitly model the interaction between nesting date and seasonal thermal pattern on the CTE. Developmental rate, *ds/dt*, is given by plugging Eq. 6 into Eq. 5. Georges (1989) exploits the fact that, because the temperature cycle is the same every day, the temperature associated with the median development rate over the whole TSP is also the temperature of median development

during a single half-day cycle. We cannot use this shortcut because the temperature cycle changes from day to day, and must instead examine development across the entire TSP to find the CTE. The CTE is therefore the temperature that makes the following equality true:

$$\sum_i \int_{a_i}^{b_i} \frac{ds}{dt} dt = \sum_j \int_{a_j}^{b_j} \frac{ds}{dt} dt \qquad \text{(Equation 7)}$$

where the time intervals (a_i,b_i) are defined as all intervals during the TSP for which temperatures are between T_0 and the CTE, and (a_j,b_j) are all time intervals with temperatures between the CTE and T_{MAX} (Fig 1 of Appendix C). The left-hand side of Eq. 7 is the total amount of development occurring below the CTE and the right-hand side is the total amount of development occurring above the CTE. The temperature for which the equality holds is, by definition, the CTE.

We developed a MATLAB algorithm to find the CTE in this model numerically (Mathworks, Inc. 2009; code available in Appendix E). The algorithm takes a specified set of parameter values (M', R, w, T_0, T_{MAX}, and length of the TSP) and finds the CTE associated with this parameter set. Any temperature between T_0 and T_{MAX} is a viable candidate for the CTE; we considered all candidate values at intervals of 0.01 $^{\circ}$C within this range. For each candidate CTE value, we solved Eq. 6 to find all the times, t, at which temperature, T, was equal to T_0, the CTE, or T_{MAX}. These times provide the limits of integration in Eq. 7. The candidate CTE value that best satisfied Eq. 7 was taken as the true CTE.

Estimation of Model Parameters and Description of Simulations

We simulated our modified CTE model under varied realistic conditions to examine the ability of painted turtles (*Chrysemys picta*) to buffer the sex ratios of their progeny from increasing environmental temperatures by advancing nesting date. Thermally-induced shifts toward earlier nesting have been documented in this species (Schwanz and Janzen 2008). For detailed methods see Appendix C. Briefly, we focused on a population located at the Thomson Causeway Recreation Area in Carroll County, IL (41º 57' N, 90º 07' W, hereafter Thomson) that has been extensively studied for the last 25 years (see Janzen 1994b, Weisrock and Janzen 1999, Warner et al. 2010 for detailed field methods). We estimated physiological model parameters using data from the literature and our previous research (T_0 = 14, T_{MAX} = 34, pivotal temp [T_{PIV}] = 28, transitional range of temperatures [TRT, range that results in mixed-sex nests] = 26.65–29.35 [ºC for all]). In addition, we used data from Thomson to describe the average nesting period (nest on 1 June and have a 30-d TSP beginning 1 July), the historic thermal profile in nests (M' = 26.3 ºC, R = 5.4 ºC, w = 0.002 ºC/2πd, Fig 2 of Appendix C), and to confirm that the CTE model accurately predicts sex ratios in this species. To estimate the parabolic shape of the thermal trend over the reproductive season, and how temperature during the TSP (M' and w) will change with warming and earlier nesting, we used historic records of soil temperature measured at a nearby weather station (Figs 2 and 3).

For all simulations, we assumed females advance nesting date such that soil temperature at nesting remains constant. If hatchling sex ratios are not buffered under this scenario, it is unlikely that smaller shifts will buffer progeny and larger shifts would likely result in eggs being placed in nests too cool for development. We further assumed that the

TSP advances the same number of days as nesting date and that TSP length remains constant. While these assumptions will not be fully accurate, adding realistic variation minimally affects our results (see Appendix C). We simulated the effects of realistic changes in slope during the TSP (w) and mean temperature at the onset of the TSP (M') on CTE values given 0–6 ºC warming (Fig 3). For comparison, we also simulated the effects of increased temperature on CTE values if females do not alter their nesting phenology. This was done by holding w at its historic value and increasing M' by the same amount as the increase in environmental temperature (0–6 ºC).

We more fully explored the model by allowing w and M' to change independently within realistic limits. Since diel thermal range might also change with future warming, we considered a wide range of R values as well. For these simulations, we calculated CTE values for all combinations of w ranging from 0–0.02 ºC/2πd (at intervals of 0.001 ºC/2πd), M' from 20–30 ºC, and R from 1–10 ºC (M' and R at intervals of 0.05 ºC). By simulating the effects of each realistic parameter value factorially, we examined 1) how sex ratios will likely respond to different uniform warming scenarios, and 2) what model parameters have the greatest effects on offspring sex ratio. Although we identified T_{MAX} = 34 °C as a reasonable estimate, this is the parameter for which we have the least direct information (see Appendix C) so we repeated our simulations with additional T_{MAX} values (30–40 ºC at 2 ºC intervals).

To ground our exploration of the simulation results to the natural history of painted turtles at Thomson, we assumed historic, pre-climate change parameter values of w = 0 ºC/2πd, M' = 26 °C, R = 5 °C, and a 30-d TSP (based on measurements at Thomson). Although we considered a range of possible temperature increases, we focus on the effects of

4 °C warming because conservative predictions from climate models suggest that the midwestern United States will experience temperature increases of this magnitude or greater over the next century (Wuebbles and Hayhoe 2004, IPCC 2007, Takle 2011). When not stated otherwise, parameters were held at their historic values for all simulations.

RESULTS

Effects of Climate Change and Earlier Nesting on TSP Temperature

Even if females adjust their phenology such that they oviposit at the same soil temperature every year, temperatures during the TSP will rise as a result of climate change (Figs 2 and 3). This results from increases in the slope of temperature change over the TSP (w) and the temperature at the onset of the TSP (M', Figs 2 and 3). Given uniform warming, a nest initiated at the historic oviposition temperature will gradually diverge from historic thermal conditions. Although average temperature during the pre-TSP period is only predicted to increase 0.81 °C with 4 °C warming (Fig 2, inset), temperature at the onset of the TSP (M') should increase ~2 °C. Slope during the TSP (w) should also increase with warming (from ~0 to 0.015 °C/2πd), such that temperatures increase an additional ~2.8 °C during the TSP (Fig 3, Table 1 of Appendix C). Together, changes in w and M' result in the mean TSP temperature rising ~3.2 °C, even when females nest ~3 weeks early (Figs 2 and 3).

Effects of Climate-change Induced Shifts in Phenology on Sex Ratios

CTE values are predicted to rise out of the TRT (temperature range that results in a mixed sex ratio) after ~1.1 ºC warming, resulting in a 100% female sex ratio and ultimately population extinction (Fig 4 solid line), even if female painted turtles advance nesting such

that they oviposit at the same temperature each year. For comparison, if females fail to shift their nesting phenology and continue to nest on 1 June, CTE values increase more rapidly and rise above the TRT after only ~0.7 ºC warming (Fig 4 dashed line). The CTE rises with nest temperature until T_{MAX} is exceeded, at which point proportionately less development occurs at high temperatures and the CTE decreases (peaks in Figs 4 and 5 curves). When nests spend > 50% of the TSP above T_{MAX}, however, the majority of development occurs at high temperatures just below T_{MAX} and the CTE again rises. While embryos can survive minor excursions above T_{MAX}, extended periods or extreme spikes should be fatal (Du et al. 2003, Angilletta 2009, Neuwald and Valenzuela 2011); therefore nests that spend more time above T_{MAX} (right of the peaks in Figs 4 and 5) should experience high mortality.

Figure 5 depicts results from the factorial simulations, showing the relationship between nest CTE and w across a realistic range of M' (Fig 5A), R (Fig 5B), and T_{MAX} (Fig 5C). It is helpful to examine predictions based on a single warming scenario to understand these results. We describe the biological effects of 4 ºC warming. Because warming and shifts in phenology should only directly affect w and M', we hold all other parameters at their historical values in this example (T_0 = 14, T_{MAX} = 34, R = 5, Fig 5A). Assume for the moment that females can alter nesting such that TSP starting temperature remains stable over time at 26 ºC and only slope increases. Following the M' = 26 line in Fig 5A from the intercept (w = 0, as in the historical data) to the dashed green line (w = 0.015, the value predicted for a 4 ºC increase in temperature) reveals that this would cause the CTE to rise from 28.5 to 29.12 ºC. While this CTE results in female bias, it is within the TRT and thus mixed-sex nests should occur. Moreover, if TSP starting temperature is slightly reduced (1–2 ºC, e.g. M' = 24 line in Fig 5A), CTE values will remain very close to T_{PIV} and thus a 1:1

sex ratio could be maintained. If we return to our assumption that females advance their nesting date to track nesting temperature, both slope and TSP starting temperature will increase (Fig 3), resulting in a CTE of 29.78 ºC (Fig 5A, M' = 28 line at w = 0.015). This is concordant with the CTE value given at 4 ºC warming in Fig 4 and should induce 100% female-biased sex ratios.

The predicted effects of other scenarios can be ascertained from Fig 5 by examining additional parameter combinations. In general, CTE values increase with each parameter until nest temperatures exceed T_{MAX}, at which point CTE values begin to decrease. Slope (w) only exerts a minor effect on the CTE, whereas TSP starting temperature (M') and diel thermal range (R) have more profound effects. The T_{MAX} value used did not affect the overall behavior of the model, except to affect the point at which CTE values began to decrease. This effect was minor for T_{MAX} values $\geq$ 34.0 ºC within realistic ranges for the other parameters.

DISCUSSION

Power of Advancing Nesting Date to Buffer Offspring from Climate Change

Even if female painted turtles alter their phenology to oviposit at the same temperature each year, temperatures during the TSP will increase if regional temperatures rise. While earlier nesting delays increases in TSP temperature, it cannot counter them. Soil temperature at oviposition fails to predict nest conditions later in development because of the parabolic shape of seasonal thermal progression. The two parameters that best describe this effect are temperature at the onset of the TSP (M') and slope of temperature change during

the TSP (*w*), both of which increase relative to oviposition temperature as nesting date advances.

Our model predicts 100% female sex ratios given realistic values for changes in mean air temperature over the 21st century (Wuebbles and Hayhoe 2004, IPCC 2007, Takle 2011), even if females alter nesting date to maintain oviposition temperature (Fig 4). This change in sex ratio could be observed after as little as ~1 °C uniform increase in temperature. Our conclusion that adjustments in oviposition phenology cannot fully compensate for warming is concordant with empirical evidence that nesting date plasticity has only minor effects on the sex ratios of Thomson painted turtles at the population level (Schwanz and Janzen 2008).

Mechanisms independent of phenology could buffer populations from climate change. Our model shows that temperature changes at the onset of the TSP (*M'*) drive variation in sex ratio. If females control *M'*, maintaining either current or slightly reduced values, mixed sex ratios could be maintained. For moderate levels of regional warming, *M'* must be reduced 2–4 °C. Nesting ever earlier will not be a viable strategy because temperatures will be near T_0 if females oviposit in the very early spring. This process lengthens the first third of development but fails to reduce *M'*. Alternatively, females might reduce *M'* by constructing nests in wetter or more shaded areas, both of which would reduce nest temperature. Numerous reptiles, including painted turtles, have shifted nesting behavior accordingly through either local adaptation or phenotypic plasticity (e.g., Doody et al. 2006, Telemeco et al. 2009, McGaugh et al. 2010). If behavioral changes prove insufficient, interannual and internest variation in temperature might rescue the population by allowing occasional influxes of males.

During the 20th century, nights warmed faster than days in the midwestern United States, reducing the diel thermal range, and climate change models predict that this trend will continue (IPCC 2007, Takle 2011). Such a reduction in diel thermal range, 2*R*, could also buffer nests from sex-ratio shifts. However, to reduce the CTE ~1 ºC, the diel thermal range must contract ~4 ºC (Fig 5B). Climate change models do not support such a large change (IPCC 2007, Takle 2011). While contracted diel thermal ranges should reduce CTE values, these reductions will be insufficient to counter the effects of increased average temperatures.

Egg mortality might increase in addition to sex-ratio shifts. Even after altering nesting date, regional warming of < 2 °C is predicted to result in nests spending much of each day above the critical thermal maximum for successful development (T_{MAX}). Brief exposure of embryos to temperatures slightly above T_{MAX} is not fatal, but prolonged exposure to these temperatures and brief exposure to more extreme temperatures are fatal (Du et al. 2003, Shine et al. 2003, Neuwald and Valenzuela 2011). Counter intuitively, because predicted CTE values initially fall when nest temperatures exceed T_{MAX}, it is theoretically plausible that exposure to high temperatures could yield male offspring (e.g., Neuwald and Valenzuela 2011). Our model only predicts this under extreme conditions, and never under scenarios where only *M'* and *w* are adjusted. Thus, increased mortality should be the primary effect of nests spending increased time above T_{MAX} (e.g., 1998 in Janzen 1994a and in responce to ENSO cycles in , Tomillo et al. 2012).

Potential Effects of Model Assumptions on Conclusions

The CTE model successfully predicted sex ratios within 40 of 46 natural painted turtle nests (see Appendix C). Model error might reflect natural among-nest variation in T_{PIV}

or the TRT, improper placement of temperature loggers within nests, or nests having TSPs outside the July data logger deployment period. Nonetheless, our results support the view that the CTE model can be a powerful tool for predicting population trends in sex ratio. Moreover, the success of our simple model with T_{MAX} reveals a general alternative to curvilinear approaches. In fact, an intermediate model, simpler than proposed curvilinear models but more realistic than our model, yielded almost identical results (see Appendix D).

Although we explored the behavior of our model for different values of the diel thermal range parameter (*R*), we assumed that *R* is constant throughout the growing season. Seasonal variation in *R* could affect the pre-TSP period and link *R* to nesting phenology, ultimately affecting offspring sex ratio. Although data collected by the Geostationary Operational Environmental Satellite (GOES-8) show that *R* constricts substantially from spring to summer, this trend appears to be an artifact of not accounting for increased cloud cover in spring (Sun et al. 2006). The trend is not observed in data from weather stations that record under all sky conditions, where instead the diel thermal range from May to August is relatively constant (Sun et al. 2006). Our assumption of a constant *R* therefore appears appropriate.

It is difficult to know how climate change will affect seasonal/annual temperature trends. Winter temperatures are increasing faster than summer temperatures globally (Balling et al. 1998, Stine et al. 2009). This disparity will dampen the slope of temperature change across spring and could increase the correlation between temperatures at nesting and at the onset of the TSP, thereby increasing the power of females to buffer offspring sex ratios by adjusting nesting date. However, this global trend is highly variable and current models have a difficult time capturing it (Balling et al. 1998). Due to this difficulty, we assumed that

spring and summer temperatures would increase uniformly. While it is evident that relative differences in summer and winter warming could change our specific results, we expect our qualitative conclusions to hold for a broad range of scenarios (Fig 5).

Our model also assumes that female phenology will shift such that oviposition occurs at the same soil temperature each year. Given a 4 °C increase in temperature, nesting date would need to advance ~3 weeks. Such shifts are plausible for reptiles (Doody et al. 2006, Tucker et al. 2008, Telemeco et al. 2009); however thermal reaction norms for female painted turtles encompass shifts in nesting phenology of only ~10 days (Schwanz and Janzen 2008). Females therefore may not be able to nest early enough to track climate change. However, nesting date plasticity appears to have little effect on TSP temperatures, and thus sex ratios (Fig 4), because slight increases in temperature should induce 100% female sex ratios regardless of nesting date.

Conclusions

Our model suggests that painted turtles will not be able to buffer their progeny from climate change by adjusting phenology alone. We considered a broad range of parameter values, thus this result should be general across species and thermally-dependent biological processes. Nesting earlier fails to buffer nest temperatures from climate change because temperatures at oviposition and during the period when embryonic sex is labile become increasingly mismatched as nesting date advances. Similar disparities have been observed in other species and are likely common among temperate species with thermally-plastic phenologies (e.g., Visser and Both 2005, Parmesan 2006, Telemeco et al. 2009). The consequences of these discordances vary. For example, mismatches in phenology can

separate development from suitable temperatures, as demonstrated here, but also can disconnect important ecological interactions such as those between predators and prey, and between pollinators and flowers (reviewed in Visser and Both 2005, Parmesan 2006).

It is plausible that high egg mortality will have earlier and greater effects on population persistence than skewed sex ratio in species with TSD. This may be especially true in long-lived species such as painted turtles where occasional influxes of the rare sex could maintain viable population sex ratios. In such cases, egg mortality might be a more pressing climate-change concern. Indeed, all species that deposit eggs terrestrially could be susceptible to these predicted increases in egg mortality. Still, temperature-sensitive species might respond such that they can persist in the face of impending climate change by controlling the temperature at the onset of the thermosensitive period, *M'*, but multiple biological responses might be necessary. Future studies on taxa observed to plastically respond to climatic variation should focus on jointly examining the effects of all responses.

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FIGURES

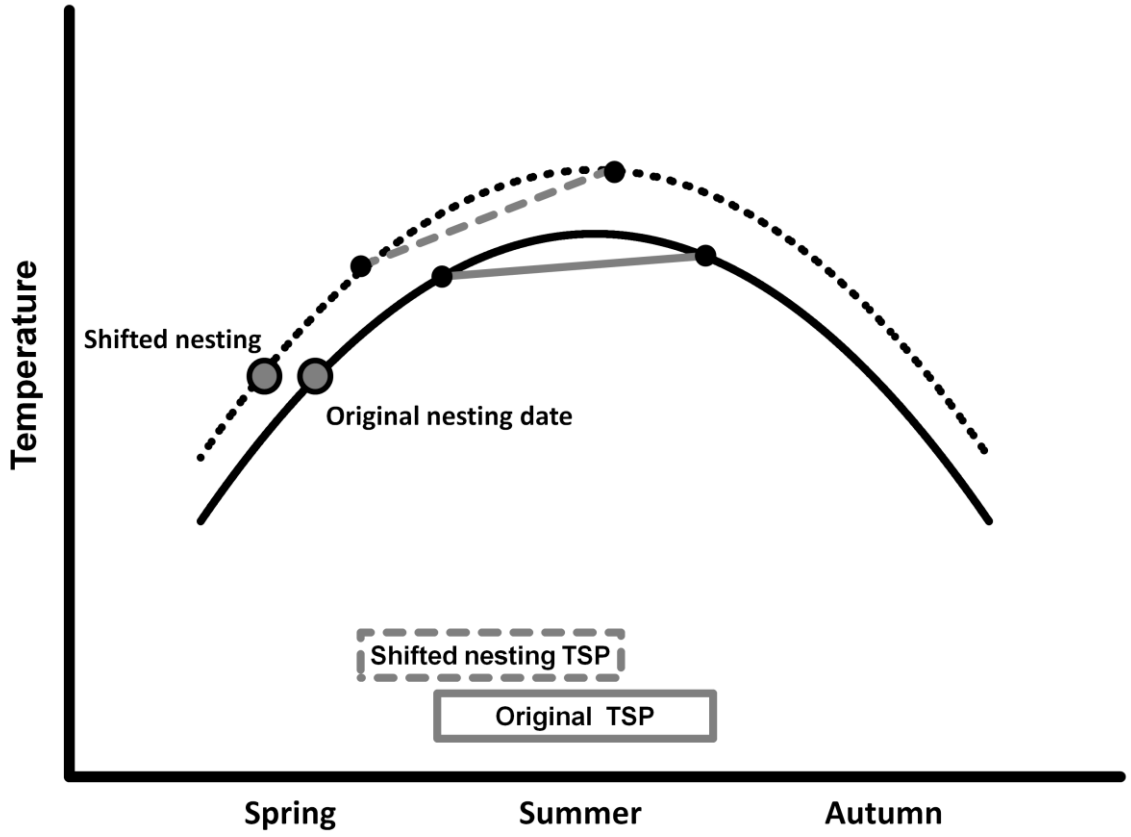

Figure 1—Hypothetical seasonal temperatures in spring, summer, and autumn before (solid line) and after (dotted line) a homogeneous increase in temperature. As females advance nesting date in response to climate warming, the slope of temperature change during the period when offspring sex is labile (thermosensitive period, TSP, horizontal boxes and corresponding lines) will increase. As a result, advancing nesting date in response to climate change such that temperature at the time of oviposition remains constant from year to year may be insufficient to keep the TSP from warming and sex ratios from becoming biased.

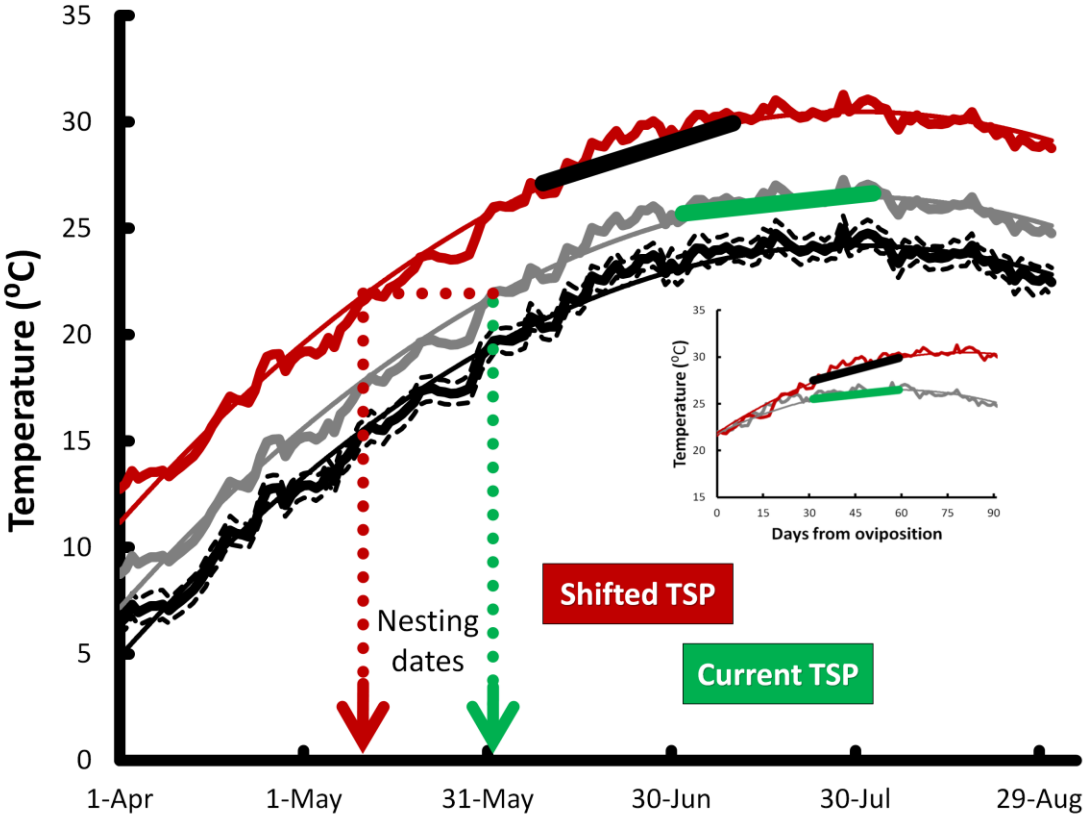

Figure 2—Predicted effects of climate change on nest temperatures during the thermosensitive period (TSP) of *Chrysemys picta* at the Thomson Causeway Recreation Area. The thick black line is mean daily 10-cm soil temperatures collected over 21 years at an Iowa City weather station and the corresponding dotted lines are ± 1.0 *s.e.* The thick grey line depicts these data after transformation to match temperatures recorded in natural nests (see Appendix C) and the thick red line depicts predicted soil temperatures after a uniform 4.0 ºC temperature increase. The smoothed lines are quadratic functions fitted to these data (prior to transformation: temperature = $-3E^{-05}(\text{time})^2 + 0.0515(\text{time}) + 4.5603$ with time in radians [day x 2π]). The green arrow shows the average historic nesting date of *C. picta* (1 June), whereas the red arrow depicts when females would need to nest in order to nest at the same temperature before and after warming (11 May, 21 d shift). The green box and corresponding line depict temperatures during the historic TSP (~July), whereas the red box and corresponding black line depict predicted temperatures during the TSP after regional warming and shifted nesting. The inset depicts the grey (pre-warming) and red (post-warming) nest temperature curves overlaid with date from oviposition on the x-axis.

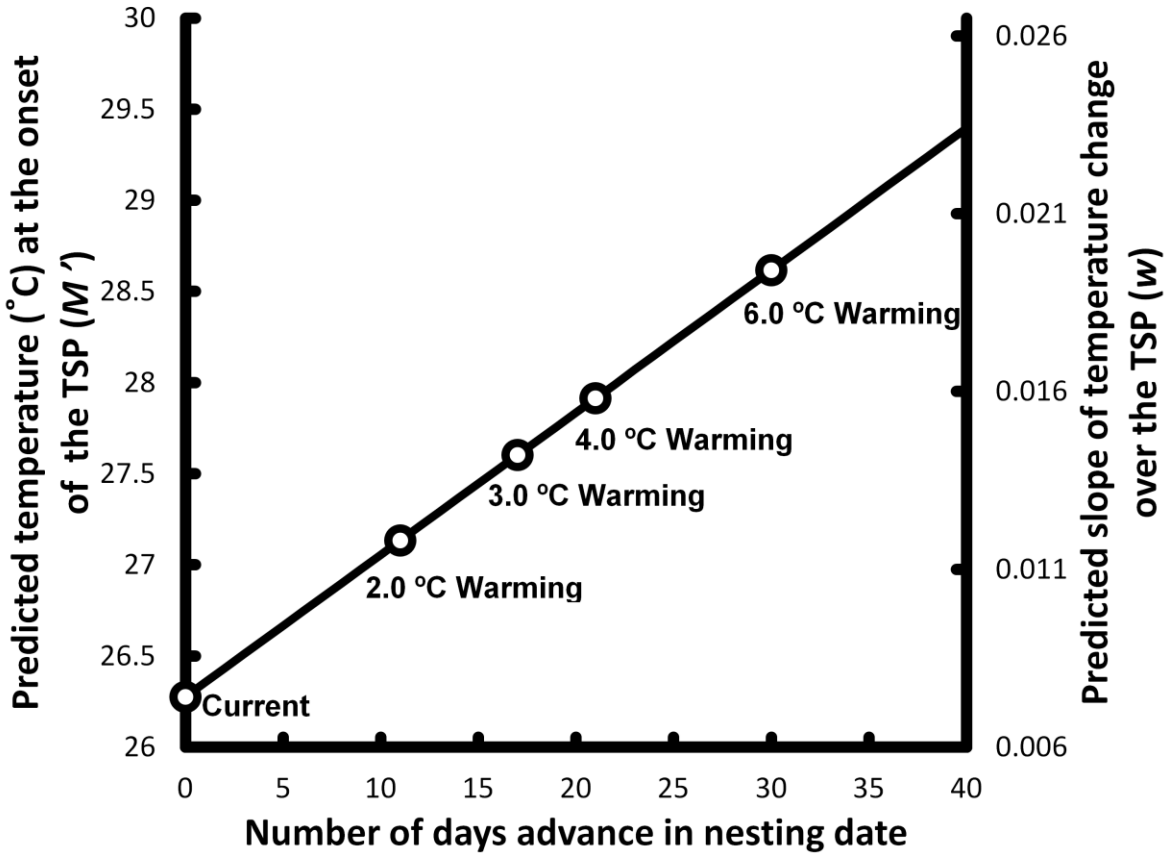

Figure 3—Effects of regional warming and shifts in *Chrysemys picta* nesting date (number of days advanced from the historic nesting date of 1 June) at the Thomson Causeway Recreation Area on temperature at the onset of the TSP (*M'*) and the slope of temperature change during the TSP (*w*), both of which are predicted to increase. Highlighted points show the amount of warming that would be necessary for corresponding shifts, given the assumption that female *C. picta* nest at the same temperature annually. Values assume uniform increases in annual temperature.

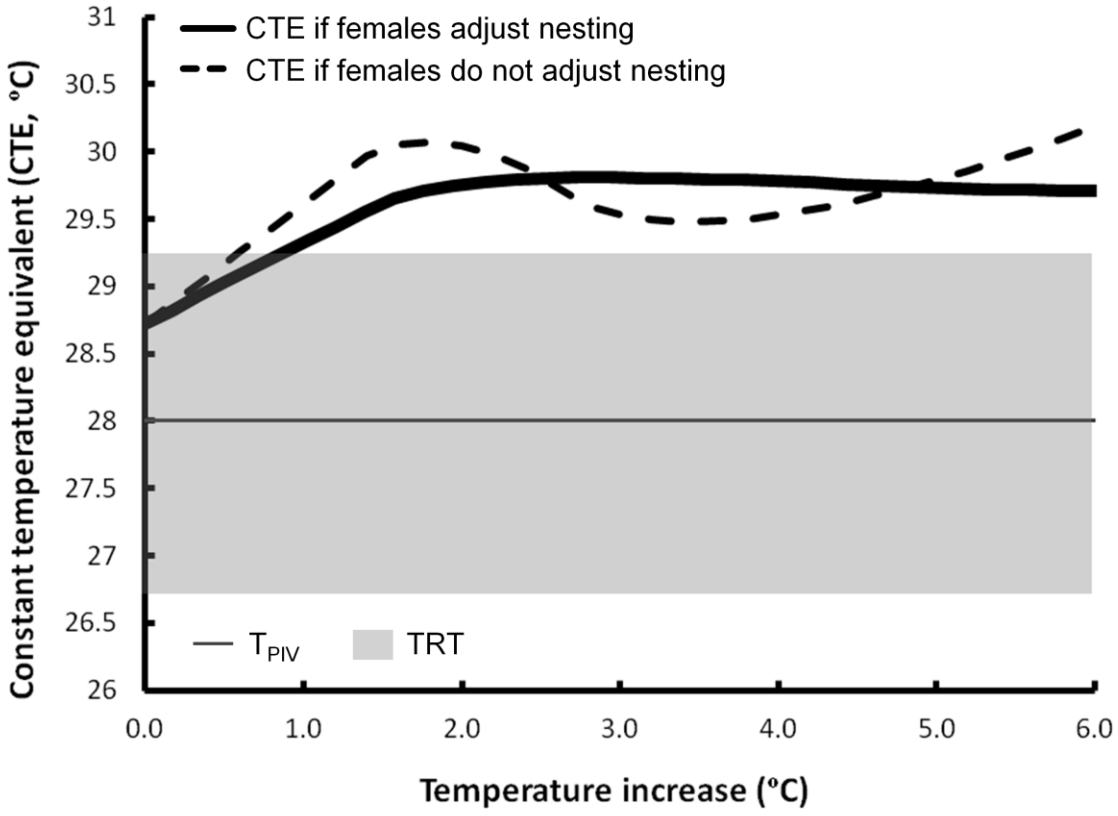

Figure 4—Predicted effects of uniform increases in environmental temperature on constant-temperature equivalent (CTE) values in painted turtle nests at the Thomson Causeway Recreation Area. The solid line depicts CTEs if females adjust their nesting date such that they always nest at the same temperature, whereas the dashed line depicts CTEs if females continue to nest on the average historic nesting date at Thomson (June 1). CTE values within the shaded transitional range of temperatures (TRT) may result in either single- or mixed-sex nests, with T_{PIV} representing the CTE that is predicted to result in a 1:1 sex ratio. To create the solid line, we simulated M' and w values shifting in tandem according to predictions from Fig 3. To create the dashed line, w was held at the historic predicted value at Thomson, while increases in environmental temperature were added to the historic M' value. All other parameters were held at their historic mean values (T_0 = 14.0 ºC, T_{MAX} = 34.0 ºC, R = 5.0 ºC, TSP length = 30 d).

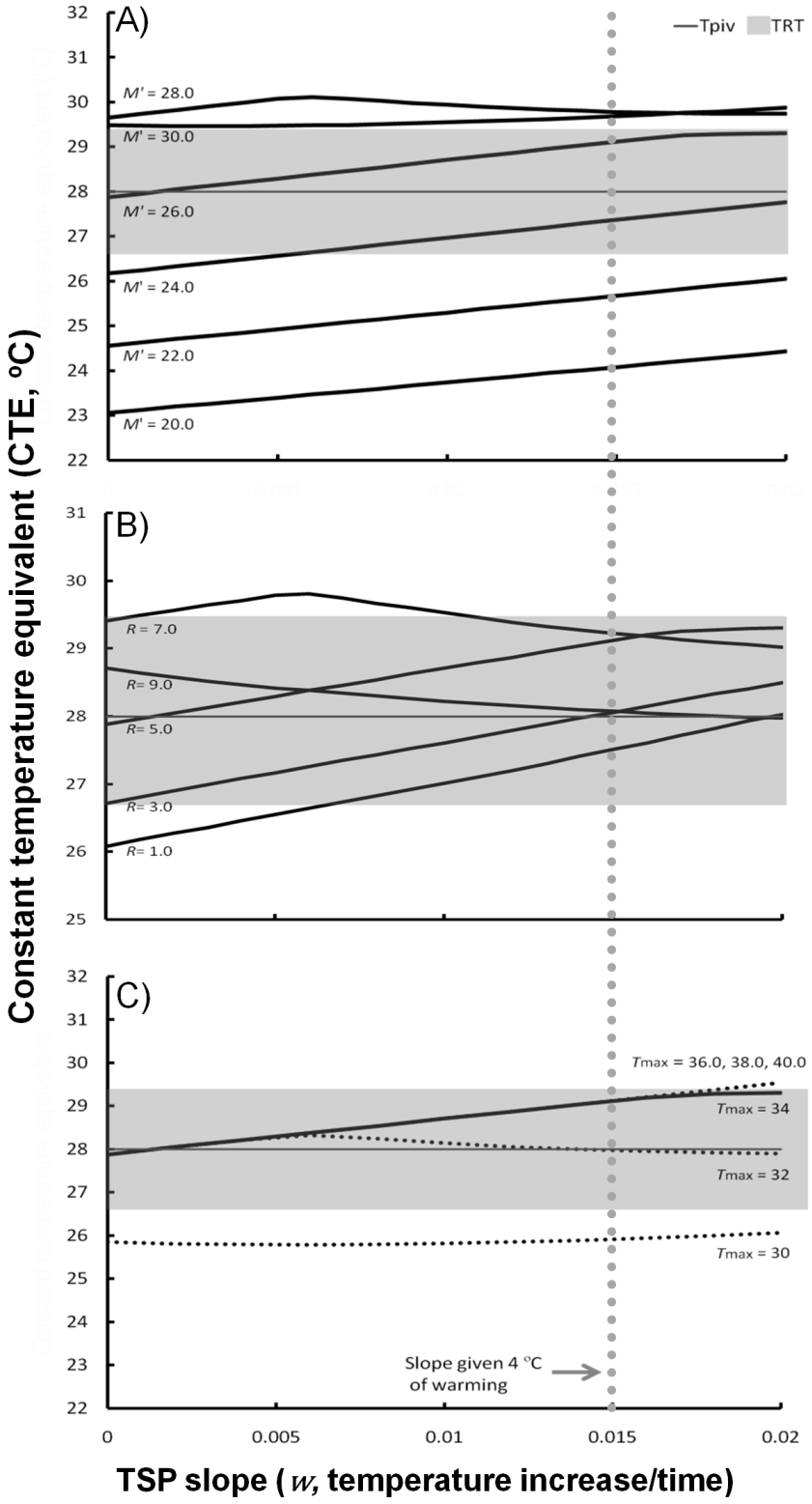

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Figure 5—Predicted effects of slope (*w*) during the thermosensitive period (TSP) on the constant-temperature equivalent (CTE) of nests for different values of A) temperature at the onset of the TSP (*M′*), B) the diel thermal range of temperature (2*R*), and C) the critical thermal maximum for development (T_{MAX}). For C), dotted lines represent when $T_{MAX} \neq 34$ to clarify where predicted CTE values overlap. When not marked otherwise, parameters were set at $M' = 26.0$ ºC, $R = 5.0$ ºC, $T_{MAX} = 34.0$ ºC, $T_0 = 14.0$ ºC, TSP length = 30 d, which are approximately the mean values from current nests. The dotted vertical line marks the slope of temperature change over the TSP that results from 4.0 ºC warming given that females advance their nesting date such that they nest at the same temperatures annually. CTE values within the shaded transitional range of temperatures (TRT) may result in either single- or mixed-sex nests, with T_{PIV} representing the CTE that is predicted to result in a 1:1 sex ratio. CTE values above the TRT are predicted to result in 100% female nests, whereas CTE values below the TRT are predicted to result in 100% male nests. To aid interpretation, a few scenarios are described in the text.

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CHAPTER 6

GENERAL CONCLUSIONS

A major goal of modern biology is to understand how rapid changes to the environment, such as those resulting from global climate change, habitat destruction, and invasive species, affect biota. It is becoming increasingly well recognized that, to understand the biotic effects of environmental change, we must first understand the mechanisms that mediate the ecological and evolutionary responses of organisms to their environment (e.g., Pearson and Dawson 2003, Buckley et al. 2010, Fuller et al. 2010, Sears and Angilletta 2011). Currently, we poorly understand these mechanisms for the vast majority of taxa. For example, we generally do not know which aspects of the environment, life-history stages, or biological attributes most limit species' distributions. My dissertation research contributes to bridging these knowledge gaps.

In Chapters 2–4, I used alligator lizards (genus *Elgaria*) as a model system to examine proximate mechanisms that mediate responses of reptiles to variation in thermal environments. Before accurate predictions for how taxa will be affected by rapid environmental change can be made, relevant taxonomic units must be identified (Atkins and Travis 2010, Pearman et al. 2010). To this end, in Chapter 2, I used an integrative approach to delineate operational taxonomic units in the *E. multicarinata–E. panamintina* species complex. This integrative approach allowed me to test predictions of alternate phylogenetic hypotheses. My data confirm the species status of *E. panamintina*, a taxon of special concern (Stebbins 1958, Jennings and Hayes 1994, Mahrdt and Beaman 2007). In addition, my data support the existence of two cryptic clades within *E. multicarinata* (as predicted by Feldman

and Spicer 2006). Additional genetic work, preferably with nuclear loci, is needed to determine whether or not these taxa are reproductively isolated biological species or are reticulating into a single taxon. Regardless, these taxa are both ecologically and genetically divergent, and thus I propose they be considered separately for management purposes. Even though my species distribution modeling suggested that these taxa have divergent tolerances (Chapters 3 and 4), I could not detect differences in the thermal physiology of these clades. Thus, the Northern and Southern *E. multicarinata* clades appear to have conserved thermal physiology, while other aspects of their physiology likely differ. Adaptive changes in behavior, either through evolution or phenotypic plasticity, may largely allow these lizards to occupy different thermal environments (Huey et al. 2003, Doody et al. 2006, Angilletta 2009, Mitchell et al. 2013). For example, variation in thermoregulatory behavior and nest site choice could largely buffer the thermal environments to which individuals are exposed, thereby reducing natural selection for divergent thermal physiologies (Huey et al. 2003, Doody et al. 2006, Angilletta 2009, Mitchell et al. 2013). Experiments examining thermoregulatory and nesting behaviors in both *E. multicarinata* clades are needed to test this hypothesis. In addition, given the importance of hydric parameters for my species distribution models, hydric physiology might largely limit the fundamental niche of these taxa. If the hydric environment has relatively low variance on a micro-habitat scale, changes in behavior will have little power to buffer individuals from inhospitable hydric conditions. Experiments examining the hydric physiology of the Northern and Southern *E. multicarinata* clades are needed to address this hypothesis.

Once relevant taxa are identified, we need to understand aspects of the environment that most limit fundamental niches. Predictive models generally account for the average

environment and ignore environmental variance (Angilletta 2009, Buckley et al. 2010). However, such variance might be more limiting, and increases in the frequency of extreme events are major predictions of climate-change models (Bloom 2010, IPCC 2013, Telemeco et al. 2013). Given that adult *E. multicarinata* and *E. coerulea* are active at virtually identical body temperatures, extreme temperatures might be more limiting for these species. In Chapter 3, I examined physiological stress of alligator lizards in response to thermal variation. As predicted, temperature affected corticosterone (CORT) levels in both species, with CORT generally increasing with temperature. While hot temperatures were stressful for both species, the species differed in their response to extreme cold temperatures, with only *E. multicarinata* displaying signs of stress. These results suggest that temperature substantially affects the stress physiology of alligator lizards and the physiological stress response might contribute to biogeographic differences in *E. multicarinata* and *E. coerulea*. Importantly, variation in the physiological stress response could affect the competitive landscape, resulting in local population declines before environmental conditions become fatal. Even so, the relative importance of the thermal-stress response for the delimiting alligator lizard biogeography compared to other factors, such as the hydric and biotic environment, is still uncertain. Further work is needed to tease apart the relative importance of these various factors.

While Chapter 3 suggests that relatively extreme environments will be more limiting for adult alligator lizards than average environmental conditions, the adult life-history stage might not be most limiting for the species. Rather, developing embryos might restrict the thermal environments where populations can persist. Indeed, Chapter 4 suggests that the thermal tolerance of *E. multicarinata* changes ontogenetically, with developing embryos

being least tolerant. Thus, the availability of suitable nest sites might limit the biogeography of this species. In particular, ontogenetic variation in thermal sensitivity may reduce *E. multicarinata* persistence in cold environments. While these results confirm that *E. multicarinata* embryos are more thermally sensitive than adults, current data are insufficient to know the full extent of this difference. I used constant temperature treatments, which provide a useful first approximation but poorly mimic natural nest conditions (Ashmore and Janzen 2003, Du and Ji 2006, Les et al. 2007, Warner and Shine 2011, Angilletta et al. 2013). How embryos respond to acute thermal stressors may have important implications for alligator lizard biogeography. Future studies are needed to examine the effects of acute thermal variance on development. Moreover, hydric conditions will interact with nest temperature in nature (Packard et al. 1988, Flatt et al. 2001, Belinsky et al. 2004). Understanding the hydric requirements of developing embryos will be necessary for understanding the full sensitivity of developing embryos to abiotic environments.

My data on the thermal ecology of alligator lizards help describe proximate mechanisms that mediate effects of thermal environments on population persistence and biogeography. My experiments highlight the importance of examining the effects of environmental variation as well as considering which life-history stage is most limiting. If we had naively constructed models predicting the effects of thermal variation on alligator lizard populations prior to these experiments, we most likely would have only considered the effects of the average thermal environment on adults. This approach would have provided poor predictions because alligator lizard adults are broadly tolerant of the average thermal environment. My results also highlight the difficulty of collecting sufficient data for constructing useful mechanistic models. While my results illuminate how the thermal

environment affects alligator lizards, they still do not provide a complete enough picture for mechanistic modeling. For example, while my results identify the importance of the embryonic life-history stage for alligator lizard population persistence, we do not know the conditions in natural nests nor the current availability of those conditions. Without this basic information, we cannot yet usefully model how nest conditions might change, how these changes will affect embryos, or how these embryonic affects will subsequently affect populations.

In Chapter 5, I constructed a mechanistic model using data from one of the few reptile species for which sufficient data are available–painted turtles (*Chrysemys picta*). I used this model to examine the potential power of altering nesting date to counteract negative consequences of climate change. Somewhat surprisingly, the model suggested that changing nesting date has little effect on offspring and thus will not substantially buffer painted turtles from climate change. This result does not imply that painted turtles will go extinct as a result of climate change, but it does suggest that shifts in phenology alone will not protect populations. The predictions of the model are likely general to any thermally-sensitive organisms that live in temperate climates. However, future work is needed to directly test this hypothesis. My results raise an interesting question: If shifting phenology generally does not buffer populations from changes in thermal environments, why are changes in spring phenology the most commonly observed biotic response to climate change (Visser and Both 2005, Parmesan 2006, Moser et al. 2009)? I hypothesize that advancing spring phenology is adaptive because it allows individuals to reproduce slightly earlier, thereby reducing the risk of reproductive failure. In species that produce multiple clutches per season, earlier nesting may also allow increased annual fecundity, and thus be even more advantageous. Under this

hypothesis, natural selection drives individuals to initiate their seasonal life-history events as soon as they are physiologically able. In the temperate zone, this physiological ability will largely depend on the timing of spring warming. Such a life-history-evolution based hypothesis could explain the broad taxonomic distribution of observed advances in spring phenology with climate change. Still, further work is needed to test this hypothesis and determine its generality.

Taken together, my dissertation illustrates the importance of experimentally investigating mechanisms that drive population responses to changing environments. Reptiles have persisted for hundreds of millions of years and it seems unlikely that they will be uniformly driven to extinction in the near future. Even so, many species and populations are on the brink (Sinervo et al. 2010, Böhm et al. 2013). My results suggest that extreme environments and embryonic life-history stages are important for determining the thermal biogeography of alligator lizards. However, this thermal physiology was largely conserved (no detectable differences between northern and southern *E. multicarinata* and limited differences between *E. coerulea* and *E. multicarinata*) and may have low evolutionary potential. Phenotypic plasticity might be more important, but no single plastic response will likely be a panacea. In painted turtles, major changes in phenology could not buffer populations from modest environmental change. None of these results were intuitively obvious, and were only achievable through experimentation and modeling.

In ancient times, the phrase "Here be dragons" warned travelers away from dangerous, poorly explored locations believed to be the realms of monsters (Kaplan 2012). Today, we know such monsters do not exist and recognize that the "dragons" we do have, reptiles, are integral components of ecosystems. We now find the ancient warning quaint; a

token of a time long-since past. Perhaps, a modern warning should be "Here *used* to be dragons," for if we do not change the rate at which we are altering environments and come to better understand the effects of already irreversible changes, soon there may no longer be "dragons" left in most places on Earth.

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APPENDIX A

SPECIMENS EXAMINED FOR MORPHOMETRIC ANALYSES IN CHAPTER 2

Table 1—Alligator lizard specimens examined for morphometric analyses. The first three letters of the Specimen ID denote the institution (CAS: California Academy of Science, LACM: Natural History Museum of Los Angeles County, MVZ: Museum of Vertebrate Zoology, University of California, Berkeley, RST: collected by the authors). Specimens for which clade were determined using mitochondrial DNA data by Feldman and Spicer (2006) are marked in the mtDNA column. All other specimens were assigned to a clade based on their collection location. Clade abbreviations are C: Coastal, SC: Southern California, NC: Northern California, SSN: Southern Sierra Nevada, and pan: *Elgaria panamintina*. Historically, C, SC, NC, and SSN were all considered *E. multicarinata*. Year is the year of collection. For all individuals, sex was determined by direct examination of the gonads. SVL is snout-vent length. Individuals for which a hemipene was analyzed are denoted with an M (linear measurements for multivariate morphometrics) and/or a G (landmark data for geometric morphometrics). Individuals for which heads were sufficiently preserved for geometric morphometric analyses of the head are denoted with a D (dorsal head data) and/or an L (lateral head data).

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_173576		C	37.61	-121.75	CA	Alameda	1989	F	11.3		D,L
CAS_178514		C	37.64	-121.49	CA	San Joaquin	1982	M	13.4	M,G	D,L
CAS_188638		C	35.62	-120.90	CA	San Luis Obispo	1988	M	12.55	M,G	D,L
CAS_188644		C	35.04	-120.04	CA	Santa Barbara	1988	M	12.8	M,G	
CAS_188646		C	37.54	-121.83	CA	Alameda	1987	F	12		D,L
CAS_188647		C	37.71	-121.55	CA	Alameda	1988	M	11.8	M,G	
CAS_188648		C	37.64	-121.77	CA	Alameda	1989	F	11.3		D,L
CAS_188676		C	37.01	-121.53	CA	Santa Clara	1976		13	M	
CAS_188680		C	37.26	-122.03	CA	Santa Clara	1976	M	13	M	

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_188684		C	37.21	-121.92	CA	Santa Clara	1976	M	11	M,G	
CAS_188697		C	37.21	-121.90	CA	Santa Clara	1981	M	12.2	M,G	D,L
CAS_188735		C	37.20	-121.56	CA	Santa Clara	1988	M	12	M	D,L
CAS_188739		C	37.01	-121.55	CA	Santa Clara	1988	M	11	M,G	D,L
CAS_188750		C	37.00	-121.53	CA	Santa Clara	1989	M	10.5	M,G	D,L
CAS_188754		C	36.96	-121.51	CA	Santa Clara	1989	M	9.6		D,L
CAS_188755		C	37.16	-121.67	CA	Santa Clara	1989	M	11.7	M,G	D,L
CAS_188758		C	37.04	-121.55	CA	Santa Clara	1989	F	8.7		D,L
CAS_188761		C	37.26	-121.78	CA	Santa Clara	1989	M	11.6	M,G	D,L
CAS_188766		C	37.06	-121.58	CA	Santa Clara	1989	M	10.5		D,L
CAS_188768		C	37.24	-121.76	CA	Santa Clara	1989	M	12.45	M,G	
CAS_188770		C	37.22	-121.74	CA	Santa Clara	1989	M	12.6	M,G	
CAS_188774		C	37.41	-121.98	CA	Santa Clara	1989	M	13.6	M,G	
CAS_188791		C	36.07	-120.93	CA	Monterey	1977	M	9.45		D,L
CAS_188792		C	36.08	-121.40	CA	Monterey	1986	M	12.55	M,G	
CAS_188796		C	36.16	-120.77	CA	Monterey	1987	M	8.7		D,L
CAS_188800		C	36.12	-121.46	CA	Monterey	1987	M	12.5	M,G	D,L
CAS_188803		C	36.35	-121.58	CA	Monterey	1988	M	10.15	M,G	D,L
CAS_197490		C	36.93	-121.78	CA	Santa Cruz	1993	M	12.4	M,G	D,L
CAS_197494		C	35.66	-121.22	CA	San Luis Obispo	1993	M	12.8	M,G	D,L
CAS_198445		C	35.81	-120.76	CA	Monterey	1994	F	9.2		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_199476		C	36.61	-121.64	CA	Monterey	1995	M	11.5	M,G	D,L
CAS_203168		C	36.77	-121.74	CA	Monterey	1997	M	12.2	M,G	D,L
CAS_203210		C	37.18	-121.75	CA	Santa Clara	1996	M	11.3	M,G	D,L
CAS_203233		C	37.42	-121.95	CA	Santa Clara	1997	F	11		D,L
CAS_205794		C	36.37	-121.56	CA	Monterey	1998	F	8.9		D,L
CAS_207093		C	37.51	-122.00	CA	Alameda	1998	F	9.4		D,L
CAS_208514	X	C	35.21	-120.25	CA	San Luis Obispo	1999	M	13.3	M,G	D,L
CAS_208515	X	C	35.21	-120.25	CA	San Luis Obispo	1999	F	13.15		D,L
CAS_208683		C	37.70	-122.02	CA	Alameda	1999	M	12.05	M,G	D,L
CAS_208948	X	C	36.14	-121.44	CA	Monterey	1999	M	13.4	M,G	D,L
CAS_226091		C	36.38	-121.55	CA	Monterey	1998	F	8.9		D,L
CAS_228224		C	36.23	-121.48	CA	Monterey	2001	F	12.9		D,L
CAS_229090		C	35.62	-121.06	CA	San Luis Obispo	2002	F	9.7		D,L
CAS_235848		C	37.59	-121.79	CA	Alameda	2006	F	9.2		D,L
CAS_241827		C	35.60	-121.07	CA	San Luis Obispo	2006	F	10.75		D,L
CAS_241848		C	35.94	-121.48	CA	Monterey	2006	M	9.1		D
CAS_241917		C	34.67	-119.92	CA	Santa Barbara	2006	F	13.2		D,L
CAS_243754		C	37.93	-122.02	CA	Contra Costa	2004	F	8.65		D,L
LACM_121964		C	34.56	-119.77	CA	Santa Barbara	1975	F	14		D,L
LACM_128472		C	36.42	-121.00	CA	San Benito	1977	M	11.55	M,G	D,L
LACM_130777		C	34.78	-119.95	CA	Santa Barbara	1979	F	14.1		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
LACM_149152		C	35.67	-121.28	CA	San Luis Obispo	2000	F	10.2		D,L
MVZ_149675		C	34.43	-119.81	CA	Santa Barbara	1977	F	10.9		D,L
MVZ_191100		C	37.94	-122.14	CA	Contra Costa	1981	M	11.5	M,G	D,L
MVZ_191125		C	37.90	-122.06	CA	Contra Costa	1981	M	9.3	M,G	D,L
MVZ_191140		C	36.27	-121.81	CA	Monterey	1982	F	10.8		D,L
MVZ_191146		C	36.27	-121.81	CA	Monterey	1982	M	12.3	M,G	D,L
MVZ_191152		C	36.52	-121.08	CA	San Benito	1983	M	11.95	M,G	D,L
MVZ_191153		C	37.40	-122.09	CA	Santa Clara	NA	F	9.55		D,L
MVZ_191316		C	37.90	-122.18	CA	Contra Costa	1984	M	9.45		D,L
MVZ_227746		C	36.51	-121.14	CA	San Benito	1988	M	13.6	M,G	D,L
MVZ_227747	X	C	36.51	-121.14	CA	San Benito	1988	F	13.5		D,L
MVZ_228811	X	C	36.70	-120.79	CA	Fresno	1998	F	11.9		D,L
MVZ_228812		C	36.33	-121.58	CA	Monterey	1998	M	10.9	M,G	D,L
MVZ_228813		C	35.38	-120.86	CA	San Luis Obispo	1998	M	9.1		D,L
MVZ_228814		C	37.12	-121.84	CA	Santa Clara	1998	F	11.35		D,L
MVZ_230557		C	37.34	-121.64	CA	Santa Clara	1999	F	9.9		D,L
MVZ_236794		C	37.87	-122.26	CA	Alameda	2002	M	8.75		D,L
MVZ_238559		C	36.38	-121.56	CA	Monterey	2003	F	8.6		D,L
MVZ_243351		C	37.96	-121.99	CA	Contra Costa	2003	M	11.5		D,L
RST_201.mw		C	36.64	-121.79	CA	Monterey	2011	F	13.1		D
RST_202.mw		C	36.65	-121.73	CA	Monterey	2011	F	11.4		D
RST_203.mw		C	36.64	-121.78	CA	Monterey	2011	M	114	M,G	D

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
RST_204.mw		C	36.64	-121.78	CA	Monterey	2011	M	11.6		D
RST_205.mw		C	36.64	-121.78	CA	Monterey	2011	M	134	M,G	D
RST_206.mw		C	36.64	-121.78	CA	Monterey	2011	M	122	M,G	D
RST_207.mw		C	36.65	-121.73	CA	Monterey	2011	M	10.7		D
RST_208.mw		C	36.63	-121.79	CA	Monterey	2011	F	13.2		D
RST_209.mw		C	36.60	-121.79	CA	Monterey	2011	M	112	M,G	D
RST_210.mw		C	36.65	-121.73	CA	Monterey	2011	F	12.7		D
RST_211.mw		C	36.64	-121.79	CA	Monterey	2011	M	113	M,G	D
RST_212.mw		C	36.64	-121.78	CA	Monterey	2011	M	123	M,G	D
RST_213.mw		C	36.64	-121.79	CA	Monterey	2011	M	113	M,G	D
RST_214.mw		C	36.63	-121.79	CA	Monterey	2011	M	135	M,G	D
RST_215.mw		C	36.63	-121.79	CA	Monterey	2011	M	134	M,G	D
RST_216.mw		C	36.63	-121.79	CA	Monterey	2011	F	12.4		D
RST_217.mw		C	36.64	-121.78	CA	Santa Cruz	2011	M	12		D
RST_227.mw		C	36.63	-121.79	CA	Monterey	2011	M	126	M,G	D
RST_228.mw		C	36.64	-121.78	CA	Monterey	2011	F	11.9		D
CAS_154649		NC	37.98	-122.55	CA	Marin	1980	F	10.5		D,L
CAS_162691		NC	39.64	-121.78	CA	Butte	1987	M	13.5	M,G	D,L
CAS_175214		NC	38.54	-121.72	CA	Yolo	1988	M	13.75	M,G	D,L
CAS_178519		NC	38.60	-121.19	CA	Sacramento	1983	M	10.2	M,G	
CAS_180336		NC	39.25	-121.28	CA	Yuba	1991	F	8.8		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_183533		NC	38.45	-122.06	CA	Solano	1991	F	12.7		D,L
CAS_186491		NC	38.41	-122.04	CA	Solano	1992	F	10.6		D,L
CAS_188658		NC	36.86	-119.12	CA	Fresno	1988	M	12.1	M,G	
CAS_201252		NC	39.61	-123.37	CA	Mendocino	1996	M	13.8	M,G	D,L
CAS_201818		NC	37.62	-122.49	CA	San Mateo	1996	M	13.6	M,G	D,L
CAS_202917		NC	39.56	-120.79	CA	Sierra	1997	F	11.1		D,L
CAS_203523		NC	39.55	-120.70	CA	Sierra	1997	F	12.1		D,L
CAS_203614		NC	39.15	-122.75	CA	Lake	1997	M	15	M,G	D,L
CAS_204625		NC	37.58	-122.42	CA	San Mateo	1998	F	12		D,L
CAS_205234		NC	39.42	-121.12	CA	Yuba	1998	M	12.2	M,G	D,L
CAS_205566		NC	39.71	-121.38	CA	Butte	1998	M	13.4	M,G	D,L
CAS_205764		NC	37.22	-122.22	CA	Santa Cruz	1998	F	10.5		D,L
CAS_205780		NC	37.57	-120.12	CA	Mariposa	1998	M	12.4	M,G	D,L
CAS_205830	X	NC	39.71	-121.38	CA	Butte	1998	M	10.6	M,G	D,L
CAS_205836	X	NC	39.71	-121.38	CA	Butte	1998	M	11.65	M,G	D,L
CAS_206368		NC	39.51	-121.35	CA	Butte	1998	M	10.2		D,L
CAS_206370		NC	39.02	-120.72	CA	Placer	1998	M	11.3	M,G	D,L
CAS_206374		NC	37.57	-122.42	CA	San Mateo	1998	F	11.9		D,L
CAS_206459	X	NC	40.25	-121.76	CA	Tehama	1998	M	11.7	M,G	D,L
CAS_207095		NC	37.53	-122.35	CA	San Mateo	1998	M	10.9	M,G	D,L
CAS_207160		NC	39.03	-122.43	CA	Colusa.	1998	M	13.9	M,G	D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_208659		NC	37.61	-122.40	CA	San Mateo	1999	M	9.05		D,L
CAS_208700		NC	39.49	-121.04	CA	Yuba	1998	F	11.15		D,L
CAS_208822	X	NC	36.92	-119.30	CA	Fresno	1999	M	14.9	M,G	D,L
CAS_208852		NC	39.83	-123.18	CA	Mendocino	1999	M	11.2	M,G	D,L
CAS_208917		NC	37.61	-119.82	CA	Mariposa	1999	F	12.75		D,L
CAS_208932		NC	37.58	-119.71	CA	Mariposa	1999	M	13.9	M,G	D,L
CAS_208947		NC	37.21	-122.21	CA	Santa Cruz	1999	F	9		D,L
CAS_209150		NC	39.20	-122.85	CA	Lake	1999	M	14.2	M,G	D,L
CAS_209176		NC	39.26	-122.94	CA	Lake	1999	F	13.5		D,L
CAS_209201		NC	37.50	-119.72	CA	Mariposa	1999	F	13.9		D,L
CAS_209204		NC	37.57	-119.88	CA	Mariposa	1999	F	14		D,L
CAS_209551		NC	39.67	-121.38	CA	Butte	1999	M	11.1	M,G	D,L
CAS_209667		NC	39.75	-121.36	CA	Butte	1999	F	11		D,L
CAS_209923		NC	39.26	-121.00	CA	Nevada	1999	F	11.1		D,L
CAS_212751	X	NC	39.29	-122.61	CA	Colusa.	2000	F	14.1		D,L
CAS_212986		NC	37.39	-119.32	CA	Madera	2000	M	10.3		D,L
CAS_218626		NC	37.70	-122.42	CA	San Mateo	2001	M	11.8	M,G	D,L
CAS_218719		NC	37.49	-122.37	CA	San Mateo	2001	M	12.45	M,G	D,L
CAS_219450		NC	39.36	-122.78	CA	Lake	2001	F	14.4		D,L
CAS_219547		NC	39.53	-122.85	CA	Lake	2001	F	10		D,L
CAS_219589		NC	39.47	-122.87	CA	Lake	2001	F	14		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_220770		NC	39.86	-123.00	CA	Mendocino	2001	M	12.2		D,L
CAS_223707		NC	39.71	-122.62	CA	Glenn	2002	F	13.2		D,L
CAS_224773		NC	39.86	-121.37	CA	Plumas	2002	F	11.55		D,L
CAS_224774		NC	40.00	-121.18	CA	Plumas	2002	M	13.4	M,G	D,L
CAS_226972		NC	40.23	-121.88	CA	Tehama	2003	M	9.35	M,G	D,L
CAS_227002		NC	40.08	-121.69	CA	Tehama	2003	M	14.5	M,G	D,L
CAS_227985		NC	38.83	-120.99	CA	El Dorado	2003	M	13.1	M,G	D,L
CAS_227986		NC	38.78	-120.92	CA	El Dorado	2003	M	13.1	M,G	D,L
CAS_228000		NC	38.09	-122.15	CA	Solano	2003	M	12.6	M,G	D,L
CAS_228311		NC	44.55	-123.40	OR	Benton	2003	F	11.3		D,L
CAS_228313		NC	43.60	-123.24	OR	Douglas	2003	M	12.4	M,G	D,L
CAS_228314		NC	43.60	-123.24	OR	Douglas	2003	M	11.6	M,G	D,L
CAS_228315		NC	45.73	-121.56	WA	Skamania	2003	M	11.2	M,G	D,L
CAS_234675		NC	38.79	-120.59	CA	El Dorado	2005	M	9.8		D,L
CAS_234679		NC	38.79	-120.65	CA	El Dorado	2005	M	9.6		D,L
CAS_235901		NC	40.41	-121.98	CA	Tehama	2006	M	13.7	M,G	
CAS_235937		NC	37.10	-119.51	CA	Fresno	2004	F	9.9		D,L
CAS_235942		NC	38.80	-120.86	CA	El Dorado	2004	F	11.3		D,L
CAS_236556		NC	38.64	-120.53	CA	El Dorado	2005	M	14.7	M,G	D,L
CAS_238468		NC	38.40	-122.81	CA	Sonoma	2005	F	9.7		D,L
CAS_238472		NC	38.37	-122.76	CA	Sonoma	2005	F	10.3		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_250301		NC	38.80	-122.82	CA	Sonoma	2012	F	12.7		D,L
CAS_250753		NC	39.39	-123.42	CA	Mendocino	2012	F	10.8		D,L
LACM_128470		NC	36.80	-119.32	CA	Fresno	1978	F	12.3		D,L
LACM_130779		NC	40.73	-122.24	CA	Shasta	1979	M	13	M,G	D,L
MVZ_150172	X	NC	38.84	-120.82	CA	El Dorado	1977	F	10.4		D,L
MVZ_162054	X	NC	38.71	-122.87	CA	Sonoma	1978	F	11.75		D,L
MVZ_162062	X	NC	40.16	-123.62	CA	Humboldt	1978	M	11.7	M,G	D,L
MVZ_162066	X	NC	38.20	-120.39	CA	Calaveras	1978	F	9.15		D,L
MVZ_175428	X	NC	39.31	-120.99	CA	Nevada	1980	F	12.3		D,L
MVZ_191127		NC	44.51	-123.43	OR	Benton	1981	M	14	M,G	D,L
MVZ_191131		NC	38.24	-120.80	CA	Calaveras	1981	F	11.75		D,L
MVZ_191137		NC	39.75	-122.53	CA	Glenn	1981	M	15.1		D,L
MVZ_196132		NC	42.25	-123.43	OR	Josephine	1985	M	12.85	M,G	
MVZ_197544		NC	37.69	-122.41	CA	San Mateo	1982	F	12		D
MVZ_205590		NC	38.31	-122.30	CA	Napa	1986	F	11.6		D,L
MVZ_207574		NC	40.92	-123.44	CA	Trinity	1981	F	12.1		D,L
MVZ_207575		NC	41.41	-123.46	CA	Siskiyou	1983	F	8.9		D,L
MVZ_227752	X	NC	36.99	-122.06	CA	Santa Cruz	1987	M	12.3	M,G	D,L
MVZ_243330		NC	37.59	-119.92	CA	Mariposa	2004	F	13.2		D,L
MVZ_245566		NC	37.72	-119.71	CA	Mariposa	2009	F	12.8		D,L
MVZ_249884		NC	37.96	-119.75	CA	Tuolumne	2005	M	15.45	M,G	D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
RST_114.mw		NC	37.13	-122.17	CA	Santa Cruz	2010	M	132	M,G	D
RST_115.mw		NC	37.13	-122.17	CA	Santa Cruz	2010	M	12.6		D
RST_116.mw		NC	37.13	-122.17	CA	Santa Cruz	2010	M	135	M,G	D,L
RST_119.mw		NC	37.13	-122.17	CA	Santa Cruz	2010	M	119	M,G	D
RST_120.mw		NC	37.13	-122.17	CA	Santa Cruz	2010	M	12.5		D
RST_121.mw		NC	39.74	-121.48	CA	Butte	2010	M	127	M,G	D
RST_122.mw		NC	39.74	-121.48	CA	Butte	2010	F	13.7		D
RST_123.mw		NC	37.03	-121.84	CA	Santa Cruz	2010	M	128	M,G	D
RST_124.mw		NC	37.03	-121.84	CA	Santa Cruz	2010	F	13.5		D
RST_125.mw		NC	38.77	-120.69	CA	El Dorado	2010	M	12.3		D
RST_218.mw		NC	37.07	-122.05	CA	Santa Cruz	2011	M	12.8		D
RST_219.mw		NC	37.07	-122.05	CA	Santa Cruz	2011	M	11		D
RST_220.mw		NC	37.07	-122.05	CA	Santa Cruz	2011	M	13.1		D
RST_221.mw		NC	37.07	-122.05	CA	Santa Cruz	2011	F	11.9		D
RST_222.mw		NC	37.07	-122.05	CA	Santa Cruz	2011	F	13.5		D
CAS_88135		pan	36.55	-117.57	CA	Inyo	1959	F	10.8		D,L
CAS_89230		pan	36.54	-117.57	CA	Inyo	1960	M	9.65	M,G	D,L
CAS_89675		pan	36.54	-117.57	CA	Inyo	1960	M	9.3		D,L
CAS_89676		pan	36.55	-117.57	CA	Inyo	1960	M	10.4	M,G	D,L
CAS_89677		pan	36.54	-117.57	CA	Inyo	1960	F	10.8		D,L
LACM_109366		pan	36.56	-117.58	CA	Inyo	1969	F	10.9		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
LACM_109367		pan	37.25	-118.18	CA	Inyo	NA	M	10.7		D,L
LACM_121969		pan	NA	NA			NA	F	13.7		D,L
MVZ_134111		pan	37.30	-118.21	CA	Inyo	1975	M	10.25	M,G	D,L
MVZ_150326		pan	37.24	-118.18	CA	Inyo	1978	M	10.6	M,G	D,L
MVZ_150327		pan	37.22	-117.99	CA	Inyo	1978	M	9.5		D,L
MVZ_191076	X	pan	36.55	-117.57	CA	Inyo	1981	M	12.15		D,L
MVZ_227761	X	pan	37.25	-118.16	CA	Inyo	1983	F	10.8		D,L
MVZ_227763	X	pan	36.67	-118.00	CA	Inyo	1985	F	10.3		D,L
MVZ_227764		pan	36.67	-118.00	CA	Inyo	1985	M	9.35		D,L
MVZ_227765		pan	36.67	-118.00	CA	Inyo	1985	M	10.8	M,G	D,L
MVZ_75918		pan	36.66	-117.86	CA	Inyo	1959	M	12.3	M,G	D,L
MVZ_77063		pan	36.11	-117.15	CA	Inyo	1962	M	9.7	M,G	D,L
CAS_180337		SC	34.34	-117.72	CA	Los Angeles	1991	F	11.4		D,L
CAS_180338		SC	34.17	-117.18	CA	San Bernardino	1991	M	12.8	M	D,L
CAS_188656		SC	33.01	-116.56	CA	San Diego	1986	F	12.15		D,L
CAS_191180		SC	33.74	-117.33	CA	Riverside	1993	M	10.2	M,G	
CAS_197500		SC	34.33	-117.64	CA	Los Angeles	1993	M	13.35	M,G	D,L
CAS_197505		SC	34.25	-117.76	CA	Los Angeles	1994	M	14	M,G	D,L
CAS_206443	X	SC	36.60	-118.11	CA	Inyo	1998	M	10.6	M,G	D,L
CAS_214887	X	SC	34.86	-119.08	CA	Kern	1999	M	12.3		D,L
CAS_214888	X	SC	34.86	-119.08	CA	Kern	1999	F	11.85		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
CAS_226089		SC	34.11	-118.77	CA	Los Angeles	1998	M	11.5		D,L
CAS_226090		SC	34.11	-118.77	CA	Los Angeles	1998	F	12.2		D,L
CAS_227987		SC	34.11	-118.77	CA	Los Angeles	2003	M	11	M,G	D,L
CAS_235680		SC	33.73	-117.73	CA	Orange	2000	M	9.8	M,G	D,L
CAS_236027		SC	33.12	-116.62	CA	San Diego	2003	M	10.5		D,L
CAS_241868		SC	36.57	-118.17	CA	Inyo	2006	M	13.2	M,G	D,L
CAS_241869		SC	36.57	-118.17	CA	Inyo	2006	F	12.85		D,L
LACM_130764		SC	34.88	-118.77	CA	Kern	1979	M	10.85	M,G	D,L
LACM_130768		SC	33.87	-118.26	CA	Los Angeles	1979	M	11.7	M,G	D,L
LACM_130769		SC	33.87	-118.26	CA	Los Angeles	1979	M	11.4	M,G	D,L
LACM_130770		SC	34.23	-118.50	CA	Los Angeles	1978	F	12.1		D,L
LACM_130771		SC	33.79	-118.38	CA	Los Angeles	1979		10.8	M,G	
LACM_130772		SC	34.01	-118.47	CA	Los Angeles	1979	M	10.1	M,G	D,L
LACM_130773		SC	34.11	-118.16	CA	Los Angeles	1979	M	9.9	M,G	D,L
LACM_130781		SC	34.42	-119.26	CA	Ventura	1979	M	10.9		D,L
LACM_131217		SC	34.22	-118.17	CA	Los Angeles	1979	M	13.1	M,G	D,L
LACM_131222		SC	30.29	-115.80	Baja		1979	F	10.55		D,L
LACM_131223		SC	30.29	-115.80	Baja		1979	F	10.7		D,L
LACM_132401		SC	34.50	-118.32	CA	Los Angeles	1980	F	9.25		D,L
LACM_132499		SC	34.02	-117.93	CA	Los Angeles	1981	M	10.6		D,L
LACM_132500		SC	34.23	-118.50	CA	Los Angeles	1981	M	11	M,G	D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
LACM_132501		SC	34.23	-118.50	CA	Los Angeles	1981	M	11.3		D,L
LACM_134421		SC	34.36	-117.91	CA	Los Angeles	1981	F	11.1		D,L
LACM_134422		SC	34.36	-117.91	CA	Los Angeles	1981	F	11.6		D,L
LACM_134423		SC	34.05	-118.69	CA	Los Angeles	1981	F	14.1		D,L
LACM_135257		SC	34.25	-118.17	CA	Los Angeles	1983	M	13	M,G	D,L
LACM_135418		SC	34.18	-117.87	CA	Los Angeles	1983	F	12.3		D,L
LACM_135431		SC	34.89	-118.75	CA	Kern	1983	F	12.7		D,L
LACM_136188		SC	34.06	-118.28	CA	Los Angeles	1983	M	9.8		D,L
LACM_136673		SC	34.50	-118.32	CA	Los Angeles	1983	M	11.6	M,G	D,L
LACM_137582		SC	34.02	-118.29	CA	Los Angeles	1988	M	10.6	M,G	D,L
LACM_148311		SC	34.17	-116.85	CA	San Bernardino	2001	M	10.3	M,G	D,L
LACM_148312		SC	34.06	-118.35	CA	Los Angeles	2001	F	11.1		D,L
LACM_149147		SC	34.24	-117.92	CA	Los Angeles	2000	M	11.6		D,L
MVZ_161393	X	SC	30.06	-115.58	Baja		1978	F	10.3		D,L
MVZ_182586		SC	34.88	-118.89	CA	Kern	1978	M	15.3	M,G	D,L
MVZ_191085		SC	34.44	-119.17	CA	Ventura	1982	F	12.55		D,L
MVZ_191128		SC	34.64	-118.35	CA	Los Angeles	1981	F	13.65		D,L
MVZ_191129		SC	34.64	-118.35	CA	Los Angeles	1981	M	13.2	M,G	D,L
MVZ_227731		SC	36.94	-118.31	CA	Inyo	1985	F	13.4		D,L
MVZ_227733	X	SC	36.81	-118.28	CA	Inyo	1986	F	12.1		D,L
MVZ_227734		SC	36.81	-118.28	CA	Inyo	1986	M	10.2	M,G	D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
MVZ_227739	X	SC	36.62	-118.19	CA	Inyo	1985	M	12.5		D,L
MVZ_227748	X	SC	34.40	-117.06	CA	San Bernardino	1987	M	11.5	M,G	D,L
MVZ_227749		SC	34.40	-117.06	CA	San Bernardino	1987	F	11.15		D,L
MVZ_229972		SC	34.23	-118.65	CA	Los Angeles	1995	F	11		D,L
MVZ_229976		SC	34.11	-119.09	CA	Ventura	1995	M	10.1		D,L
MVZ_233381		SC	34.40	-117.06	CA	San Bernardino	1987	M	12.3	M,G	D,L
RST_101.mw		SC	32.67	-117.05	CA	San Diego	2010	M	135	M	D
RST_104.mw		SC	32.67	-117.05	CA	San Diego	2010	F	13.2		D
RST_106.mw		SC	32.67	-117.05	CA	San Diego	2010	M	113	M	D
RST_108.mw		SC	32.75	-116.45	CA	San Diego	2010	F	13.7		D
RST_109.mw		SC	32.75	-116.45	CA	San Diego	2010	F	14.4		D
RST_110.mw		SC	32.67	-117.05	CA	San Diego	2010	F	12.6		D
RST_111.mw		SC	32.67	-117.05	CA	San Diego	2010	M	12.7		D
RST_112.mw		SC	32.67	-117.05	CA	San Diego	2010	F	11.2		D
RST_301.mw		SC	34.07	-118.91	CA	Los Angeles	2012	M	13.5		D
RST_302.mw		SC	34.18	-118.10	CA	Los Angeles	2012	M	117	M,G	D
RST_303.mw		SC	34.18	-118.10	CA	Los Angeles	2012	M	125	M,G	D
RST_304.mw		SC	34.07	-118.91	CA	Los Angeles	2012	F	13.9		D
RST_306.mw		SC	34.14	-118.52	CA	Los Angeles	2012	M	130	M,G	D
RST_307.mw		SC	34.14	-118.52	CA	Los Angeles	2012	M	140	M,G	D
RST_309.mw		SC	34.18	-118.10	CA	Los Angeles	2012	M	13.4		D

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
RST_310.mw		SC	34.14	-118.52	CA	Los Angeles	2012	M	133	M,G	D
RST_311.mw		SC	34.14	-118.52	CA	Los Angeles	2012	F	12.8		D
CAS_188632		SSN	35.48	-118.73	CA	Kern	1986	F	10.4		D,L
CAS_206440	X	SSN	35.71	-118.74	CA	Kern	1998	M	14.15	M,G	D,L
CAS_214889	X	SSN	35.07	-118.48	CA	Kern	1999	F	9.8		D,L
CAS_219611		SSN	36.02	-118.47	CA	Tulare	2001	M	14	M,G	D,L
CAS_220864		SSN	36.20	-118.76	CA	Tulare	2001	F	12.7		D,L
CAS_220884		SSN	36.19	-118.67	CA	Tulare	2001	M	14.3	M,G	D,L
CAS_220908		SSN	35.86	-118.63	CA	Tulare	2001	M	8.8		D,L
CAS_220920		SSN	35.72	-118.61	CA	Kern	2001	M	9.75		D,L
CAS_223541		SSN	35.85	-118.63	CA	Tulare	2001	F	10.75		D,L
CAS_224765		SSN	35.65	-118.60	CA	Kern	2002	M	15.8		D,L
CAS_224872		SSN	36.30	-118.79	CA	Tulare	2002	F	13.3		D,L
CAS_236172		SSN	36.17	-118.70	CA	Tulare	2005	M	11.2	M,G	D,L
CAS_236212		SSN	35.53	-118.62	CA	Kern	2005	M	9		D,L
CAS_247436		SSN	35.50	-118.56	CA	Kern	2009	M	14.4	M,G	D,L
CAS_247437		SSN	35.51	-118.56	CA	Kern	2009	F	13.7		D,L
MVZ_128421		SSN	36.39	-118.87	CA	Tulare	1975	F	8.75		D,L
MVZ_137822		SSN	35.53	-118.66	CA	Kern	1976	F	11.4		D,L
MVZ_137823		SSN	35.51	-118.69	CA	Kern	1976	M	9.3		D,L
MVZ_147929		SSN	35.31	-118.56	CA	Kern	1976	M	9.7		D,L

Appendix A Table 1 Continued

Specimen ID	mtDNA	Clade	Latitude	Longitude	State	County	Year	Sex	SVL (cm)	Hemipene	Head
MVZ_147930		SSN	35.31	-118.56	CA	Kern	1976	M	9.5		D,L
MVZ_149588		SSN	35.55	-118.59	CA	Kern	1977	F	14		D,L
MVZ_172785		SSN	35.59	-118.06	CA	Kern	1978	M	13	M,G	D,L

APPENDIX B

SEX DETERMINATION IN THE SOUTHERN ALLIGATOR LIZARD (*ELGARIA MULTICARINATA*; ANGUIDAE)

A paper intended for publication as a shorter communication in a herpetological journal

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ABSTRACT

Sex in many reptile species is determined by temperature during development (temperature-dependent sex determination, TSD) rather than by an individual's genotype (genotypic sex determination, GSD). TSD has numerous ecological and evolutionary implications for species, and whether or not species display TSD is of high conservation concern because substantial habitat and climate change have the potential to skew sex ratios in these species. It is therefore important to describe the means by which sex is determined in reptile species. To date, the sex determination mechanism is not known for any species within the Anguidae, a diverse lizard family that is globally distributed. I used controlled incubation experiments to test the hypothesis that an anguid lizard, the Southern Alligator Lizard (*Elgaria multicarinata*) displays TSD. Offspring sex was assessed by direct examination of the gonads. Developmental temperature did not affect offspring sex,

suggesting that *E. multicarinata* displays GSD, as is most common in vertebrates. Nonetheless, further work with additional anguid species is clearly needed.

INTRODUCTION

Unlike most organisms where sex is determined by an individual's genotype (genotypic sex determination, GSD), the sex of many reptiles is determined by temperature during development (temperature-dependent sex determination [TSD], Bull 1980, Janzen and Paukstis 1991, Valenzuela and Lance 2004). TSD is thought to be adaptive when developmental temperature differentially affects the fitness of the sexes. Under this scenario, TSD can allow production of the more-fit sex under all thermal conditions (Charnov and Bull 1977, Janzen and Phillips 2006, Warner and Shine 2008). However, TSD could be maladaptive when one sex is overproduced (Fisher 1930, Janzen and Phillips 2006). Species with TSD are therefore of conservation concern as a result of rapid habitat and directional climate change. Small increases in environmental temperature have the potential to strongly skew sex ratios in species with TSD, thereby decreasing effective population sizes and increasing extinction risk (e.g., Janzen 1994, Hawkes et al. 2007, Mitchell et al. 2008). Given the ecological and evolutionary implications of TSD, describing the sex-determination mechanism of species is of high import.

The Anguidae is a diverse family of lizards with a global distribution (Macey et al. 1999, Vitt and Caldwell 2009). Even though the family includes many large, charismatic taxa as well as endangered species (Vitt and Caldwell 2009, IUCN 2012), the sex-determination system is not known for any anguid lizard (Harlow 2004). Early work with the Southern Alligator Lizard (*Elgaria multicarinata*), suggested that this species might display TSD

(Langerwerf 1984). However, this study neither reported temperature treatments nor sex ratios, and the results are generally questioned (e.g., Harlow 2004). As part of a larger study on the thermal ecology of alligator lizards, I used controlled incubation experiments to test the hypothesis that *E. multicarinata* displays TSD.

The sexes are difficult to assign using morphological characters in *E. multicarinata*. Adults display weak sexual dimorphism: males have larger heads than females, and females have more "pear-shaped" bodies than males (Stebbins 2003, Beck 2009), but each trait overlaps extensively between the sexes. In addition, females possess enlarged hemiclitores (personal observation, described in varanid lizards by Böhme 1995) that are difficult to distinguish from the hemipenes of males. From my experience, only examination of the gonads allows 100% diagnosis of the sexes, even in adult *E. multicarinata*. Therefore, to assign sex, I directly examined the gonads of juvenile lizards via both gross morphology and histology.

MATERIALS AND METHODS

I obtained eggs (n = 85 fertile) from 9 wild-caught Southern Alligator Lizards within 24 h of oviposition. Each female produced only one clutch for the present study. With assistance, I collected gravid females in California (San Diego, San Mateo, Santa Cruz, and Monterey counties) during the 2010 and 2011 activity seasons (females per year: N = 5 and N = 4, respectively) and transported them to a captive colony at Iowa State University. I housed the lizards individually in conditions suitable for oviposition and checked enclosures daily for eggs.

Upon discovery, I placed an egg individually in a 140-mL glass jar approximately 2/3 filled with moist vermiculite (water potential = -150 kPa). I gently pressed eggs into the vermiculite such that they were 1/2–2/3 buried. Throughout this process, I took care to not roll the eggs. I then covered each jar in clear plastic wrap and sealed it with a rubber band to prevent evaporation (Warner et al. 2012). I distributed eggs from each clutch evenly among temperature treatments in incubators. In 2010, I placed eggs (n = 47) in one of three constant temperature treatments: 26.0, 28.0, and 30.0 ºC. I divided eggs produced in 2011 (n = 38) among these temperature treatments and two new treatments, 24.0 and 32.0 ºC. I chose these temperatures because previous work suggested that development progresses well at 27–28 ºC (Langerwerf 1984). In addition, these temperatures span the range of temperatures where hatching success is high (see Chapter 4). I placed iButton data loggers (Maxim Integrated, San Jose, CA.) in the incubators for the duration of the experiment to confirm temperatures. To control for thermal gradients within the incubators, I rotated egg position within each chamber three times per week. I checked eggs daily for hatching.

At hatching, I removed juveniles from their incubation jars, permanently marked them via toe clipping, and housed them in groups of five in conditions otherwise identical to those for adults. In 2010, I initially attempted to identify sex of offspring by ascertaining the presence/absence of hemipenes in 7-d old hatchlings (Harlow 1996). This was unsuccessful, so I resorted to a reliable assessment of sex using gross gonadal morphology (e.g., Brooks 1906) after euthanizing lizards with a lethal injection of sodium pentobarbitol once lizards were at least 6 mo of age. In 2011, I euthanized all hatchlings by decapitation at 30-d of age and excised their gonads. I formalin fixed, dehydrated, and embedded these gonads in paraffin wax according to standard histological protocol (e.g., Ross and Pawlina 2006). I then

latitudinally sectioned gonads at 7.0 μm and stained them with hematoxylin and eosin (H and E stain). I ascertained sex by examining the prepared sections under a light microscope and looking for seminiferous tubules or developing follicles (as in Doddamani 1994, 2006). No individuals incubated at 32 °C successfully hatched; however, I could assess the sex of one advanced stillborn embryo via histology.

I attempted to ascertain the sex of all juvenile *E. multicarinata* born in the 2010 and 2011 cohorts. For analyses, however, I only included individuals for which I was able to confidently identify sex either by histology in 2011 or by gross examination of the gonads in 2010 (n = 21, mean N per treatment ± 1 SD = 5 ± 2.9). I used logistic regression to examine the effect of incubation temperature on offspring sex. I did not include maternal identity in this analysis because most mothers only produced one offspring for which I was able to positively determine sex per incubation treatment. I performed this analysis both including the extreme temperature treatments (24 and 32) and excluding them. Models with and without these extreme temperatures were examined because these temperatures are likely outside the bounds of normal incubation in *E. multicarianta* and sample sizes at these temperatures were very small (Table 1). The residual and q-q plots suggested that the assumptions of parametric tests were met (Zuur et al. 2009). I performed the analyses using the program, R version 2.15.2 (R Core Team 2013).

RESULTS

Examination of hemipenes in 7-d old individuals via the method of Harlow (1996) was not an effective way to ascertain sex in *E. multicarinata*, as all individuals appeared to have hemipenes (hemipenes and hemiclitores were indistinguishable). Noticeable divergence

of the gonads between the sexes varied with age of the lizards. Up to 30-d of age, the sexes were only identifiable by histological examination of the gonads (Figs 1A, 1B). Gross gonadal morphology was very similar between the sexes; the gonads were smooth, white orbs just posterior to the adrenal glands, which were yellowish and had coarser surface texture (Figs 1C, 1D). Even so, re-examination after the sexes were known through histology revealed gross gonadal differences that might be diagnostic: ovaries were slightly smaller than testes (≈0.85 mm L x 0.37 mm W versus 1.1 mm L x 0.53 mm W, respectively) and oviducts were visible in females, although these structures were very delicate (Figs 1C, 1D). For individuals at least 6-mo of age, the gonads were readily differentiated morphologically. The gonads were much larger (ovary: ≈4.1 mm L x 2.5 mm W, and testis: ≈3.0 mm L x 0.95 mm W, in 1 yr olds) and developing follicles in the ovaries were easily visible (Figs 1E, 1F).

Information on individuals for which I was able to positively identify sex is given in Table 1. I was unable to confidently determine the sex of all individuals that hatched because, in 2010, some individuals died when they were still too young for sex determination by examination of gross gonadal morphology and, in 2011, I was unable to see obvious seminiferous tubules/developing follicles for some individuals. For the entire sample of sexable individuals, sex ratio was male biased (71.4 % male, $\chi^2 = 3.86$, df = 1, $P = 0.05$). However, sex ratio was not affected by temperature treatment, regardless of inclusion/exclusion of the extreme treatments (All treatments included: $\chi^2 = 4.14$, df = 4, $P = 0.39$, deviance explained = 16.5%; Only 'optimal' treatments included [26, 28, and 30 ºC]: $\chi^2 = 1.79$, df = 1, $P = 0.18$, deviance explained = 7.8%).

DISCUSSION

Incubation temperature did not affect offspring sex ratio in *E. multicarinata*. These data contradict the conclusion of Langerwerf (1984) that *E. multicarinata* displays TSD. Langerwerf's (1984) conclusion is questionable because neither the temperature treatments used, the method of sexing juvenile lizards, nor the sex ratio were presented (further discussed in Harlow 2004). External morphological characters were probably used to assign sex, and such characters are unreliable in this species (see above). I examined the gonads both morphologically and histologically to assign sex, and thus sex identification should be accurate.

A power analysis suggests that my model excluding extreme temperature treatments could detect an effect size of 0.66 with 80% power given my sample size (model with all treatments could detect an effect size of 0.75). My results are therefore sufficient to reject the hypothesis that *E. multicarinata* display strict TSD (100% males produced within a range of temperatures and 100% females within another range). Moreover, both sexes were produced at each temperature within the optimal temperature range (26-30). However, my sample size does not provide sufficient power to rule out the possibility that offspring sex ratio in *E. multicarinata* is in some way temperature sensitive (e.g., Quinn et al. 2007, Radder et al. 2008)(e.g., Quinn et al., 2007; Radder et al., 2008). Thermal sensitivity better describes what Langerwerf (1984) originally observed, as he states that *E. multicarinata* "... produces more males at 27–28 ºC, but this is not very obvious." Even so, the most parsimonious conclusion is that *E. multicarinata* displays GSD, like most other vertebrates.

Elgaria multicarinata is the only species within the Anguidae for which sex determination has been examined to date. Therefore, current data suggest that the Anguidae

display GSD, not TSD. Moreover, within the super-family Anguimorpha (families: Shinisauridae, Lanthanotidae, Varanidae, Helodermatidae, Xenosauridae, and Anguidae, from Wiens et al. 2012) there currently appears to be no convincing evidence for TSD (reviewed in Harlow 2004), suggesting that the entire clade displays GSD. However, further work with additional species is clearly needed.

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TABLES

Table 1—Sex of southern alligator lizard, *Elgaria multicarinata*, offspring from eggs incubated at five constant temperatures. Also displayed for each treatment are the number of clutches represented by sexed individuals, the total number of eggs incubated, and the total number of eggs that hatched. Thermal effects on traits other than offspring sex are presented in Chapter 4.

Incubation Temperature	Male	Female	Total Sexed	Clutches	Eggs Incubated	Eggs Hatched
24.0 ºC	2	0	2	1	6	2
26.0 ºC	7	2	9	6	24	19
28.0 ºC	4	2	6	3	24	21
30.0 ºC	1	2	3	3	25	11
32.0 ºC	1	0	1	1	6	0

FIGURES

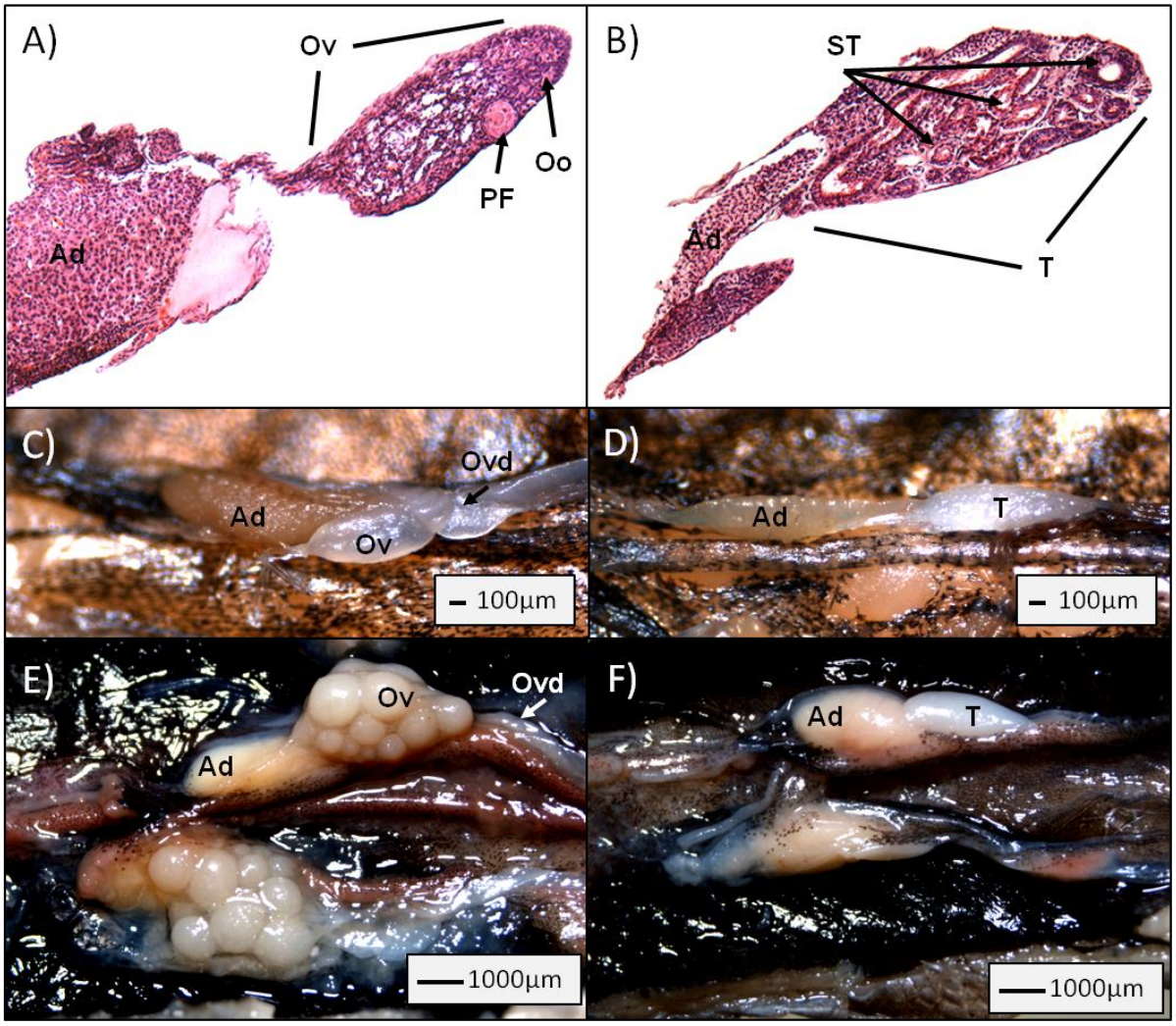

Figure 1—Gonads from 30-d old (A, B, C, D) and ≈1-yr old (E, F) Southern Alligator Lizards, *Elgaria multicarinata*. A and B are representative histological preparations of latitudinal sections at 100X magnification, and C, D, E, and F are representative views of gross morphology under a dissection microscope. A, C, and E display females whereas B, D, and F display males. Left is anterior for all. Abbreviations are: Ad = adrenal, PF = primordial follicle, Oo = oocyte, Ov = ovary, Ovd = oviduct, ST = seminiferous tubule, and T = testis.

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APPENDIX C

ADDITIONAL INFORMATION ON CTE MODELING

Online appendix to Telemeco et al. 2013. *The American Naturalist* 181: 637-648

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ESTIMATION OF MODEL PARAMETERS IN PAINTED TURTLES

General Field Methods

Female turtles were monitored annually as they came on land to nest in May and June. After nesting, thermal data loggers (HOBO XT, Onset Computer Corporation, Pocasset, MA, and iButton, Dallas Semiconductor, Dallas, TX) were placed in the center of a subset of these nests for the duration of the TSP. The nests were excavated in September, before offspring naturally emerge, and hatchlings were transported to Iowa State University. Because assessing the sex of painted turtle neonates is destructive, we only sexed a subset of offspring from nests. On average, 94% of hatchlings were sexed per nest and all nests had greater than 50% of hatchlings sexed. All surviving hatchlings were hibernated in the laboratory and released the following spring.

TSD in Painted Turtles

In painted turtles, T_{PIV} is ~28.0 °C (Janzen and Paukstis 1991, Les et al. 2007), and the range of constant temperatures that produces both sexes (TRT) at Thomson is 2.7 ± 0.7 °C wide (Morjan 2003a). We therefore considered models successful if predicted CTE values were 1) above 28.0 ºC and nests were entirely female, 2) below 28.0 ºC and nests were entirely male, or 3) between 26.65 and 29.35 ºC (i.e., the TRT) and nests contained both sexes. Importantly, CTE values within the TRT were considered successful predictors of sex ratios when nests were either mixed or of the appropriate single sex. This approach is valid because the TRT is a population-level parameter that describes the range within which mixed-sex nests can occur but need not always occur (Mrosovsky and Pieau 1991, Morjan 2003a). In addition, this approach accounts for the possibility that our subsampling of hatchlings could result in misclassification of some mixed-sex nests as single sex.

As in other species, the TSP of painted turtles is approximately the middle third of development (Janzen and Paukstis 1991). At Thomson, nesting begins in late May/early June (Schwanz and Janzen 2008, Warner et al. 2010) with oviposition at the gastrula stage (Mahmoud et al. 1973) and eggs hatch in late August/early September. July, therefore, roughly corresponds with the middle third of development (Janzen 1994, Schwanz and Janzen 2008). Thus, we assumed a historic nesting date of 1 June (Schwanz and Janzen 2008, Warner et al. 2010) and a 30-day TSP beginning on 1 July.

Critical Thermal Minimum and Maximum for Painted Turtle Development

T_0 for painted turtles is ~14.0 °C (Les et al. 2007). T_{MAX} may be as low as 32 °C because eggs incubated at constant temperatures ≥ 32 °C fail to hatch (F.J. Janzen,

unpublished data). However, brief exposure to temperatures slightly above 32 °C is not fatal and Neuwald and Valenzuela (2011) suggest that developmental rate in painted turtles is actually highest near 34 °C. We therefore considered several possibilities (T_{MAX} = 32, 33.5, 34, 34.5, 36, or even ∞ °C) and examined the ability of our CTE model to predict the sex ratios of 46 natural nests in each case. The data comprised 13 all-female nests, 17 all-male nests, and 16 mixed-sex nests collected between 1996 and 2004 with $\geq$ 3 offspring sexed per nest (data available in the Dryad repository, doi:10.5061/dryad.rk571).

Using nest-specific thermal parameters (that is *w*, *M'*, and *R* fitted to information from the data loggers within each nest), the T_{MAX} = 34 model outperformed the others by correctly predicting the sex ratios of 87% of the nests (Table 2). In addition to providing an estimate for T_{MAX}, this high success rate confirms the ability of our model to reproduce observed patterns in sex ratio. By comparison, if we were to take a coarse view and use the mean nest temperatures as the CTE, we would successfully predict sex ratios for only 56.5% of nests.

Thermal Profile of Natural Nests

To examine the general ability of females to buffer the sex ratios of their progeny by shifting nesting date, we need historical values for *w*, *M'*, and *R* that are general baselines for comparison. To obtain these, we examined TSP (July) temperatures within 16 mixed-sex nests laid between 1996 and 2002 (mean nests/yr ± 1.0 *s.e.* = 4.25 ± 1.25; 11 of these nests were also used above, differences result from some nests not having sufficient data for both analyses and/or being constructed more than 10 d from 1 June). Because they lead to a ~1:1 sex ratio, temperatures in these mixed-sex nests should represent the long-term conditions to

which this population is adapted (e.g., Fisher 1930, Bull and Charnov 1988, Janzen and Phillips 2006). These data are available in the Dryad repository (doi:10.5061/dryad.rk571).

Daily nest temperatures measured during the TSP are shown in Fig 2. The slope of temperature change was 0.002 ºC/2πd. The grand mean (± 1.0 s.e.) temperature and diel thermal range was 26.3 ± 0.1 ºC and 10.8 ± 0.9 ºC. Because the slope of temperature change was approximately zero, the grand mean temperature and diel thermal range give historic values of M' and $2R$, respectively. As expected given the data were derived from mixed-sex nests, these values (w = 0.002, M' = 26.3, and R = 5.4 with a 30-day TSP, T_0 = 14 and T_{MAX} = 34) result in a CTE of 28.5 ºC, which is close to T_{PIV} (~28.0 ºC). We use these parameter values as baselines against which we compare the results under climate-change scenarios.

Predicting General Effects of Climate Change and Phenology on Sex Ratios

Effects of changes in climate and phenology on nest temperature

How climate change and nesting behavior interact to affect nest temperatures will largely depend on the parabolic shape of the thermal trend over the reproductive season (Fig 1 in Chapter 5). To estimate this thermal trend for Thomson, we used historic records of soil temperature measured at a depth of 10 cm from the nearest weather station with these data (Iowa City, IA, 41º 38' 56" N, 91º 31' 58" W, ~120 km from Thomson; NOAA National Climate Data Center). Painted turtle nest depth is ~10 cm (Morjan 2003b). Because Iowa City is at similar latitude to Thomson, thermal profiles should be comparable. Data were available for 21 years (1982-1997 and 2006-2010) and temperatures were recorded twice per day (08:00 and 17:00, and 07:00 and 17:00, respectively) under sod in full sun (conditions similar to many natural painted turtle nests). From these data, we calculated grand mean

temperatures for each day from 1 April–31 August and fitted a quadratic function (r^2 = 0.9898; Fig 2 in Chapter 5 black lines). The weather station soil temperatures were systematically cooler than our natural nest data, perhaps because soil temperatures were not sampled during the warmest part of the day (i.e., 15:00–16:00). Such sampling would reduce daily mean temperature estimates but should not affect the seasonal pattern of thermal progression. Therefore, for analyses, we transformed the quadratic function derived from the weather station records to better match our natural nest data by raising it 2.29 ºC (Fig 2 in Chapter 5 grey lines). After transformation, the mean 1 July (TSP onset) temperature and the slope of temperature change during July were 26.28 ºC and 0.007 ºC/2πd, respectively, very closely matching the values for M' and w estimated above from nest data (M' = 26.3 ºC, w = 0.002 ºC/2πd).

We assume that females will adjust their phenology in response to increasing temperatures and continue to nest at the historical 1 June soil temperature (22.01 ºC). We further assume that the TSP starting date will advance from 1 July (average onset of the TSP at Thomson) the same number of days that the nesting date advances from 1 June. Although this is unlikely to be fully accurate, it is a valid assumption because temperatures during the pre-TSP period are not predicted to increase significantly, even after a 4.0 ºC increase in environmental temperature (average pre-TSP temperature increase = 0.81 ºC, Fig 2 inset from Chapter 5). Given a Q_{10} of 2.2 (maximum value measured in developing painted turtles, Du et al. 2010), the onset of the TSP could only advance 1.98 days relative to nesting (6.6% increase in developmental rate), resulting in a negligible change in the temperature range experienced during the TSP. Similarly, we assumed that the TSP is always 30-d long. While increased temperature will shorten the TSP, realistic changes in TSP length should not

affect the CTE because daily temperatures are highly autocorrelated. The fact that July air temperatures are strongly correlated with offspring cohort sex ratio at Thomson (r2 = 0.83, Janzen 1994), even though annual temperature and exact nesting dates vary, is evidence that natural shifts in the length and timing of the TSP minimally affect TSP temperature and sex ratio.

From these assumptions, we can use the gray curve in Fig 2 (from Chapter 5) to determine how shifts in nesting date in response to uniform warming will affect our thermal parameters, *w* and *M'*, during the TSP. For example, if regional temperatures increase 4.0 ºC, nesting will need to advance 21 days for temperature at nesting to be constant. By raising the thermal trend 4.0 ºC (as shown by the red curve in Fig 2 from Chapter 5), we can project 30 days from this new nesting date and determine the expected mean temperature (*M'*) at the start of the TSP, as well as the change in the daily mean temperature (*w*) during the TSP. Fig 3 from Chapter 5 shows how each of these parameters is expected to change with different advances in nesting date.

TABLES

Table 1—Relationship between slope (*w*) across the thermosensitive period (TSP) and temperature increase over the TSP. Temperature increase was calculated as: $Temp = w \times 2\pi \times 30$ (TSP length, days).

Slope (*w*)	Temperature increase (ºC)
0.000	0.00
0.001	0.19
0.005	0.94
0.010	1.88
0.015	2.83
0.020	3.77
0.050	9.42
0.100	18.85

Table 2—Number of nests for which sex ratio was correctly predicted by mean temperature and CTE models with differing values for T_{MAX}. Each model was tested using data from 46 nests. The sex ratios within six nests were not accurately predicted by any models. All sex ratio types were represented by these six nests: four were mixed-sex, one was 100% female, and one was 100% male. For these nests, predicted CTE values from the T_{MAX} = 34 ºC model were off by ± 0.63 ºC, on average, from values that would have given the right prediction, and were too warm or too cool for three nests each.

Model	**Nests correctly predicted**	**Notes**
Mean temperature	26	
$T_{MAX} = \infty$	39	Equivalent to the original Georges (1989) model
T_{MAX} = 32	32	
T_{MAX} = 33.5	39	
T_{MAX} = 34	40	Successfully predicted sex ratios in all nests correctly predicted by any model as well as 1 additional nest.
T_{MAX} = 34.5	39	
T_{MAX} = 36	39	Identical to $T_{MAX} = \infty$ model because 36 ºC was rarely exceeded.

FIGURES

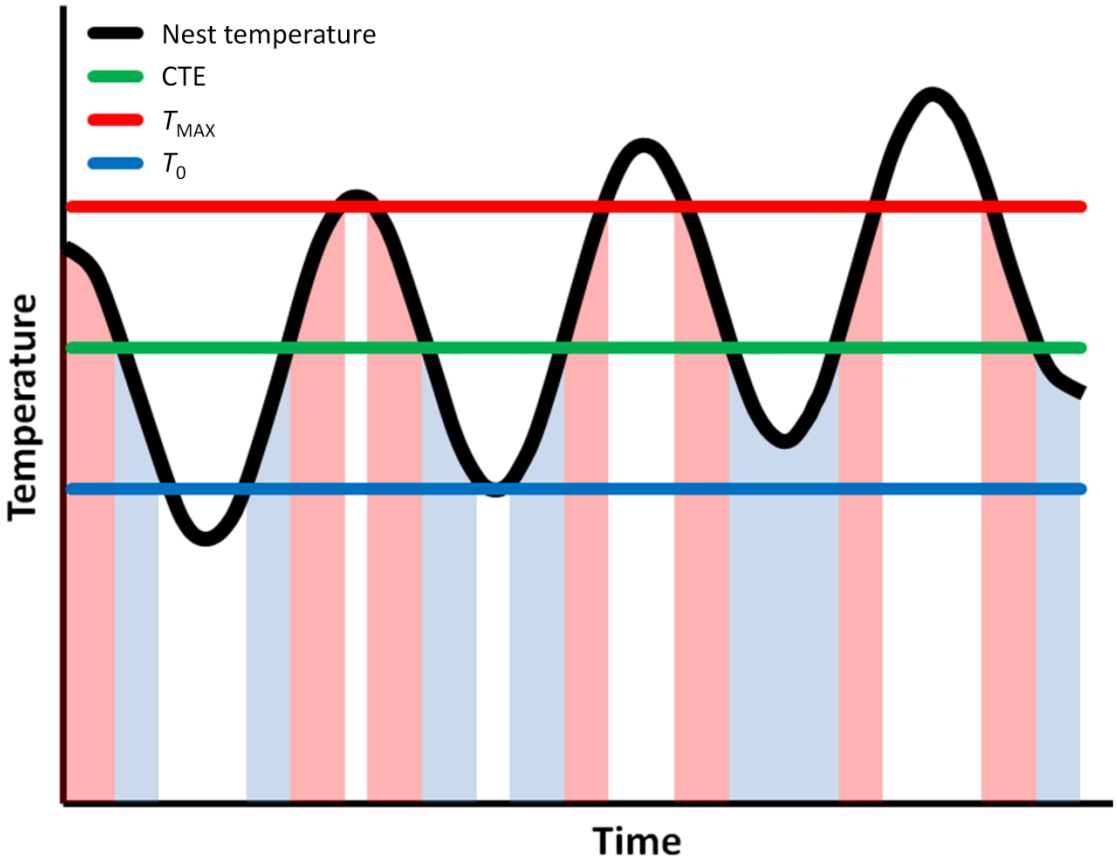

Figure 1—Diagrammatic representation of the CTE slope model, showing where nest temperature crosses the CTE, T_0 (minimum developmental temperature), and T_{MAX} (maximum developmental temperature) during the first three days of the thermosensitive period (TSP). Each day is represented by one cycle of the cosine function (2π). The blue shaded regions depict the area under the curve when temperature is between T_0 and the CTE, and the red shaded regions depict the area under the curve between the CTE and T_{MAX}. The start and end times of these intervals are the integration limits (a_i,b_i) and (a_j,b_j), respectively, in Eq 7 (in Chapter 5). The CTE is the temperature that equalizes the amount of development occurring in the red- and blue-shaded areas.

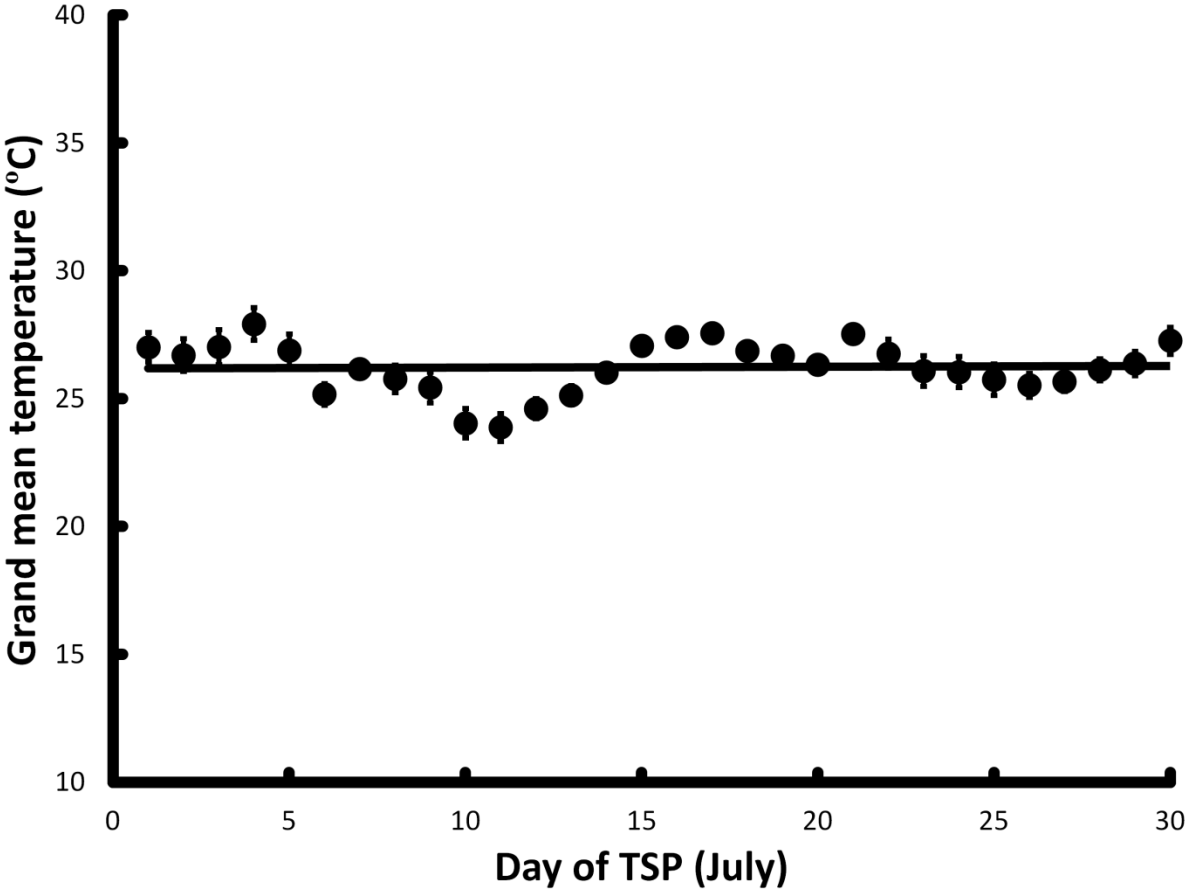

Figure 2—Daily grand mean temperatures (± 1.0 *s.e.*) during the thermosensitive period (TSP) from 16 natural *Chrysemys picta* nests that produced mixed sex ratios at the Thomson Causeway Recreation Area. The line of best fit used to derive the historic slope ($w = 0.002$) of temperature change over the TSP is shown.

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APPENDIX D

AN ALTERNATE CTE MODEL

Supplemental material for Telemeco et al. 2013. *The American Naturalist* 181: 637-648

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ALTERNATIVE MODEL FOR DESCRIBING THE RELATIONSHIP BETWEEN DEVELOPMENTAL RATE AND TEMPERATURE.

For the modified CTE model we present in the manuscript, we assumed that developmental rate increases linearly with temperature until a critical temperature is reached (T_{MAX}), at which point developmental rate becomes zero (Eq. 5 from Chapter 5, Fig 1A). However, the actual relationship between developmental rate and temperature is curvilinear (Sharpe and DeMichele 1977, Georges et al. 2005). Through much of the relevant thermal range, developmental rate increases linearly with temperature, but developmental rate falls toward zero along a curve at extreme low and extreme high temperatures. Multiple curvilinear models have been developed describing this relationship (Sharpe and DeMichele 1977, Georges et al. 2005, Neuwald and Valenzuela 2011). However, the parameters in these models that describe the curved portion of the relationship are difficult to estimate because they require incubating eggs for various amounts of time at temperatures that generally result in egg death. To examine how a more realistic model might affect our

simulation results, we developed a hybrid model. While this model is not fully curvilinear, it captures the major feature of curvilinear models: a gradual decline in developmental rate above some threshold temperature. Furthermore, it is simple enough that we are able to estimate parameter values with our nest temperature data.

This model allows developmental rate to increase with temperature as before. However, instead of developmental rate dropping directly to zero when some maximum point is reached, it now drops linearly toward zero with a negative slope (Fig 1B). The equation is:

$$\frac{ds}{dt} = \begin{cases} 0 & T < T_0 \\ A(T - T_0) & T_0 < T < T_{PEAK} \\ \frac{A(T_{PEAK} - T_0)}{T_{MAX} - T_{PEAK}}(T_{MAX} - T) & T_{PEAK} < T < T_{MAX} \\ 0 & T > T_{MAX} \end{cases} \quad \text{(Equation 1)}$$

where T_{PEAK} is the temperature at which developmental rate is maximized (Fig 1B). The slope of change in developmental rate above T_{PEAK} is determined by the distance between T_{PEAK} and T_{MAX}. Developmental rate is now given by plugging Eq. 6 (from Chapter 5) into Eq. 1 and the CTE is estimated as before.

We used the data from our 46 natural nests for which we had both July temperature data and offspring sex ratios to estimate T_{PEAK} and T_{MAX} for painted turtles at Thomson. To do this, we factorially adjusted both parameters within realistic ranges (T_{PEAK} = 32–34 ºC, T_{MAX} = T_{PEAK} + 3–7 ºC). The success of the model at predicting the sex ratios of nests with each parameter value is displayed in Table 1. The best-performing model was the T_{PEAK} = 32 ºC, T_{MAX} = 36 ºC model (successfully predicted the sex ratios of 42/46 nests). All models

had identical success predicting sex ratios of nests that were either 100% male or 100% female with the exception of the T_{PEAK} = 32 ºC, T_{MAX} = 35 ºC model, which misclassified an additional 100% female nest as male or mixed. All models misclassified the same one 100% female, one 100% male, and two mixed-sex nests. Because these nests spent little if any time at extreme high temperatures, it is unlikely that any model of this form will make accurate predictions for these nests. The remaining variation in accuracy came from variable success in classifying the same three mixed-sex nests.

Because the T_{PEAK} = 32 ºC, T_{MAX} = 36 ºC model had the highest predictive power with our test data set, we re-ran our simulations with this model. All other aspects of the simulations were identical to the simulations described in Chapter 5. The results are displayed in Figs. 2 and 3 in the current appendix and are virtually identical to Figs. 4 and 5 from Chapter 5, respectively; suggesting that using a more complicated model for the relationship between temperature and developmental rate will not affect our conclusions. The only noticeable differences are that the transition from a positive to a negative relationship between the CTE and slope, and the dynamism in the relationship between the CTE and increases in environmental temperature, are somewhat dampened in this model. However, these effects are minor.

Our comparison of these models suggests that our simple T_{MAX} model will likely be sufficient for many applications. Unless full physiological realism is required, the addition of the extra parameters needed for full curvilinear models might frequently be unnecessary and unjustified.

TABLES

Table 1—Number of nests for which sex ratio was correctly predicted by CTE models with differing values for T_{PEAK} and T_{MAX}. Each model was tested using data from 46 nests.

T_{PEAK} (ºC)	ºC between T_{PEAK} and T_{MAX}				
	3	4	5	6	7
32	41	42	41	40	39
33	39	39	39	39	39
34	39	39	39	39	39

FIGURES

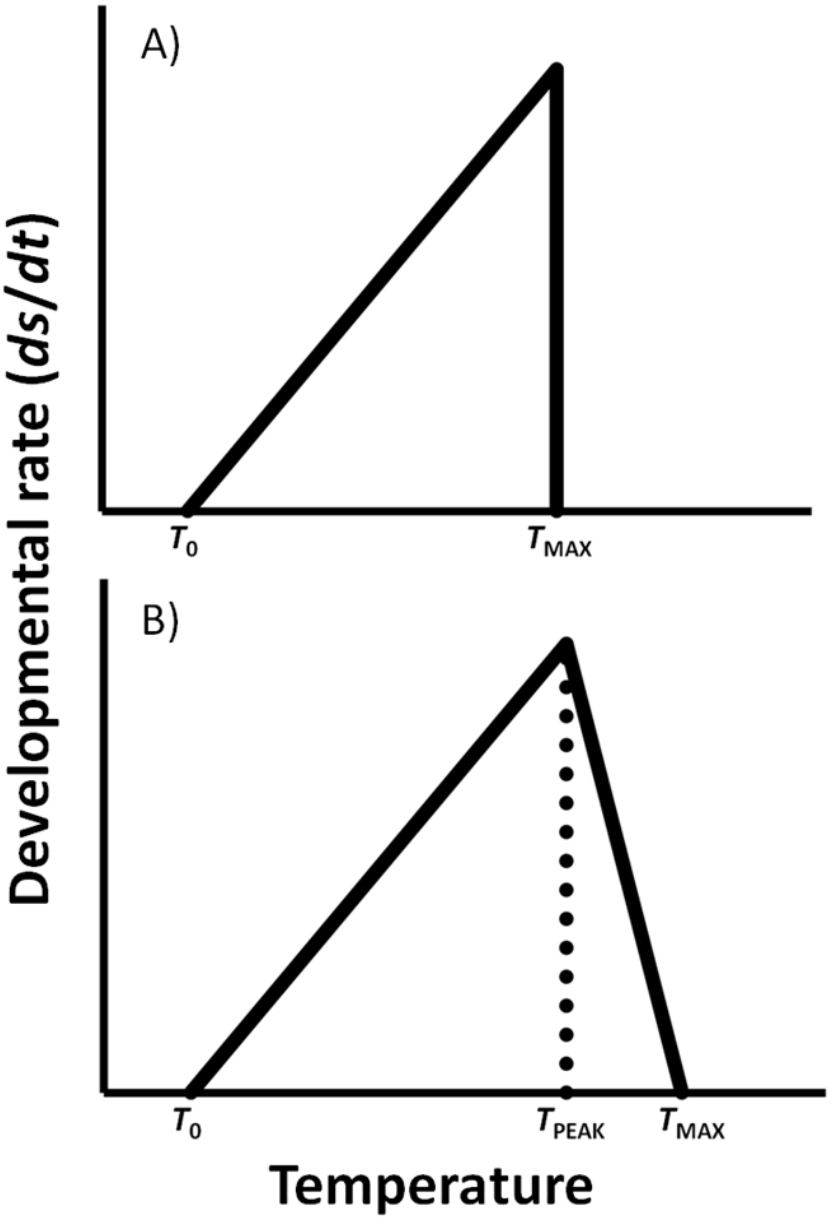


Figure 1—Models for how temperature affects developmental rate. A) Developmental rate increases linearly from a critical thermal minimum (T_0) until a critical thermal maximum (T_{MAX}) is reached, at which point developmental rate drops directly to zero (Eq. 5 from Chapter 5). B) Developmental rate increases as before from T_0 until the temperature where developmental rate is maximized is reached (T_{PEAK}). Temperature then drops linearly toward T_{MAX}, where developmental rate is zero (Eq. 1).

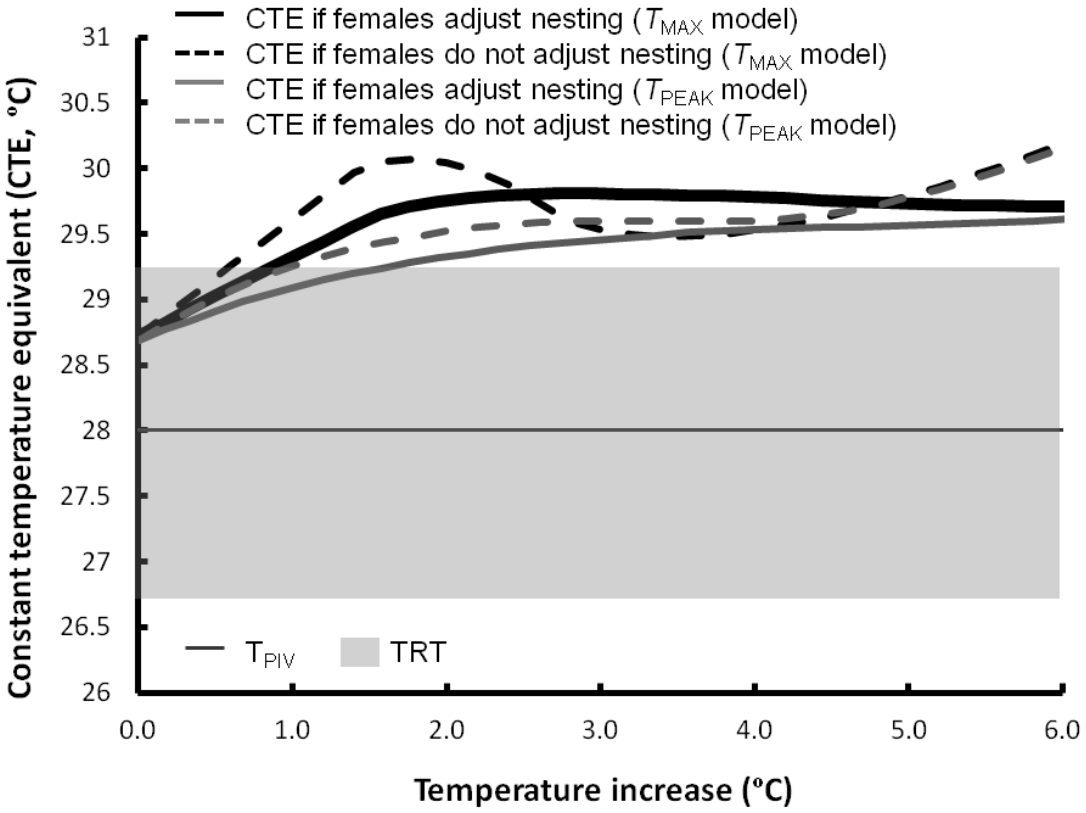
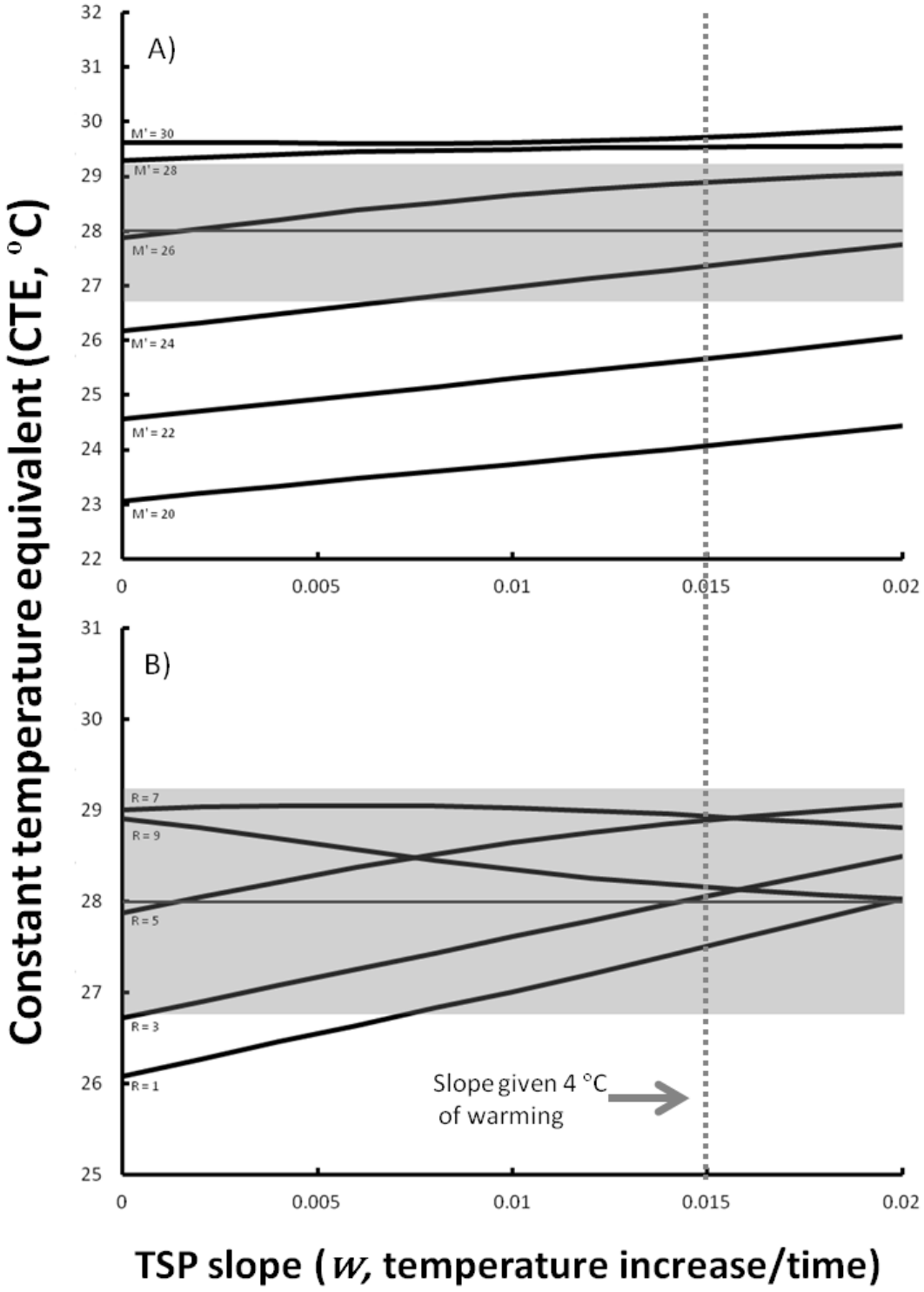


Figure 2—Predicted effects of uniform increases in environmental temperature on constant-temperature equivalent (CTE) values in painted turtle nests at the Thomson Causeway Recreation Area. The solid lines depict CTEs if females adjust their nesting date such that they always nest at the same temperature, whereas the dashed lines depict CTEs if females continue to nest on the average historic nesting date at Thomson (June 1). Black lines depict results from simulations using the T_{MAX}= 34.0 ºC model, whereas grey lines depict results from using the T_{PEAK} = 32.0 ºC, T_{MAX} = 36.0 ºC model. CTE values within the shaded transitional range of temperatures (TRT) may result in either single- or mixed-sex nests, with T_{PIV} representing the CTE that is predicted to result in a 1:1 sex ratio. To create the solid lines, we simulated *M′* and *w* values shifting in tandem according to predictions from Fig. 3 (from Chapter 5). To create the dashed lines, *w* was held at the historic predicted value at Thomson, while increases in environmental temperature were added to the historic *M′* value. All other parameters were held at their historic mean values (T_0 = 14.0 ºC, T_{MAX} = 34.0 ºC, R = 5.0 ºC, TSP length = 30 d).



Legend for Figure 3 is on the following page

Figure 3—Predicted effects of slope (*w*) during the thermosensitive period (TSP) on the constant-temperature equivalent (CTE) of nests for different values of A) temperature at the onset of the TSP (*M′*) and B) the diel thermal range of temperature (2*R*). CTE values were calculated using our more realistic model (see text for a description) with T_{PEAK} = 32.0 and T_{MAX} = 36.0 ºC. When not marked otherwise, other parameters were set at *M′* = 26.0 ºC, *R* = 5.0 ºC, and T_0 = 14.0 ºC, which are approximately the mean values from current nests. For comparison, axes are identical to those in Figs 5A and 5B from Chapter 5. The vertical dotted line marks the slope of temperature change over the TSP that results from 4.0 ºC warming given that females advance their nesting date such that they nest at the same temperatures annually. CTE values within the shaded transitional range of temperatures (TRT) may result in either single- or mixed-sex nests, with T_{PIV} representing the CTE that is predicted to result in a 1:1 sex ratio. CTE values above the TRT are predicted to result in 100% female nests, whereas CTE values below the TRT are predicted to result in 100% male nests.

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APPENDIX E

MATLAB CODE FOR CALCULATING THE CTE WHEN AVERAGE TEMPERATURE INCREASES LINEARLY OVER THE THERMOSENSITIVE PERIOD (TSP)

Supplemental material for Telemeco et al. 2013. *The American Naturalist* 181: 637-648

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PRIMARY PROGRAM

This code allows for the automated calculation of CTE's when T_{MAX}, M', R, and w are varied factorially. Green text following the % symbol designates annotations describing the code's function.

```
clear all


T0 = 14; % Tmin (minimum developmental temperature)
days = 30; % length of the thermosensitive period in days
b = days*2*pi;
% create a text file for storing the results, using fopen command
CTEfile = fopen('filename.txt', 'w');
% (optional) print column headings using fprintf command
```

```
fprintf(CTEfile,'%s\t%s\t%s\t%s\t%s\t%s\n','Tmax','M','R','w','CTE','avgT'
);
 for Th = 30:2:40; % Tmax (maximum developmental temperature)
    for M = 20:2:30; % TSP starting temperature
        for R = 1:2:9; % 1/2 the diel thermal range
            for w = 0:0.002:0.02; % slope over the thermosensitive period.
% When w=0 and M+R<Th, this model is identical to Georges' original CTE
model
                bestbigT = -9999;
% if there is an error in the program for a given set of parameters -9999
% will appear in the results text file
                [Th, M, R, w]
%MATLAB will print these parameters in the command window so that you can
see %the program running
                TotalAreaUnderCurve = (R*sin(b)+(M-T0)*b + w*(b^2)/2);
% this is the total area under the curve ds/dt, to be divided up
% into regions where development is occurring at temps above T' (the CTE)
% and regions where temps are below T'.

% this next section sets up the trial values of T' that MATLAB
% will use to find the CTE.
% The trial values (stored in Tvector) range from the lowest
% to the highest temperatures experienced during development,
% and includes all values in between at intervals of "stepsize".
                minT = T0; % minimum temp during development
                maxT = Th; % highest temp during development
                stepsize = 0.01;
                Tvector = minT:stepsize:maxT;
```

```
% for each trial value of T', matlab will find all the values of t (time)
% where Rcos(t) + M + wt = T'.  We'll use fzero for this, using the
program  % crossT.m (see "Additional Program" below)

% Temperature is predicted to cross T' twice a day almost every
% day, so fzero will look for a crossing point in every half-day interval.
                HalfDayIntervals = 0:pi:b;
                crosspoints = zeros(1,length(HalfDayIntervals)-1);
                crosspoints2 = zeros(1,length(HalfDayIntervals)-1);


              for j = 1:length(HalfDayIntervals)-1
                    if sign(R*cos(HalfDayIntervals(j)) + M +
w*HalfDayIntervals(j) - Th) ~= sign(R*cos(HalfDayIntervals(j+1)) + M +
w*HalfDayIntervals(j+1) - Th)
                    crosspoints2(1,j) = fzero(@(x)
crossT(x,R,M,w,Th),[HalfDayIntervals(j) HalfDayIntervals(j+1)]);
                    end
                end

% Now, have MATLAB ignore any t-values that are within "mininterval" of a
% value it already found.
                mininterval = 2*pi/(24*60); % time interval of 1 minute
 % Now, we need a place to store the minimum difference
% between (area where temp is > T') and (area where temp is < T').
% We'll update this minimum as we go and use the T' that
% gives us the closest match between the two areas.  We'll
% start with a value that will surely be bested: the worst
```

```
% case scenario where the whole area is either above or
% below T':
                minAreaDiff = TotalAreaUnderCurve;

% Now, take trial T' values one at a time:
                for k = 1:length(Tvector)
                    bigT = Tvector(k);
% for the given T', find all the t values where Rcos(t) + M + wt = T'.
% Do this by cycling through all the half-day intervals
                for i = 1:length(HalfDayIntervals)-1

% the next chunk of code checks to see if the temperature crosses T'
during  % this half-day interval.  If it does, it uses fzero to find the
exact time  % where that cross occurs.
                    if sign(R*cos(HalfDayIntervals(i)) + M +
w*HalfDayIntervals(i) - bigT) ~= sign(R*cos(HalfDayIntervals(i+1)) + M +
w*HalfDayIntervals(i+1) - bigT)
                        crosspoints(1,i) = fzero(@(x)
crossT(x,R,M,w,bigT),[HalfDayIntervals(i) HalfDayIntervals(i+1)]);
                    end
                end

% Next, toss out any t's that are either negative or > b, then sort
results  % in ascending order

                crosspoints =
sort(crosspoints(crosspoints>0&crosspoints<=b));
```

```
% Specify all the limits of integration: the t values found, plus the
% endpoints 0 and b

                integlims = unique(sort([0, crosspoints, crosspoints2,
b]));

% Next, keep track of the areas where ds/dt > A(T'-T0) and where ds/dt <
% A(T'-% T0) -- these should be equal when the correct value of T' is
found.  % Start by setting both to 0, then add to each running sum as
areas are      % calculated.

                AreaAbove = 0;
                AreaBelow = 0;

% for each interval between points where the temperature crosses T',
% calculate the integral (i.e. area under the temperature curve)

                for j = 1:(length(integlims)-1)
                    lowlim = integlims(j); % lower integration limit
                    uplim = integlims(j+1); % upper integration limit
                    Area = (R*sin(uplim) - R*sin(lowlim) + (M-T0)*(uplim-
lowlim) + (w/2)*((uplim^2)-(lowlim^2)));

% If the temperature at the midpoint between lowlim & uplim is > T', count
% this integral toward the total area where ds/dt > A(T'-T0):
```

```
                    if R*cos((uplim+lowlim)/2) + M + w*((uplim+lowlim)/2)
> bigT && R*cos((uplim+lowlim)/2) + M + w*((uplim+lowlim)/2) < Th
                            AreaAbove = AreaAbove + Area;

% otherwise, count this integral toward the total area where ds/dt < A(T'-
T0)

                    elseif R*cos((uplim+lowlim)/2) + M +
w*((uplim+lowlim)/2) < bigT
                            AreaBelow = AreaBelow + Area;
                    end
                end

% if the areas above and below are the closest to equal we've found so
far,   % classify T' as "bestbigT" (closest thing to the CTE so far):
                if abs(AreaAbove - AreaBelow) < minAreaDiff
                   minAreaDiff = abs(AreaAbove - AreaBelow);
                   bestbigT = bigT;
                end
                end
% Calculate the average nest temperature for comparison with the CTE
                avgT = M + w*b/2;
% Place results in the file created at the very beginning ("CTEfile")
% same format as above, but now change the %s to %f, to put in actual
numbers % (instead of text for the column headings)
% this will print the current values for M, R, w, bestbigT (the CTE), and
% avgT with tabs in between, then start a new line
fprintf(CTEfile,'%f\t%f\t%f\t%f\t%f\t%f\n',Th,M,R,w,bestbigT,avgT);
```

```
            end
        end
    end
end
fclose(CTEfile);
Finished = 1 %this will print in the command window to indicate that the
program has finished running
```

ADDITIONAL PROGRAM

The primary program uses this additional program. Importantly, this code must be saved in an m-file entitled "crossT.m" for MATLAB to access it when running the primary code.

```
function f = crossT(x,R,M,w,bigT)

f = R*cos(x) + M + w*x - bigT;
```