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# From the cradle to the grave: how the embryonic environment shapes responses to climate throughout the life cycle

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FROM THE CRADLE TO THE GRAVE: HOW THE EMBRYONIC ENVIRONMENT  
SHAPES RESPONSES TO CLIMATE THROUGHOUT THE LIFE CYCLE

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A Thesis  
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the Graduate School of  
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Master of Science  
Biological Sciences

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by  
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## ABSTRACT

With rapidly warming climates, biologists are challenged to predict organismal responses to environmental variation, changes in biodiversity, and shifts in species distributions. In many cases, organismal responses differ across life stages that exhibit differences in physiological tolerances, habitat requirements, and resource allocations strategies. For instance, mobile life stages can alter behavior in response to changes in the thermal environment, whereas immobile life stages are often vulnerable to warming temperatures due to behavioral constraints and the limited microclimatic conditions experienced over small spatial extents (e.g., an embryo in an egg). Unfavorable developmental conditions can reduce growth, alter developmental rates, and increase mortality. Additionally, the developmental environment can influence subsequent life stages via changes in maturation rates, reproductive success, and survival. Therefore, organismal responses to changing environmental conditions can depend heavily on the physiology of immobile life stages as well as the behavioral and physiology capacities of mobile stages.

The first empirical chapter of this thesis (Chapter Two) concerns organismal responses to warming during early ontogeny and the ecological implications of such responses for a widespread ectotherm, the Eastern fence lizard (*Sceloporus undulatus*). Through a series of field and laboratory experiments, I examined the effects of recurrent sublethal warming on growth, survival, and distribution of *S. undulatus*. I then incorporated that data into an ecological model that accounts for variation in responses to warming through ontogeny and across geography. Combining empirical studies with mechanistic

species distribution modeling, we demonstrated that organisms with thermally sensitive life stages do not have to experience lethal temperatures to undergo negative changes at the individual and population levels.

The second empirical chapter (Chapter Three) concerns how the capacity for adaptation to mitigate negative impacts of future climates may vary across species ranges. Using a series of field observations and laboratory-based reciprocal transplant experiment, we examined how geographic variation in maternal behavior and thermal physiology underlies patterns of growth and development across the range of the eastern *S. undulatus* clade. By demonstrating the extents to which genetic background and environmental conditions affect thermal biology across geography, my data serves to increase understanding of the capacities for populations to persist under climate warming.

## DEDICATION

This work is dedicated to my family and friends who have shaped me into the person I am today. First, to my wife, Amy, for her undying love, patience, and understanding, and for her confidence in me even when I find it hard to believe in myself. Without her, none of this would have been possible. To my parents, Jeanine and Charlie, for raising me to be confident in life and to never give up. To my brothers, Matthew, Marc, Justin, and Christopher, for toughening me up for what life wants to throw at me and for always having my back. To my in-laws, Michelle, Ed, Eddie, and Sam, for welcoming me into their family with open arms and for their never-ending enthusiasm. To my grandparents, for their unconditional love and support. And to my best friends, Hughey, Jeff, and Megan, for always knowing how to bring me up when I am down and making sure I enjoy life when I am up. There are many other family members and friends who are important in my life, and I ask their forgiveness for not taking the time to list them here. I could honestly write another book about all of the wonderful people I am privileged to know and love.

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## CHAPTER ONE

### INTRODUCTION

#### Background and Objectives

As rapid climate change alters local conditions in ecosystems across the globe, the capacity to tolerate change depends upon responses to warming throughout all stages of the life cycle (Ådahl *et al.* 2006; Morris *et al.* 2008; Radchuk *et al.* 2013; Levy *et al.* 2015). Organisms with complex life cycles have distinct stages that may differ in response to climate according to physiological tolerances, habitat requirements, and allocation of resources to different processes (McConnaughay & Coleman 1999; Crozier *et al.* 2008; Kingsolver *et al.* 2011). Yet, few studies consider impacts of environmental change at each life stage of an organism, instead focusing predominantly on mature life stages. For instance, most species distribution projections are based on adult biology (e.g., Buckley 2008; Buckley *et al.* 2010; Sinervo *et al.* 2010; Kearney 2012), but they may underestimate vulnerability to climate warming by overlooking sensitive stages of development. Warming to deleterious temperatures during vulnerable stages of early ontogeny can have drastic effects on lifetime fitness and population dynamics (Crozier *et al.* 2008; Radchuk *et al.* 2013; Levy *et al.* 2015). Thus, predictive understanding of the ecological impacts of climate change will rely on examination of the complexity of organismal responses to environmental fluctuations throughout the life cycle.

The effects of climate warming begin in development, during early life stages when organismal responses to environmental change may be constrained (Feder 1997;

McConnaughay & Coleman 1999). Mobile organisms can use behavioral thermoregulation to buffer effects of environmental changes (Huey 1974), but sessile stages of early ontogeny are particularly vulnerable to warming (Feder 1997; O'Steen & Janzen 1999). For example, many reptiles lay eggs in shallow underground nests where developing embryos are exposed to thermal variation driven by heat flux from the soil surface. Without the ability to behaviorally thermoregulate, immobile embryos must rely on physiological responses to ambient conditions (Feder 1997; O'Steen & Janzen 1999). Deleterious developmental temperatures can reduce growth, alter development rates, and increase mortality (e.g., Albon *et al.* 1983; Castro *et al.* 2005; Georges *et al.* 2005; Hepp *et al.* 2006; Potter *et al.* 2011; Levy *et al.* 2015). Additionally, the developmental environment can influence subsequent life stages via changes in maturation rates, reproductive success, and survival (e.g., Haywood & Perrins 1992; Lumey & Stein 1997; Warner & Andrews 2002a; DuRant *et al.* 2010; Larios *et al.* 2014). Loss of performance during early ontogeny can limit population abundances and increase risk of extinction (Neilson *et al.* 2005; Crozier *et al.* 2008; Woods 2013), even if performance improves during later life stages (Levy *et al.* 2015). Lethal temperatures during early ontogeny have demographic consequences (e.g., Angilletta *et al.* 2013; Sánchez *et al.* 2014; Motani & Wainwright 2015); however, repeated exposure to moderate warming can also decrease fitness, even when conditions are not acutely lethal (Woods 2013; Bowden *et al.* 2014). Thus, impacts of changing thermal fluctuations in early ontogeny must be examined to further our understanding of species responses to warming.

Neglecting the influence of sublethal temperature fluctuations may underestimate the ecological consequences of climate change. Ecological responses to climate are partially driven by lethal conditions but are more likely to begin during sublethal warming (Petes *et al.* 2007; Woodin *et al.* 2013). Recurrent sublethal extremes can lead to chronic stress (Campbell *et al.* 1998; Badyaev 2005), which can reduce fitness through negative effects on growth and survival (Davison *et al.* 1993; De Kogel 1997; Shine & Elphick 2001; Marshall & Sinclair 2011). Thus, warming daily maximum temperatures may reduce survival and inhibit growth due to chronic stress from repeated exposure to temperatures near the limits of embryonic tolerances. Unfortunately, data on responses to sublethal environmental fluctuations are not sufficiently available beyond a few well-studied systems, such as corals (e.g., Edmunds 2005; Olsen *et al.* 2014; Maynard *et al.* 2015), intertidal mussels (e.g., Petes *et al.* 2007; Miller *et al.* 2009; Fly & Hilbish 2013), and some insect species (e.g., Potter *et al.* 2011; Ma *et al.* 2015; Marshall & Sinclair 2015). Climate-driven changes in population growth have been implicated in range contractions and decreased biodiversity of coral along the Great Barrier Reef (Edmunds 2005), northern range expansions and southern contractions of European butterfly species (Parmesan *et al.* 1999; Hill *et al.* 2002) and Pan-Arctic vegetation (Sturm *et al.* 2001; Tape *et al.* 2006), and elevational contractions of alpine plants (Grabherr *et al.* 1994) and tropical amphibians (Pounds *et al.* 1999). If even moderate rates of warming can impede recruitment and decrease mean fitness, ecological consequences of climate change may intensify and exceed previous predictions, unless populations can respond to mitigate those consequences.

The persistence of many species under climate change may rely heavily on adaptation to mitigate the impacts of rapid warming (Parmesan 2006; Merilä & Hendry 2014; Urban *et al.* 2016). In response to climate change, organisms must disperse, acclimate, or adapt to avoid extinction (Holt 1990, Fuller *et al.* 2010). Migration has become improbable for many species at a pace to match rates of warming, particularly due to increasing obstacles to long-distance dispersal due to anthropogenic activities (Opdam & Wascher 2004; Loarie *et al.* 2009). Among widespread ectotherms, adaptations of thermally sensitive traits have enabled persistence across environments with differing thermal regimes (Huey & Stevenson 1979; Angilletta *et al.* 2004b). For instance, thermal adaptation has altered *S. undulatus* embryo physiology along latitudinal gradients to speed growth and development in colder environments (Oufiero & Angilletta 2006). However, as warming pushes conditions at nest sites closer to the limits of embryo tolerances, the susceptibility of populations of species to climate change will likely rely on the capacity for adaptive plasticity (Ghalambor *et al.* 2007; Urban *et al.* 2014). That capacity may vary across geography for wide-ranging species, which consist of populations with different genetic backgrounds due to local adaptation (Pelini *et al.* 2010; Urban *et al.* 2016). Therefore, it is essential to consider ontogenetic variation in responses to fluctuating thermal regimes as well as how those responses may vary along environmental gradients that occur across species ranges.

In this publication, I present my work to examine variation in thermal biology through ontogeny and across geography, as well as the implications of those variations for the persistence of a widespread North American lizard under climate change. The aims of

this thesis are: to examine how recurrent sublethal thermal stressors in early ontogeny affect development, growth, and survival, and how those effects may extend to later life stages; to incorporate ontogenetic variation of thermal biology into an ecological model to refine projections under climate change; and to examine geographic variation in nesting behavior and embryonic thermal physiology to better understand the potential for persistence of populations across a wide species range under future climates. In the first empirical chapter (Chapter Two), I present a series of field and laboratory experiments to examine the effects of recurrent sublethal warming on growth, survival, and distribution of the Eastern fence lizard (*Sceloporus undulatus*). I incorporated the laboratory data into a mechanistic species distribution model to assess how consideration of ontogenetic variation in responses to warming impacts predictions of species distributions. In the second empirical chapter (Chapter Three), I present a series of field observations and a laboratory-based reciprocal transplant experiment to examine how geographic variation in nesting behavior and thermal physiology may underlie patterns of growth and development across latitudes. I leveraged the results to assess the capacity for adaptation to enable population persistence under climate change. Altogether, this thesis represents integrative research to examine how impacts of environmental change in early ontogeny can shape organismal responses to climate and the implications of ontogenetic variation in thermal biology in a warming world.

### A brief introduction to the Eastern fence lizard (*Sceloporus undulatus*)

In seeking a system to work within for my thesis research, I decided on *Sceloporus undulatus* for several reasons, not the least of which because it has served as a model organism for decades in physiological, behavioral, demographic, and evolutionary studies (e.g., Tinkle & Ballinger 1972; Ferguson & Brockman 1980; Stearns & Crandall 1981; Ferguson & Talent 1993; Niewiarowski & Roosenburg 1993; Andrews *et al.* 2000; Angilletta *et al.* 2002; Warner & Andrews 2002a, b; Niewiarowski *et al.* 2004; Sears & Angilletta 2004; Angilletta *et al.* 2004b; Oufiero & Angilletta 2006; Parker & Andrews 2007; Niewiarowski & Angilletta 2008; Angilletta *et al.* 2013; Levy *et al.* 2015), particularly in the realm of thermal biology. Additionally, distinct life stages of *S. undulatus* exhibit differences in physiological tolerances, behavioral capacities, and habitat requirements (covered within Chapters Two and Three) that provide opportunities to design experiments examining ontogenetic variation in thermal biology. The Eastern fence lizard represents the eastern clade of the widespread monophyletic *S. undulatus* species group, which ranges across the eastern two-thirds of the United States and northern Mexico (Leaché & Reeder 2002; Leaché 2009). The range of the eastern clade stretches up the eastern US from central Florida to northern New Jersey and extends west to the Mississippi River (Leaché 2009; Fig. 1.1). It exhibits patterns of life-history characteristics across its range that are consistent with the temperature-size rule (Niewiarowski *et al.* 2004; Angilletta *et al.* 2004a, b; Oufiero & Angilletta 2006; Niewiarowski & Angilletta 2008). In southern populations mortality is higher and individuals mature younger at smaller sizes and produce fewer offspring, whereas northern populations experience lower mortality,

and individuals delay reproduction to mature at larger sizes, lay larger eggs, and produce more offspring (Tinkle & Ballinger 1972; Niewiarowski 1992; Niewiarowski *et al.* 2004; Angilletta *et al.* 2004a, b). Early demographic studies across the species range suggested that geographic variation in growth and development could be explained by differences in the biophysical environment, particularly differences in the lengths of available activity periods across habitats (Tinkle & Ballinger 1972; Ferguson & Brockman 1980). However, more recent studies have begun to investigate the relative impacts of local adaptation and environmentally induced plasticity on life-history phenotypes across *S. undulatus* populations (e.g., Niewiarowski & Roosenburg 1993; Oufiero & Angilletta 2006; Niewiarowski & Angilletta 2008).



**Figure 1.1** The range of the Eastern fence lizard (*Sceloporus undulatus*), which represents the eastern clade of the *S. undulatus* species group (Leaché & Reeder 2002; Leaché 2009). The map in is modified from (Leaché 2009).



In regards to general characteristics of morphology and behavior, *Sceloporus undulatus* is a rough-scaled, spiny lizard with strong sexual dimorphism in both body size and coloration. Females tend to grow to larger body sizes than males (i.e., longer snout-vent-length; Warner 2001). In general, females tend to have stronger dorsal patterning, and adult males are easily spotted by the patches of blue coloration along their ventral sides and throat during the breeding season (Fig. 1.2; though, some females may have smaller and less vivid patches of blue; Swierk & Langkilde 2013). Lizards in the eastern *S. undulatus* clade prefer access to edges in mixed deciduous and pine forests. As an ectotherm skilled at behavioral thermoregulation, it requires access to sunlight. So, it is more often found in forested areas with woody debris and rocks, including forests that have been disturbed by humans (Warner 2001). The *S. undulatus* breeding season begins in Spring as early as mid-April and continues through Summer (Tinkle & Ballinger 1972). Nesting typically begins in May and continues into July, though nesting can begin in late April and extend later into August in populations inhabiting warmer climates (Carlo, unpublished data). Another benefit of utilizing this species in my thesis research is that it produces relatively large clutches of eggs (typically, 6-10 eggs per clutch, Warner 2001; this thesis, Chapters Two and Three and references therein; although, I have personally witnessed a single-egg clutch and a 16-egg clutch), which enabled me to split clutches for manipulations of embryo temperatures under multiple treatments while controlling for maternal effects. Hatching typically begins approximately two months after the first clutches are laid and continues through September, though the timing of hatching may vary across the species range.



**Figure 1.1** Dorsal and ventral views of female (upper lizard in images) and male (lower lizard in images) *Sceloporus undulatus*. These lizards were captured in the Long Cane Ranger District of Sumter National Forest in Edgefield County, South Carolina, US).

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## CHAPTER TWO

### RECURRENT SUBLETHAL WARMING REDUCES EMBRYONIC SURVIVAL, INHIBITS JUVENILE GROWTH, AND ALTERS SPECIES DISTRIBUTIONS UNDER CLIMATE CHANGE

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#### Abstract

The capacity to tolerate climate change often varies across ontogeny in organisms with complex life cycles. Recently developed species distribution models incorporate traits across life stages; however, these life-cycle models primarily evaluate effects of lethal change. Here, we examine impacts of recurrent sublethal warming on development and survival in ecological projections of climate change. We reared lizard embryos in the laboratory under temperature cycles that simulated contemporary conditions and warming scenarios. We also artificially warmed natural nests to mimic laboratory treatments. In both cases, recurrent sublethal warming decreased embryonic survival and hatchling sizes. Incorporating survivorship results into a mechanistic species distribution model reduced annual survival by up to 24% compared to models that did not incorporate sublethal

warming. Contrary to models without sublethal effects, our model suggests that modest increases in developmental temperatures influence species ranges due to effects on survivorship.

### Introduction

For organisms with complex life cycles, ecological consequences of climate change may be driven by responses to warming that vary across ontogeny (Kingsolver *et al.* 2011; Radchuk *et al.* 2013). With rapid warming, a major goal for ecologists is to determine thermally-sensitive processes that underlie shifts in range dynamics (Pacifiçi *et al.* 2015; Urban *et al.* 2016). Recent advances in species distribution models (SDMs) incorporate biological mechanisms to predict climate-driven range shifts (Helmuth *et al.* 2005; Buckley *et al.* 2010; Riddell *et al.* 2017) but often rely upon adult life stages to make predictions (e.g., Sykes *et al.* 1996; Buckley 2008; Deutsch *et al.* 2008; Randin *et al.* 2009; Kearney 2013). Downstream effects from early life have consequences for growth, survival, and reproduction (reviews in Lindström 1999; Podolsky & Moran 2006; Harrison *et al.* 2011). Thus, ecological projections might hinge on responses across ontogeny for many species (Lindström 1999; De Block & Stoks 2005).

Sensitive stages of early ontogeny drive ecological responses to environmental change (Radchuk *et al.* 2013). Sessile stages are sensitive to fluctuating conditions due to limited behaviors and the small range of microclimatic conditions experienced over small spatial extents (e.g., an egg; Refsnider & Janzen 2010; Telemeco *et al.* 2016; but see Du & Shine 2015). Embryos consequently rely on physiological responses to developmental

conditions that can alter growth and development rates and increase mortality (e.g., Castro *et al.* 2005; Georges *et al.* 2005; Hepp *et al.* 2006; Oufiero & Angilletta 2006; Potter *et al.* 2011). In turn, developmental conditions may influence population dynamics through changes in maturation rates, reproductive success, and survival (e.g., Haywood & Perrins 1992; Lumey & Stein 1997; Warner & Andrews 2002; DuRant *et al.* 2010; Larios *et al.* 2014), particularly in short-lived species (Tinkle 1969; Overall 1994). Downstream effects of warming also increase risk of extirpation by reducing reproductive performance and survival (Edmunds 2005; Neilson *et al.* 2005; Crozier *et al.* 2008). Impacts of thermal fluctuations in early ontogeny should thus be considered in the development of physiologically-explicit models (Levy *et al.* 2015; Urban *et al.* 2016).

The lasting effects of warming during early ontogeny may be underestimated by ignoring impacts of fluctuating thermal conditions. Recurrent sublethal stressors—exposures to suboptimal conditions that are not acutely lethal—are increasingly likely as climate warming increases daily temperature variance and frequencies of extreme weather events (Meehl & Tebaldi 2004; IPCC 2013). Modest increases in temperature can benefit growth and development (Angilletta *et al.* 2004b; Refsnider & Janzen 2010), particularly in environments where low temperatures limit growth (Deutsch *et al.* 2008; Randin *et al.* 2009; Paaijmans *et al.* 2013). However, in warmer environments, increased incubation temperatures may result in recurrent sublethal extremes that lead to chronic stress (Campbell *et al.* 1998; Badyaev 2005), which can inhibit development, increase embryo mortality, and influence lifetime fitness (e.g., Shine & Elphick 2001; Fly & Hilbish 2013; Marshall & Sinclair 2015). Recent SDMs incorporate ontogenetic variation of

thermotolerance for some well-studied species (e.g., Crozier *et al.* 2008; Levy *et al.* 2015). Clearly, lethal thresholds influence fitness; however, physiologically-explicit SDMs based solely on lethal limits ignore consequences of recurrent sublethal fluctuations (Woodin *et al.* 2013). Unfortunately, the preponderance of constant-temperature treatments in physiological studies has left little focus on fluctuating developmental regimes (Niehaus *et al.* 2012; Bowden *et al.* 2014). Constant incubation temperatures have advanced research by elucidating thermal sensitivities of phenotypes across many oviparous taxa (reviews in Deeming & Ferguson 1991a; Booth 2006; Bowden *et al.* 2014). However, the applicability of that data to development under natural conditions is limited. By overlooking acute and recurrent thermal stressors, incubation under constant temperatures poorly predicts development under natural cycles (reviews in Bowden *et al.* 2014; Warner 2014; Wu *et al.* 2015). Thermal stress on anurans and *Manduca sexta* larvae reared under constant temperatures resulted in reaction norms that poorly predicted growth and development under naturalistic regimes (Niehaus *et al.* 2012; Kingsolver *et al.* 2015). Thermal impacts on development underscore the importance of experimental conditions for the embryonic environment.

Here, we use naturalistic thermal cycles to examine consequences of recurrent sublethal warming during incubation on embryonic and post-hatching phenotypes. We integrate these findings to predict the species distribution of *Sceloporus undulatus*, a widespread North American lizard. Maternal behavior of *S. undulatus* suggest that females nest in the warmest parts of their environment, digging shallow nests where embryos experience diel thermal cycles (Fig. 2.1a,b; Angilletta *et al.* 2000; Angilletta *et al.* 2009).



Increases in temperature means and variances of *Sceloporus* embryos can speed growth and development without affecting survival (e.g., Sexton & Marion 1974; Andrews *et al.* 2000; Angilletta *et al.* 2000; Oufiero & Angilletta 2006). However, our study is the first to warm embryos throughout incubation beyond regimes experienced at contemporary nest sites in this system. In the laboratory, we reared embryos under treatments that simulated contemporary and potential future thermal conditions. In a complementary field experiment, we artificially warmed natural nests to simulate similar sublethal warming. We integrated embryonic responses to warming into a SDM using a life-cycle submodel of population dynamics (Levy *et al.* 2015). Model projections indicate that moderate warming during early ontogeny can limit species ranges. Our study highlights consequences of transient, but recurrent, exposure to warmer nests that may harm embryos and hatchlings, shaping ecological responses to environmental change.

## Methods

### Laboratory Methods

#### *Collection & husbandry*

To examine impacts of sublethal warming during incubation, we conducted experiments using *S. undulatus* eggs from females collected in Edgefield County, South Carolina (SC) in May and June 2014 (UTM Easting 396467.43, Northing 3753517.85, Zone 17S). We housed adult lizards at Clemson University in terraria (8.48L; 30x19.5x14.5cm) with moist sphagnum for oviposition. Programmable environmental chambers (I-36VL; Percival Scientific, Perry, Indiana, USA) maintained 14:10-hour

light:dark cycles and kept lizards at preferred daytime (32°C) and approximate nighttime (24°C) temperatures (Niewiarowski 1992; Angilletta 2001). We replenished water daily and offered crickets *ad libitum* every two days.

Collection and care of eggs minimized exposure to conditions outside of treatment designs. We checked terraria hourly 0700-2100 to immediately weigh and place eggs in individual containers (59mL; 3cm-height-by-5cm-diameter) with a 1:100 water-to-silica-sand mixture (Angilletta *et al.* 2000). Environmental chambers (I-36VL; Percival Scientific) maintained eggs at 80% relative humidity and temperatures per treatment designs. We replaced water lost from containers every 3 days to maintain hydric conditions throughout incubation. We rotated treatment groups between chambers and rotated shelves in a balanced randomized design to control for potential effects of chamber or shelf location. Hatchlings were transferred to containers (474mL; 7.5cm-height-by-9cm-diameter) under the same conditions as adults, except pinhead crickets were offered daily.

### *Treatment design*

We designed the treatments to create naturalistic thermal regimes based on soil temperatures recorded in simulated nests in Edgefield County, SC (Angilletta & Sears, unpublished data), which were constructed assuming nesting conditions consistent with those observed by Angilletta *et al.* (2009). The treatments included a thermal regime that estimated contemporary SC nest temperatures and two regimes that increased daily maximum temperature ( $T_{\max}$ ) to simulate warming scenarios (Fig. 2.1a). Angilletta *et al.* (2013) suggested that exposure to high  $T_{\max}$  was not necessarily harmful to *S. undulatus*

embryos below a lethal threshold ( $\sim 41^{\circ}\text{C}$ ). However, they measured effects of acute exposure. To examine impacts of recurrent exposures to high  $T_{\text{max}}$  throughout incubation, we increased  $T_{\text{max}}$  in the warming treatments by  $3.5^{\circ}$  and  $7.0^{\circ}\text{C}$  relative to the contemporary treatment ( $32.0^{\circ}\text{C}$ ). Thus, embryo  $T_{\text{max}}$  increased to suboptimal levels without reaching the lethal threshold. Though climate warming also increases nighttime minima (Donat & Alexander 2012; IPCC 2013), we held daily minimum temperature ( $T_{\text{min}}$ ) at  $19.0^{\circ}\text{C}$  across treatments to specifically examine effects of increasing  $T_{\text{max}}$ . From 12 clutches (clutch size  $7.67 \pm 0.39$  (SEM), range 6-10), 29 embryos were reared under the contemporary treatment, 33 under  $+3.5^{\circ}\text{C}$ , and 31 under  $+7.0^{\circ}\text{C}$ .

To control for maternal effects, we randomly distributed each clutch evenly among treatments. In *S. undulatus*, oviposition occurs at about 18-26% of embryonic development (Sexton & Marion 1974; Parker *et al.* 2004). We maintained females under common conditions in the laboratory. So, assuming females maintained similar field body temperatures ( $T_b$ ), embryos experienced the same temperatures *in utero*. Therefore, embryos were exposed to maternal  $T_b$  during the earliest stages of embryogenesis and to experimental temperatures during mid-to-late-development.

### *Embryonic survival & hatchling growth*

We monitored survival daily by checking for heartrates using an infrared sensor (Buddy Egg Monitor; Avitronics, Cornwall, UK). If no heartrate was detected for three consecutive days, we marked the embryo as deceased on the first day. We measured hatchling mass to  $0.1\text{mg}$  and snout-vent length (SVL) to  $0.1\text{mm}$ . We then calculated scaled

mass indices (SMI) from standard regressions of mass-to-SVL as outlined in Peig & Green (2009; 2010) to estimate hatchling body conditions. We chose SMI as a less biased measure than other indices (e.g., Fulton's index:  $\text{mass} \cdot \text{length}^{-3}$ ) that do not account for changing allometry across growth stages (see Appendix A for details).

To examine downstream effects of warming treatments, we calculated juvenile growth rates. We repeated body size measurements for the first three weeks post-hatching. Then, we used the approach described by Dunham (1978) and Schoener & Schoener (1978) to estimate characteristic growth rates ( $r$ ) for the interval form of von Bertalanffy growth models. We used SVL instead of mass to minimize potential variation due to nutritional state (Dunham 1978; Sears 2005). We fitted the growth model using Levenberg-Marquardt nonlinear least-squares regression from the *minpack.lm* library in R (Elzhov *et al.* 2015).

## Field Methods

### *Tracking & collection*

In May and June 2015, we tracked gravid females using radio telemetry to locate nests. We attached radio transmitters (Model BD-2X; Holohil Systems Ltd., Carp, Ontario, Canada) weighing <5% of a female's body mass to the dorsum with surgical adhesive. We located 8 nests (82 eggs, clutch size  $10.2 \pm 0.36$ , range 9-12) and assigned clutches laid within five days of each other to nesting groups, within which we reciprocally transplanted eggs to control for maternal effects. We carefully excavated eggs and placed them in individual containers as in the laboratory methods for transport to Clemson University. We incubated eggs at 15°C for up to five days to allow collection of multiple clutches. This

method suspends development without affecting growth and survival after development resumes (Christian *et al.* 1986; Andrews *et al.* 1997). We then reconstructed nests to contain a random sample including at least one egg from each clutch in the nesting group and totaling the original clutch size laid in that nest. iButton loggers (DS1922L; Maxim Integrated, San Jose, California, USA) recorded hourly temperatures at mean nest depth.

### *Treatment design*

We randomly assigned half the nests to a warming treatment, for which a 0.09m<sup>2</sup> section of black thermoplastic (TerraTexSF-D; Hanes Geo, Winston-Salem, North Carolina, USA) was stapled against the soil surface to decrease solar reflectance. There were 44 embryos among the natural nests and 38 among warmed. The material consisted of woven 2.0mm-wide-by-0.15mm-thick polypropylene filaments, forming a porous surface that increased daytime nest temperatures without retaining excess heat overnight and allowed for water and gas exchange. To ensure this method did not influence soil moisture or oxygen availability, we performed a validation experiment in which we measured soil temperatures, moisture, and oxygen in a grid of mock nests randomly assigned to the warmed or natural treatment (see Appendix A and Table B1 for details).

### *Embryonic survival & hatchling size*

We monitored nests daily for emerging hatchlings. Steel wire cages with 3.0mm spacing placed over nests enabled collection. We calculated survival by counting

hatchlings and confirmed results through excavation to count nonviable eggs and empty shells. We measured hatchling mass and SVL and calculated SMI as described above.

## Data Analysis

We conducted statistical analyses in R v3.3.1 (R Core Team 2016). To test effects of laboratory warming treatments on embryonic survival, we used a Cox proportional hazard model from the *survival* library (Therneau 2014), which included an estimator of variance attributable to maternal identity to control for correlation of responses among siblings. To test effects of laboratory treatments on development time, hatchling sizes, SMI, and  $r$ , we constructed linear mixed effects (LME) models using the *lme* function (Pinheiro *et al.* 2016) with treatment as a categorical variable and maternal identity as a random effect. We added hatchling SVL as a continuous variable for  $r$  and initial egg mass as a continuous variable for development time and hatchling sizes. For the field data, we constructed LME models with treatment as a categorical variable and with assigned nest and nesting group as random effects for  $T_{\max}$ ,  $T_{\min}$ , embryonic survival, development time, hatchling body sizes, and SMI. We could not include maternal identity in analyses of field data due to the reciprocal transplants. For each parameter in an LME model, we calculated effect sizes ( $\omega^2$ ) to determine the proportion of explained variance of each parameter included in an ANOVA (Olejnik & Algina 2003):

$$\omega^2 = (SS_{\text{treatment}} - (df_{\text{treatment}} \cdot MS_{\text{error}})) / (SS_{\text{total}} + MS_{\text{error}}) \quad [1]$$

where  $SS_{\text{treatment}}$  = sum of squares,  $df_{\text{treatment}}$  = degrees of freedom,  $MS_{\text{error}}$  = mean square error, and  $SS_{\text{total}}$  = total sum of squares.

## Life-Cycle Model of Population Dynamics

### *Modeling embryonic and juvenile survival*

We developed a SDM to explore how inclusion of our results affects projections of embryonic survival and population growth in North America. Our model was based on a population dynamic model developed by Buckley (2008) to incorporate biology of free-living *Sceloporus* life stages into population growth projections under climate change and extended to include embryonic development and juvenile survival as in Levy *et al.* (2016b). Parameterization followed previous simulations, except where noted below.

We simulated activity by predicting  $T_b$  for female lizards of average size (10.7g; Angilletta 2001) across the geographic range on surfaces with 0-100% shade. We calculated  $T_b$  from operative temperatures (steady state temperature in a microclimate; Bakken 1992) using hourly microclimates (Levy *et al.* 2016a) covering the USA at 36x36-km resolution for the past (1980-2000) and future (2080-2100, assuming radiative forcing of +8.5W/m at year 2100). See Table B2 and Appendix A for parameter values and additional details. We assumed that lizards are active when  $T_b$  falls within the preferred range (central 80% of field body temperatures; Table B2) and that reproductive season begins after temperatures enable 30 days of activity (Tinkle & Ballinger 1972; Angilletta 2001). On each day of the reproductive season, we simulated oviposition by allocating nests to microhabitats with each combination of shade (0, 25, 50, 75, or 100%) and depth (3, 6, 9, or 12cm), which captured the range of microhabitats for natural nests (Angilletta *et al.* 2009; this manuscript).

Based on our empirical observations, we evaluated the impacts of warming nest temperatures on embryonic survival and population growth rates by comparing results of the model with and without effects of sublethal warming. We parameterized embryonic survival in the sublethal model using our laboratory survivorship results to provide conservative estimates based on experiments in which we controlled hydric conditions across treatments to isolate the impacts of incubation temperatures. See Appendix A for further details.

### *Modeling population growth*

We computed population growth rates ( $r_0$ , lizards/day) per Buckley (2008):

$$r_0 = m \cdot e_{net} - \mu, \quad [2]$$

where  $e_{net}$  = net energy gain by an adult,  $\mu$  = daily mortality ( $197.36 \cdot 10^{-5}$  lizards/day; Buckley 2008), and  $m$  = eggs produced per Joule ( $3.2 \cdot 10^{-4}$  eggs/J; Buckley 2008) multiplied by probability of surviving to adulthood. Net energy gain was estimated as the difference between energy gained from feeding and digestion and energy expended during resting and activity. For each location, we calculated the survival to adulthood component of  $m$  as the product of embryonic and juvenile survivorships (Levy *et al.* 2015). We then compared projections of population growth with and without effects of sublethal warming. See Appendix A for additional information.

To test how exposure of embryos to recurrent sublethal warming may alter projections through effects on later life stages, we ran the model with different hatchlings sizes and juvenile growth rates to calculate time to maturity. Assumptions built into the



model—juvenile survivorship, juvenile growth, and size at maturity do not vary across geography, and all lizards mature by the next reproductive cycle—prevent incorporation of predicted time to maturity into projections. So, we estimated changes in intrinsic growth rates due to delayed maturity using life tables for northern (New Jersey (NJ)) and southern (SC) populations. See Appendix B for details.

## Results

### Laboratory & Field Experiments

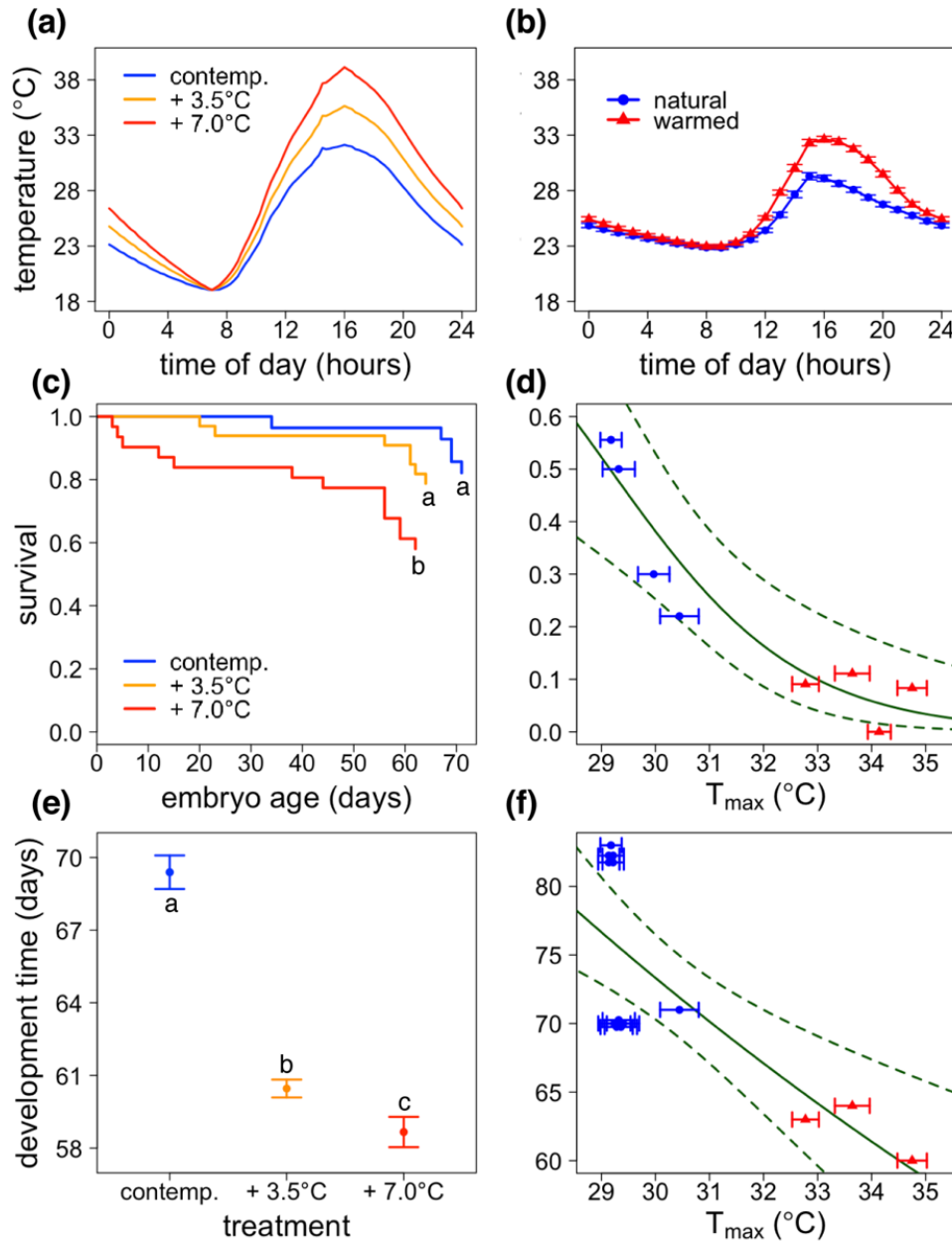
The field warming treatment increased  $T_{\max}$  among warmed nests by  $4.21 \pm 0.26^{\circ}\text{C}$  compared to natural nests and did not alter  $T_{\min}$  across treatments (Fig. 2.1b, Table 2.1). We used degree-day calculations (Zalom *et al.* 1983) to compare the magnitudes of warming experienced by embryos due to changes in means and variances between treatments in both experiments (see Appendix A for details). Embryos under laboratory warming treatments accrued averages of 257.87 and 336.65 degree-days above the  $T_{\max}$  of the contemporary treatment. In the field, embryos under the warming treatment accrued an average of 309.99 degree-days above the mean  $T_{\max}$  of natural nests. Although absolute temperatures differed between experiments, the field warming treatment induced a magnitude of warming similar to that applied in the laboratory.

Recurrent sublethal warming increased embryonic mortality in both experiments. In the laboratory, embryonic survival decreased with increased warming (Fig. 2.1c). The proportional hazard model estimated 82.1% survival for the contemporary treatment versus 78.8% for  $+3.5^{\circ}\text{C}$  and 58.1% for  $+7.0^{\circ}\text{C}$ . Embryos in the  $+7.0^{\circ}\text{C}$  treatment had lower

survival probability than both the contemporary ( $\beta=-2.84\pm1.05$ ,  $z=2.81$ ,  $p=0.005$ ) and  $+3.5^{\circ}\text{C}$  ( $\beta=-1.01\pm0.47$ ,  $z=2.12$ ,  $p=0.034$ ) treatments. Though survivorship decreased from the contemporary to the  $+3.5^{\circ}\text{C}$  treatment, there was no significant difference between those survivorship curves ( $\beta=-1.84\pm1.07$ ,  $z=1.60$ ,  $p=0.110$ ). Embryonic survival in the field also decreased under warming with  $36.9\pm9.3\%$  survival among natural nests (typical of nest survivorship in SC, Tinkle & Ballinger 1972) versus  $7.1\pm4.9\%$  among warmed nests (Fig. 2.1d, Table 2.1). Lower survivorship in the field than in the laboratory was likely due to differences in hydric conditions. We maintained consistent hydric conditions in the laboratory, whereas embryos in the field experience natural variations in soil moisture that can impact survival (Tracy 1980; Packard *et al.* 1982).

Sublethal warming also led to shorter incubation times and smaller hatchling sizes in both experiments, lower body conditions of hatchlings in the field, and slower post-hatching growth in the laboratory. In the laboratory, hatchlings emerged 12.9% earlier from the  $+3.5^{\circ}\text{C}$  treatment ( $n=26$ ,  $-8.93\pm0.37$  days) and 15.4% earlier from  $+7.0^{\circ}\text{C}$  ( $n=18$ ,  $-10.72\pm0.63$  days) compared to the contemporary treatment ( $n=23$ ,  $69.39\pm0.69$  days; Fig. 2.1e, Table 2.1). In the field, hatchlings from warmed nests emerged 17.6% earlier ( $n=3$ ,  $-13.30\pm1.20$  days) than from natural nests ( $n=11$ ,  $75.64\pm1.90$  days; Fig. 2.1f, Table 2.1). Lizards from laboratory warming treatments hatched at shorter SVL (contemporary:  $n=17$ ,  $24.91\pm0.22\text{mm}$ ;  $+3.5^{\circ}\text{C}$ :  $n=19$ ,  $24.40\pm0.19\text{mm}$ ;  $+7.0^{\circ}\text{C}$ :  $n=13$ ,  $23.80\pm0.27\text{mm}$ ; Fig. 2.2a, Table 2.1), though hatchling mass and SMI did not differ (contemporary:  $n=17$ ,  $0.48\pm0.01\text{g}$ ,  $0.486\pm0.025$  SMI;  $+3.5^{\circ}\text{C}$ :  $n=19$ ,  $0.49\pm0.01\text{g}$ ,  $0.485\pm0.023$  SMI;  $+7.0^{\circ}\text{C}$ :  $n=13$ ,  $0.47\pm0.02\text{g}$ ,  $0.473\pm0.028$  SMI; Fig. 2.2c, Table 2.1). In the field, hatchlings emerged

from warmed nests at shorter SVL and lighter mass (natural:  $n=11$ ,  $25.60\pm0.10\text{mm}$ ,  $0.53\pm0.01\text{g}$ ; warmed:  $n=3$ ,  $24.83\pm0.16\text{mm}$ ,  $0.45\pm0.01\text{g}$ ; Fig. 2.2b,d, Table 2.1), which led to lower SMI (natural:  $0.534\pm0.019$ , warmed:  $0.447\pm0.046$ ; Table 2.1). The growth model predicted 6.4% lower  $r$  from the  $+3.5^\circ\text{C}$  treatment ( $n=8$ ,  $7.51\pm0.19\mu\text{m/day}$ ) and 10.5% lower from  $+7.0^\circ\text{C}$  ( $n=4$ ,  $7.18\pm0.14\mu\text{m/day}$ ) compared to contemporary ( $n=6$ ,  $8.02\pm0.22\mu\text{m/day}$ ; Fig. 2.2e, Table 2.1).



**Figure 2.1** Thermal treatments in laboratory and field experiments and impacts of treatments on embryo development time and survival. Error bars indicate  $\pm 1$  SE. (a) Laboratory treatments simulated contemporary thermal conditions at *S. undulatus* nest sites and warming scenarios designed to introduce recurrent sublethal thermal stressors via increased  $T_{max}$ . (b) In the field, the warming treatment induced sublethal warming of daytime nest temperatures without altering overnight minima. Recurrent sublethal warming reduced embryonic survival in (c) the laboratory and (d) the field. Among lizards that survived to hatching, development time (days from oviposition to hatching) decreased with increased warming in (e) the laboratory and (f) the field. For panels c and e, letters denote statistical relationships such that data with different letters are significantly different ( $p < 0.05$ ). In panel f, overlapping points are offset. See Table 2.1 for summary statistics.

**Table 2.1** Summary statistics for analyses of laboratory and field data using mixed effects ANOVA. Laboratory data include (a) time to hatching, hatchlings sizes in (b) SVL and (c) mass, (d) hatchling body conditions, and (e) characteristic growth rate derived from the Von Bertalanffy growth models. Laboratory analyses were performed using maternal identity as a random effect. Field data include (f) maximum and (g) minimum daily nest temperatures, (h) embryonic survival, (i) time to hatching, hatchling sizes in (j) SVL and (k) mass, and (l) hatchling body conditions. Analyses of field data included assigned nest and nesting group as a random effect. Bolded values indicate statistical significance.

Response	Parameter	F	p	$\omega^2$
<i>Lab Experiment</i>				
(a) development time	treatment	<b>108.71</b> <sub>2,63</sub>	<b>&lt; 0.001</b>	<b>0.7521</b>
	initial egg mass	<b>5.01</b> <sub>1,63</sub>	<b>0.029</b>	0.0140
(b) hatchling SVL	treatment	<b>7.16</b> <sub>2,45</sub>	<b>0.002</b>	<b>0.1653</b>
	initial egg mass	<b>14.22</b> <sub>1,45</sub>	<b>&lt; 0.001</b>	<b>0.1774</b>
(c) hatchling mass	treatment	0.32 <sub>2,45</sub>	0.725	0.0000
	initial egg mass	<b>19.40</b> <sub>1,45</sub>	<b>&lt; 0.001</b>	<b>0.2784</b>
(d) hatchling SMI	treatment	0.34 <sub>2,45</sub>	0.713	0.0000
(e) characteristic growth rate ( <i>r</i> )	treatment	<b>3876</b> <sub>2,14</sub>	<b>&lt; 0.001</b>	<b>0.3226</b>
	hatchling SVL	<b>16259</b> <sub>1,14</sub>	<b>&lt; 0.001</b>	<b>0.6769</b>
<i>Field Experiment</i>				
(f) T <sub>max</sub>	treatment	<b>438.65</b> <sub>1,792</sub>	<b>&lt; 0.001</b>	<b>0.3553</b>
(g) T <sub>min</sub>	treatment	1.35 <sub>1,792</sub>	0.245	0.0004
(h) embryonic survival	treatment	<b>14.93</b> <sub>1,6</sub>	<b>0.008</b>	<b>0.6351</b>
(i) development time	treatment	<b>12.35</b> <sub>1,12</sub>	<b>0.004</b>	<b>0.4477</b>
(j) hatchling SVL	treatment	<b>14.14</b> <sub>1,12</sub>	<b>0.003</b>	<b>0.4842</b>
(k) hatchling mass	treatment	<b>16.38</b> <sub>1,12</sub>	<b>0.002</b>	<b>0.5235</b>
(l) hatchling SMI	treatment	<b>24.11</b> <sub>1,12</sub>	<b>&lt;0.001</b>	<b>0.6228</b>

$\omega^2$ , effect size (Olejnik & Algina 2003)

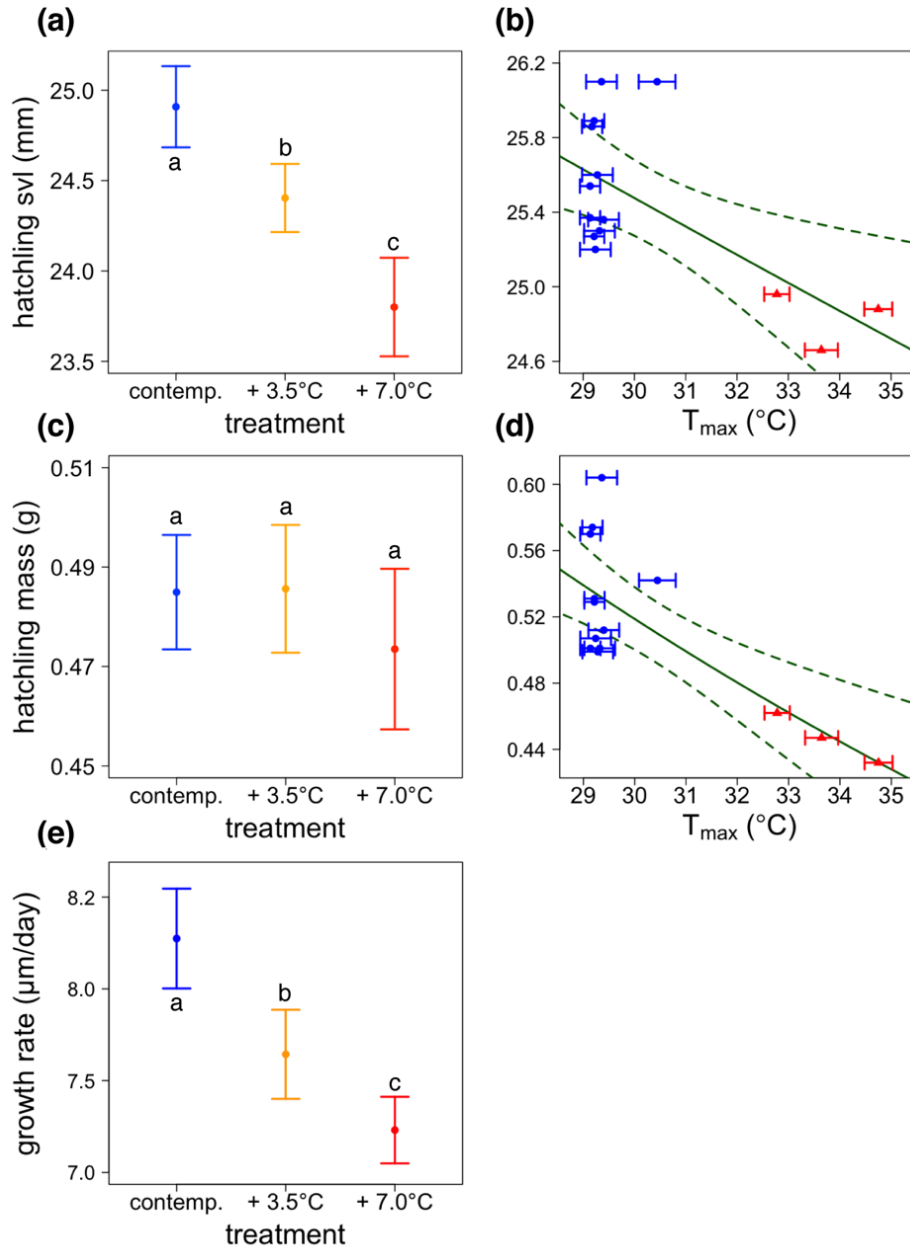
SVL, snout-vent-length

SMI, scaled mass index (Peig & Green 2009; 2010)

*r*, post-hatching growth rate

T<sub>max</sub>, maximum daily temperature

T<sub>min</sub>, minimum daily temperature



**Figure 2.2** Impacts of warming treatments on post-hatching sizes and projected growth rates. Error bars indicate  $\pm 1$  SE. Hatchling SVL decreased with increased warming (a) in the laboratory and (b) in the field. Hatchling mass decreased with warming nest temperatures (d) in the field, but there was no significant difference in hatchling mass among (c) laboratory treatments. (e) In the laboratory, characteristic growth rates derived from von Bertalanffy growth models decreased with increased warming. For panels a, c, and e, letters denote statistical relationships such that data with different letters are significantly different ( $p < 0.05$ ). See Table 2.1 for summary statistics.

## Model of Population Dynamics

Our SDM (herein “sublethal model”) predicts more severe consequences of climate warming than those of a model based solely on lethal limits of embryonic thermotolerances (herein “lethal model”). The sublethal model accounts for the fact that nesting conditions avoiding lethal extremes still experience recurrent thermal stressors (Fig. 2.3; Fig. B1-B14). By accounting for moderate warming, we demonstrate that even small changes in temperature can lead to increased risk of extirpation under contemporary and future climates.

Predicted embryonic survival decreases under contemporary and future climates when incorporating our empirical observations. Under typical nesting conditions in July (6cm-depth and 50%-shade, Angilletta *et al.* 2009; 4.4-8.0cm and 51.6-63.5%, this manuscript), the sublethal model predicts lower survival across 82.6% of the species range by -2.2% on average and by as much as -12.0% in locales that experience lower temperature variance, including portions of the southeast, the central plains, and the southwest (Fig. 2.4c). The magnitude and distribution of differences in predicted survival varies with nest depth, shade, and timing of oviposition (Fig. 2.4a-i, Fig. 2.5, Fig. B15-B42). For instance, incorporating the effects of sublethal warming alters survival across 96.8% of the range by -7.8% on average and by as much as -23.8% for nests laid in July at 12cm depth and 50% shade (Fig. 2.4i). Reduced embryonic survival then leads to decreased projected population growth.

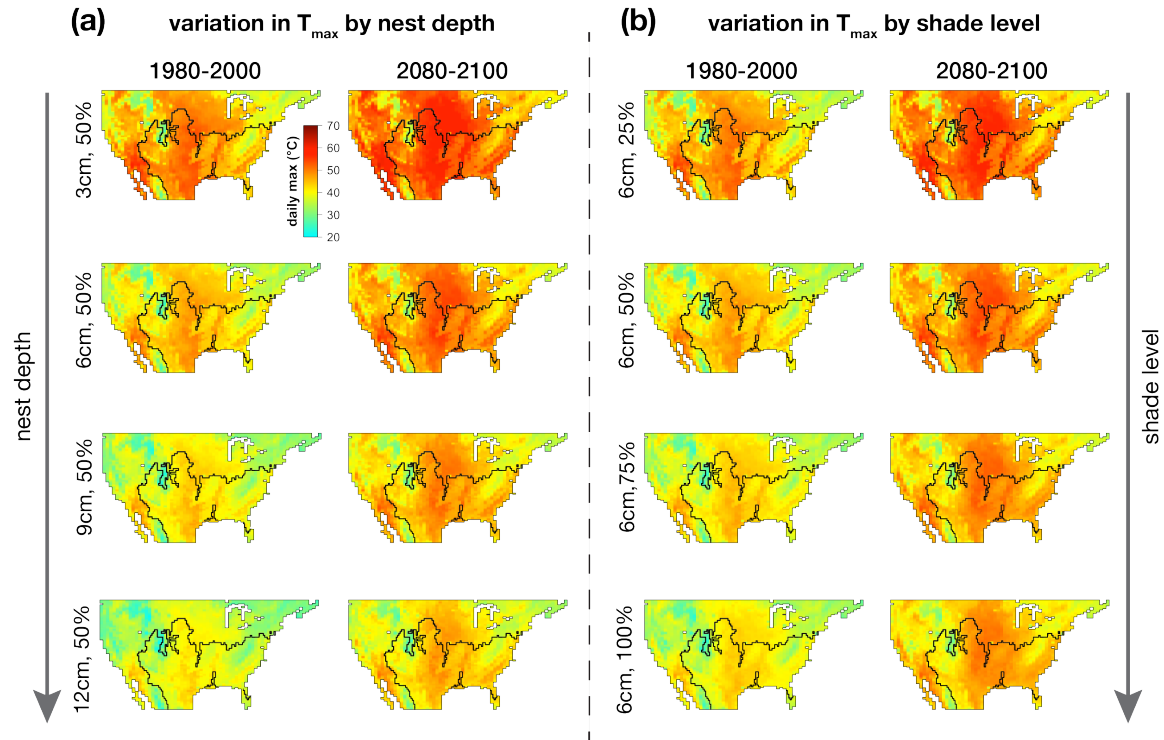
Recurrent sublethal warming during incubation leads to decreased projected population growth. Both models show positive population growth across 96.0% of the

species range under contemporary nesting conditions. Yet, when accounting for sublethal warming, the majority (84.7%) of those areas with positive growth experience increased risk of extirpation due to reduced population growth rates. Both models also agree on the geographic area of decreases in population growth under future warming (e.g., 51.4% and 50.5% of the range from the lethal and sublethal models respectively for typical nesting conditions). However, the magnitudes of reduced growth differ between the models. By overestimating embryonic survival, the lethal model underestimates negative impacts on population growth across 92.7% of the species range by 3.2% on average and by as much as 12.2% in locales that experience lower temperature variance (Fig. 2.4). Differences in population growth projections vary with nest depth, shade, timing, and geography similarly to embryonic survival (Fig. 2.4j-r, Fig. B43-B46).

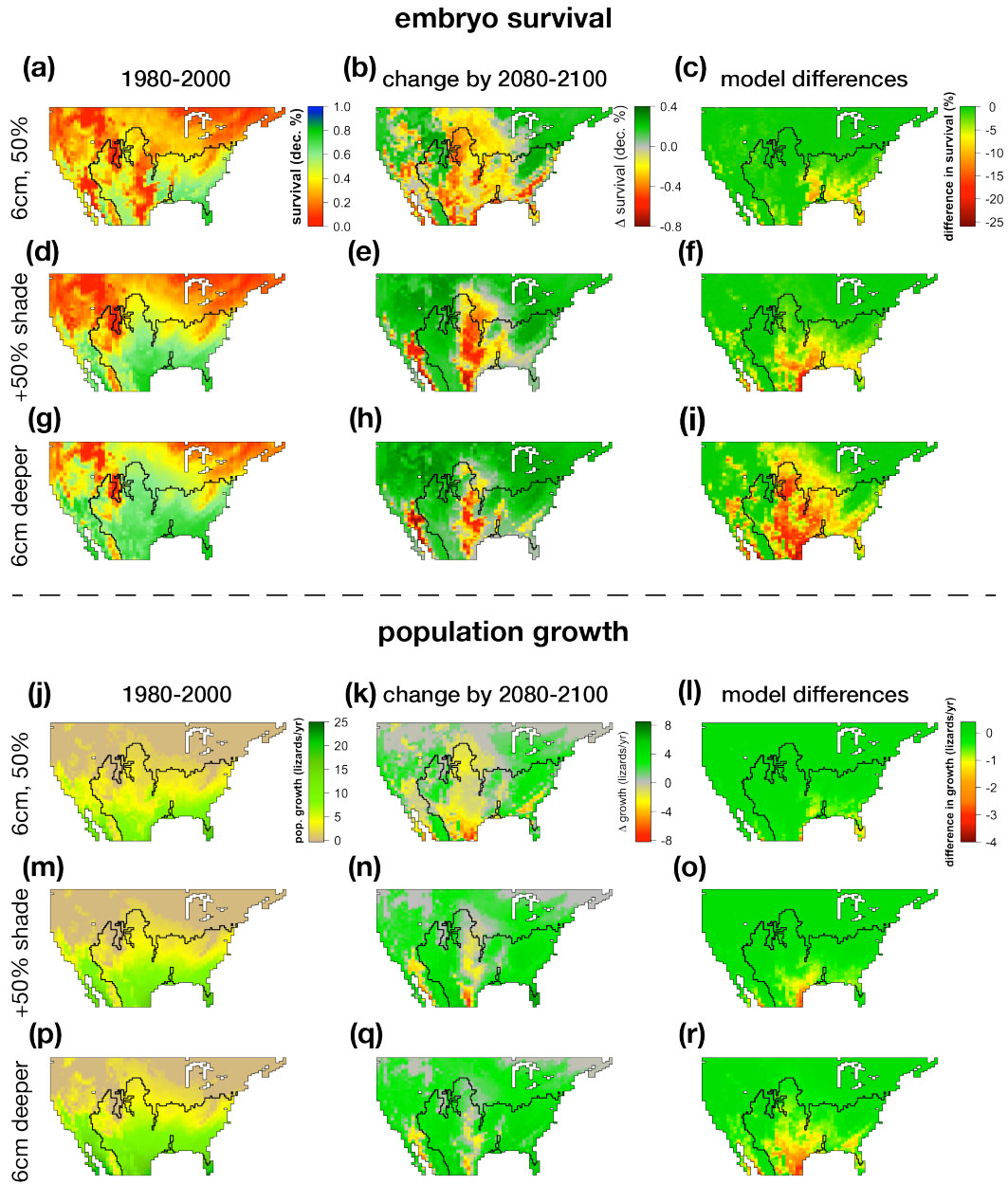
Sensitivity analyses examined how changes in hatchling sizes and juvenile growth rates affected projections of population growth via changes time to maturity. The growth model indicated increased age at maturity by  $32.4 \pm 7.6$  days across the species range when incorporating slowed juvenile growth (Fig. B48). In SC, a predicted 26-day delay in maturity could reduce population growth rates up to an additional 39.7% over the 24.4% predicted by the sublethal model. In NJ, population growth rates could decrease by an additional 80.1% due to a 29-day delay in maturity, which would lead to population decline and likely extirpation. These results demonstrate potentially severe impacts of sublethal warming during incubation on population dynamics via downstream effects through ontogeny.



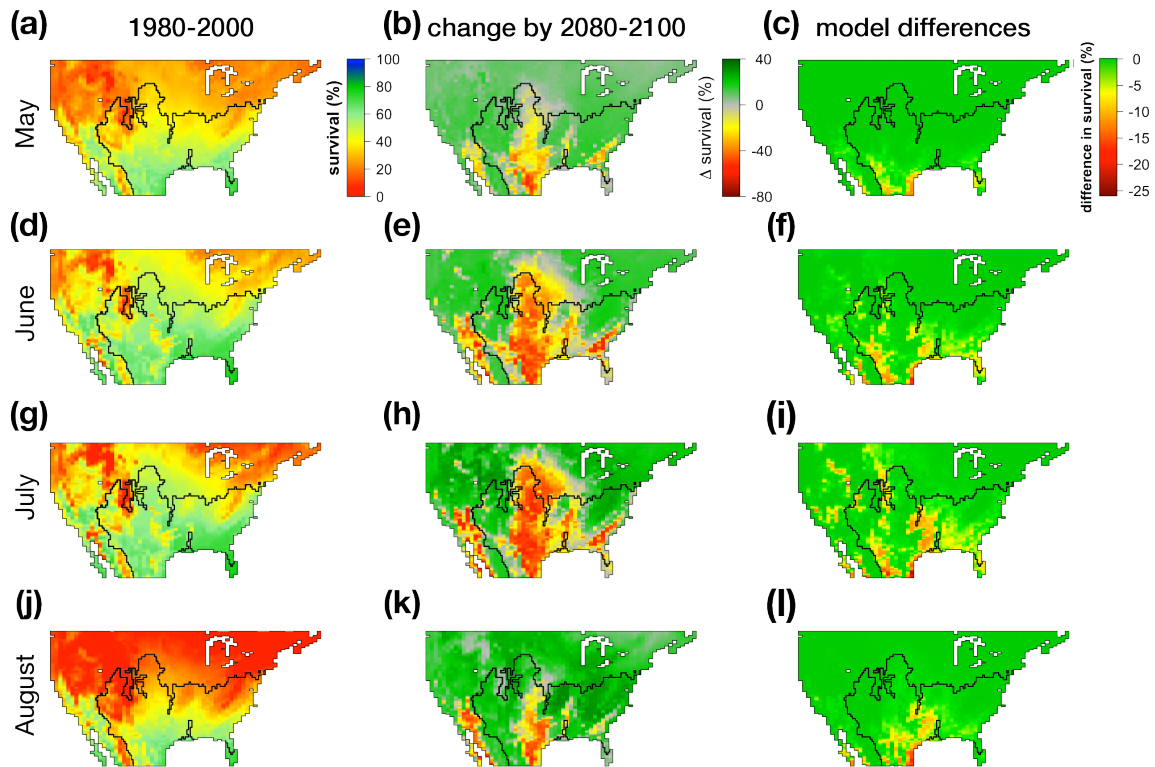
After comparing projections, we evaluated how well predictions match the contemporary species distribution. Both models predict the contemporary extent of the species range equally well if we treat positive embryonic survival and population growth as the only criteria. We also calculated sensitivity indices (proportion of presences predicted with positive survival, Manel *et al.* 2001; Buckley *et al.* 2010) and found no differences (see Appendix A for details). However, embryonic survival under the sublethal model decreased across 74.4% of occurrences to rates more consistent with demographic data (Tinkle & Ballinger 1972; Vinegar 1975; Tinkle & Dunham 1986). Thus, consideration of fluctuating developmental conditions reveals vulnerability to climate change that is not apparent without examination of sublethal warming.



**Figure 2.3** Spatial distributions of average maximum daily temperatures ( $T_{\max}$ ) during the month of July for the period 1980-2000 and predicted for the period 2080-2100. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Variation in  $T_{\max}$  is displayed across (a) increasing nest depths under 50% shade and (b) across increasing shade levels at 6cm nest depth. See Fig. B1-B14 for plots based on all other combinations of nest depth (3, 6, 9, or 12cm) and shade (0, 25, 50, 75, or 100%) and for nests laid in April, May, June, August, September, and October.



**Figure 2.4** Spatial distributions of embryonic survival and population growth rates generated by the sublethal model for the period 1980-2000, changes by 2080-2100, and differences between these projections and those generated by the lethal model. Negative model differences indicate the degree to which predictions are reduced by incorporating effects of moderate warming. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Results are shown at three scenarios of nesting behavior: (a-c, j-l) 6cm depth and 50% shade typical of *S. undulatus* (Angilletta *et al.* 2009; this manuscript), (d-f, m-o) nest sites with 50% more shade, and (g-i, p-r) nests dug 6cm deeper. Survival results are based on simulations for nests laid in July. See Fig. B15-B42 for survival plots at all other combinations of nest depth (3, 6, 9, or 12cm) and shade (0, 25, 50, 75, or 100%) and for nests laid in April, May, June, August, September, and October. Also, see Fig. B43-B46 for population growth plots based on all other combinations of nest depth and shade.



**Figure 2.5** Spatial distributions of predicted embryonic survival generated by the sublethal model for the period 1980-2000, predicted changes by 2080-2100, and differences between these projections and those generated by the lethal model. Negative model differences indicate the degree to which predictions are reduced by incorporating effects of moderate warming. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Results are shown across months in the breeding season to illustrate differences based on the timing of oviposition. These results are based on simulations for nests laid at 9cm depth and 50% shade. See Fig. B15-B42 for survival plots based on all other combinations of nest depth (3, 6, 9, or 12cm) and shade (0, 25, 50, 75, or 100%) and for nests laid in April, September, and October.

### Discussion

We have demonstrated that organisms with thermally sensitive life stages do not have to experience lethal temperatures to undergo negative changes at the individual and population levels. Explicitly testing the effects of increasing  $T_{\max}$  showed decreased embryonic survival under recurrent sublethal warming. The effects of warming extended through later life stages via reduced body condition and slowed growth. By integrating

survivorship results into a SDM, we show that consideration of moderate warming during vulnerable life stages alters predicted impacts of climate change. Shifts in distributions result from both lethal conditions (Jones *et al.* 2010; Wetthey *et al.* 2011; Levy *et al.* 2015) and chronic exposure to sublethal fluctuations (Fly & Hilbish 2013; Woodin *et al.* 2013; Maynard *et al.* 2015). Numerous studies demonstrate that changing mean incubation temperatures affect phenotypes of oviparous ectotherms (e.g., reviews in Deeming & Ferguson 1991a; Booth 2006; Bowden *et al.* 2014), and variance of incubation temperatures affects traits across ontogeny as strongly or more than increasing means (e.g., Shine & Harlow 1996; Paaijmans *et al.* 2013). In the *Sceloporus* system, warming of constant and fluctuating incubation regimes can speed development without impacting hatchling sizes (review in Angilletta *et al.* 2004b). However, studies using fluctuating temperatures did not reach stressful highs (except Levy *et al.* 2015, but see below). In this study, survival decreased as the mean and variance of embryonic temperatures increased beyond that experienced in contemporary nests. We cannot partition the effects of temperature means and variances in our experiments. Yet, biological impacts of climate warming likely result from interactions between thermal means and variances, which are presumably not independent of one another in natural microclimates (Shine & Harlow 1996; Paaijmans *et al.* 2013; Bozinovic *et al.* 2015). By utilizing naturalistic thermal regimes, we demonstrate how impacts of warming on sensitive periods of ontogeny can affect ecological predictions.

Our SDM indicates that moderate warming during incubation can lead to reduced population growth compared to model predictions that do not incorporate sublethal

fluctuations. Interestingly, the differences in laboratory survivorship that altered model predictions stemmed primarily from mortality in the first weeks post-oviposition. Running the survival analysis for the first 25% of the incubation period showed lower survival probability under the +7.0°C treatment before any mortality events in the other treatments. Levy *et al.* (2015) suggested similar levels of warming had no effect on *S. undulatus* embryo survival, but they did not begin treatments until halfway through incubation. Our results suggest increased sensitivity to thermal stress in the earliest stages post-oviposition, during which incidences of developmental abnormalities increase as incubation temperatures near the lethal limits for reptiles and other ectotherms (reviews in Deeming & Ferguson 1991b; Farmer 2000). Therefore, *in situ* examinations of plasticity in nesting behavior could be critical to predicting the susceptibility of many ectotherms to climate change.

Plasticity of maternal behavior could buffer embryos from negative effects of climate change (Telemeco *et al.* 2009; Levy *et al.* 2015). However, the benefit of compensatory nesting behavior diminishes when accounting for effects of sublethal warming. Our model examines scenarios of altered nesting behavior by simulating oviposition across ranges of nest depths, shades, and days of the year beyond that exhibited among contemporary *S. undulatus* populations (Tinkle & Ballinger 1972; Niewiarowski 1994; Angilletta *et al.* 2009; this manuscript). Per the sublethal model, embryonic survival will decrease across much of the species range regardless of phenology (Fig. 2.5; though see Levy *et al.* 2016b). Nests with lower temperature variance could reduce negative impacts of warming by avoiding lethal extremes, but the impacts of sublethal warming may

constrain that mitigation. For instance, if females nest 3cm deeper than contemporary averages, the sublethal model predicts a 17.4% lower increase in embryonic survival at the end of this century than the 179.2% benefit predicted by the lethal model. Repeated exposure to sublethal highs can be more detrimental to fitness than acute exposure to extreme temperatures for some species (Kearney *et al.* 2012; Marshall & Sinclair 2015). Thus, the effects of sublethal warming drive responses to warming through impacts on development and stage-specific mortality.

We demonstrate that warming during incubation could have significant impacts on demography via stage-specific survival and growth. Recurrent sublethal warming decreased embryo survival. Additionally, it led to smaller hatchlings and slowed juvenile growth, which could decrease survival to maturity via increased predation risk and reduced foraging success (Sinervo 1993; Stearns 2000; Sears & Angilletta 2004). One could argue that a longer growing season under warming mean temperatures could compensate for slowed juvenile growth. However, increased temperature variance would likely counteract such benefits via constrained activity time and more frequent potential for heat stress (Kingsolver *et al.* 2013; Levy *et al.* 2016b). Additionally, epigenetic effects could compensate for negative impacts of incubation conditions, such that exposure to warming during early ontogeny increases survival and performance of later stages. Though that is beyond the scope of this study, we incorporated predictions of embryonic survival and time to maturity into life tables to examine how slowed juvenile growth could negatively impact population persistence. Though assumptions in our model preclude life-history variation across geography, our life tables include such differences and highlight potentially severe

downstream consequences of recurrent sublethal warming during incubation; results indicate particularly strong effects in northern populations that already exhibit delayed maturity compared to southern populations (Tinkle & Ballinger 1972, Niewiarowski 1994). Future integration of geographic variation of life-history traits will further improve model predictions.

According to life-history theory, faster growth should occur in environments where juveniles experience low survivorship (Stearns 2000), and *S. undulatus* juveniles grow more quickly and experience higher mortality at more southern latitudes (Angilletta *et al.* 2004a; Sears & Angilletta 2004). Our novel nest temperature data demonstrate a counterintuitive pattern wherein southern embryos experience cooler temperatures than their northern conspecifics (Angilletta *et al.* 2009). Considering our results, one could hypothesize that variation in nest characteristics may be a mechanism underlying geographic variation in life-history traits in this species. Further research, such as reciprocal transplants of *S. undulatus* embryos across latitudes, could address hypotheses concerning plasticity of life-history traits (e.g., Stearns & Koella 1986) and elucidate impacts of nesting behavior and embryo thermal physiology on such variation. Accordingly, our work demonstrates the need for increased focus on ontogenetic and spatiotemporal variation of organismal responses to environmental fluctuations.

Our results should motivate researchers to expand efforts to examine life-cycle responses to local climates. If moderate warming during development can impede recruitment and decrease mean fitness, species in locations with lower thermal variance and relatively low frequencies of extreme events may suffer more than previously thought



under climate warming. Unfortunately, data on responses to sublethal extremes are not sufficiently available to inform models beyond a few well-studied systems, such as corals (e.g., Edmunds 2005; Maynard *et al.* 2015), intertidal mussels (e.g., Miller *et al.* 2009; Fly & Hilbish 2013), and some insect species (e.g., Crozier & Dwyer 2006, Potter *et al.* 2011; Marshall & Sinclair 2015). The enduring impacts of sublethal environmental fluctuations is a largely unaddressed problem in ecological modeling. Future studies should examine responses to spatiotemporal variation in developmental conditions to further elucidate adaptive processes by which organisms handle environmental fluctuations.

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### Author Contributions

MAC and MWS designed the lab and field studies with consultation from EAR. OL designed the species distribution model. MAC collected data and analyzed model output. MAC wrote the first draft, and all authors contributed to revisions.

### Data Accessibility

Data supporting the results in this paper are archived at Dryad (doi:10.5061/dryad.pr1h0).

### Supporting Information

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library ([www.ecologyletters.com](http://www.ecologyletters.com)).

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## CHAPTER THREE

### GEOGRAPHIC VARIATION IN NESTING BEHAVIOR AND THERMAL PHYSIOLOGY ACROSS THE *SCELOPORUS UNDULATUS* RANGE: IMPLICATIONS FOR ADAPTIVE RESPONSES TO CLIMATE CHANGE

#### Abstract

The capacity for populations within a species to evolve in pace with rapid climate may vary due to local adaptation. However, we lack the understanding to fully explain the impacts of genetic and environmental influence on phenotypic variation for most species. Here, we using a series of field observations and a laboratory-based reciprocal transplant to address the potential for evolutionary responses of populations to keep pace with climate change. We examined how geographic variation in maternal behavior and thermal physiology underlie patterns of growth and development across the range of a widespread North American lizard. Field observations revealed a pattern of nesting behavior that creates countergradient variation in nest temperatures across latitudes. Using a space-for-time substitution, reciprocal transplants showed that the thermal sensitivity of embryonic development and post-hatching phenotypes vary across populations due to local adaptation. By demonstrating the extents to which genetic background and environmental conditions affect thermal biology across geography, this study serves to increase understanding of the capacities for populations to persist under climate warming.



## Introduction

Persistence under climate warming may rely heavily on the capacity for adaptation to mitigate negative impacts of future climates (Parmesan 2006; Merilä & Hendry 2014a; Urban *et al.* 2016). In the absence of natural or human-assisted dispersal to track favorable habitats, evolutionary change is likely key to the survival of many species (Hoffmann & Sgrò 2011). Recent studies have demonstrated rapid evolution in some species (e.g., Huey *et al.* 2000; Franks *et al.* 2007; Charmantier *et al.* 2008; Hendry *et al.* 2008; Whitney & Gabler 2008), suggesting the potential for adaptation under climate change. However, species are ordinarily made up of populations with different genetic backgrounds due to local adaptation (Pelini *et al.* 2010; Urban *et al.* 2016). Questions remain as to what extent environmental tolerances vary across species ranges (Violle *et al.* 2012; Buckley *et al.* 2015) and whether locally adapted populations can evolve to match forecasted rates of climate change (Jump & Penuelas 2005; Merilä & Hendry 2014b).

Spatial variation in vulnerability to climate warming depends on the relative impacts of local adaptation and environmental conditions on thermally sensitive traits across species ranges (Etterson & Shaw 2001; Hoffmann & Sgrò 2011; Clusella-Trullas & Chown 2013). Despite the prevalence of phenotypic variation across species ranges, we lack the understanding to explain why certain patterns occur along environmental gradients (Sears 2005; Urban *et al.* 2016). Phenotypes are the consequences of both genetic background and environmental conditions (Conover & Schultz 1995; Metcalfe & Monaghan 2001). Given the variations in thermal conditions encountered across large ranges, many species have managed to spread over wide geographic areas due to

adaptations in behavioral, physiological, and life-history phenotypes (Conover & Schultz 1995; Qualls & Shine 1998; Angilletta *et al.* 2004b; Urban *et al.* 2014). For species with complex life cycles, such adaptations may be necessary within each life stage for populations to persist across environmental gradients (Angilletta *et al.* 2004b; Kingsolver *et al.* 2011). However, studies of thermally-sensitive traits have mainly examined responses within single life stages (e.g., focus on adult life stages without considering early ontogeny, Lindström 1999; De Block & Stoks 2005) and have largely focused on responses to the most basic environmental manipulations (e.g., constant instead of naturalistic thermal regimes, Niehaus *et al.* 2012; Bowden *et al.* 2014). Even among studies that have examined responses to naturalistic regimes through multiple stages of ontogeny, few have done so across populations (e.g., Qualls & Shine 1998; Etterson & Shaw 2001; Niewiarowski & Angilletta 2008). This gap in information limits our ability to elucidate the full effects of environmental variation and differing genetic backgrounds on phenotype for most organisms (Urban *et al.* 2016).

Mechanistic understanding of thermally-sensitive phenotypes across species ranges is particularly lacking for organisms with complex life cycles because different life stages often exhibit distinct responses to environmental change due to differences in physiological tolerances, habitat requirements, and resource allocation strategies (McConnaughay & Coleman 1999; Crozier *et al.* 2008; Kingsolver *et al.* 2011). Mobile life stages may exhibit behavioral and physiological adaptations to environmental heterogeneity, which enable persistence across wide ranges. For instance, juveniles and adults of many ectotherms can alter behavior to regulate body temperatures (Huey & Slatkin 1976; Adolph & Porter 1993;

Kearney *et al.* 2009). However, immobile life stages are limited to microclimatic conditions over the small spatial extents that they experience due to constrained behavioral capacities (e.g., an embryo within an egg; Refsnider & Janzen 2010; Telemeco *et al.* 2016). Hence, organismal responses to changing environmental conditions can depend heavily on the physiology of sessile life stages as well as the behavioral and physiology capacities of mobile stages. For example, when an oviparous female chooses a nest, she determines the conditions experienced by developing embryos (Angilletta *et al.* 2009; Li *et al.* 2017; Fialho *et al.* 2018); her embryos are exposed to environmental fluctuations in the nest, particularly under a lack of parental care. Thus, to resolve the impacts of environmental variation on phenotypes across geography, we must consider how biological responses to thermal conditions vary through ontogeny and effects of responses that may carry across life stages (De Block & Stoks 2005; Ådahl *et al.* 2006; Harrison *et al.* 2011; Urban *et al.* 2016).

Our understanding of the underlying causes of phenotypic patterns over environmental gradients will improve through examination of the differences in ontogenetic variation of responses to environmental change across locally adapted populations. In ectotherms, recent work has done much to increase understanding of how physiological responses to the thermal environment vary through stages of ontogeny (e.g., Shine & Harlow 1996; De Block & Stoks 2005; Gilbert & Lattanzio 2016; Carlo *et al.* 2018; Lockwood *et al.* 2018) and across geography (e.g., Niewiarowski & Roosenburg 1993; Oufiero & Angilletta 2006; Niewiarowski & Angilletta 2008; Du *et al.* 2010; 2012; MacLean *et al.* 2016; Lockwood *et al.* 2018). Decades of research in the Eastern fence

lizard (*Sceloporus undulatus*) system demonstrates life-history patterns consistent with the temperature-size rule (Angilletta *et al.* 2004a, b; Oufiero & Angilletta 2006; Niewiarowski & Angilletta 2008). In southern populations mortality is higher and individuals mature younger at smaller sizes and produce fewer offspring, whereas northern populations experience lower mortality, and individuals delay reproduction to mature at larger sizes, lay larger eggs, and produce more offspring (Tinkle & Ballinger 1972; Niewiarowski 1992; Niewiarowski *et al.* 2004; Angilletta *et al.* 2004a, b; Table 3.1). Additionally, *S. undulatus* exhibit local adaptations in embryonic development and juvenile growth along latitudinal clines (Niewiarowski & Roosenburg 1993; Angilletta *et al.* 2004b; Oufiero & Angilletta 2006; Du *et al.* 2010; 2012; Table 3.1). Yet, even in this well-studied system, there is sparse data on thermal regimes in early ontogeny. Consistent with our criticisms above, most studies either fail to utilize naturalistic temperatures, focus on a single part of the life cycle, or are limited to studying individuals from one location. To improve understanding of the potential for adaptive responses to changing climates, more studies are required that utilize naturalistic thermal regimes experienced at different life stages and that examine variation (or similarities) of responses to thermal conditions among populations.

Here, we conducted a series of field observations and a laboratory-based reciprocal transplant experiment to address the potential for evolutionary responses of populations to keep pace with climate change (using a space-for-time substitution). We examined how geographic variation in maternal behavior and thermal physiology may underlie patterns of growth and development across the *S. undulatus* range, which extends across the eastern United States (Niewiarowski *et al.* 2004; Leaché 2009). Additionally, we collected novel

data on latitudinal differences in embryonic temperatures experienced in *S. undulatus* nests. Environmental data at spatial scales experienced by individuals in different stages of ontogeny is limited at best for most species, and *S. undulatus* is no exception. Field body temperatures of juvenile and adult *S. undulatus* have been well studied (e.g., Crowley 1985; Niewiarowski 1992; Andrews 1998; Angilletta 2001; Angilletta *et al.* 2002). Yet, until now, maternal nesting behavior and nest thermal regimes had only been recorded in the field within one population at the northern edge of the species range in New Jersey (Angilletta *et al.* 2009; though temperatures in one nest were recorded in Virginia by Andrews *et al.* 2000). Using laboratory-based reciprocal transplants of embryos and juveniles, we then examined how individuals with different genetic backgrounds respond to natural variation in nest thermal regimes across a latitudinal cline. We expected individuals from northern latitudes to hatch more quickly and to be less susceptible to the negative effects of higher mean and variance of nest temperatures (smaller size at hatching, slowed juvenile growth; Carlo *et al.* 2018) than southern individuals due to local adaptation. By illuminating the extents to which genetic and environmental influences underlie geographic variation in *S. undulatus* growth and development, this study serves to increase understanding of the potential for populations within a species to persist in a rapidly warming world.

## Methods

### Maternal behavior and nest thermal regimes

To examine the potential for geographic variation in nesting behavior and its impacts on embryonic thermal environments, we compared field observations between southern and northern *S. undulatus* populations. We monitored females via radio telemetry during the summer breeding season in Edgefield County, South Carolina, USA (33.915°N, -82.121°W), which is in the southern half of the species range. We then compared our SC data to a far northern population in New Jersey, USA (Angilletta *et al.* 2009; Fig. 3.1a). The habitat in SC was similar to that of Angilletta *et al.* (2009): a heterogeneous forest consisting of open patches of pine with a sparse understory and denser patches of mixed tree species with a thick understory, and a sand and gravel access road bisecting the site. We captured gravid females from late April through mid-June and attached miniature radio transmitters (Model BD-2X, Holohil Systems Ltd., Carp, Ontario, Canada; Model SOPR-2011, Wildlife Materials International, Inc., Murphysboro, Illinois, USA) to the dorsum using surgical adhesive. Each transmitter weighed <5% of a female's body mass and had a nominal battery life of 24 days, enabling us to track movements before, during, and after nesting. We monitored 10 females in 2015 and 15 females in 2016 (3 of which were recaptures from 2015). In 2016, by replacing transmitters after three weeks, we monitored 5 females through their first two clutches.

Our tracking protocols were designed to ensure thorough observations of nesting behaviors. We used a handheld receiver (TRX-1000S; Wildlife Materials Inc., Murphysboro, Illinois, USA) to locate females once every 2-3 hours and a handheld GPS

(Samsung Galaxy S4 running the application *GPS Essentials*) to record locations to the nearest  $1 \times 10^{-6}$  decimal degree. Contrary to the consistent nighttime nesting observed in NJ (Angilletta *et al.* 2009), females in this population nest during daytime and nighttime (Carlo, pers. obs.). Without an observer witnessing oviposition, a specific female's nest cannot be located. Therefore, gravid females were tracked with increasing frequency as their eggs grew larger to increase the likelihood of locating nest sites. If a female was still active after sunset, tracking continued until that individual either nested or ceased activity for at least two hours. Most females were tracked for at least 1 week before and after nesting (2 lost to predation within 5 days of nesting; 2 dropped trackers within 3 days of nesting; 2 killed by invasive fire ants (*Solenopsis invicta*) while attempting to nest in ant mounds; 2 killed by *S. invicta* within 3 days of nesting).

During incubation, we recorded canopy cover and hourly soil temperatures at nest sites (2015, n=10; 2016, n=20) and at randomly selected sites throughout the habitat (2015, n=60, 2016, n=50). We recorded hourly temperatures (°C) using miniature loggers (DS1922L; Maxim Integrated, San Jose, California, USA) placed at mean nest depth for nest sites and at random depths from 0.1 to 15.9 cm for random sites. We recorded canopy cover (% shade) as the average of four measurements using a spherical densiometer (Model A; Forestry Suppliers, Inc., Jackson, Mississippi, USA) facing north, south, east, and west. To compare environmental conditions at nest sites versus random sites, we performed randomization tests following the procedure outlined by Angilletta *et al.* (2009).

To compare maternal nesting behavior within and among SC females and to compare behavior between females in SC and NJ, we estimated nesting ranges and home

ranges for each female. Nesting ranges were calculated using coordinates during each nesting period, which we delineated beginning with stereotypical patterns of movement in the days prior to nesting and ending on the day after nesting (Angilletta *et al.* 2009). Home ranges were calculated using tracking coordinates collected outside of nesting periods. To calculate nesting and home ranges (area in hectares), we estimated utilization distributions using the *adehabitatHR* package in R (Calenge 2006) to determine 95% fixed kernel ranges with unit variance standardization and least squares cross-validation (Worton 1989; Seaman *et al.* 1996). We then used QGIS v. 2.18.16 (QGIS Development Team n.d.) to calculate the percent of each nesting range that overlapped with a female's home range and to calculate the distance (m) from each female's nest site(s) to the center of her home range. Similar to Angilletta *et al.* (2009), we reasoned that spatial and environmental differences between nesting and home ranges would indicate ways in which *S. undulatus* use distinct (or similar) microhabitats for nesting versus other activities.

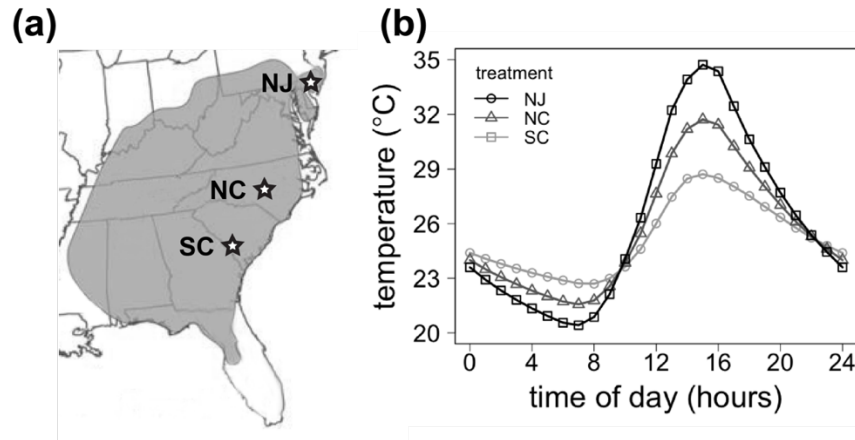
#### Laboratory-based reciprocal transplants

After observing differences in nesting behavior and nest thermal regimes across latitudes, we conducted a laboratory-based reciprocal transplant experiment in 2017 to examine geographic variation in phenotypic responses to nest temperatures. We collected gravid *S. undulatus* females across a latitudinal gradient (Fig. 3.1a): n=20 from Edgefield County, SC, USA (33.915°N, -82.121°W); n=21 from Montgomery County, North Carolina, USA (35.998°N, -79.978°W, n=12; 35.362°N, -79.862°W, n=9); and n=19 from Burlington and Atlantic Counties in NJ, USA (39.741°N, -74.724°W, n=8; 39.899°N, -



74.586°W, n=11). Females were transported to Clemson University, where they were allowed to lay eggs without inducing. For details on husbandry, see Carlo *et al.* (2018).

We collected eggs immediately post-oviposition and reared them under treatments designed to simulate nest thermal regimes at the collection sites in SC, NC, and NJ (Fig. 3.1b). To control for maternal effects, eggs (SC eggs n=155; NC n=187; NJ n=126) from each clutch were placed in individual containers (59mL) with a 1:100 water-to-silica-sand mixture (Angilletta *et al.* 2000), then distributed evenly and randomly among the treatments. Environmental chambers (I-36VL; Percival Scientific, Perry, Indiana, USA) maintained eggs at temperatures per treatment designs and, following the approach described by Riddell *et al.* (2017), varied relative humidity to maintain constant vapor pressure deficits. We based the NJ regime on Angilletta *et al.* (2009) and the SC regime on daily nest temperatures collected in our field work. Since no data exists on NC nest temperatures, we estimated nest thermal regimes at that mid-latitude location based on a set of hourly microclimates covering the USA at 36x36-km resolution (Levy *et al.* 2016), assuming *S. undulatus* females at the NC location construct nests at depths consistent with observations across the species range and at shade levels intermediate to observed SC and NJ nesting behaviors (Angilletta *et al.* 2009; Carlo *et al.* 2018 this study).



**Figure 3.1** The range of the Eastern fence lizard (*Sceloporus undulatus*) and average daily nest temperatures at three locations. The map in [a] is modified from (Leaché 2009) and shows the three locations where gravid females were captured and nest temperatures were either recorded (South Carolina, this study; New Jersey, Angilletta *et al.* 2009) or estimated from a set of hourly microclimates (Levy *et al.* 2016). The plot in [b] shows the treatments based on those nest temperatures that were used for laboratory-based reciprocal transplants in this study.

We reared embryos under assigned treatments (SC treatment  $n=160$ , NC  $n=151$ , NJ  $n=157$ ) for the duration of incubation and monitored embryonic survival daily by checking for heart rates each morning using an infrared sensor (Buddy Egg Monitor; Avitronics, Cornwall, UK). If no heart rate was detected for three consecutive days, we marked the embryo as deceased on the first day. We recorded incubation time as the number of days from oviposition to hatching. To examine effects of nest thermal regimes on post-hatching phenotypes, we transferred hatchlings to environmental chambers programmed to simulate day lengths at the sites of their assigned treatments using preferred daytime (33°C) and approximate nighttime (24°C) temperatures (Niewiarowski 1992; Angilletta 2001). Upon hatching, we recorded snout-vent length (SVL) to 0.1 mm and mass to 0.1 mg, then estimated relative hatchling body conditions using scaled mass indices (SMI) calculated from standard regressions of mass-to-SVL (Peig & Green 2009; 2010), following the

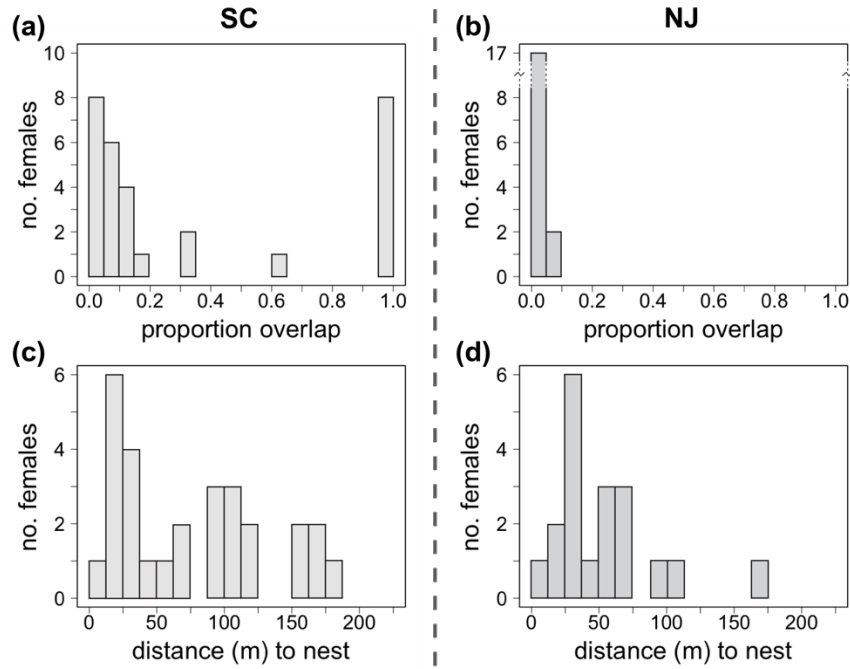
approach described in Carlo *et al.* (2018). We repeated size measurements every 4 days for 32 days post-hatching. We then estimated characteristic growth rates ( $r$ ) for the interval form of von Bertalanffy growth models using the approach described by Dunham (1978) and Schoener & Schoener (1978). We used SVL instead of mass to minimize potential variation in size measurements due to nutritional state (Dunham 1978; Sears 2005), and we varied the free parameter of asymptotic size according to maternal origins using adult SVLs reported across the species range (Niewiarowski *et al.* 2004). Growth models were fitted using Levenberg-Marquardt nonlinear least-squares regression from the *minpack.lm* library in R (Elzhov *et al.* 2015).

We conducted statistical analyses in R v3.4.2 (R Core Team 2017). To test for effects of source population and laboratory treatments on embryonic and juvenile survival, we constructed Cox proportional hazard models from the *survival* library (Therneau 2014) with maternal identity as an estimator of variance to control for correlation of responses among siblings. To test for effects of laboratory treatments on incubation times, hatchling SVL, hatchling mass, hatchling SMI, and  $r$  within and among source populations, we constructed linear mixed effects (LME) models using the *lme* function (Pinheiro *et al.* 2016). We included treatment and source population as interacting categorical variables with maternal and individual identities as nested random effects. We added initial egg mass as a continuous variable for hatchling SVL, mass, SMI, and  $r$ . We also added hatchling SMI as a continuous variable for  $r$ . We then calculated effect sizes ( $\omega^2$ ) to determine the proportion of explained variance of each LME parameter included in an ANOVA (Olejnik & Algina 2003).

## Results

### Maternal behavior and nest thermal regimes

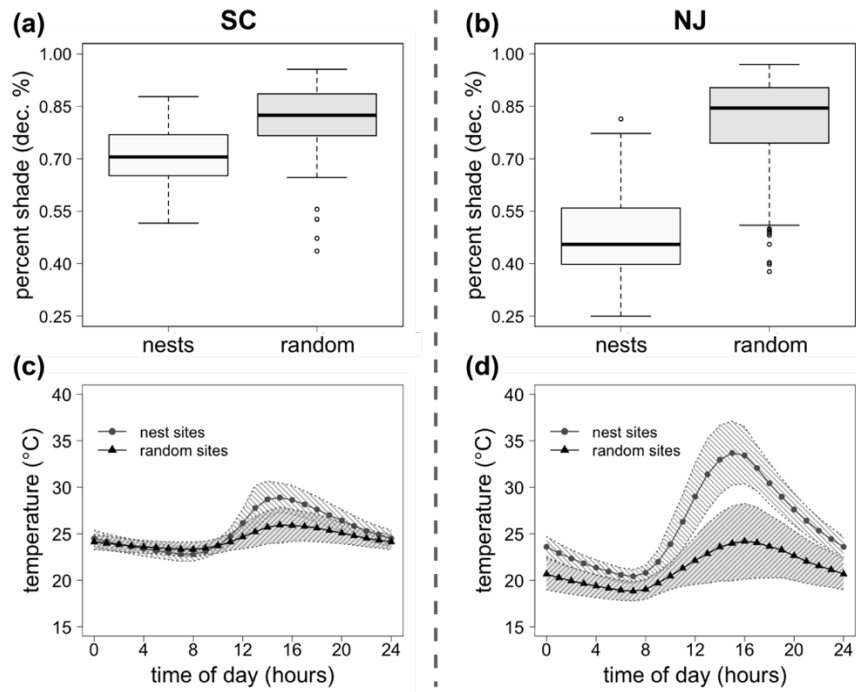
We observed variations in *S. undulatus* habitat use and nesting behavior both within and among individuals in SC that were markedly different than the consistent pattern observed by Angilletta *et al.* (2009). In NJ, females consistently exhibited a shift in microhabitat use during nesting, traveling outside their home ranges to nest at night in a common area of the habitat (distance from center of home range to nest:  $n = 19$ , mean =  $53.98 \pm 37.98$  m ( $\pm$  SD), range = 12.98-167.74 m; Fig. 3.2d). However, in SC, females nested during the daytime and nighttime, and they exhibited individual variation in nesting behavior; 8 females laid 12 nests within their home ranges, and another 18 nests were laid by 15 lizards that traveled far outside their home ranges ( $n = 30$ , mean =  $76.32 \pm 72.97$  m, range = 0.0-210.3 m; Fig. 3.2c). On average, 35.1% ( $n = 30$ , SD: 41.7%, range: 0-100%; Fig. 3.2a) of the area used by SC females during nesting overlapped with their home ranges, compared to merely 1.1% average ( $n = 30$ , SD: 2.0%, range: 0-6.6%) overlap in NJ (Fig. 3.2b). Estimated home range sizes did not differ between SC ( $n = 23$ , mean =  $0.169 \pm 0.080$  ha, range = 0.022-0.334 ha) and NJ ( $n = 19$ , mean =  $0.169 \pm 0.174$  ha, range = 0.015-0.627 ha). In NJ, all females converged on the same narrow area of the habitat to nest, an abandoned railway that bisected the site, which was adjacent to most of their home ranges. In contrast, SC females did not converge on a common area of the habitat; nests both within and outside of home ranges were spread throughout the forest.



**Figure 3.2** Frequency distributions of distances that *S. undulatus* females traveled to nest sites and the proportions of overlap between nesting and home ranges in SC (this study) and NJ (Angilletta *et al.* 2009). The proportions of females' nesting ranges that overlapped with their home ranges were greater on average and more highly varied among [a] SC lizards than among [b] NJ lizards. Additionally, we observed greater variation in the distances (m) that females traveled from the centers of their home ranges to their nest sites among [c] SC lizards than among [d] NJ lizards.

The differences in nesting behaviors between northern and southern populations led to distinct nest thermal regimes. There was no difference between nest depths in SC and NJ, resulting in similar mean nest temps (SC: 25.34°C, NJ: 25.85°C). However, the shade levels at nest sites differed between SC and NJ (Fig. 3.3a-b), leading to significantly higher daily temperature variance in the sunnier NJ nests (mean = 25.85±4.47°C,  $T_{\min}$  = 20.42±0.74,  $T_{\max}$  = 33.68±3.42) than in the shadier SC nests (mean = 25.34±2.36°C,  $T_{\min}$  = 22.81±0.75°C,  $T_{\max}$  = 28.90±1.55°). Additionally, comparisons of nest thermal regimes to randomly selected locations differed across populations (Fig. 3.3c-d). In NJ, all females nested in an area with some of the most open sites available (nests: n = 21, 49.28±13.81%

shade; random:  $n = 100$ ,  $79.03 \pm 15.30\%$ ), resulting in warmer and more variable nest temperatures than if they had nested randomly throughout the habitat (nests:  $25.85 \pm 4.47^\circ\text{C}$ ; random:  $21.32 \pm 1.80^\circ\text{C}$ ). Conversely, in SC, the differences between shade levels at nest sites versus randomly selected sites were much less pronounced (nests:  $n = 30$ ,  $70.76 \pm 9.07\%$  shade; random:  $n = 110$ ,  $80.60 \pm 11.57\%$ ), leading to mean nest temperatures closer to random (nests:  $25.28 \pm 3.31^\circ\text{C}$ ; random:  $24.16 \pm 1.90^\circ\text{C}$ ). On average, SC nests were only slightly warmer than random sites for 12 hours a day (1100-2300 hours,  $p < 0.001$ ), whereas NJ nests were warmer than random sites at all hours (Angilletta *et al.* 2009). The distinct nest thermal regimes found in SC and NJ populations reveal a counterintuitive pattern in which embryos from warmer southern latitudes experience cooler nest temperatures than their northern conspecifics. Though there was no difference in mean nest temperatures, the higher variance of nest temperatures in northern nests led to embryos experiencing warming incubation regimes than southern embryos. Using degree-day calculations (Zalom *et al.* 1983) to compare temperatures experienced by embryos due to differences in thermal variances, an average SC nest accrues 0.742 degree-days per day above the mean nest temperature compared to 2.318 degree-days per day in an average NJ nest. The differences in thermal variance within nests across latitudes should be considered in future analyses because the biological impacts of warming likely result from interactions between thermal means and variances, which are presumably not independent in natural microclimates (Shine & Harlow 1996; Paaijmans *et al.* 2013; Bozinovic *et al.* 2015).



**Figure 3.3** Distributions of vegetative cover (percent shade) and hourly soil temperatures (°C) at nest sites and randomly selected sites within the habitats in SC (this study) and NJ (Angilletta *et al.* 2009). Nest sites in [a] SC were shadier than those in [b] NJ, which were significantly more open than random sites. As a result, nests in [c] SC experienced lower mean and variance of daily nest temperatures than [d] NJ nests. Additionally, SC nest temperatures only warmed higher than random sites in the second half of the day, while NJ nests were warmer than random sites at all hours. Lines and shaded regions in the soil temperatures plots show mean  $\pm$  SD.

In addition to the comparative behavioral data between northern and southern populations, we tracked 5 females through sequential clutches in 2016 and 3 females during sequential breeding seasons in SC. Of the 5 lizards tracked through clutches within a season, 4 nested within their home ranges for their first clutch and then traveled beyond their home ranges for their second clutch (mean distance: 118.6 m, range: 89.9-181.7 m). The remaining lizard nested outside of her home range for both clutches, still traveling much further for her second clutch (73.9 and 150.9 m, respectively). None of the females

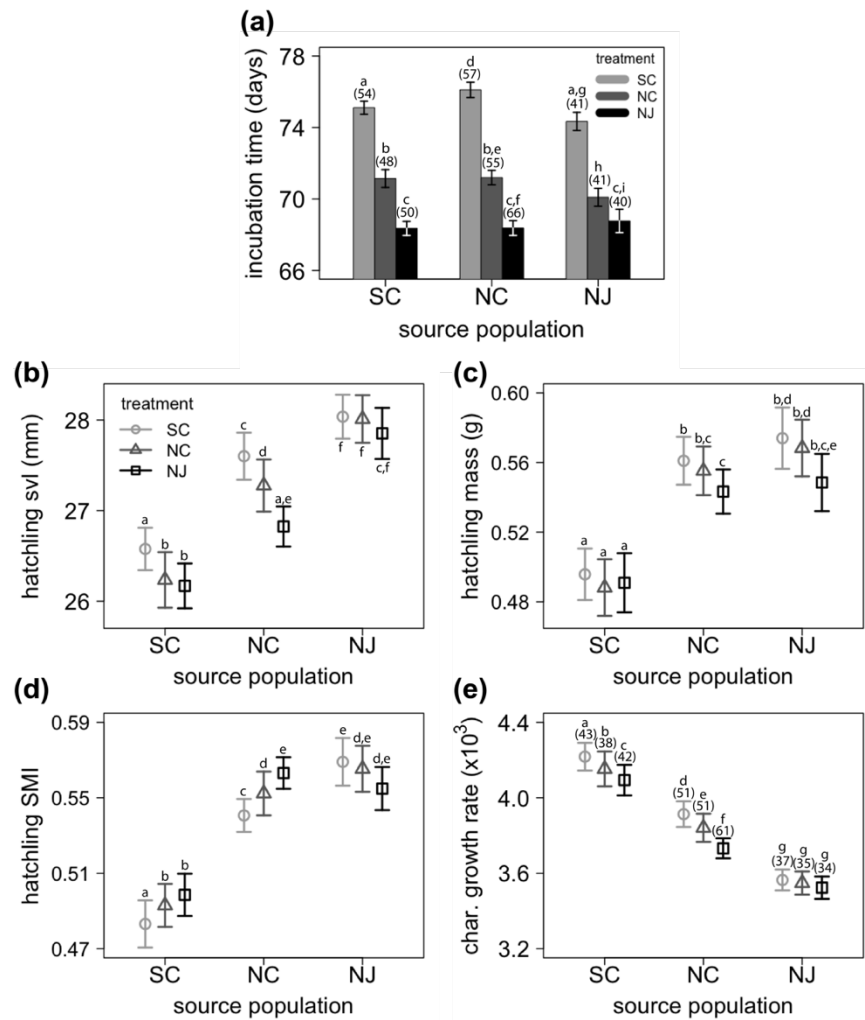
tracked through multiple clutches revisited the same nest site within a season; however, the 2 that we recaptured for their first clutches in 2015 and 2016 returned to the exact same nest sites (73.9 and 210.3 m outside their home ranges), indicating that nest site fidelity may contribute to inter-annual patterns of nesting behavior. The other recaptured lizard was observed for her first clutch in 2015 and her second clutch in 2016, exhibiting the same pattern of nesting behavior as most of the lizards monitored for sequential clutches within a season (within home range for first clutch, outside home range for second clutch).

#### Laboratory-based reciprocal transplants

The reciprocal transplants affected incubation times and post-hatching phenotypes in ways that differed according to source population, and our results were consistent with previously documented geographic variation across latitudinal clines (Table 3.1). There was no effect of the treatments on survival among embryos from any of the source populations ( $p = 0.632$ ). Lizards from all source populations hatched earlier under warmer treatments that simulated more northern nest conditions (Fig. 3.4a; Table 3.2). Hatchlings from NJ emerged earlier than southern hatchlings under the SC ( $p < 0.001$ ) and NC ( $p = 0.002$ ) treatments, but there were no differences according to source population between incubation times under the NJ treatment ( $p = .429$ ). Overall, hatchlings from SC and NC emerged smaller and grew more slowly under more northern treatments, whereas hatchlings from NJ emerged at equivalent lengths but decreased mass and exhibited no differences in growth across treatments (Fig. 3.4 b-d; Table 3.2). Hatchling SVL decreased under warmer treatments among lizards from SC ( $p = 0.009$ ) and NC ( $p < 0.001$ ), but there



was no difference in SVL of NJ hatchlings among treatments ( $p = 0.353$ ; Fig. 3.4b, Table 3.2). Hatchling mass decreased with warming among NC ( $p = 0.012$ ) and NJ ( $p < 0.001$ ) hatchlings, but body mass did not differ among treatments for hatchlings from SC ( $p = 0.216$ ; Fig. 3.4c, Table 3.2). As a result of the differences in hatchling sizes across source populations and treatments, hatchling SMI increased with warming among SC ( $p = 0.025$ ) and NC ( $p = 0.003$ ) hatchlings, but there was no significant difference among treatments in SMI of NJ hatchlings ( $p=0.183$ ; Fig. 3.4d, Table 3.2). After continuing to rear hatchlings in chambers that simulated day lengths under their assigned treatments, the growth model predicted slower growth of SC ( $p < 0.001$ ) and NC ( $p < 0.001$ ) juveniles reared under more northern thermal conditions, and it predicted no difference in growth among treatments for NJ juveniles ( $p = 0.258$ ; Fig. 3.4e, Table 3.2). Considering the results of our study on maternal nesting behavior and nest thermal regimes across latitudes, the effects of reciprocal transplants on post-hatching phenotypes indicate that lizards from southern latitudes are more sensitive to increases in the mean and variance of developmental temperatures, such as those predicted to occur under climate change (Levy *et al.* 2016), whereas lizards from far northern latitudes may be more resilient to changing thermal conditions.



**Figure 3.4** Impacts of laboratory-based reciprocal transplants on incubation times and post-hatching phenotypes. Among embryos from each source population, [a] incubation times (days from oviposition to hatching) decreased with warming thermal regimes that simulated more northern nest conditions. The impacts of reciprocal transplants differed according to source population for [b] hatchling snout-vent length (SVL; mm), [c] hatchling body mass (g), [d] hatchling scaled-mass index (SMI), and [e] juvenile growth estimated as the free parameter of characteristic growth rate from von Bertalanffy growth models using juvenile SVL. Error bars indicate 95% confidence intervals. Letters denote statistical relationships such that data with different letters are significantly different ( $p < 0.05$ ). Parenthetical values denote sample sizes, which are the same for panels a-d.

**Table 3.1.** Summary of life-history characteristics from *Sceloporus undulatus* populations across the eastern United States. Values are pooled means  $\pm$  SD from the sources listed for each location. Values in parentheses are means  $\pm$  SD from this study, for which the post-hatching data is only from individuals reared under treatments simulating conditions at their locations of origin. Only one other source (Carlo *et al.* 2018), calculated characteristic growth rates of juveniles. So, to compare growth data with other sources, we recalculated juvenile growth rates from this study and from Carlo *et al.* (2018) as mm/day in SVL for the first month post-hatching.

Location	Clutch size	Egg mass (g)	Hatchling SVL (mm)	Hatchling mass (g)	Juvenile growth (mm/day)	Sources
New Jersey	8.4 $\pm$ 1.5 (9.7 $\pm$ 1.8)	.40 $\pm$ .05 (.41 $\pm$ .06)	27.4 $\pm$ 1.4 (27.9 $\pm$ 0.9)	.54 $\pm$ .05 (.55 $\pm$ .05)	.16 $\pm$ .03 (.14 $\pm$ .03)	Niewiarowski & Roosenburg 1993; Niewiarowski 1994; 1995; Angilletta <i>et al.</i> 2000; 2001; Niewiarowski <i>et al.</i> 2004; Oufiero & Angilletta 2006; Niewiarowski & Angilletta 2008
Indiana	7.1 $\pm$ 1.3	.41 $\pm$ .07	25.9 $\pm$ 1.2	.55 $\pm$ .06	.10 $\pm$ .04	Oufiero & Angilletta 2006; Du <i>et al.</i> 2010; 2012
Virginia	8.1 $\pm$ 1.5	.40 $\pm$ .06	24.7 $\pm$ 0.9	.55 $\pm$ .04	.12 $\pm$ .04	Andrews <i>et al.</i> 2000; Warner & Andrews 2002a; 2003; Oufiero & Angilletta 2006; Parker & Andrews 2007; Niewiarowski & Angilletta 2008
North Carolina	(10.9 $\pm$ 1.6)	(.44 $\pm$ .05)	(27.3 $\pm$ 1.1)	(.56 $\pm$ .05)	(.16 $\pm$ .04)	
South Carolina	7.4 $\pm$ 1.6 (9.8 $\pm$ 2.0)	.36 $\pm$ .05 (.38 $\pm$ .04)	25.6 $\pm$ 1.0 (26.6 $\pm$ 0.9)	.52 $\pm$ .05 (.50 $\pm$ .06)	.18 $\pm$ .04 (.19 $\pm$ .04)	Tinkle & Ballinger 1972; Ferguson & Brockman 1980; Niewiarowski 1995; Oufiero & Angilletta 2006; Niewiarowski & Angilletta 2008; Carlo <i>et al.</i> 2018
Mississippi	9.4 $\pm$ 1.4	.36 $\pm$ .07	25.1 $\pm$ 0.8	.51 $\pm$ .05	.18 $\pm$ .07	Parker 1994; Du <i>et al.</i> 2010; 2012
Florida	6.8 $\pm$ 1.2	.32 $\pm$ .09	24.4 $\pm$ 1.0	.49 $\pm$ .06	0.09 $\pm$ .04	Oufiero & Angilletta 2006; Du <i>et al.</i> 2010; 2012

**Table 3.2.** Summary statistics for analyses of data from the laboratory-based reciprocal transplant experiment using mixed effects ANOVA. Data include [a] time (days) from oviposition to hatching, [b] hatchling snout-vent lengths (SVL; mm), [c] hatchling body masses (g), [d] hatchling scaled-mass indices (SMI) calculated as described in Peig and Green (2009, 2010) and Carlo *et al.* (2018), and [e] the free parameter of characteristic growth rate ( $r$ ) derived from von Bertalanffy growth models using juvenile SVL. Analyses included: maternal and individual identities as nested random effects; initial egg mass as a continuous variable for SVL, mass, SMI, and  $r$ ; and SMI as a continuous variable for  $r$ .

Response	Parameter	F	p	$\omega^2$
(a) incubation time	treatment	718.52 <sub>2,441</sub>	< 0.001	0.7505
	source pop.	3.07 <sub>2,441</sub>	0.048	0.0022
	treatment x source pop.	6.70 <sub>4,441</sub>	< 0.001	0.0118
(b) hatchling SVL	treatment	15.24 <sub>2,432</sub>	< 0.001	0.0430
	source pop.	14.92 <sub>2,432</sub>	< 0.001	0.0420
	egg mass	4.27 <sub>1,432</sub>	0.039	0.0049
	treatment x source pop.	2.40 <sub>4,432</sub>	0.049	0.0084
	treatment x egg mass	55.91 <sub>2,432</sub>	< 0.001	0.1657
	source pop. x egg mass	13.01 <sub>2,432</sub>	< 0.001	0.0362
	treatment x source pop. x egg mass	4.39 <sub>4,432</sub>	0.002	0.0204
(c) hatchling mass	treatment	6.76 <sub>2,432</sub>	0.001	0.0110
	source pop.	88.89 <sub>2,432</sub>	< 0.001	0.1676
	egg mass	3.97 <sub>1,432</sub>	0.047	0.0029
	treatment x source pop.	1.33 <sub>4,432</sub>	0.259	0.0012
	treatment x egg mass	138.18 <sub>2,432</sub>	< 0.001	0.2616
	source pop. x egg mass	54.98 <sub>2,432</sub>	< 0.001	0.1030
	treatment x source pop. x egg mass	7.15 <sub>4,432</sub>	< 0.001	0.0234
(d) hatchling SMI	treatment	4.58 <sub>2,432</sub>	0.011	0.0084
	source pop.	162.54 <sub>2,432</sub>	< 0.001	0.3828
	egg mass	0.07 <sub>1,432</sub>	0.799	0.0000
	treatment x source pop.	3.28 <sub>4,432</sub>	0.012	0.0108
	treatment x egg mass	13.97 <sub>2,432</sub>	< 0.001	0.0308
	source pop. x egg mass	14.24 <sub>2,432</sub>	< 0.001	0.0314
	treatment x source pop. x egg mass	1.28 <sub>4,432</sub>	0.276	0.0013
(e) characteristic growth rate ( $r$ )	treatment	22.24 <sub>2,369</sub>	< 0.001	0.0487
	source pop.	9.27 <sub>2,369</sub>	< 0.001	0.0190
	egg mass	6.29 <sub>1,369</sub>	0.013	0.0060
	hatchling SMI	92.05 <sub>1,369</sub>	< 0.001	0.1044
	treatment x source pop.	0.84 <sub>4,369</sub>	0.500	0.0000
	treatment x egg mass	111.57 <sub>2,369</sub>	< 0.001	0.2537
	source pop. x egg mass	38.87 <sub>2,369</sub>	< 0.001	0.0869
	source pop. x hatchling SMI	5.94 <sub>2,369</sub>	0.003	0.0113
	treatment x source pop. x egg mass	6.06 <sub>4,369</sub>	< 0.001	0.0232

$\omega^2$ , effect size (Olejnik & Algina 2003)

## Discussion

We have demonstrated differences in *S. undulatus* nesting behavior and thermal physiology with implications for the persistence of populations under climate warming. Latitudinal differences in maternal behavior produced nest thermal regimes that vary idiosyncratically across latitudes such that embryos at warmer southern latitudes experience cooler daily temperatures than their northern conspecifics. Through laboratory-based reciprocal transplants, we then showed population differences in embryonic development and post-hatching phenotypes in response to changing nest temperatures. Contrary to previous research in this system (Oufiero and Angilletta 2006), *S. undulatus* from the northern locality in NJ did not hatch earlier than those from more southern populations under all treatments, likely because of the lower variance of nest temperatures in our cooler treatments compared to the cooler regime that Oufiero and Angilletta (2006) used to simulate high-elevation nests in Virginia. Ours is the first study to compare nest thermal regimes across the *S. undulatus* range. So, at the time that Oufiero and Angilletta (2006) conducted their research, they understandably analyzed data under the assumption that embryos experience warmer environments with decreasing latitude. Similar to Carlo *et al.* (2018), the effects of warming daytime nest temperatures affected post-hatching phenotypes via smaller hatchling body sizes and reduced juvenile growth among individuals from southern populations. However, embryos from the northern NJ populations did not experience reduced hatchling SVL or juvenile growth as a result of nest temperatures, suggesting that northern *S. undulatus* are adapted to the consistently higher mean and variance of nest temperatures at those latitudes. Though, across incubation

regimes, juvenile growth rates declined with increasing latitude of origin. Consequently, we hypothesize that local adaptation of maternal behavior and embryonic physiology contribute significantly to the phenotypic variation observed across the *S. undulatus* range (at least, in the eastern clade).

Our field studies demonstrate that female *S. undulatus* exhibit different patterns of nesting behavior across latitudes. In NJ, females nest in open areas at forest breaks and margins (Angilletta *et al.* 2009), whereas in SC, females tend to nest in the cooler forest understory. The warmer, sunnier NJ nest sites enable earlier hatching, which allows juveniles to fully develop and grow before brumation; if NJ lizards laid in the forest understory, nests would not likely warm sufficiently to enable hatching or development in the shorter northern growing season (Angilletta *et al.* 2009). Counterintuitively, in the hotter SC environment, average nest  $T_{\max}$  is  $\sim 4.8^{\circ}\text{C}$  cooler than in NJ, and the variance of nest temperatures is significantly lower. Decreased thermal variance in the shadier understory can buffer embryos from high daytime temperature fluctuations that may be lethal thermal stressors (Angilletta *et al.* 2013) or recurrent sublethal stressors (Carlo *et al.* 2018) in sunnier locations. Additionally, females in SC exhibited individual variation in habitat use and nesting behavior. Conversely, NJ females consistently traveled beyond their home ranges to converge on the hottest, most open area of the habitat to nest, leading Angilletta *et al.* (2009) to propose that philopatry may be a strong driver of nesting behavior in that population. We observed females in SC returning to nest sites between seasons, demonstrating the capacity for nest site fidelity; however, they spread clutches across distant sites within a season. It is likely that, as the eastern *S. undulatus* clade diverged and

spread north during the Miocene (~11.29 mya; Leaché & Sites 2009), behavioral plasticity was constrained to enable persistence at the spreading edge of the species range (Ghalambor *et al.* 2007; Chevin *et al.* 2010), which could have led to canalization in the thermal physiology of early life stages as suggested by the results of our laboratory-based reciprocal transplant experiments.

Latitudinal differences in nesting behavior could have contributed to the countergradient variation in growth and development observed across the *S. undulatus* range. Life-history theory holds that such variation across wide species ranges is typically due to (and maintained by) differences in resource quality and abundance or by differences in biotic interactions (Roff 1992; Stearns 1992; Sears & Angilletta 2003). Theory predicts that faster growth should occur in environments where juveniles experience low survivorship and that lizards should delay maturation to achieve larger body sizes, maximizing fecundity and offspring quality (Stearns & Crandall 1981; Stearns 2000; Sears & Angilletta 2004). Indeed, *S. undulatus* from warmer southern latitudes grow more quickly but reach smaller adult sizes than those from northern latitudes (Tinkle & Ballinger 1972; Niewiarowski 1992; Niewiarowski *et al.* 2004; Angilletta *et al.* 2004a, b), and *S. undulatus* juveniles in southern populations do experience lower survivorship than more northern populations (Tinkle & Ballinger 1972; Niewiarowski *et al.* 2004; Angilletta *et al.* 2004b). However, considering our revelations concerning patterns of nesting behavior across latitudes, geographic variation in life-history characteristics was also likely driven by responses to contra-gradient variation in nest thermal regimes. Thus, what was thought to be countergradient variation in growth and development across the species range

(Oufiero & Angilletta 2006; Niewiarowski & Angilletta 2008) is actually co-gradient variation when the nest thermal environment is taken into account. In more northern populations, evolution of thermal physiology would have been necessary to enable rapid growth and development under increased nest temperatures without incurring the negative impacts of warmer incubation regimes experienced by individuals from southern populations (Carlo *et al.* 2018; Fig. 3.4). Such adaptation would likely have contributed to the more rapid embryonic development and the larger hatchling sizes observed among *S. undulatus* from higher latitudes. In more southern populations, less constrained nesting behavior and longer growing seasons likely enabled the cooler nest thermal regimes that serve to buffer embryos from potentially stressful fluctuations in daily nest temperatures, that protect against decreased hatchling sizes or slowed juvenile growth. These hypotheses may hold for the evolutionary history of the species, but the question remains what the future holds for populations under climate change.

As climate warming alters local thermal conditions, geographic variation in maternal behavior may have implications for the persistence of populations across the *S. undulatus* range. In the face of rapid climate change, organisms must migrate, acclimate, or adapt to avoid extinction (Holt 1990; Fuller *et al.* 2010). Migration is improbable for small organisms with short dispersal distances at a pace to match projected rates of warming, especially in a fractured landscape with ever-increasing anthropogenic barriers to long-distance dispersal (Opdam & Wascher 2004; Loarie *et al.* 2009). Rapid evolution necessary under climate change may be hindered within populations by low trait heritabilities, overriding effects of plasticity, and highly fluctuating selection pressures



(Calosi *et al.* 2008; Hoffmann & Sgrò 2011; Buckley *et al.* 2015). Lethal limits of embryonic thermal physiology are conserved among *S. undulatus* populations (Angilletta *et al.* 2013), which suggests low adaptive potential for upper thermal tolerances in developing fence lizards. Therefore, the susceptibility of *S. undulatus* populations to climate warming may rely heavily on the plasticity of maternal behavior. Given that southern females already tend to nest in the forest understory, the potential benefits of altered nesting behavior are likely only available to more northern populations, as long as northern *S. undulatus* have not lost that capacity. Many species of ectotherms exhibit adaptive plasticity in nesting behavior. Female lizards tend to construct nests under thermal and hydric conditions that improve offspring survival and fitness (Warner & Andrews 2002b; Doody *et al.* 2006; Telemeco *et al.* 2009; Li *et al.* 2017). Telemeco *et al.* (2017) failed to induce a plastic response in nesting behavior of *S. tristichus* from Arizona, and Angilletta *et al.* (2009) suggested that *S. undulatus* behavior may be limited by philopatry, indicating that the behavioral plasticity necessary to compensate for climate warming may not exist in some *Sceloporus* populations. However, knowledge of fence lizard nesting behavior is limited, and we did observe individual variation in female behavior within and among southern *S. undulatus* in this study. Additionally, if aspects of nesting behavior are heritable, any existent variation could allow for an evolutionary response. Thus, while southern populations may be under greater threat under climate change, northern *S. undulatus* may be able to persist via adaptive plasticity. Future work is needed to test that possibility.

Our results should encourage further experiments examining geographic patterns of ontogenetic variation in responses to microhabitat conditions. Advances in life-history theory and other aspects of evolutionary biology will follow from more thorough investigations of the relative impacts of genetic background and environmental pressures on biological responses to the environment throughout the life cycle. Such investigations require integration of field and laboratory work that increases the resolutions of natural abiotic regimes and biotic interactions across species ranges as well as the differences in responses to natural conditions at different life stages. Furthermore, incorporation of molecular techniques will begin to reveal the genetic mechanisms underlying differential responses to microhabitat conditions across life stages and populations, leading to improved genome-to-phenome understanding of trait variation across environmental gradients.

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#### Author Contributions

MAC and MWS designed the study. All authors contributed to data collection and analysis. MAC and MWS wrote the first draft, and all authors contributed to revisions.

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## APPENDICES

## Appendix A

### Chapter One: Supplementary methods for lab and field experiments, parameters within the life-cycle model of population dynamics, and sensitivity analyses for model projections

#### *Scaled mass index calculations for hatchling body conditions*

As a measure of hatchling body conditions in the laboratory and field experiments, we calculated scaled mass index (SMI) as proposed by Peig & Green (2009; 2010). We chose SMI due to its proposal as a more reliable measure than other indices that do not account for changing relationships between mass and length at different body sizes and growth stages. Calculations were made using body mass and snout-vent length (SVL) measurement of fresh hatchlings. We calculated

$$SMI = M_i * (L_0 / L_i)^{b_{SMA}} \quad [S1]$$

where  $M_i$  and  $L_i$  = mass and SVL of the  $i^{th}$  hatchling,  $L_0$  = standardized body size (in this case, the arithmetic mean of sample SVLs), and  $b_{SMA}$  is a scaling component calculated from a standardized regression axis of mass on SVL. In this calculation, SMI is the predicted value of mass for a hatchling with  $L_0$ . We calculated a different  $b_{SMA}$  for the laboratory and field experiments. For each experiment, we calculated  $b_{SMA}$  for each treatment group using the *lmodel2* packaged for R (Legendre 2014) and used the scaling component with the highest strength of fit ( $r^2$ ).

### *Validation experiment for nest warming methods in the field*

For the validation experiment, we established 16 mock nests in a 2.3 m<sup>2</sup> grid at a level section of the field site approximating soil type and average vegetative cover of nests in this study. We randomly warmed half the mock nests and left the remaining exposed. We recorded hourly temperatures using iButton loggers (DS1922L; Maxim Integrated) buried 6 cm in the center of each mock nest. We recorded soil moisture immediately before applying treatments and again after 2 weeks, measured as percent volume to the nearest 0.1% using a portable moisture probe (ML2x; Dynamax Inc., Houston, Texas, USA) and meter (HH2; Delta-T Devices, Cambridge, UK). Before the second moisture readings, we measured soil oxygen availability using an oxygen meter (EOM-PG2-PS3; Presens Precision Sensing, Regensburg, Germany) inserted to 6 cm.

Results of the validation experiment demonstrated that the warming treatment increased daytime nest temperatures without altering minimum daily temperature, soil moisture, or soil oxygen availability (Table B1).

### *Calculating degree days to compare warming treatments between experiments*

To compare the impacts of changing means and variances of embryo temperatures under warming treatments in the laboratory and field experiments, we calculated degree-days above the  $T_{\max}$  and the mean temperatures of the contemporary (laboratory) and natural (field) treatments. Degree-days (°D) serve as a unit that combines temperature and time to approximate the amount of heat received by an organism (Zalom *et al.* 1983). Iterating through each hour of the day for the length of incubation, if embryo temperature

was greater than the threshold temperature, we used the following equation to find the sum of °D (if embryo temperature was equal to or less than the threshold temperature,  $D=0$  at that iteration):

$$D = \left( \sum_{day=1, hour=0}^{day=i, hour=j} (t_h - t_0)_{i,j} \right) / 24 \quad [S2]$$

where  $D$  =sum of °D,  $t_h$ =temperature (°C) at time  $i$  on day  $j$ ,  $t_0$ =threshold temperature Zalom *et al.* (1983). For analyses of the laboratory treatments,  $t_0$  was set to either the  $T_{max}$  or the mean temperature of the contemporary treatment. For the field treatments,  $t_0$  was set to the  $T_{max}$  or the mean temperature recorded in natural nests.

When using  $T_{max}$  as the threshold temperature, embryos under the warming treatments in the laboratory experienced averages of 257.87°D (+3.5°C) and 336.65°D (+7.0°C) above the  $T_{max}$  of the contemporary treatment. In the field, embryos in the four nests under the warming treatment experienced an average of 309.99°D above the mean  $T_{max}$  of natural nests. Due to natural variation in daily temperatures, embryos in the four nests under the natural treatment did experience an average of 10.08°D above the mean  $T_{max}$ .

When using mean temperature as the threshold, embryos under warming treatments in the laboratory experienced averages of 295.95°D (contemporary), 818.48°D (+3.5°C), and 1133.03°D (+7.0°C) above the mean temperature of the contemporary treatment. In the field, embryos experienced averages of 107.24°D (natural) and 964.93°D (warmed) above the mean temperature among of natural nests.

In comparing the °D calculations using both  $T_{max}$  and mean temperatures of the contemporary and natural treatments in our two experiments, we can conclude that the field

warming treatment induced a magnitude of warming similar to that applied in warming treatments in the laboratory. Additionally, the combined impacts of changes in temperature means and variances was similar in the application of warming treatments in both experiments in the relative change above the contemporary (laboratory) and natural (field) nest conditions. In both experiments, embryos under the warmed treatments spent similar amounts of physiological time (combination of time and temperature, Zalom *et al.* 1983) under temperatures higher than those experienced in the control treatments.

### *Modeling lizard body temperatures*

Body temperatures were predicted from operative temperatures, which were derived from air temperatures and radiative loads in each microhabitat. For each time step  $t$ , we calculated the body temperature ( $T_{b,t}$ ) by solving heat-exchange equations in Fei *et al.* (2012):

$$T_{b,t} = T_{b,t-1} + \Delta T_b. \quad [\text{S3}]$$

The parameters and equations used are described in Table B2. We selected a small value for  $\Delta t$  (2 min) to yield small values of  $\Delta T_b$ , which enhanced the stability of the model. We simulated behavioral thermoregulation by assuming that lizards could select between exposed or shaded microhabitats, and either a laying or standing posture. Thus, we calculated  $T_{b,t}$  for each of these conditions and assumed that a lizard maintains its preferred body temperature (33.1°C; Angilletta 2001) if the temperature is within the four options. Otherwise, we assigned the lizard the closest available temperature. During the night, when

the absence of solar radiation precluded thermoregulation, lizards were assigned a body temperature equivalent to the operative temperature of a laying animal in 100% shade.

### *Modeling the energy balance of lizards*

We tracked thermoregulation, feeding, and digestion of adults and juveniles based on the predicted body temperatures ( $T_b$ ). Lizards foraged whenever their body temperatures were in the range required for activity. We assumed that lizards could engage in activity whenever an operative temperature between 29.4° and 36.3° occurs (central 80% of field body temperatures; Angilletta 2001). To determine the energy gain for each hour of foraging, we first calculated the maximal velocity ( $v$ , m s<sup>-1</sup>) of the lizard as

$$\log_{10}(v) = 0.044 + 0.2 \cdot \log_{10}(M_b), \quad [\text{S4}]$$

based on published observations where  $M_b$  equaled the mass of a lizard (adult - 10.y g, juvenile - 1.1g; Van Damme & Vanhooydonck 2001). Then, assuming lizards forage at 70% of their maximal velocity (Irschick & Losos 1998), we calculated the distance traveled ( $d$ , m) in one second as  $0.7v$ . As in Buckley's analysis (2008), we assumed that the energy content of an insect equals 30.12 J, the rate of insect encounter assuming foraging along a line equals 0.005 insects m<sup>-1</sup> s<sup>-1</sup> (Jones *et al.* 1987; Niewiarowski & Roosenburg 1993), 50% of insects encountered are captured by a foraging lizard, and lizards assimilate 76% of ingested energy (Angilletta 2001). Hence, at each hour, the energy intake ( $e_{i,h}$ ) was

$$e_{i,h} \text{ (J h}^{-1}\text{)} = 30.12 \text{ (J insect}^{-1}\text{)} \cdot 0.005 \text{ (insect m}^{-1} \text{ s}^{-1}\text{)} \cdot 0.5 \cdot 0.76 \cdot d \text{ (m)} \cdot 3600 \text{ (s h}^{-1}\text{)} \quad [\text{S5}]$$

For each hour, we calculated the digestive efficiency as

$$DE_h = \left\{ \sin \left[ \frac{\pi(85.34 - 0.05 \cdot T_b + 0.000074 \cdot T_b^3)}{180} \right] \right\}^2. \quad [\text{S6}]$$

At the end of the day, the mean digestive efficiency ( $DE$ , dec %) and the daily energy intake ( $e_i$ , J d<sup>-1</sup>) were used to calculate the mean energy derived from foraging ( $e_f$ , J d<sup>-1</sup>) as

$$e_f = DE \cdot e_i, \quad [S7]$$

We estimated the energy lost while resting and foraging from experimental studies of metabolic rates. A study of resting metabolic rate (Angilletta 2001) yielded the following model:

$$\ln(\text{RMR}, \text{J s}^{-1}) = -10.0 + 0.51 \cdot \log(M_b) + 0.12 \cdot T_b, \quad [S8]$$

where  $M_b$  equaled the mass of an adult lizard (10.7 g). We multiplied RMR by 1.5 to yield the resting metabolic rate of a digesting animal,  $e_r$ , (Roe *et al.* 2005) and by 2 to yield the cost of pursuing prey,  $e_p$  (Bennett 1982).

Using the above estimates, we calculated the daily net energy gain as

$$e_{\text{day}} = e_f - t_f \cdot e_p - t_r \cdot e_r, \quad [S9]$$

where  $t_f$  and  $t_r$  equal durations of foraging and resting (h d<sup>-1</sup>), respectively, and  $e_f$ ,  $e_p$ , and  $e_r$  equal daily energy derived from foraging (J d<sup>-1</sup>), and the mean energy lost while foraging and resting, respectively (J h<sup>-1</sup>). We estimated daily net energy gains of juveniles to simulate storage and depletion of fat reserves, and annual net energy gains of adults to estimate rates of population growth.

In modeling the energy balance of lizards, we do make assumptions about the rate of encounter of prey, which is based largely on prey abundance. For an examination of the sensitivity of the model's bioenergetics predictions to changes in those assumptions, please refer to Levy *et al.* (2017), which examines how shifts in activity time due to climate change affect feeding and assimilation rates of lizards from the *Sceloporus undulatus*

complex. There, the authors demonstrate that lizards are limited by digestion rather than feeding as activity time shifts under climate change, and there was no significant difference in the results when the authors varied the abundance of insects (and thus the encounter rate for feeding lizards); it took more time foraging for lizards to fill their guts, but the limiting factor was still the relationship between thermal opportunity and digestion.

### *Calculating embryonic survival*

Based on our empirical observations, we evaluated the impacts of warming nest temperatures on embryonic survival and population growth rates by comparing results of the Levy *et al.* (2015) model to a model that includes effects of sublethal warming (this manuscript). First, per Levy *et al.* (2015), we assumed soil temperatures ( $T_{\text{soil}}$ ) above 44°C are lethal and eggs that cool below 25°C have a chance of survival according to lowest  $T_{\text{soil}}$  during incubation:

$$\text{logit}(\text{survival}) = -2.19 + 0.14 \cdot T_{\text{soil,lowest}} \quad [\text{S10}]$$

Eggs that neither warmed to 44°C nor cooled below 25°C were assigned 80% chance of hatching, based on mean survivorship in experiments (Oufiero & Angilletta 2006). Then, we ran the model with effects of recurrent sublethal warming, as found in this study; eggs that experienced mean  $T_{\text{max}}$  above 32°C have a chance of survival according to mean  $T_{\text{max}}$  during incubation:

$$\text{logit}(\text{survival}) = 7.63 - 0.18 \cdot T_{\text{soil,max}} \quad [\text{S11}]$$

We parameterized this function using the laboratory survivorship results to provide conservative estimates from experiments in which we controlled hydric conditions across



treatments to isolate the impacts of changing incubation temperatures. Surviving embryos developed at an hourly rate ( $D$ , dec %) described as

$$D = (0.00081 + 0.00067 \cdot T_{soil})/24 \quad [S12]$$

This function was parameterized with development rates from Angilletta *et al.* (2000).

### *Calculating sensitivity indices to compare models*

To compare the performance of the lethal and sublethal models in predicting presences of *S. undulatus*, we calculated sensitivity indices as described in Manel *et al.* (2001) and Buckley *et al.* (2010) as the percent of occurrences correctly predicted. Our occurrence data was downloaded from 11,676 records (VertNet 2016; H.E.R.P. 2017; HerpMapper 2017), which we narrowed down to 4,060 occurrences with location descriptions and with coordinates to at least  $1 \times 10^{-3}$  decimal degrees that matched the described locations (Fig. B47). From both models, we examined the predictions of contemporary embryonic survival under typical nesting conditions ( $\sim 6$  cm depth and  $\sim 50\%$  shade, Angilletta *et al.* 2009;  $\sim 4$ -8 cm depth and  $\sim 50$ -64% shade, this manuscript) averaged across the summer breeding season; both models predicted 100% of occurrences. When we examined predictions separately for each month of the season (April through October), both models predicted at least 99.4% of occurrences.

### *Calculating time to maturity*

We performed sensitivity analyses to test how changes in hatchling sizes and juvenile growth rates could affect p population growth via delayed maturity. To predict age at maturity, we ran the model with mean hatchling SVLs and characteristic growth rates ( $r$ ; estimated from the von Bertalanffy growth models) from the contemporary (SVL=24.91 mm;  $r$ =8.02 $\mu$ m/day) and +7.0°C (SVL=23.80 mm;  $r$ =7.18 $\mu$ m/day) laboratory treatments. Juvenile growth was calculated following the approach described by Dunham (1978) and Schoener & Schoener (1978):

$$L = A \cdot \left[ 1 - \frac{L_0}{A} \cdot e^{-r \cdot t} \right] \quad [S13]$$

where  $L$  = SVL (mm) at time  $t$ ,  $A$  = asymptotic SVL (mm),  $L_0$  = hatchling SVL (mm),  $r$  = characteristic growth rate (mm/day), and  $t$  = days since hatching. Asymptotic SVL was set at the size of adult females used in the model (67 mm). Juvenile growth was assumed to pause in periods of inactivity during overwintering. We then solved for  $L=55$  (55-mm minimum SVL at maturity; Tinkle & Ballinger 1972) to find the time to maturity for lizards across the map (Fig. B48).

### *Estimating intrinsic population growth rates from life tables*

The population growth model within our “sublethal” model includes assumptions that prevent incorporation of time to maturity into calculations of population growth rates. So, to examine how delayed maturity may affect estimates of population growth rates, we calculated intrinsic rates of increase from life tables. We combined projections of embryonic survival and time to maturity from the model with demographic data from the

literature to populate life tables for *S. undulatus* populations in South Carolina (SC) and New Jersey (NJ) (see Tables B3-B8). We chose these populations because (1) detailed demographic data is available for those locations and (2) they represent southern and northern reaches along the latitudinal cline of the species range. Juveniles typically mature within 12 months of hatching in SC (Tinkle & Ballinger 1972) and in about 20 months in NJ (Niewiarowski *et al.* 2004), resulting in differences between the structure of age classes between northern and southern populations regarding fecundity and survivorship.

Our model predicted that slowed juvenile growth would delay maturity in SC and NJ by approximately 26.14 and 28.94 days, respectively, assuming climatic conditions simulated in the model by the end of the century. To examine how such delays in maturity may impact predictions of population growth, we varied fecundity values in the first reproductive season, assuming in the most severe case that delayed maturity would cause an individual to miss reproductive opportunities equivalent to laying one less clutch in that season. In assigning fecundities, we assumed 1:1 sex ratios within clutches. In each case, we calculated survival probabilities and fertility to construct Leslie matrices as described in Gotelli (2008). Intrinsic rates of increase were then calculated as the natural logarithm of the dominant eigenvalue from each Leslie matrix.

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## Appendix B

### Chapter One: Supplementary tables and figures

#### Supplementary tables for field experiment, model parameters, and life tables

**Table B1** Summary statistics for analyses of data from the validation experiment, including (a) daytime nest temperatures, (b) minimum daily temperature ( $T_{\min}$ ), (c) soil moisture, and (d) soil oxygen. Results show the warming method was effective in raising daytime nest temperatures without altering  $T_{\min}$ , soil moisture, or soil oxygen availability.

Response	Parameter	F	p	$\eta^2$
(a) daytime temp.	treatment	<b>8.14</b> <sub>1,6030</sub>	<b>0.004</b>	0.0012
(b) $T_{\min}$	treatment	0.26 <sub>1,40</sub>	0.613	0.0000
(c) soil H <sub>2</sub> O	treatment	0.06 <sub>1,62</sub>	0.802	0.0000
(d) soil O <sub>2</sub>	treatment	0.85 <sub>1,14</sub>	0.374	0.0000

$\omega^2$ , effect size, (Olejnik and Algina 2003)

$T_{\min}$ , minimum daily temperature



**Table B2** Lizard parameters used to calculate changes in body temperature ( $\Delta T_b$ ) of a *S. undulatus* lizard.

Description	Value (units)	Source
Size of adults	0.067 (m)	(Niewiarowski <i>et al.</i> 2004)
Body mass	$SVL^3 * 3.55 * 10^{-8}$ (kg)	(Tinkle & Ballinger 1972)
Surface area	$0.0314\pi M_b^{2/3}$ (m <sup>2</sup> )	(Fei <i>et al.</i> 2012)
Projected area for direct and scattered solar radiation	0.4 A <sub>L</sub> (m <sup>2</sup> )	(Porter <i>et al.</i> 1973)
Projected area facing toward the ground	0 (m <sup>2</sup> ) – laying, 0.4 A <sub>L</sub> (m <sup>2</sup> ) – standing	(Porter <i>et al.</i> 1973)
Projected area that touched the ground	0.35 A <sub>L</sub> (m <sup>2</sup> ) – laying, 0.05 A <sub>L</sub> (m <sup>2</sup> ) – standing	(Barlett & Gates 1967)
Area facing toward the sky	0.6 A <sub>L</sub> (m <sup>2</sup> )	(Barlett & Gates 1967)
Area that is exposed to air	0.9 A <sub>L</sub> (m <sup>2</sup> )	(Fei <i>et al.</i> 2012)
Thermal absorptivity	0.965 (dec. %)	(Barlett & Gates 1967)
Convective heat transfer coefficient	10.45 (W m <sup>-2</sup> K <sup>-1</sup> )	(Porter <i>et al.</i> 1973)
Emissivity of skin	0.965	(Barlett & Gates 1967)
Thermal conductivity	0.5 (W K <sup>-1</sup> m <sup>-1</sup> )	(Porter <i>et al.</i> 1973)
Thickness (diameter)	0.02 (m)	our approximation
Heat capacity	3762 (J kg <sup>-1</sup> )	(Porter <i>et al.</i> 1973)

**Table B3** Parameters used in life tables for a representative SC population during the period 1980-2000. Survivorship ( $g(x)$ ) of embryos is from the sublethal model in this manuscript, assuming lizards nest at 6-cm depth and 50% shade. Fecundities ( $b(x)$ ) were calculated assuming a 1:1 sex ratio among total numbers of eggs produced in a reproductive season (Tinkle & Ballinger 1972; Niewiarowski *et al.* 2004). Juvenile and adult  $g(x)$  are from Tinkle & Ballinger (1972).

Age class (x)	b(x)	g(x)
0	0	.5244
.25	0	.44
.83	3.7	.49
1.83	11.1	.49
2.83	11.1	.49
3.83	11.1	0

**Table B4** Parameters used in life tables for a representative SC population during the period 2080-2100 without the effect of delayed maturity due to smaller hatchling size and slowed juvenile growth. Survivorship ( $g(x)$ ) of embryos is from the sublethal model in this manuscript, assuming lizards nest at 6-cm depth and 50% shade. Fecundities ( $b(x)$ ) were calculated assuming a 1:1 sex ratio among total numbers of eggs produced in a reproductive season (Tinkle & Ballinger 1972; Niewiarowski *et al.* 2004). Juvenile and adult  $g(x)$  are from Tinkle & Ballinger (1972).

Age class (x)	b(x)	g(x)
0	0	.4095
.25	0	.44
.83	3.7	.49
1.83	11.1	.49
2.83	11.1	.49
3.83	11.1	0

**Table B5** Parameters used in life tables for a representative SC population during the period 2080-2100 with the effect of delayed maturity due to smaller hatchling size and slowed juvenile growth. Survivorship ( $g(x)$ ) of embryos is from the sublethal model in this manuscript, assuming lizards nest at 6-cm depth and 50% shade. Fecundities ( $b(x)$ ) were calculated assuming a 1:1 sex ratio among total numbers of eggs produced in a reproductive season (Tinkle & Ballinger 1972; Niewiarowski *et al.* 2004). Juvenile and adult  $g(x)$  are from Tinkle & Ballinger (1972).

Age class (x)	$b(x)$	$g(x)$
0	0	.4095
.25	0	.44
.91	0	.49
1.83	11.1	.49
2.83	11.1	.49
3.83	11.1	0

**Table B6** Parameters used in life tables for a representative NJ population during the period 1980-2000. Survivorship ( $g(x)$ ) of embryos is from the sublethal model in this manuscript, assuming lizards nest at 6-cm depth and 50% shade. Fecundities ( $b(x)$ ) were calculated assuming a 1:1 sex ratio among total numbers of eggs produced in a reproductive season (Niewiarowski *et al.* 2004). Juvenile and adult  $g(x)$  are from Niewiarowski (1994) and Niewiarowski *et al.* (2004).

Age class (x)	$b(x)$	$g(x)$
0	0	.4624
.67	0	.23
1.67	8.9	.44
2.67	8.9	.44
3.67	8.9	0

**Table B7** Parameters used in life tables for a representative NJ population during the period 2080-2100 without the effect of delayed maturity due to smaller hatchling size and slowed juvenile growth. Survivorship ( $g(x)$ ) of embryos is from the sublethal model in this manuscript, assuming lizards nest at 6-cm depth and 50% shade. Fecundities ( $b(x)$ ) were calculated assuming a 1:1 sex ratio among total numbers of eggs produced in a reproductive season (Niewiarowski *et al.* 2004). Juvenile and adult  $g(x)$  are from Niewiarowski (1994) and Niewiarowski *et al.* (2004).

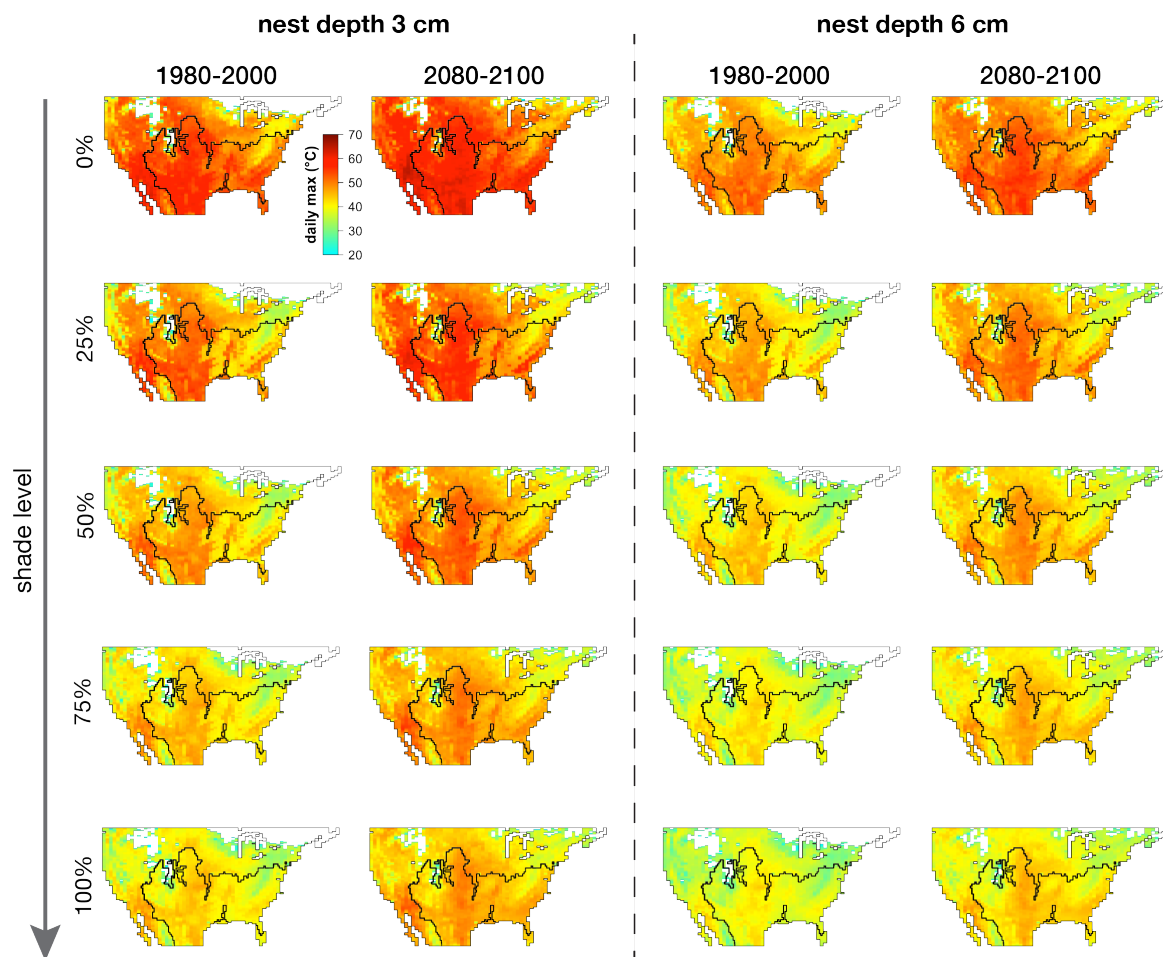
Age class (x)	$b(x)$	$g(x)$
0	0	.3895
.67	0	.23
1.67	8.9	.44
2.67	8.9	.44
3.67	8.9	0

**Table B8** Parameters used in life tables for a representative NJ population during the period 2080-2100 with the effect of delayed maturity due to smaller hatchling size and slowed juvenile growth. Survivorship ( $g(x)$ ) of embryos is from the sublethal model in this manuscript, assuming lizards nest at 6-cm depth and 50% shade. Fecundities ( $b(x)$ ) were calculated assuming a 1:1 sex ratio among total numbers of eggs produced in a reproductive season (Niewiarowski *et al.* 2004). Juvenile and adult  $g(x)$  are from Niewiarowski (1994) and Niewiarowski *et al.* (2004).

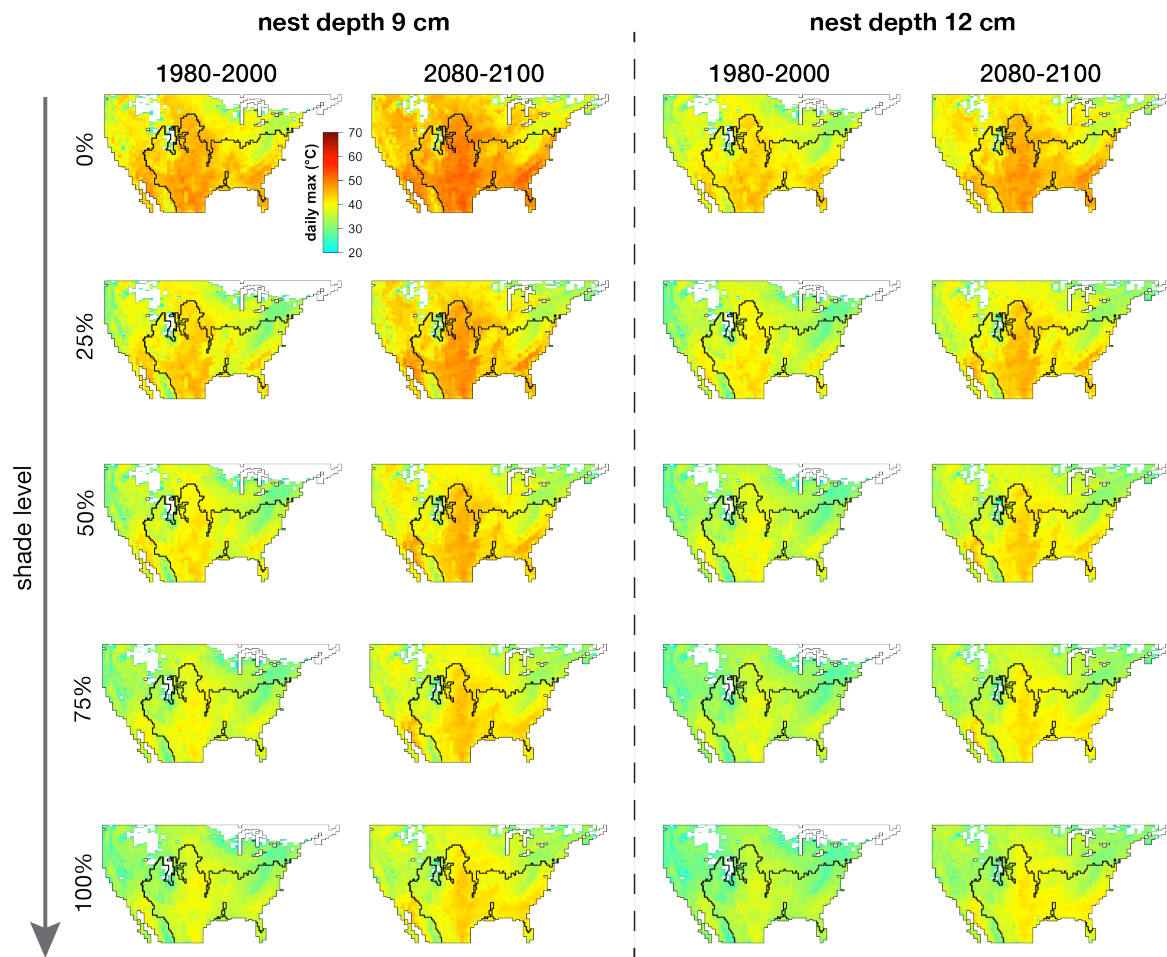
Age class (x)	$b(x)$	$g(x)$
0	0	.3895
.67	0	.23
1.75	4.5	.44
2.67	8.9	.44
3.67	8.9	0

Supplementary figures (Fig. B1-B14) for maximum soil temperatures

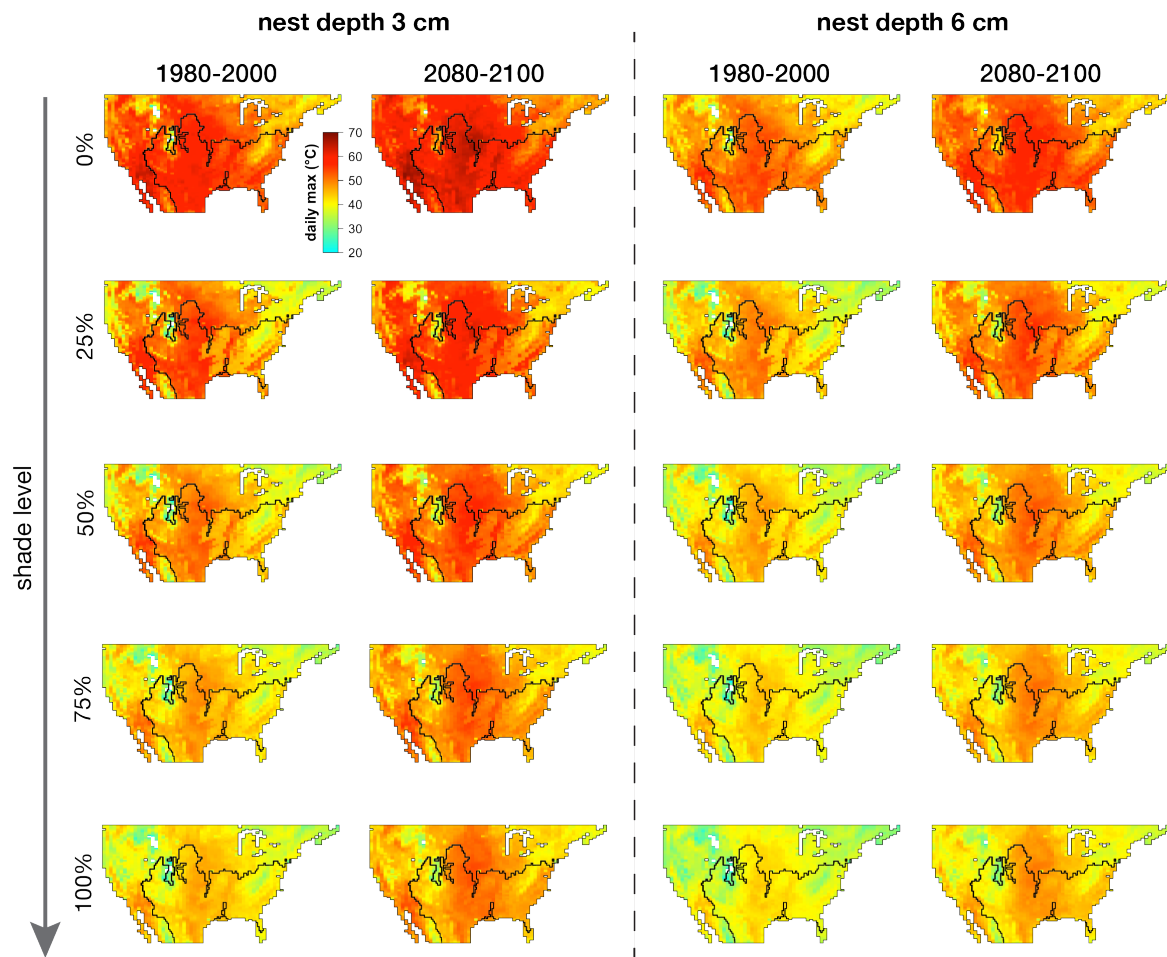
**Figure B1** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in April at depths of 3 cm and 6 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.



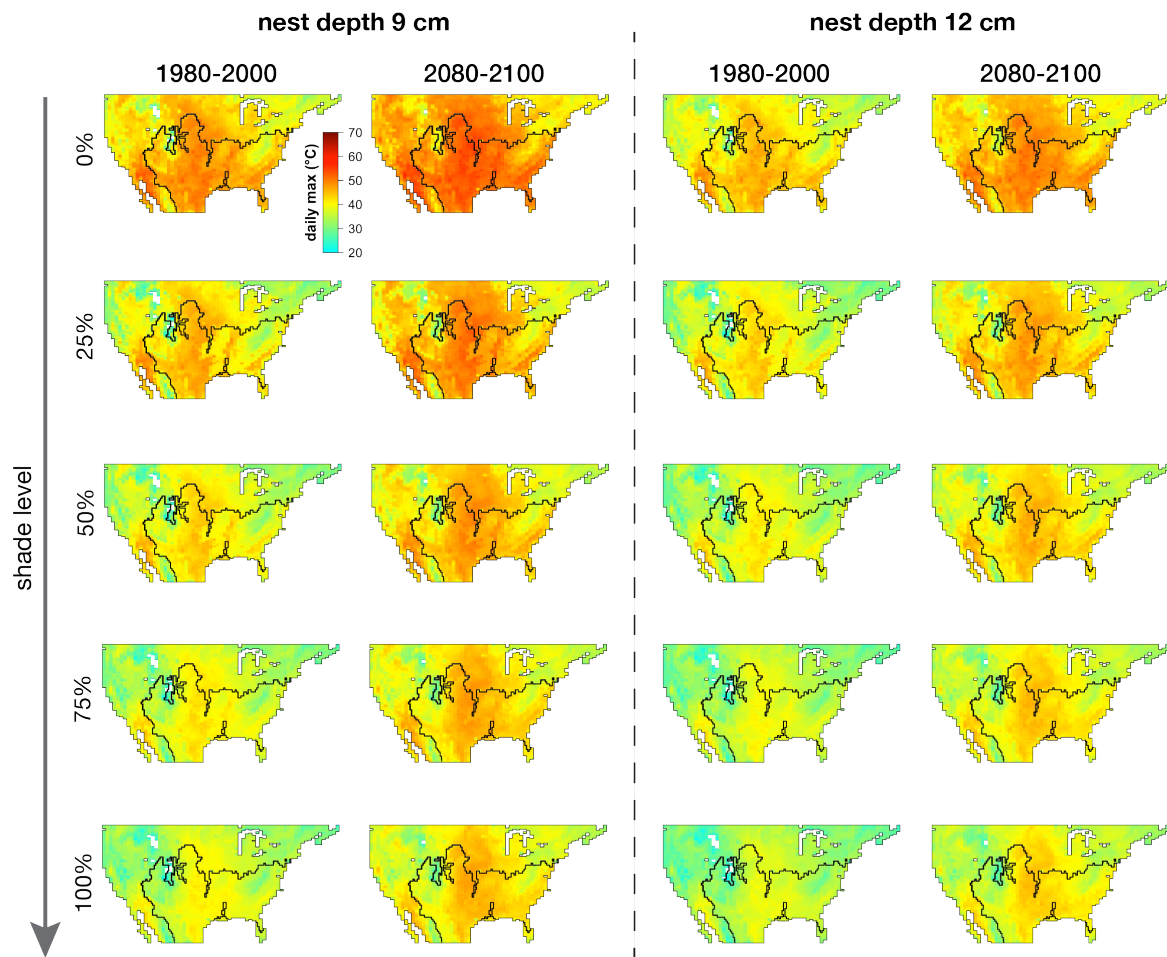
**Figure B2** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in April at depths of 9 cm and 12 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.



**Figure B3** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in May at depths of 3 cm and 6 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

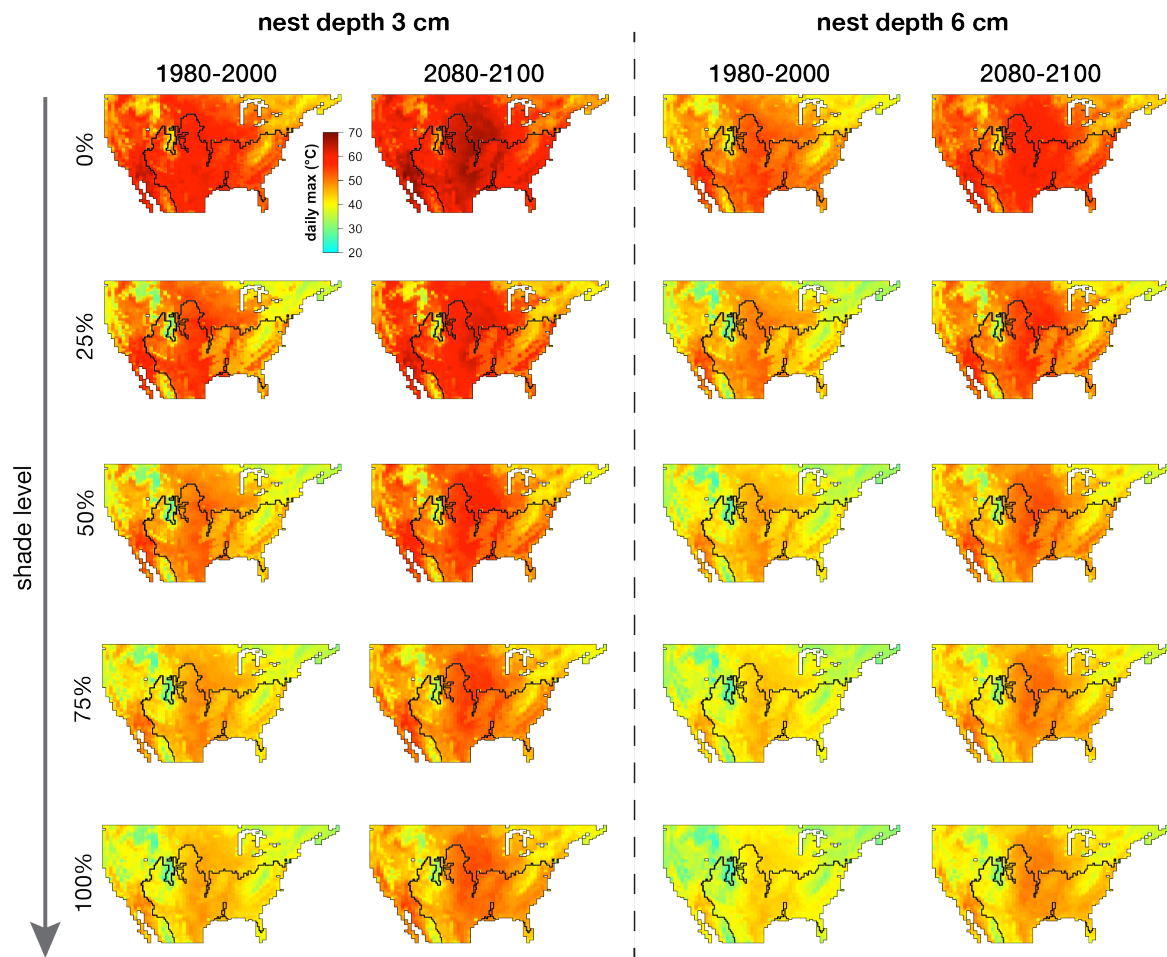


**Figure B4** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in May at depths of 9 cm and 12 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

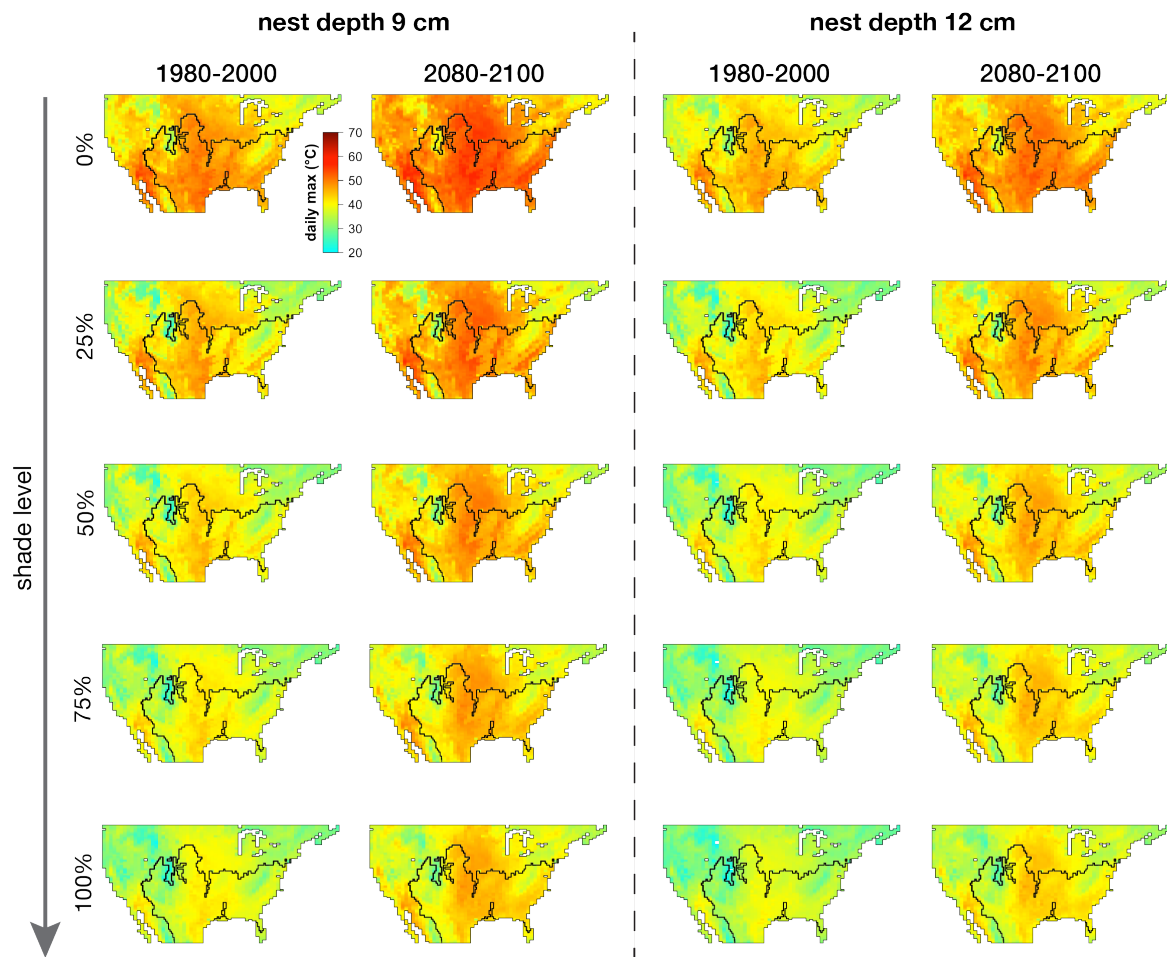




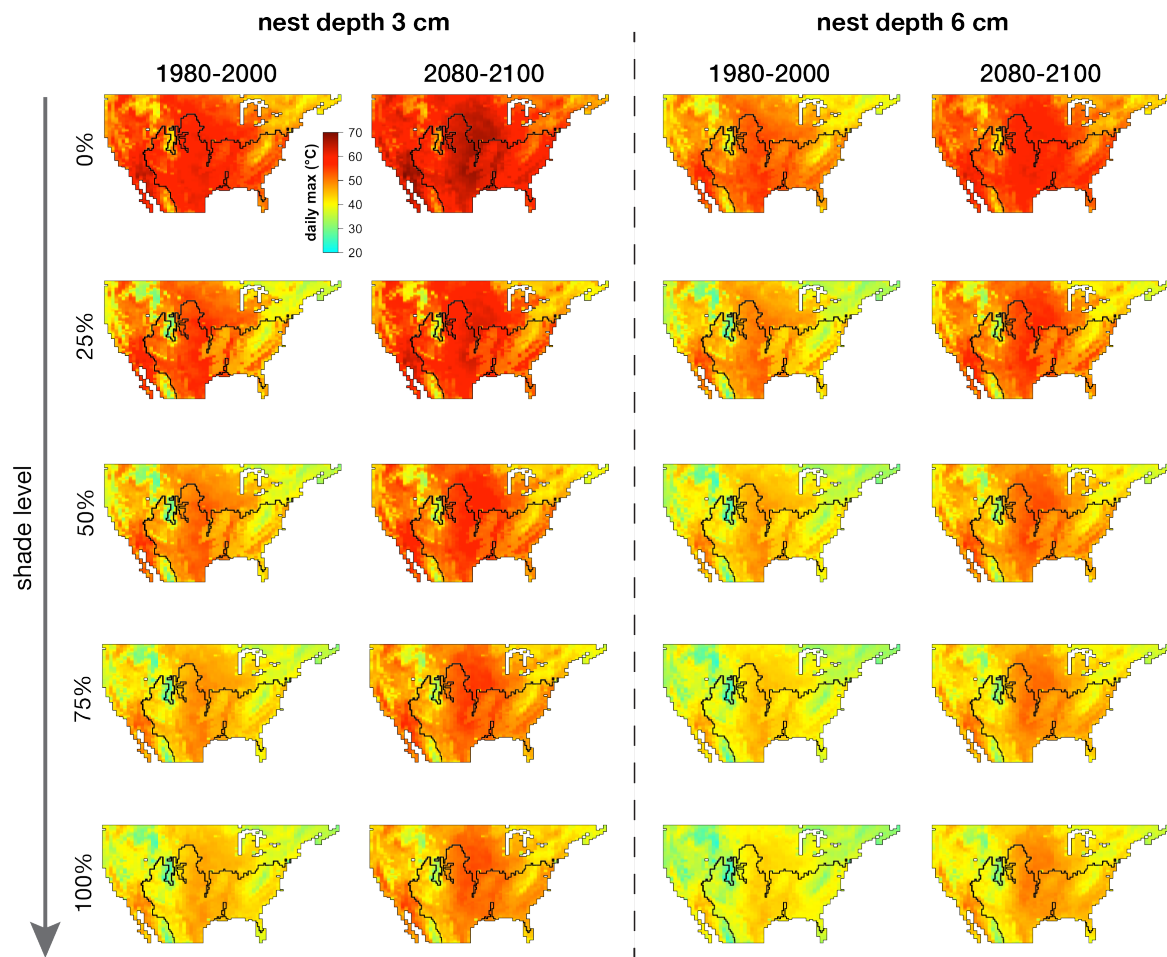
**Figure B5** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in June at depths of 3 cm and 6 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.



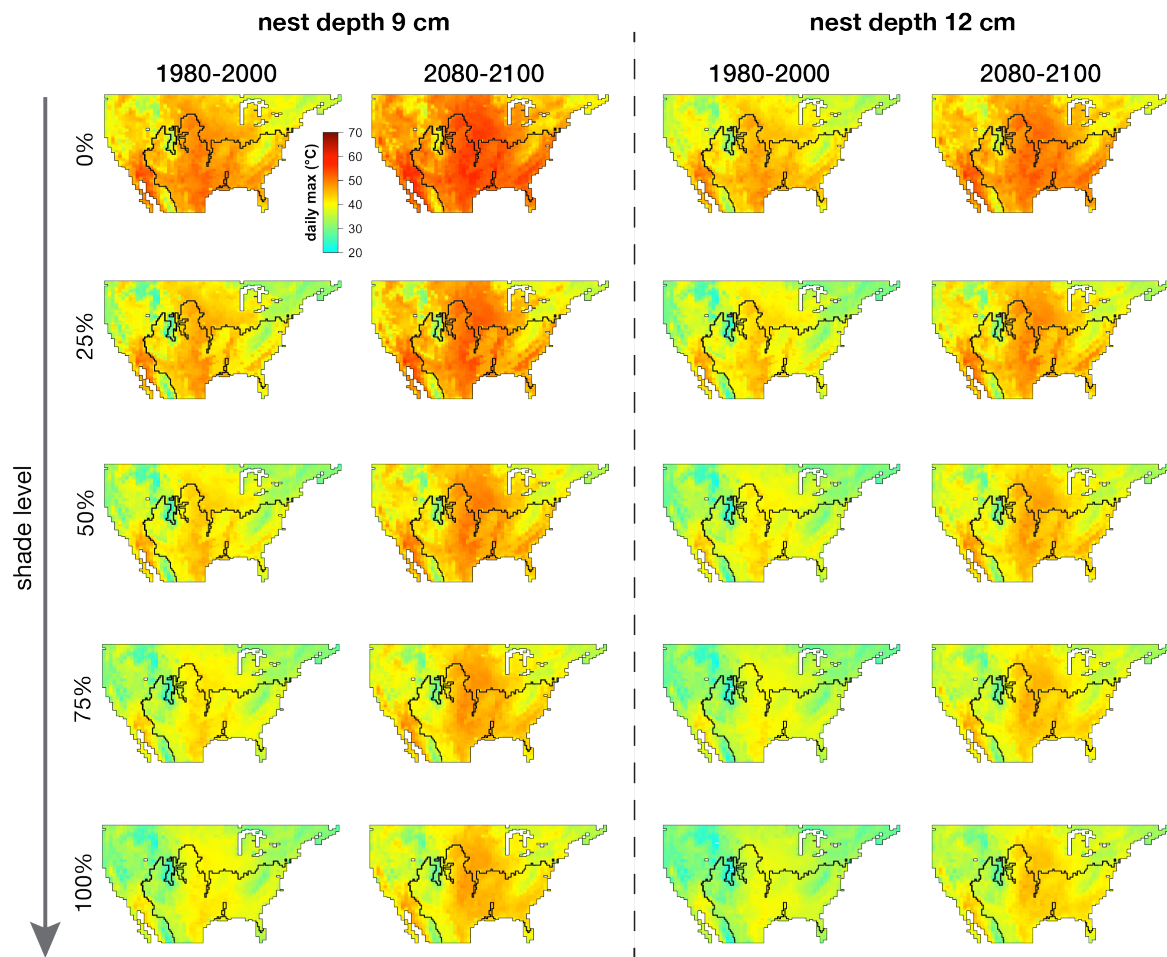
**Figure B6** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in June at depths of 9 cm and 12 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.



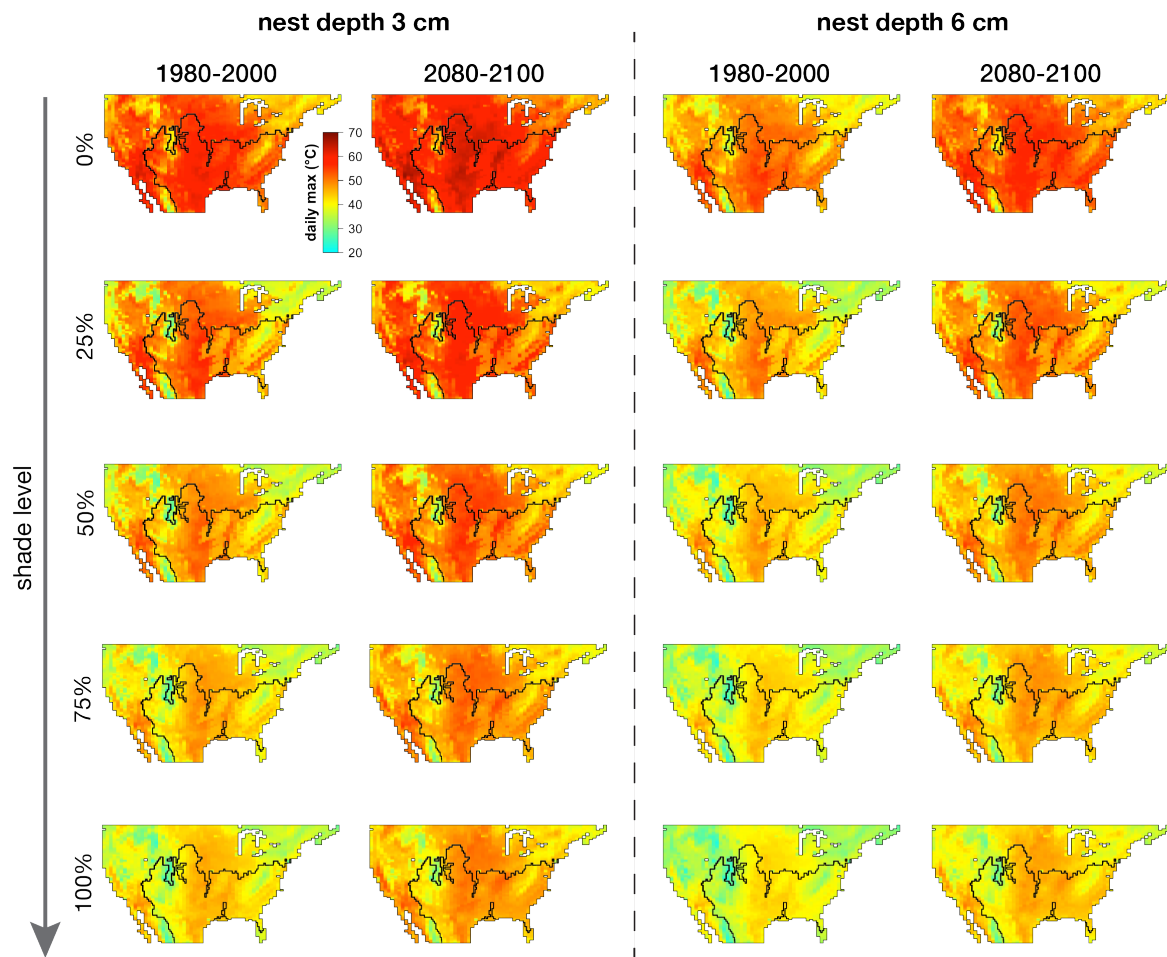
**Figure B7** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in July at depths of 3 cm and 6 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.



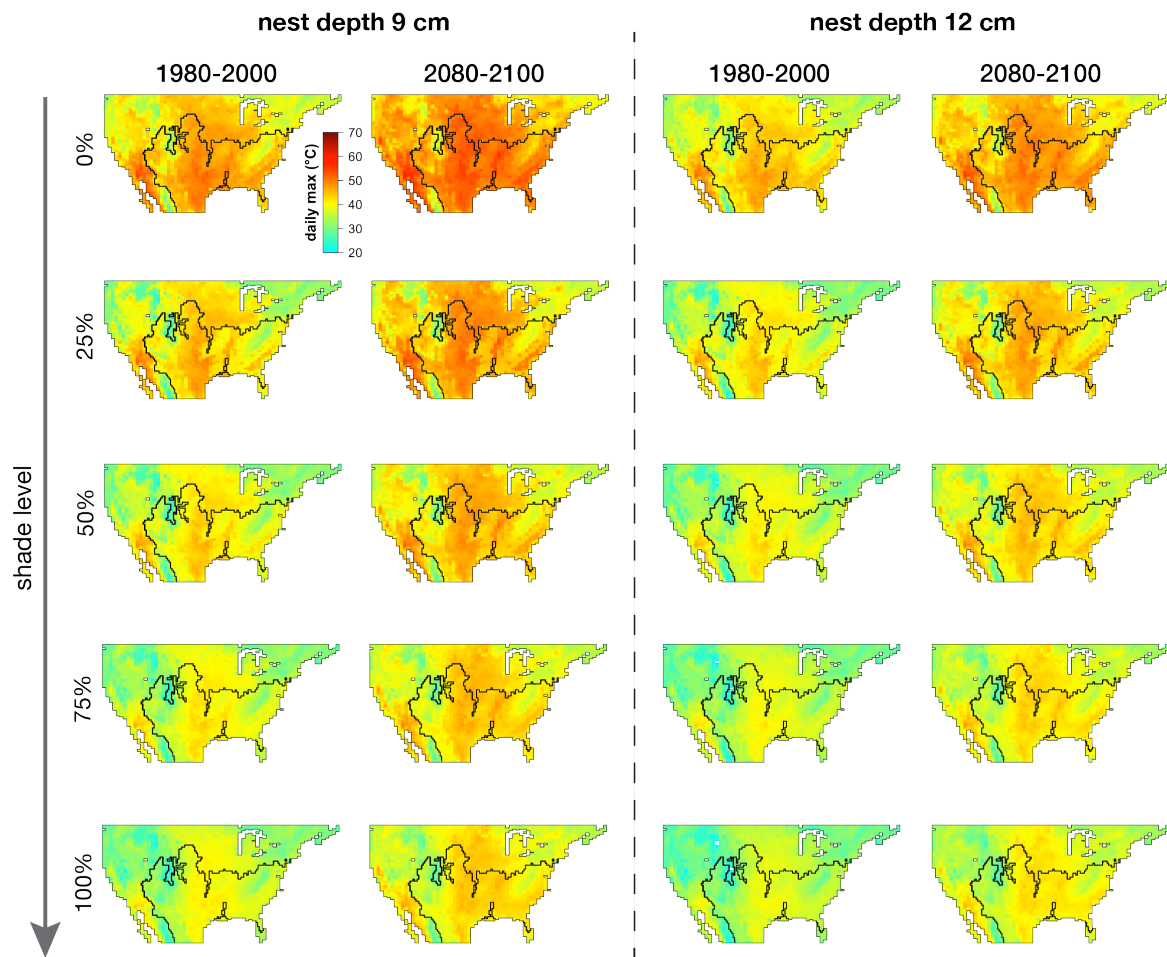
**Figure B8** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range. (IUCN 2017) Data are presented for nests laid in July at depths of 9 cm and 12 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.



**Figure B9** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in August at depths of 3 cm and 6 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

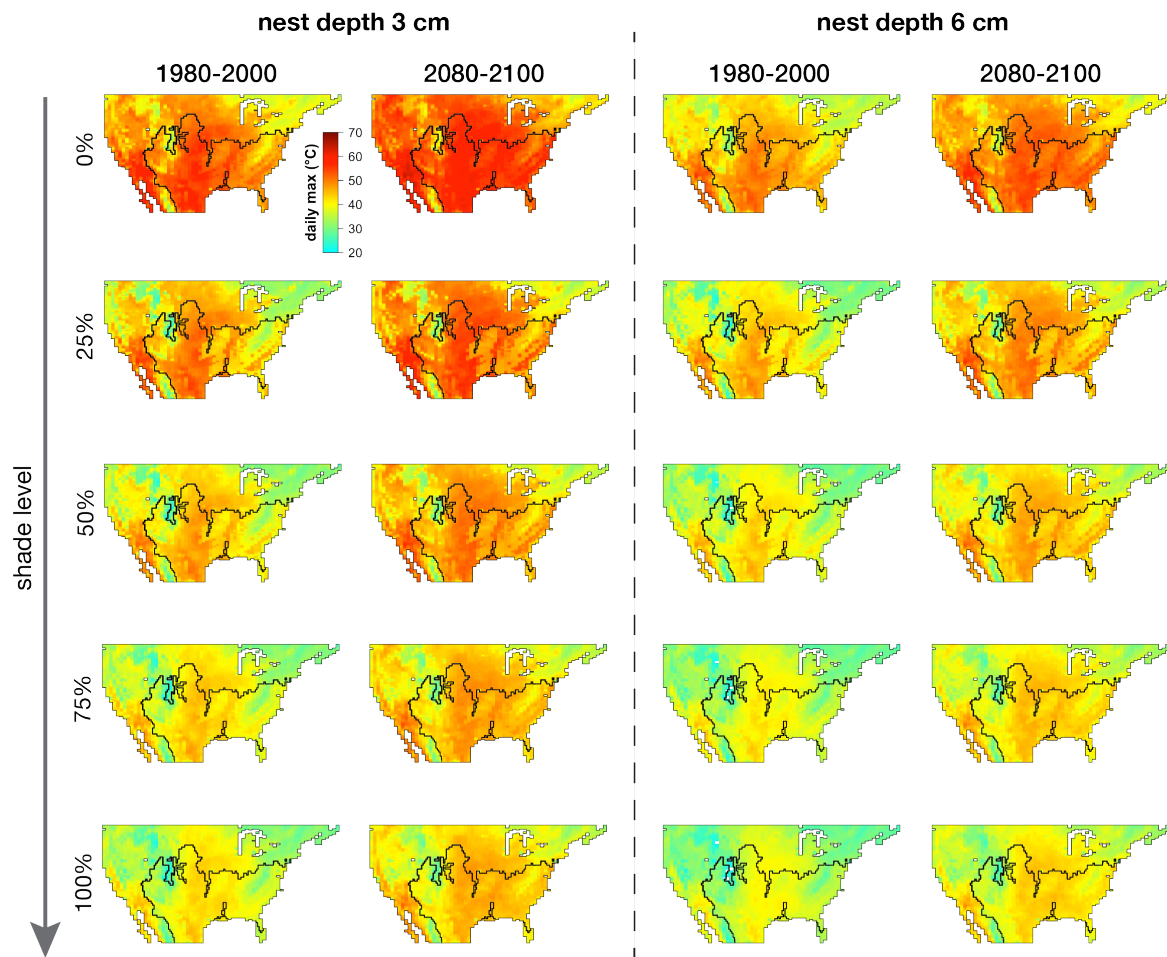


**Figure B10** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in August at depths of 9 cm and 12 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

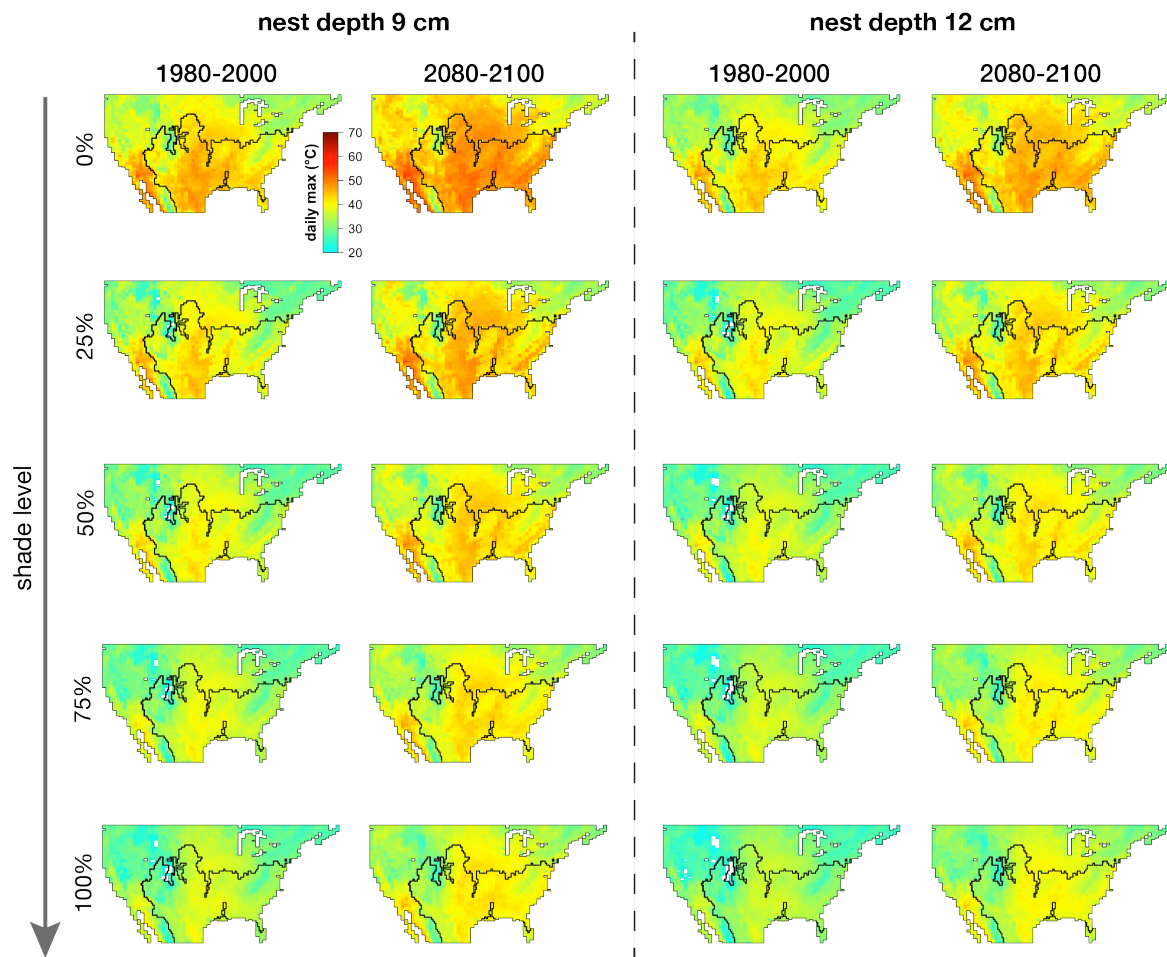




**Figure B11** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in September at depths of 3 cm and 6 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

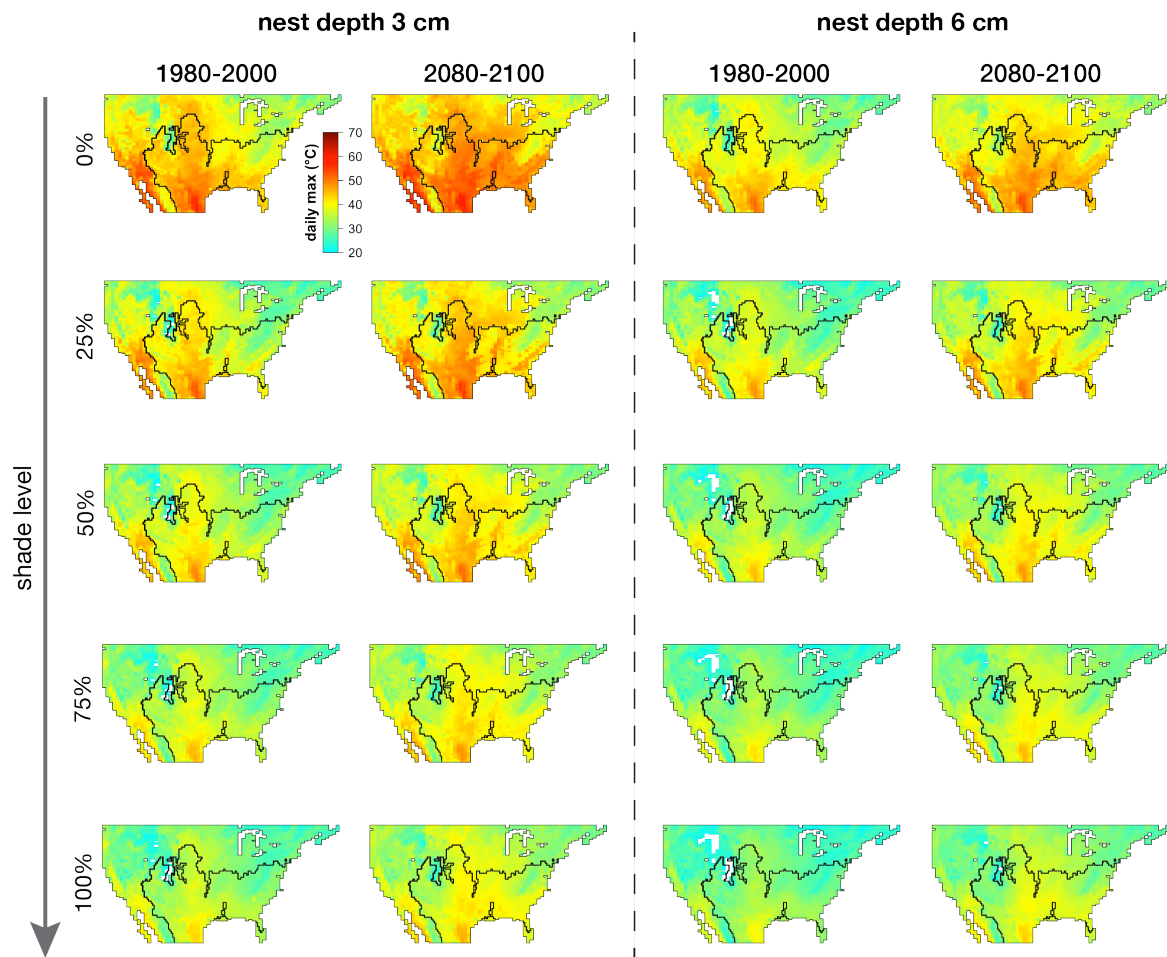


**Figure B12** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in September at depths of 9 cm and 12 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

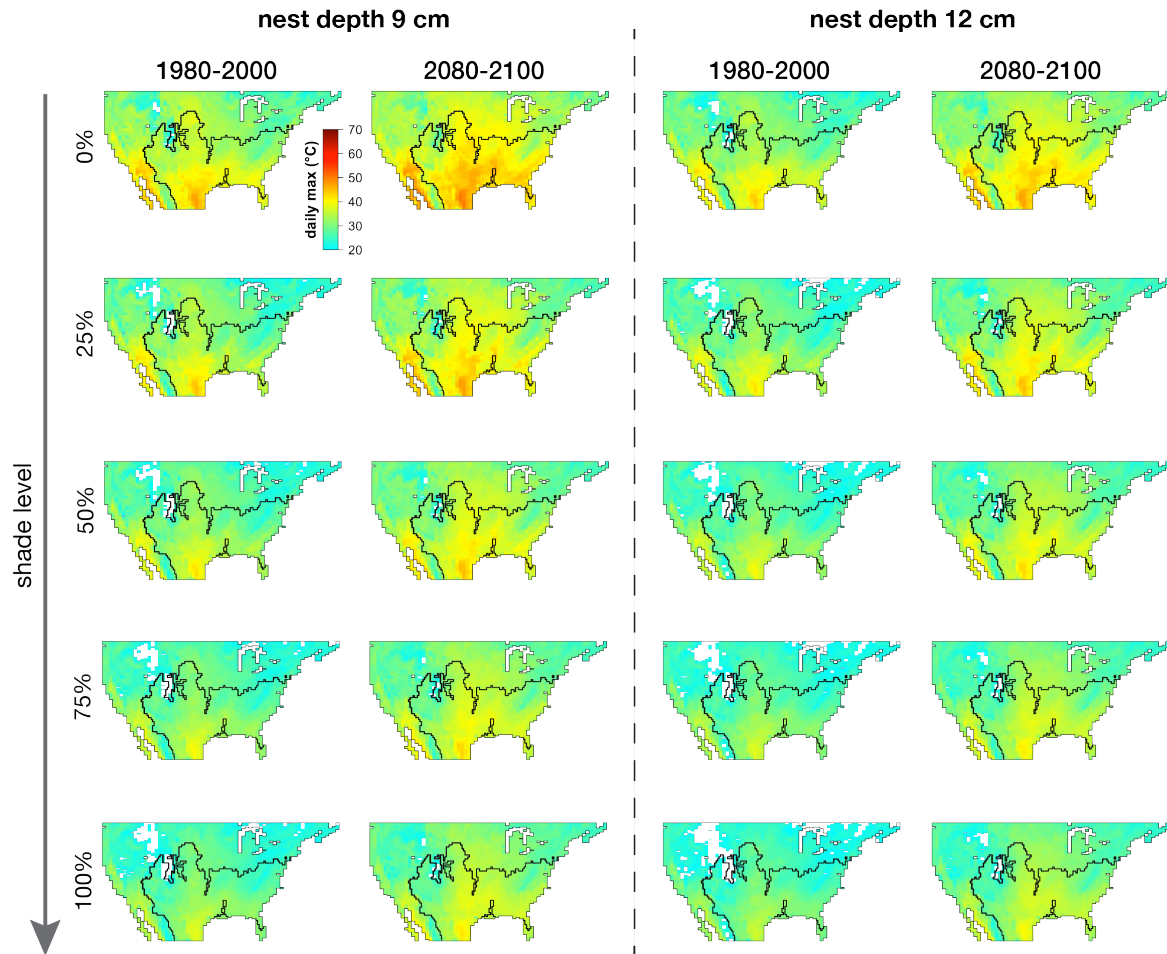




**Figure B13** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in October at depths of 3 cm and 6 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

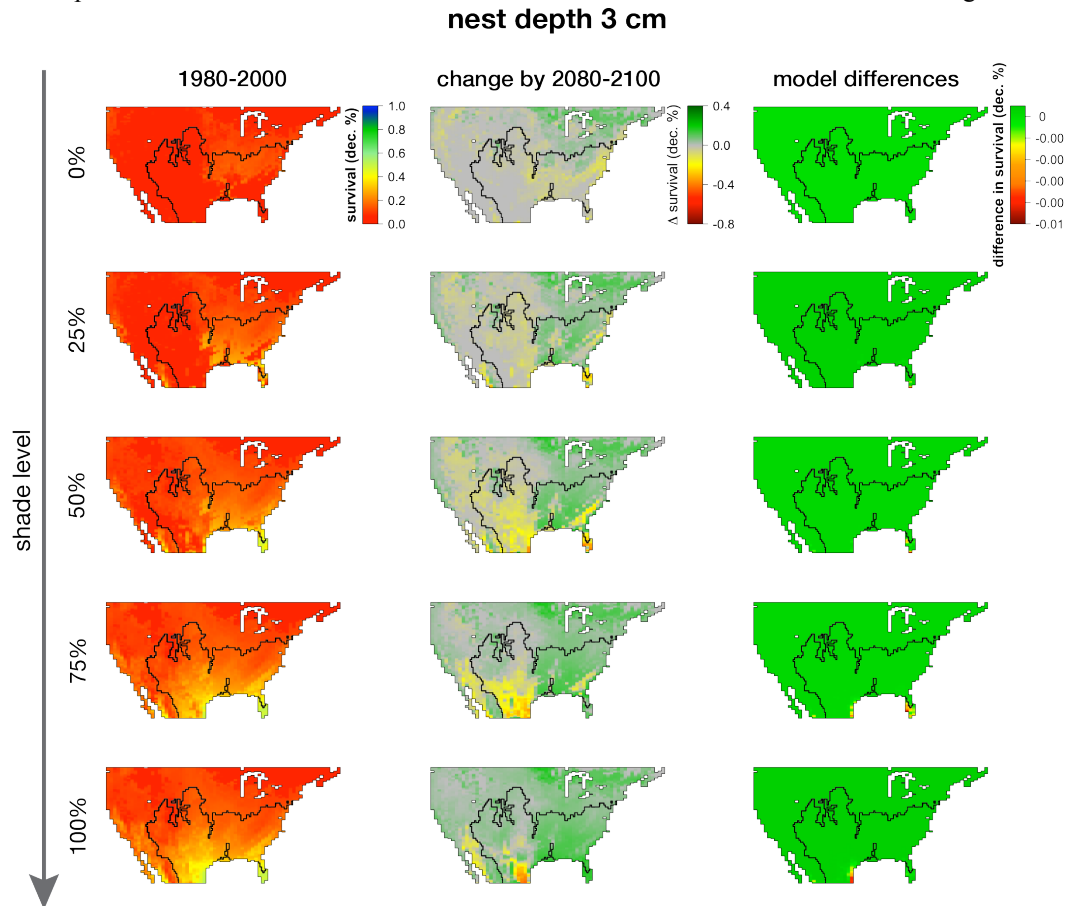


**Figure B14** Maximum soil temperature during nesting under past climate (1980-2000) and predicted change in the future (2080-2100). Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Data are presented for nests laid in October at depths of 9 cm and 12 cm under different shade conditions. White areas represent locations for which climate conditions did not enable enough activity to promote reproduction. Color scales are the same for figures S1-S14 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels.

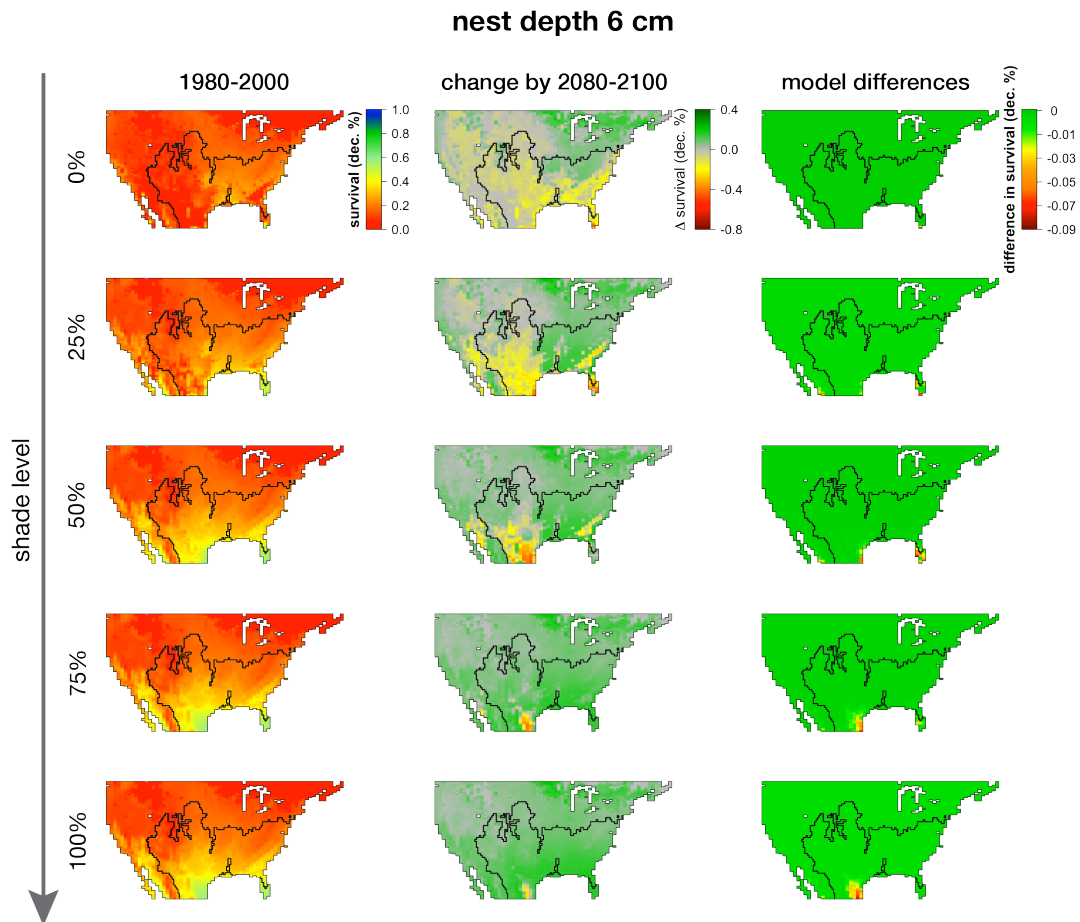


Supplementary figures (Fig. B15-B42) for embryo survival

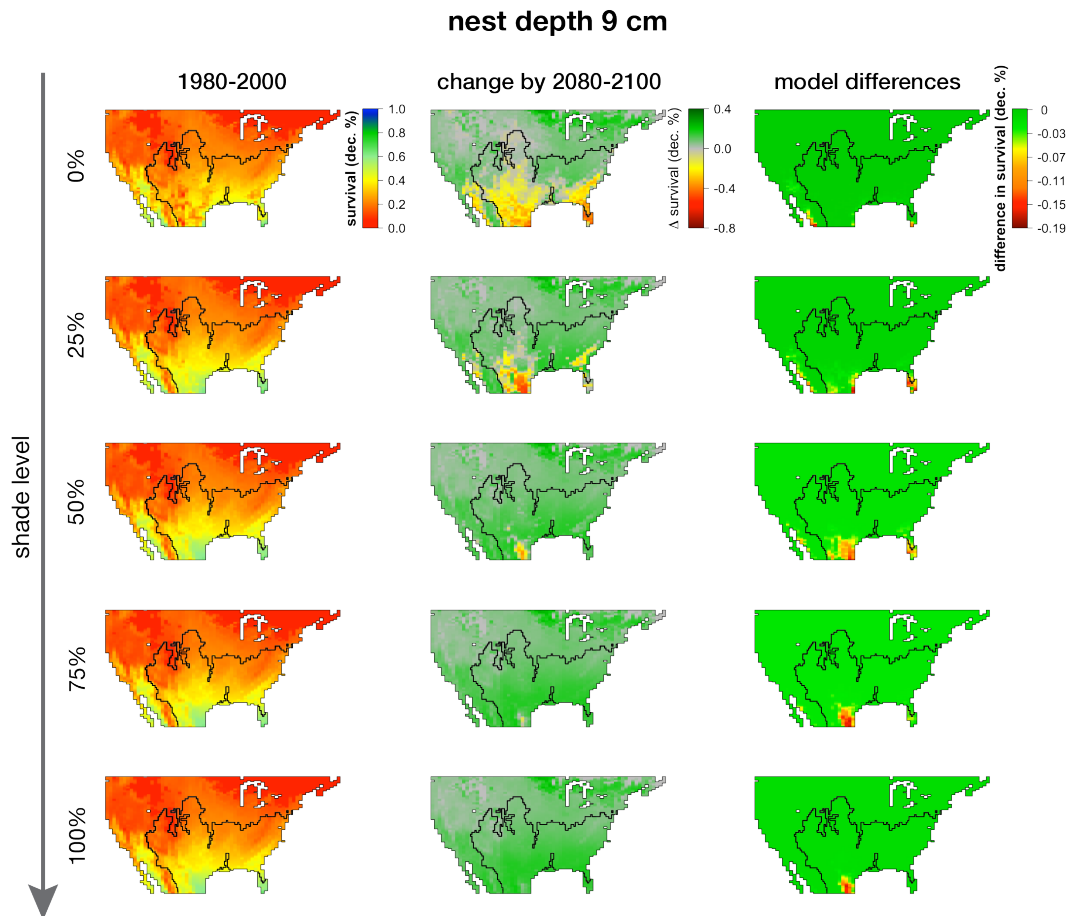
**Figure B15** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in April at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



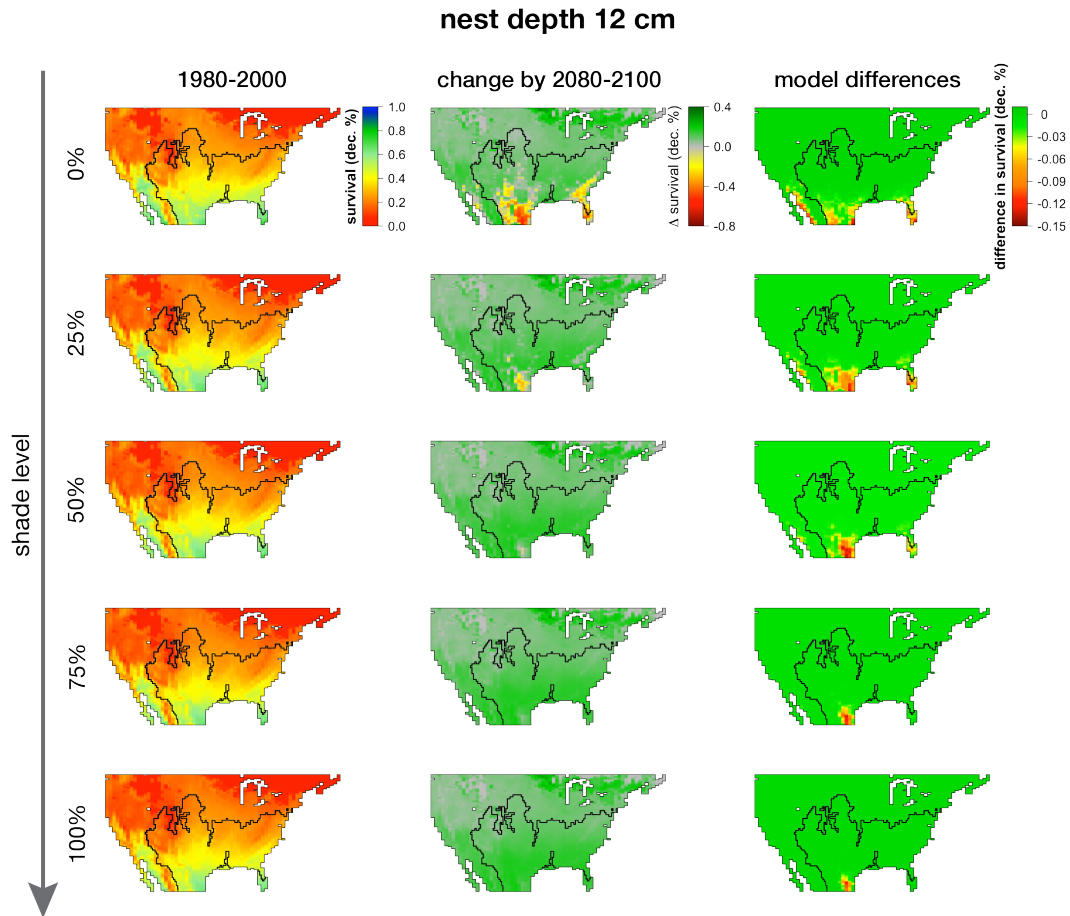
**Figure B16** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in April at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



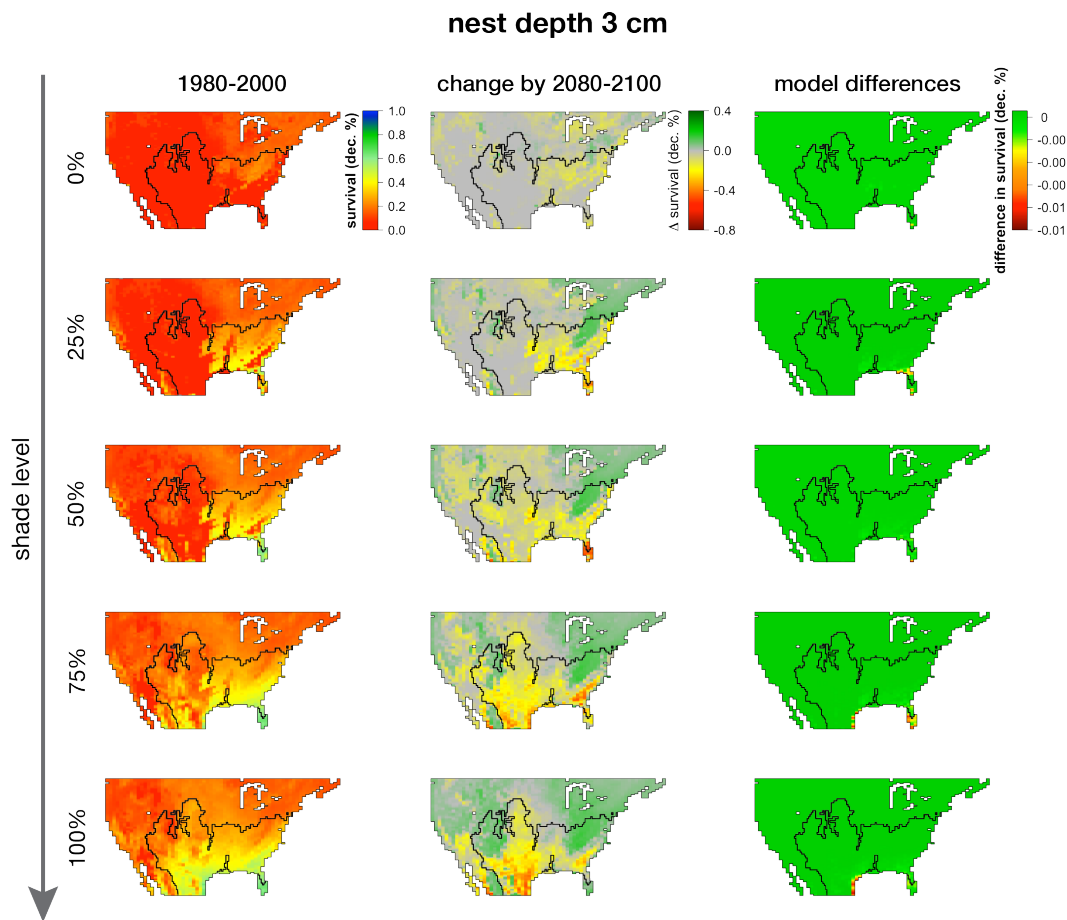
**Figure B17** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in April at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



**Figure B18** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in April at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

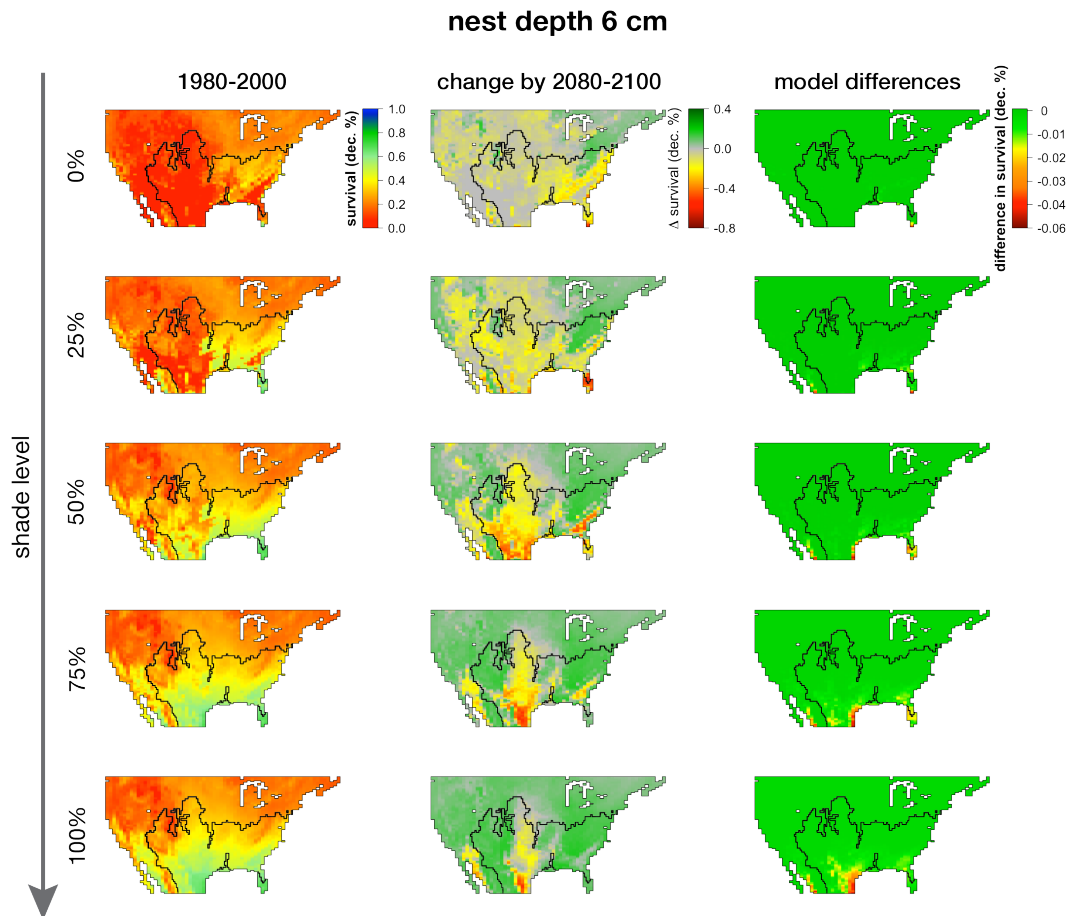


**Figure B19** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in May at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales for embryo survival and change by 2080-2100 are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



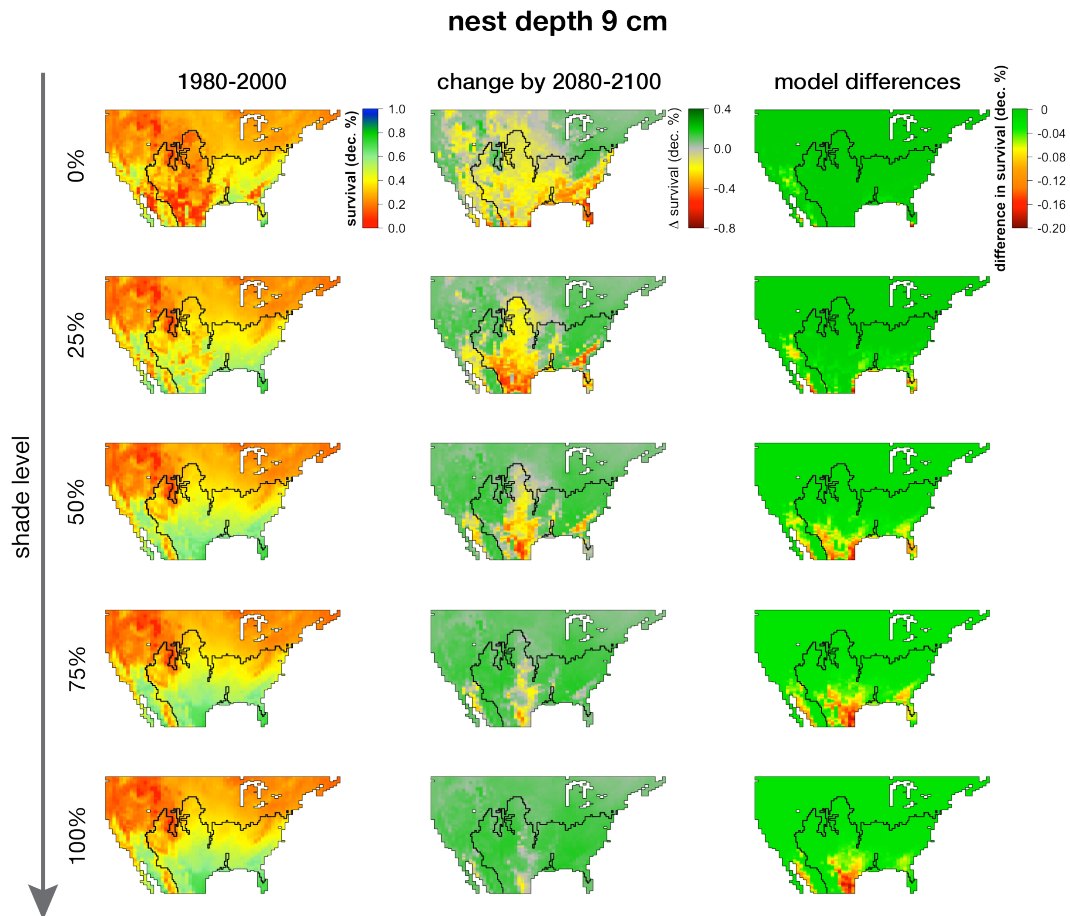


**Figure B20** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in May at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

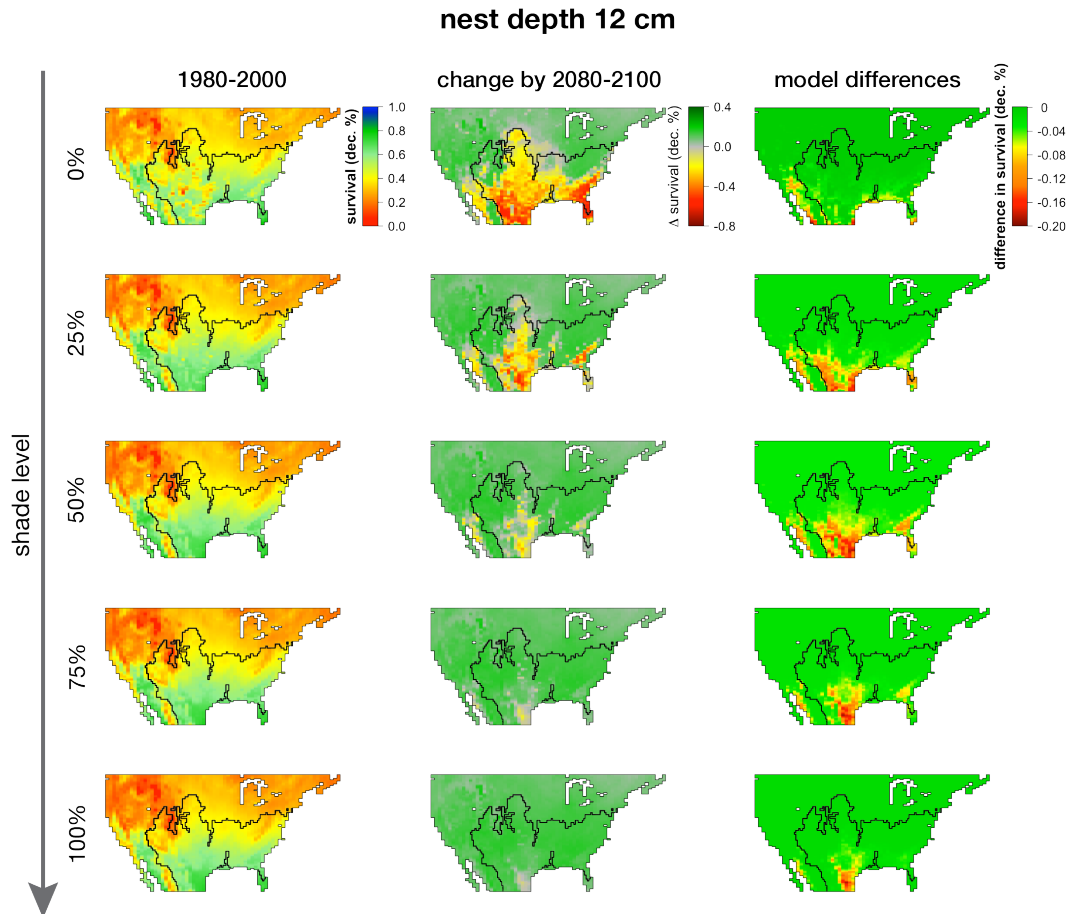




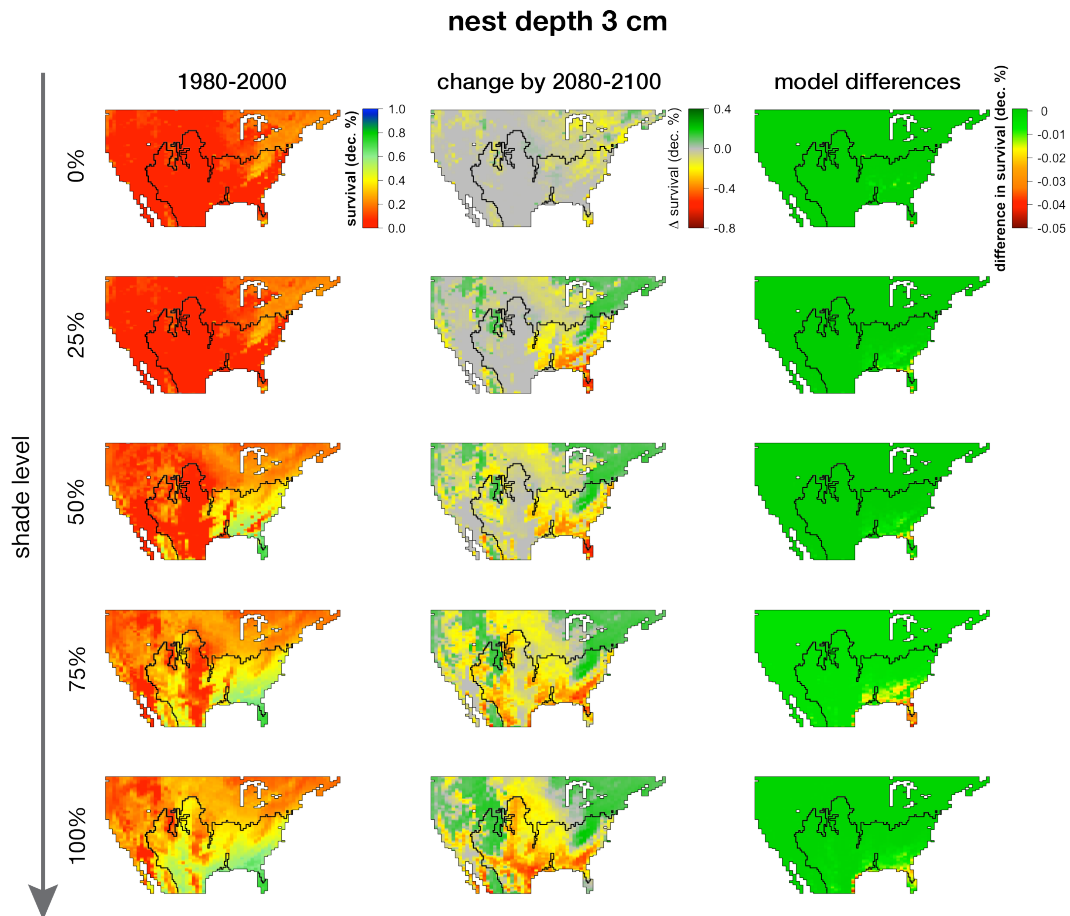
**Figure B21** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in May at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



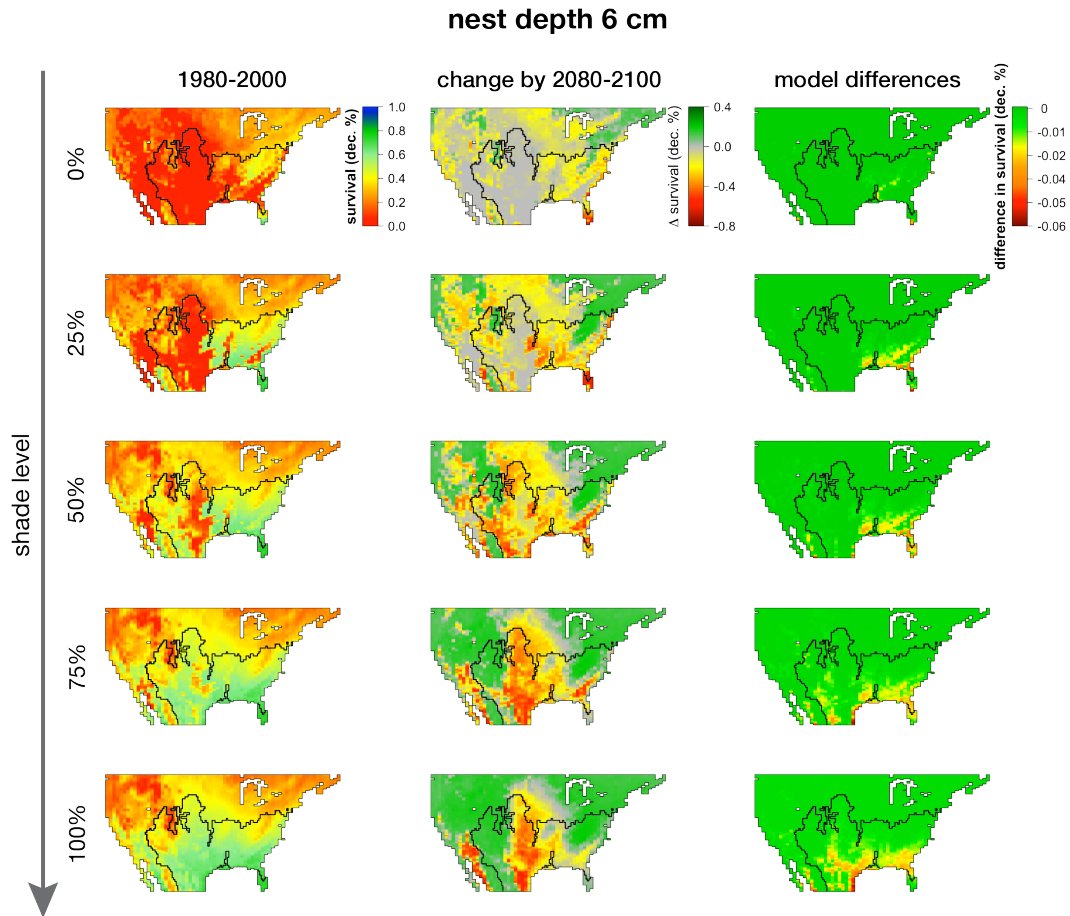
**Figure B22** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in May at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



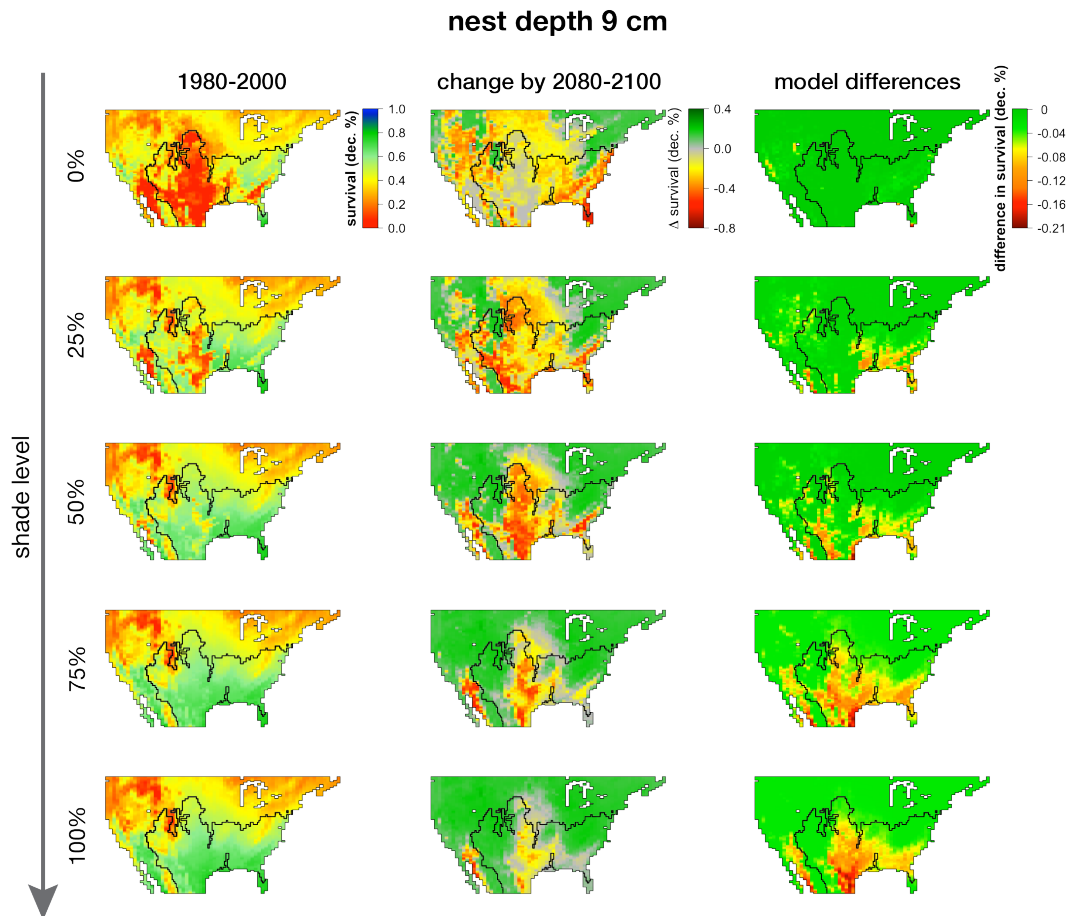
**Figure B23** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in June at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



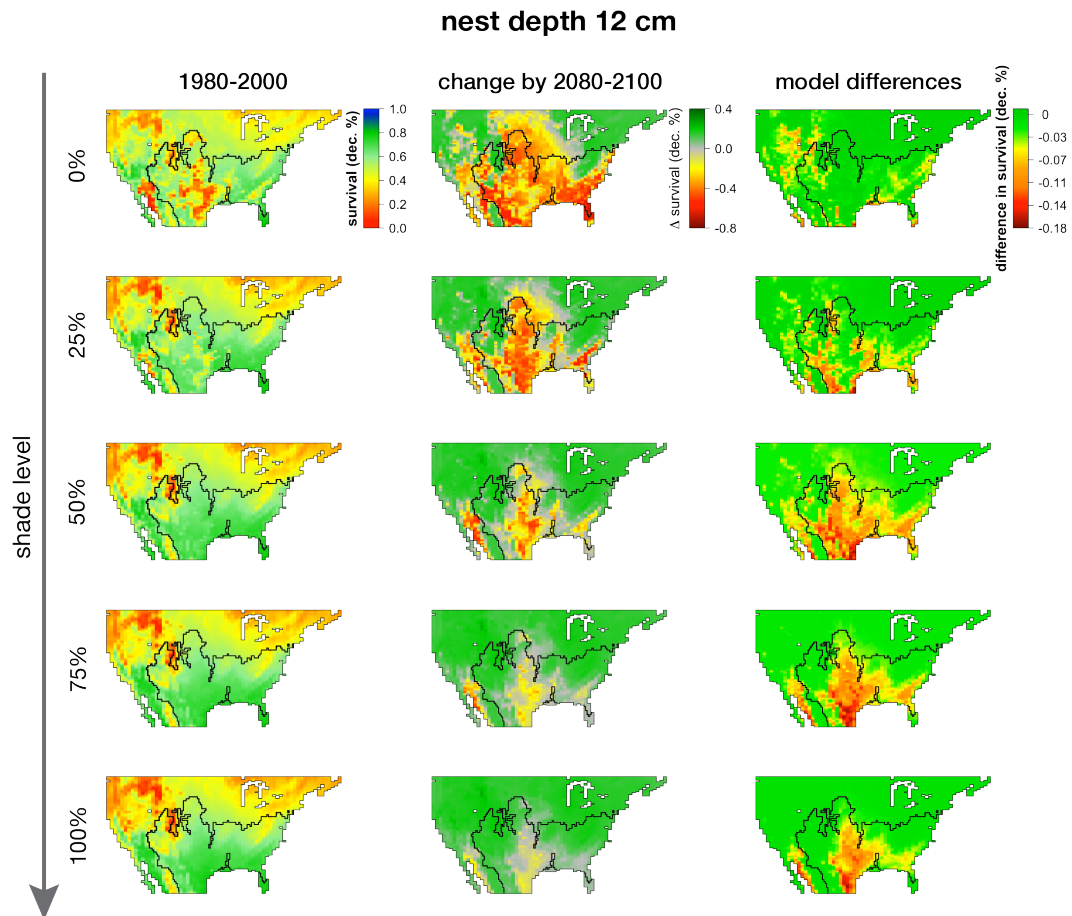
**Figure B24** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in June at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



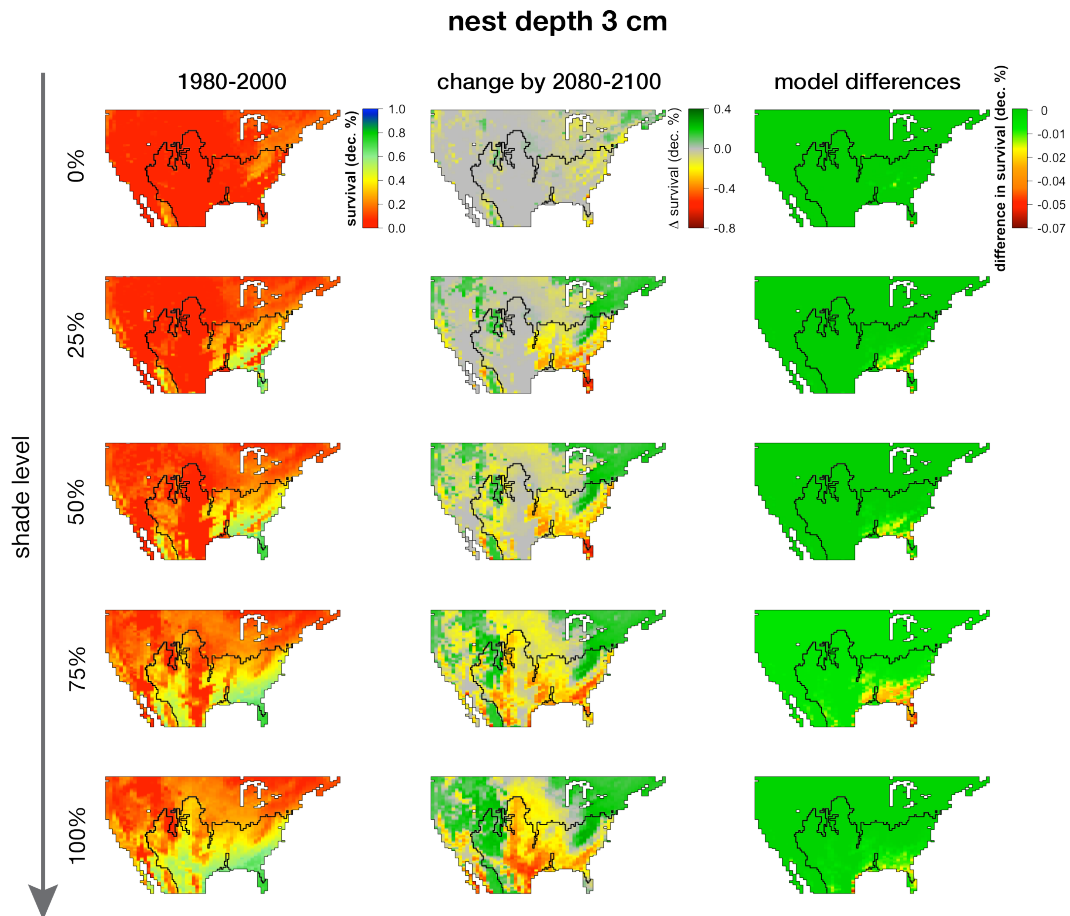
**Figure B25** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in June at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



**Figure B26** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in June at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

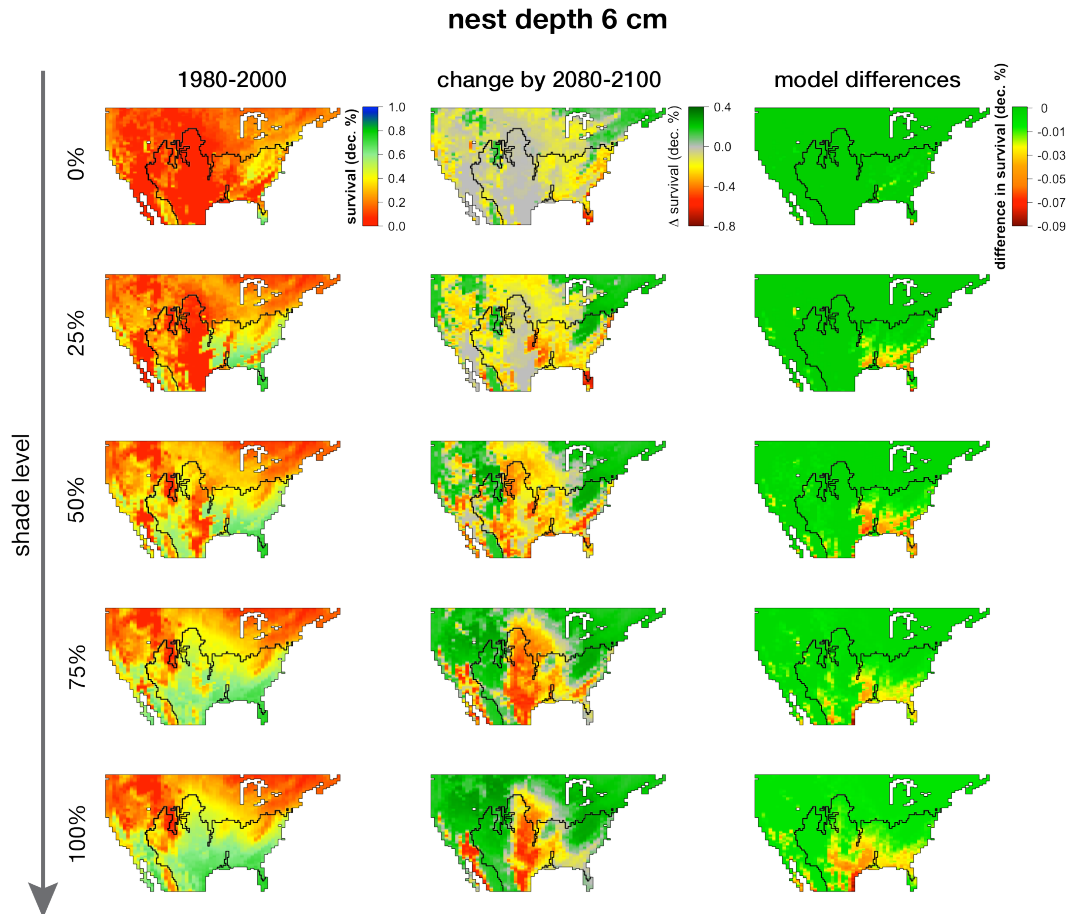


**Figure B27** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in July at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



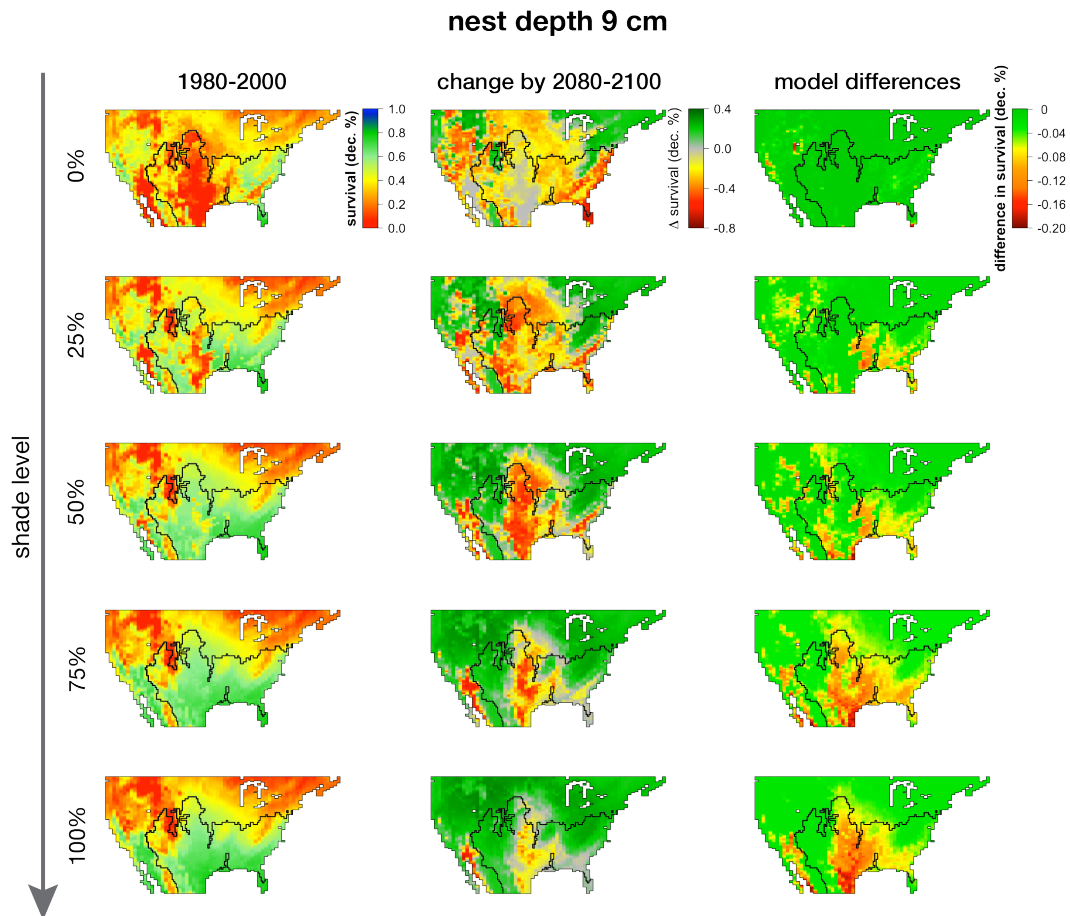


**Figure B28** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in July at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

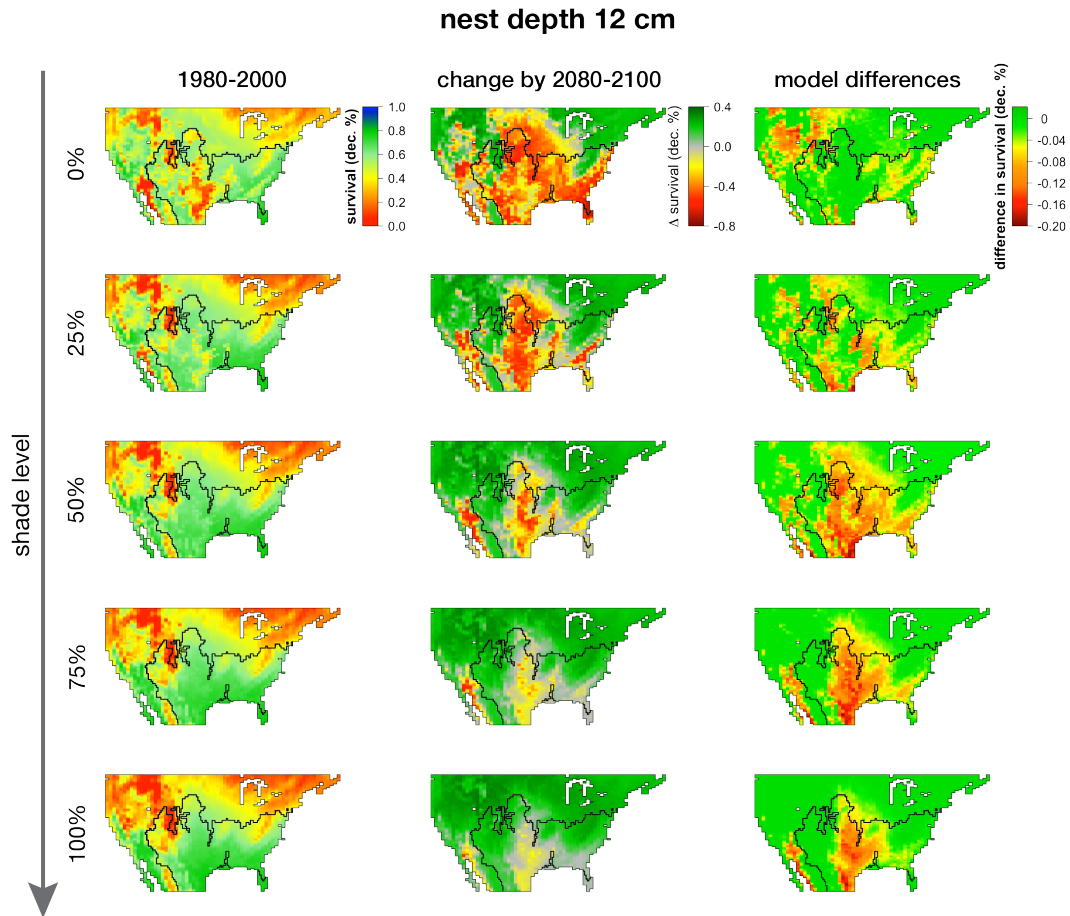




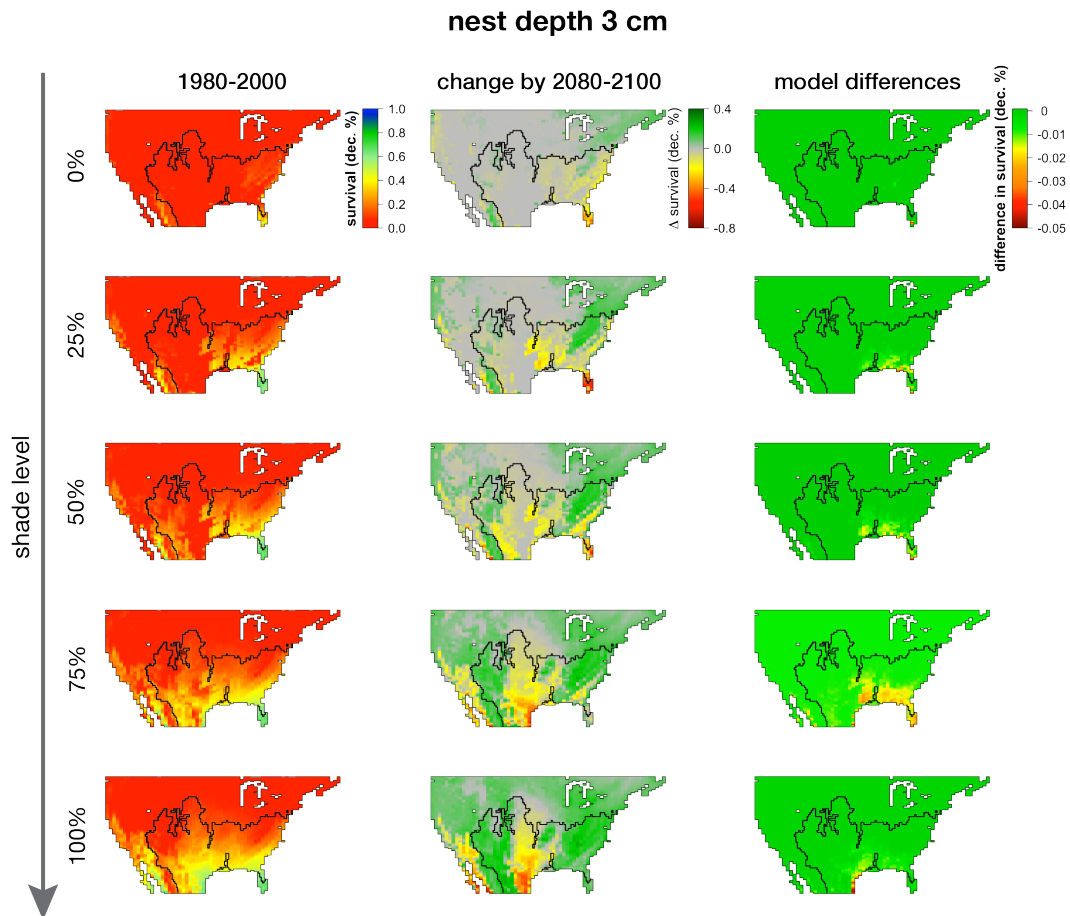
**Figure B29** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in July at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



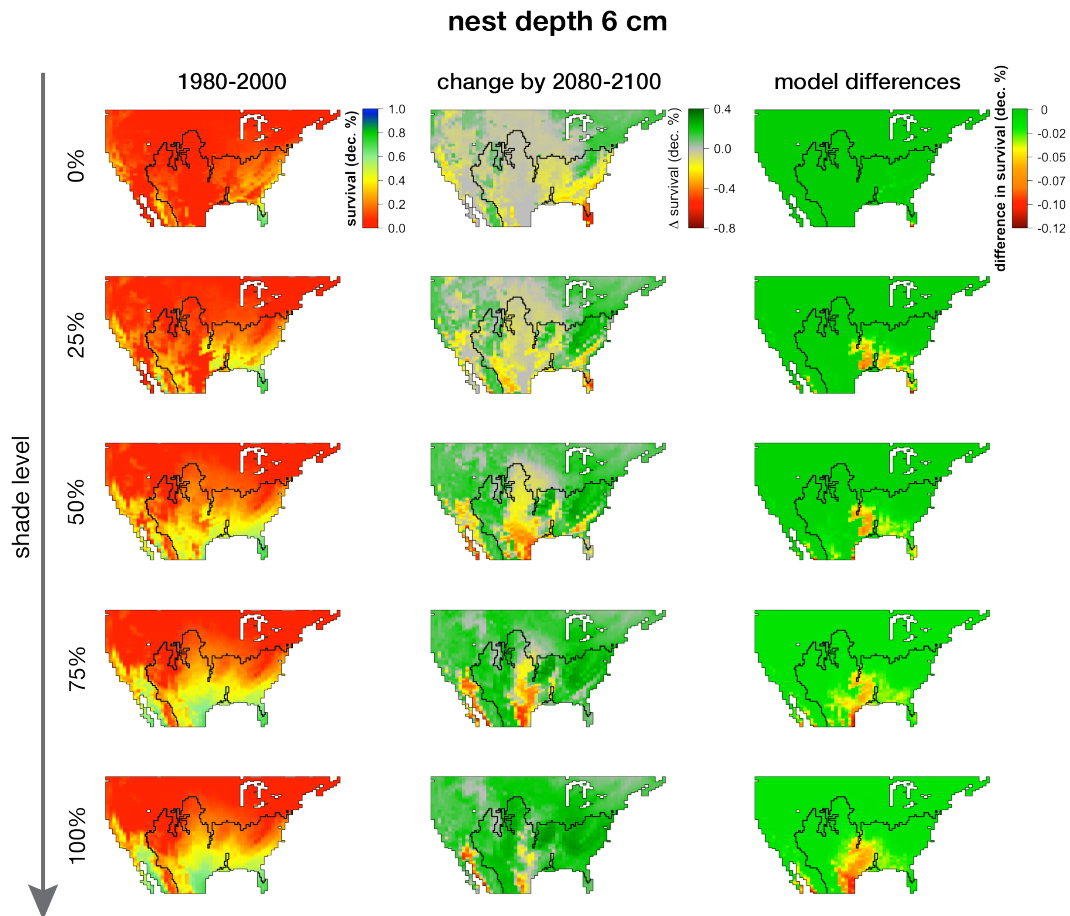
**Figure B30** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in July at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



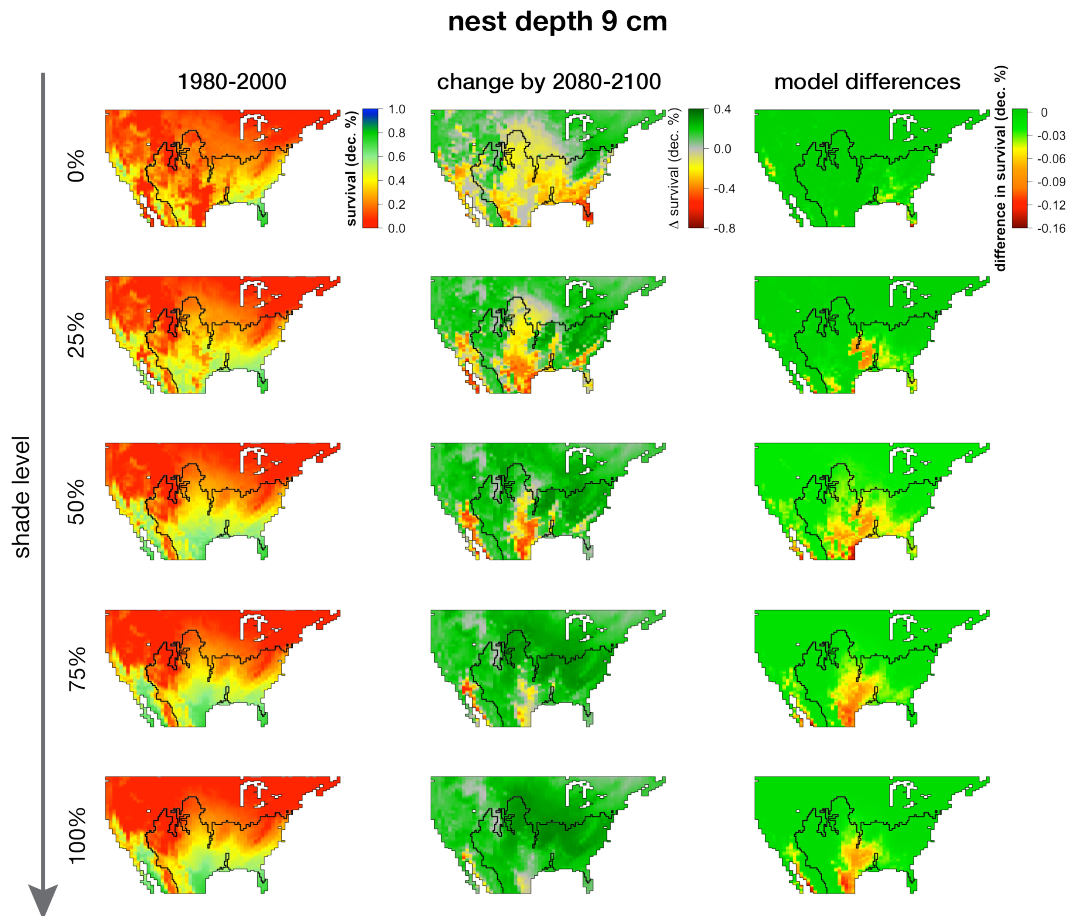
**Figure B31** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in August at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



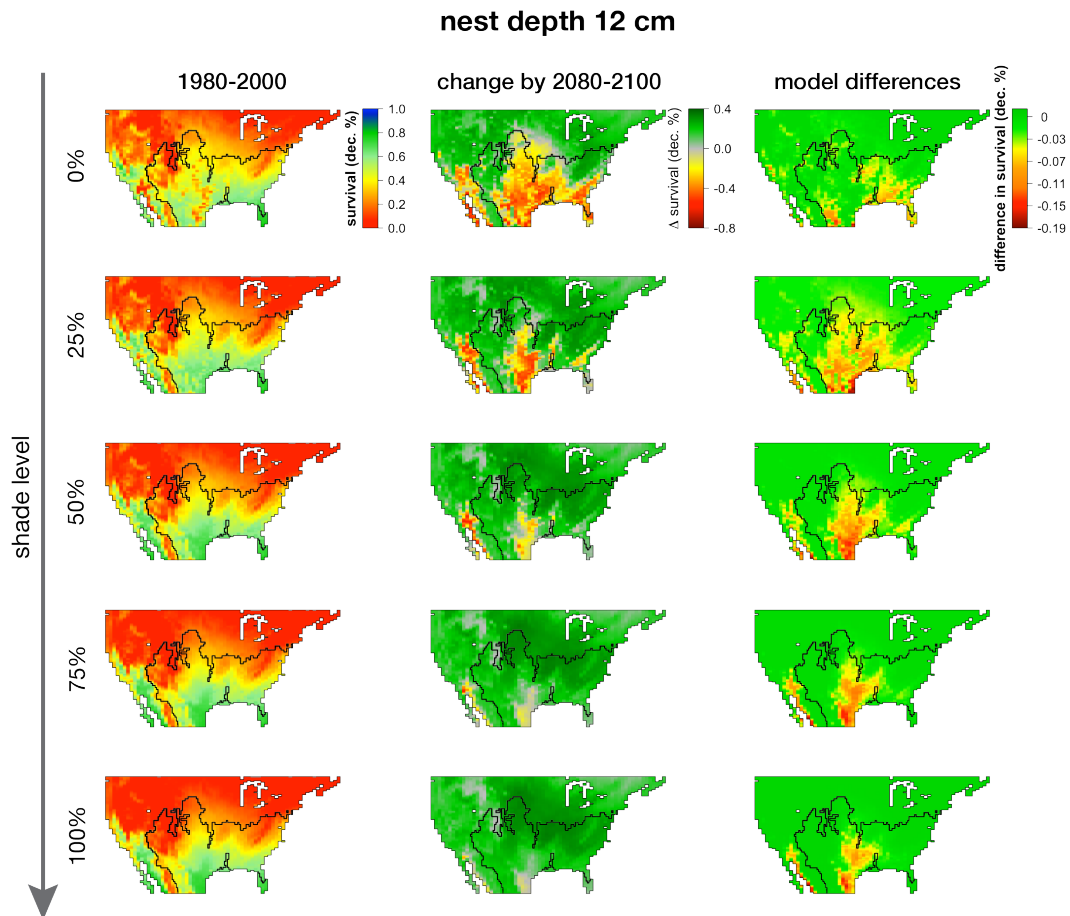
**Figure B32** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in August at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



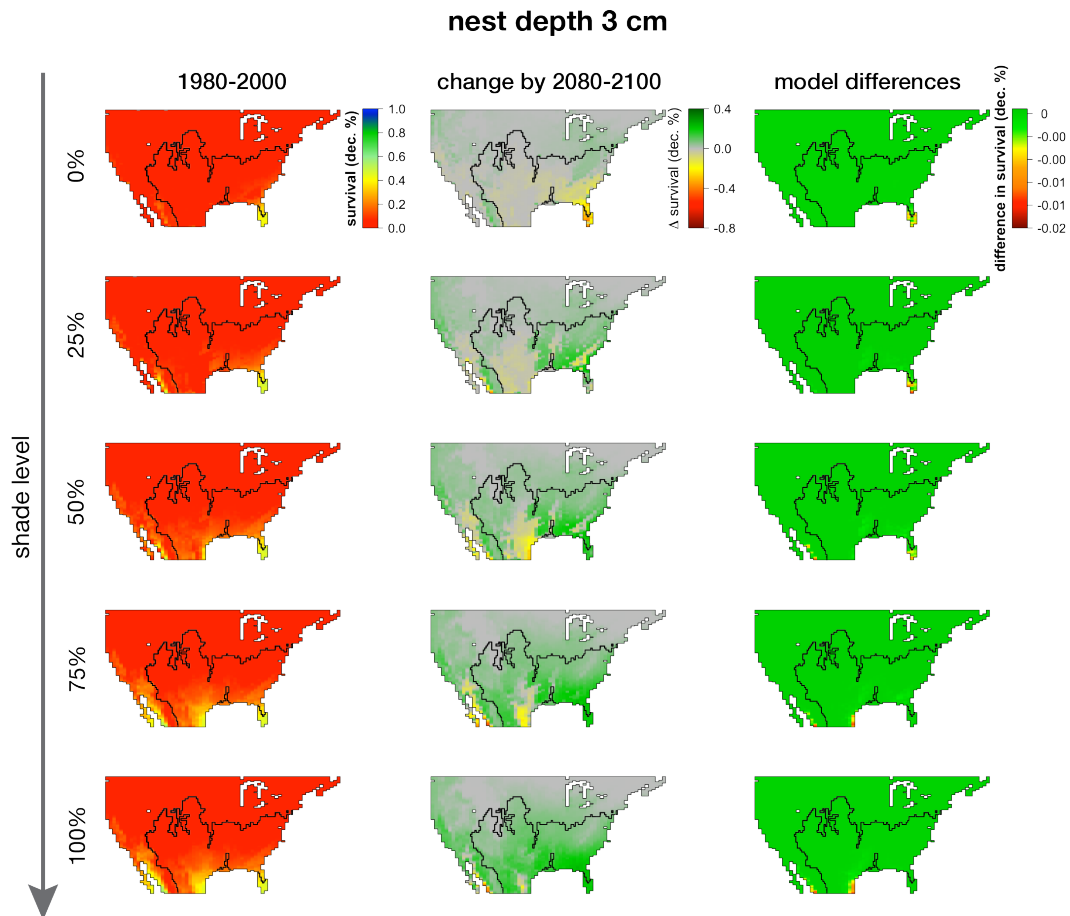
**Figure B33** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in August at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



**Figure B34** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in August at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

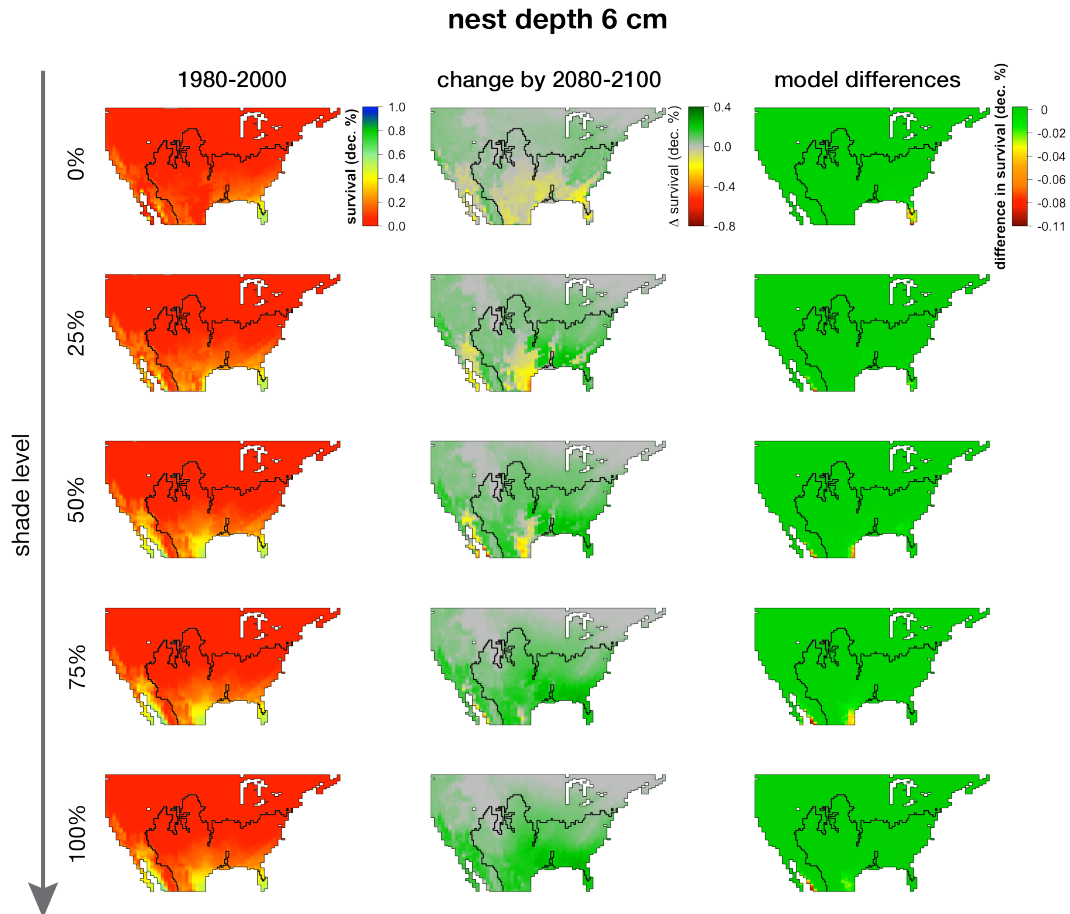


**Figure B35** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in September at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



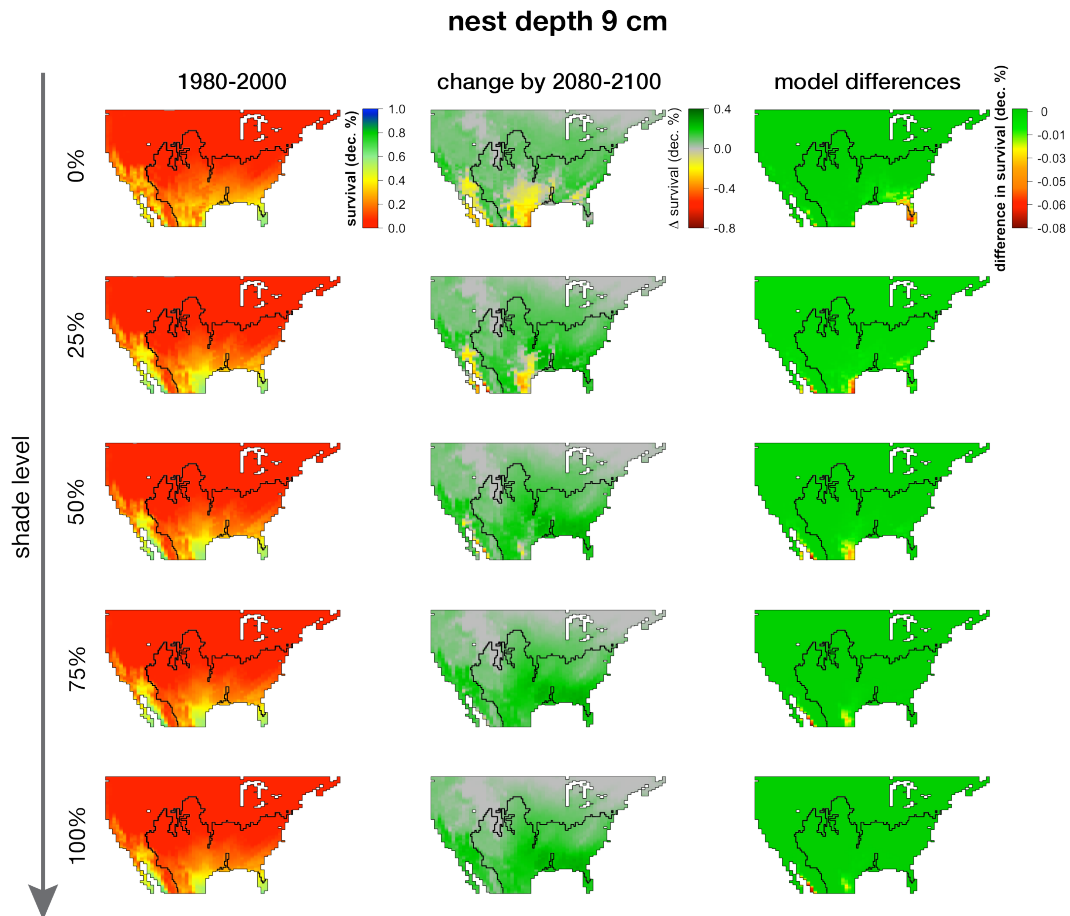


**Figure B36** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in September at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

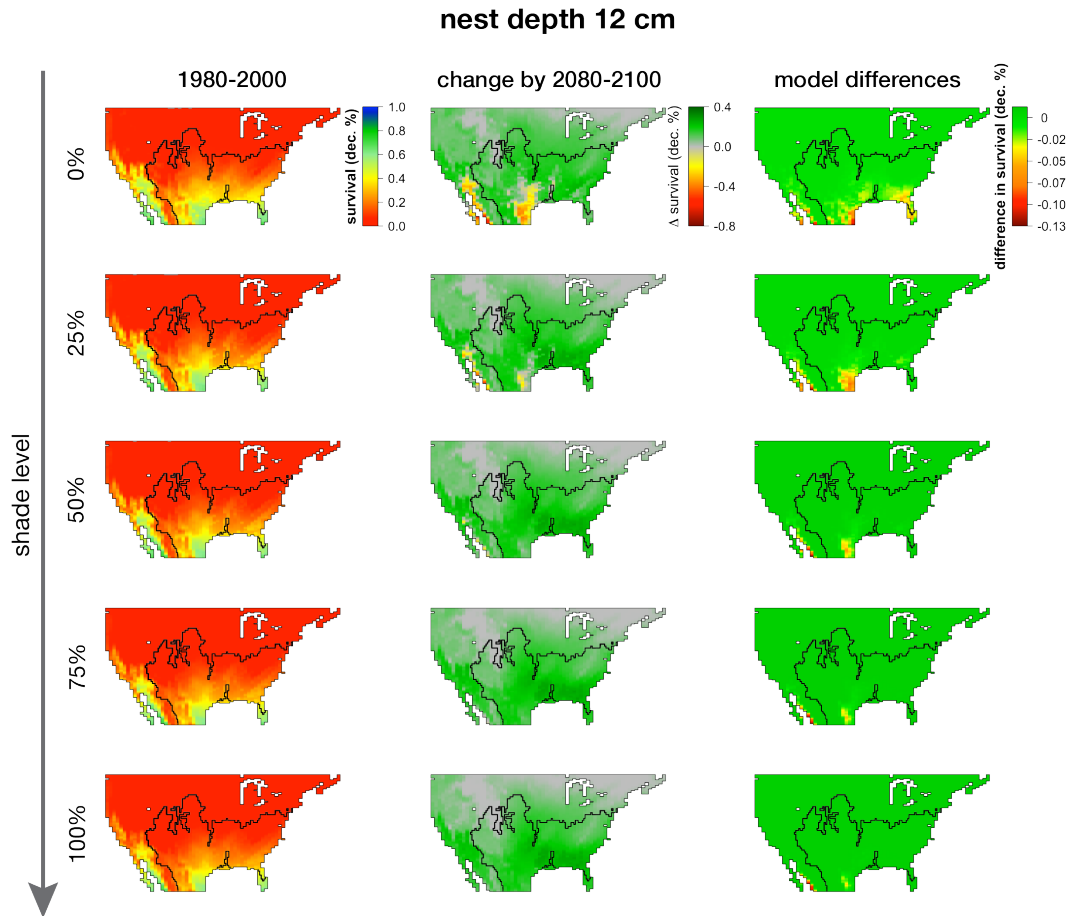




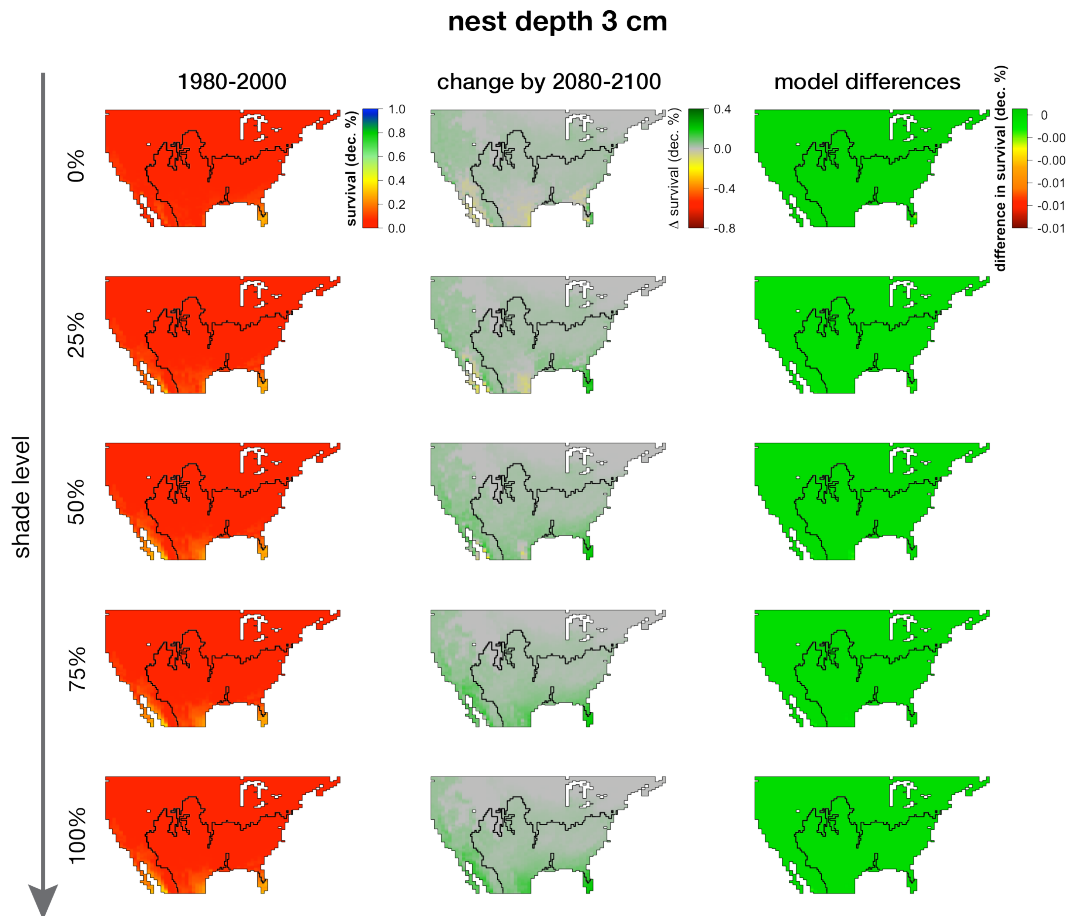
**Figure B37** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in September at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



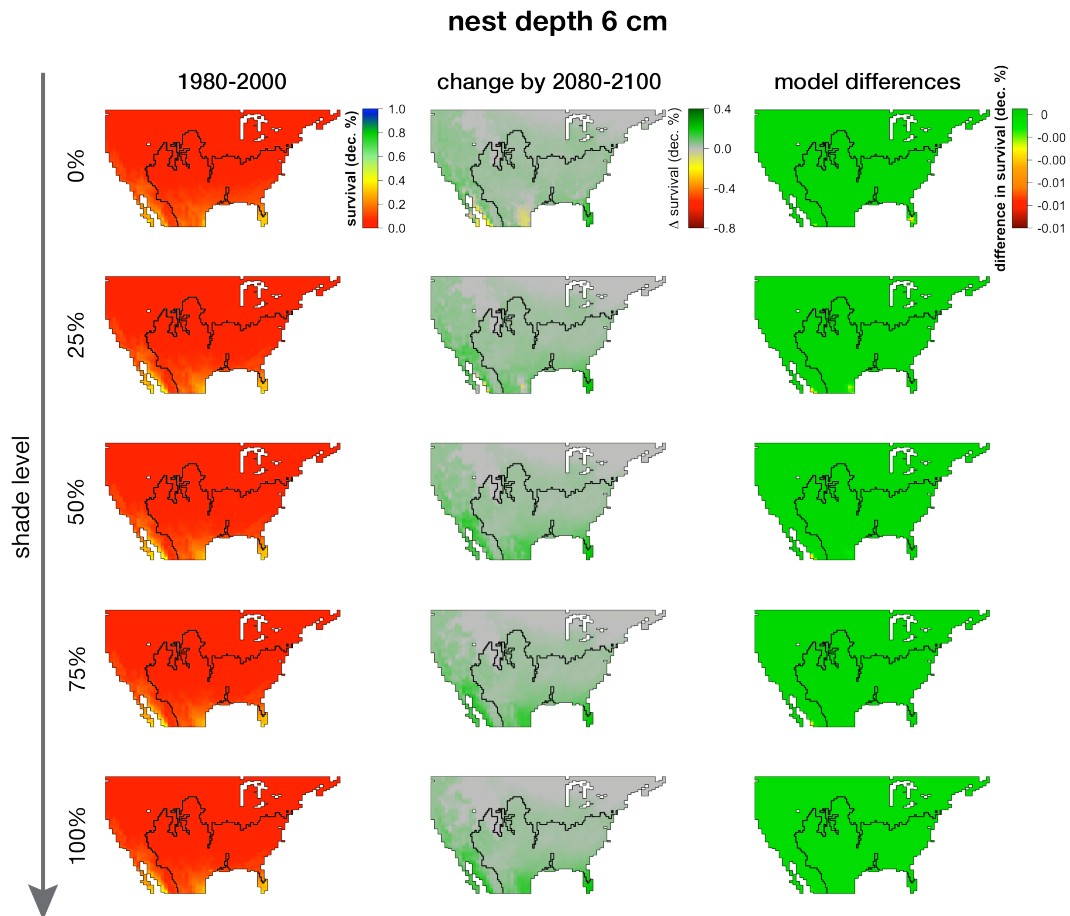
**Figure B38** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in September at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



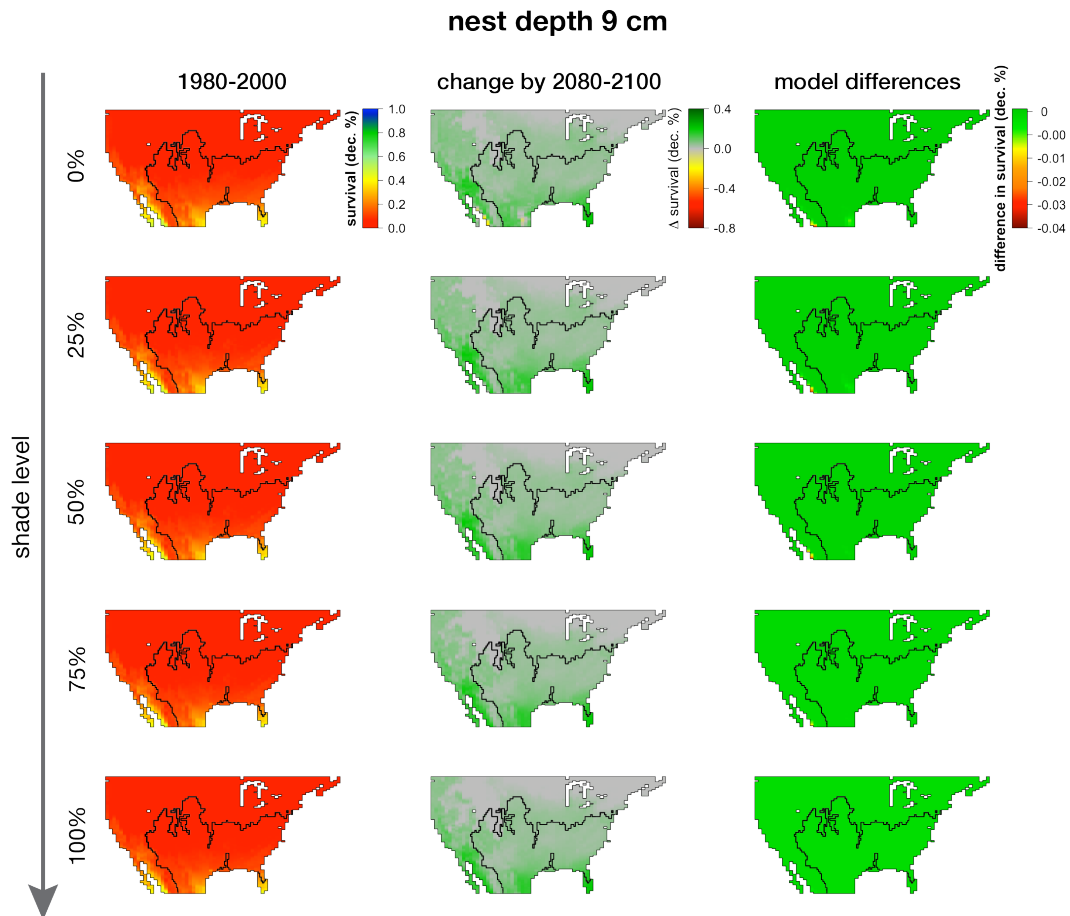
**Figure B39** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in October at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



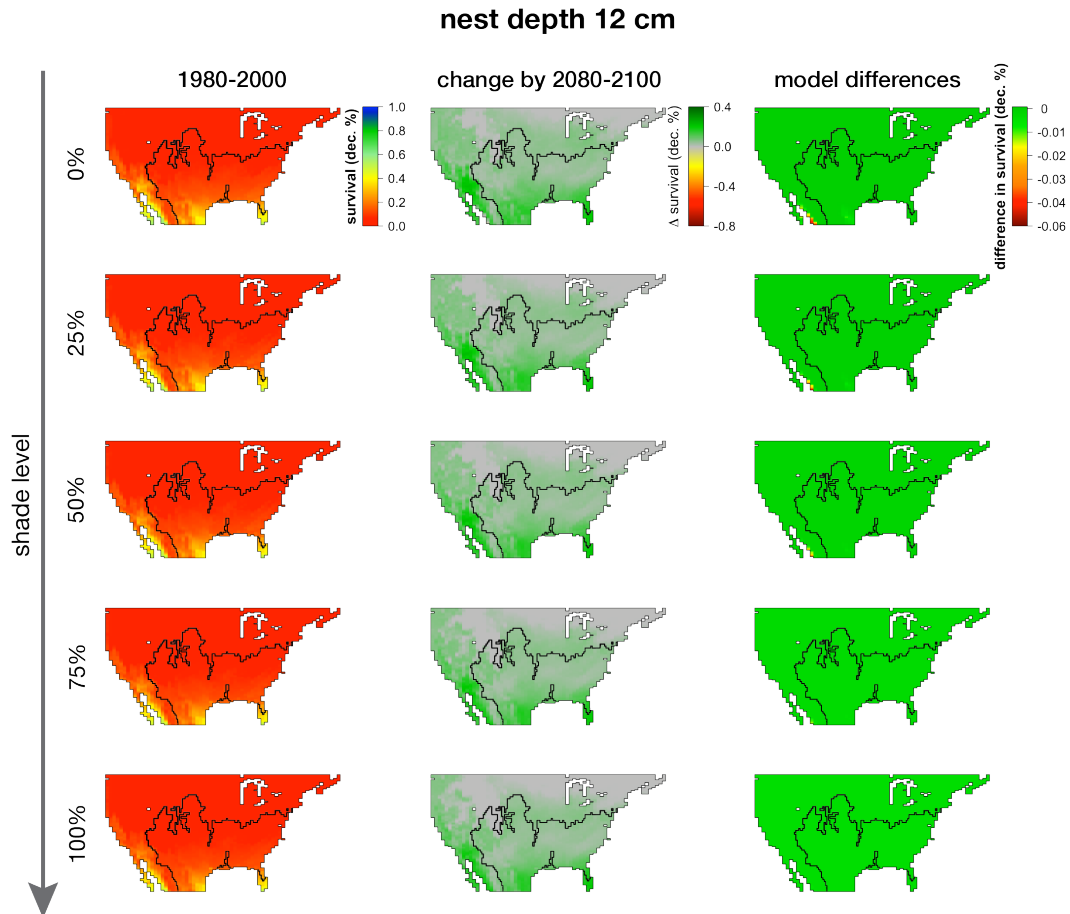
**Figure B40** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in October at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.



**Figure B41** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in October at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

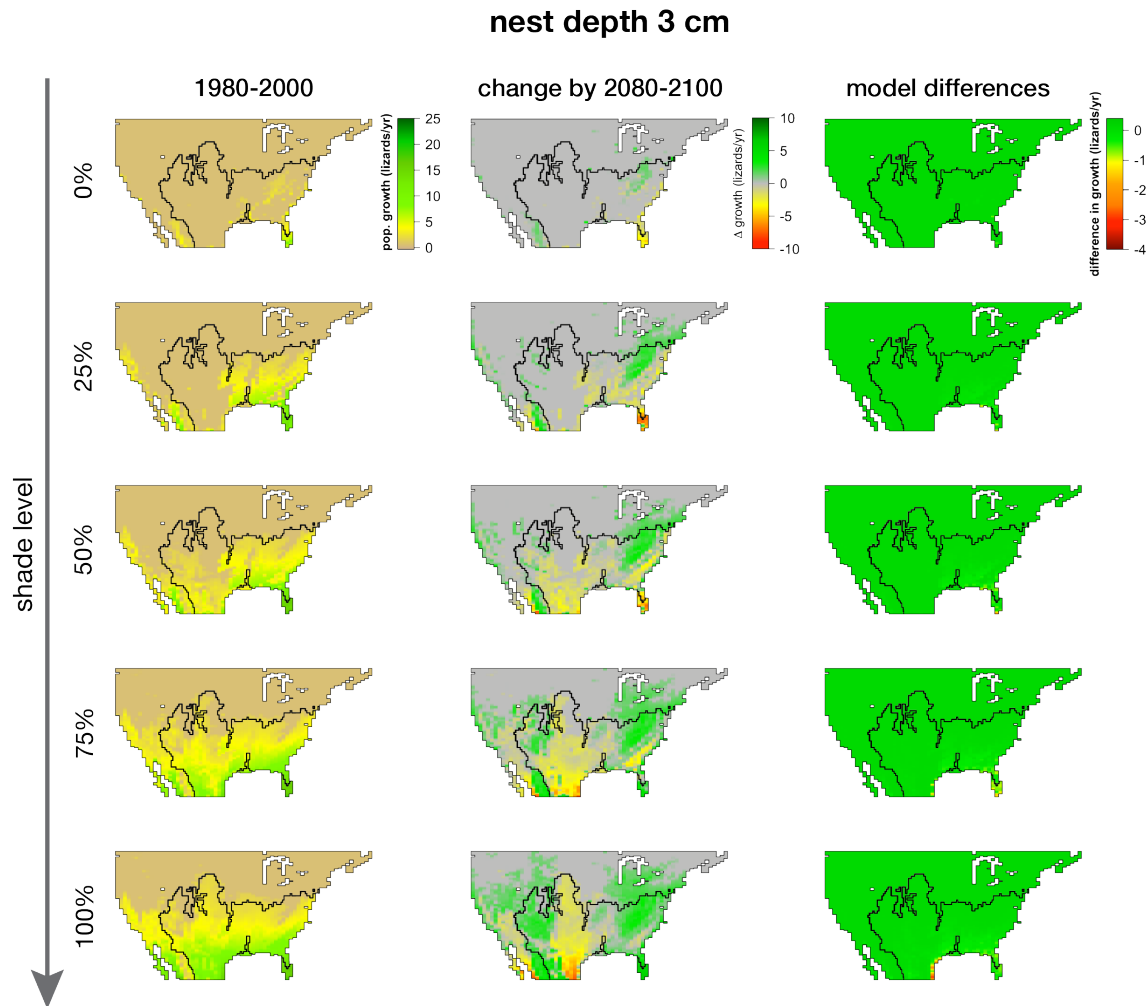


**Figure B42** Predictions of embryo survival in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models for embryos that were laid in October at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of survival by the lethal model. Plots of embryo survival and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S15-S42 to enable visual comparison between different combinations of oviposition months, nests depths, and shade levels. Color scales for model differences are the same within figures but differ between months and depths to enable visual comparison for situations in which the differences in survival are different orders of magnitude.

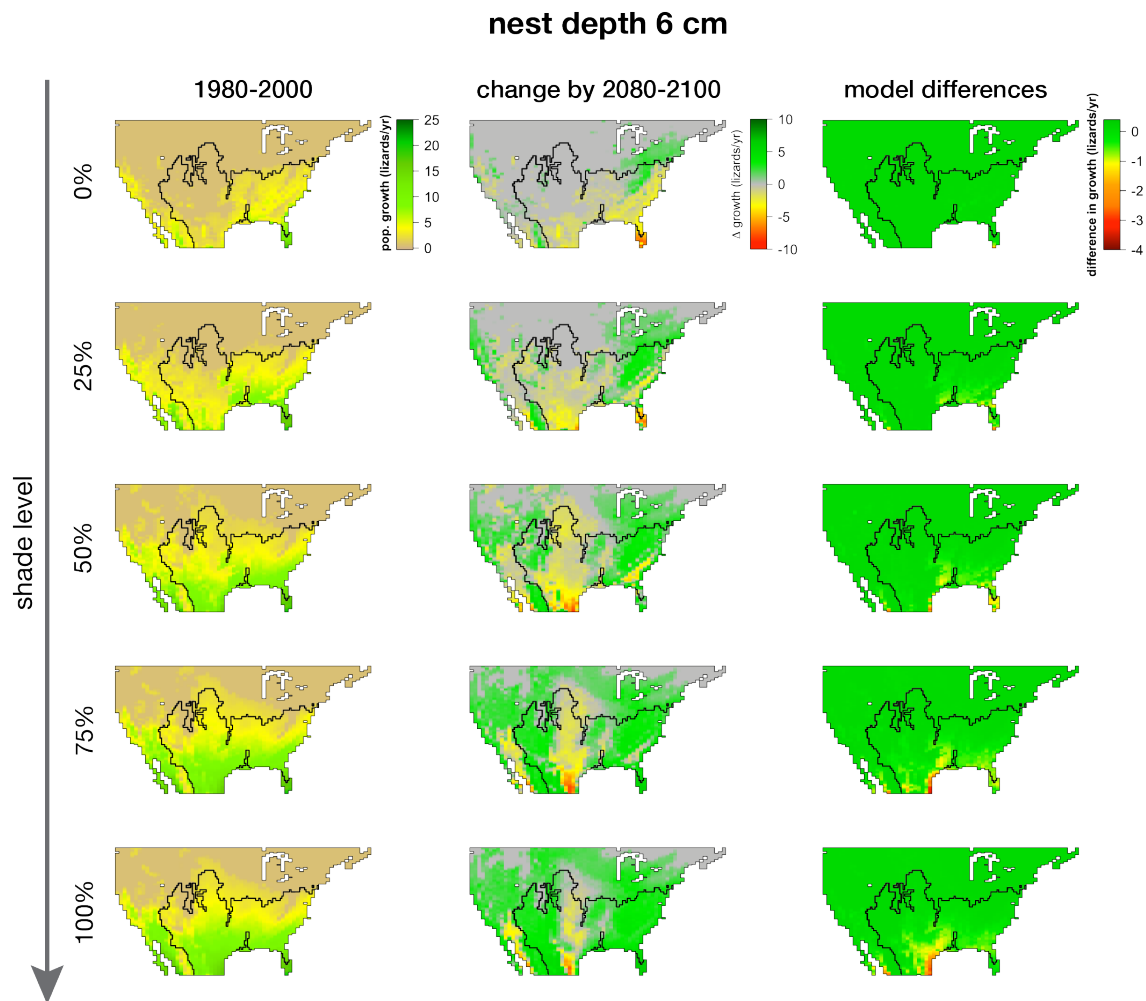


Supplementary figures (Fig. B43-B46) for population growth rates

**Figure B43** Predictions of population growth rates in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models if females dig nests at 3 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of population growth rates by the lethal model. Plots of population growth and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S43-S46 to enable visual comparison between different nesting behavior (nest depth and shade above the nest site).

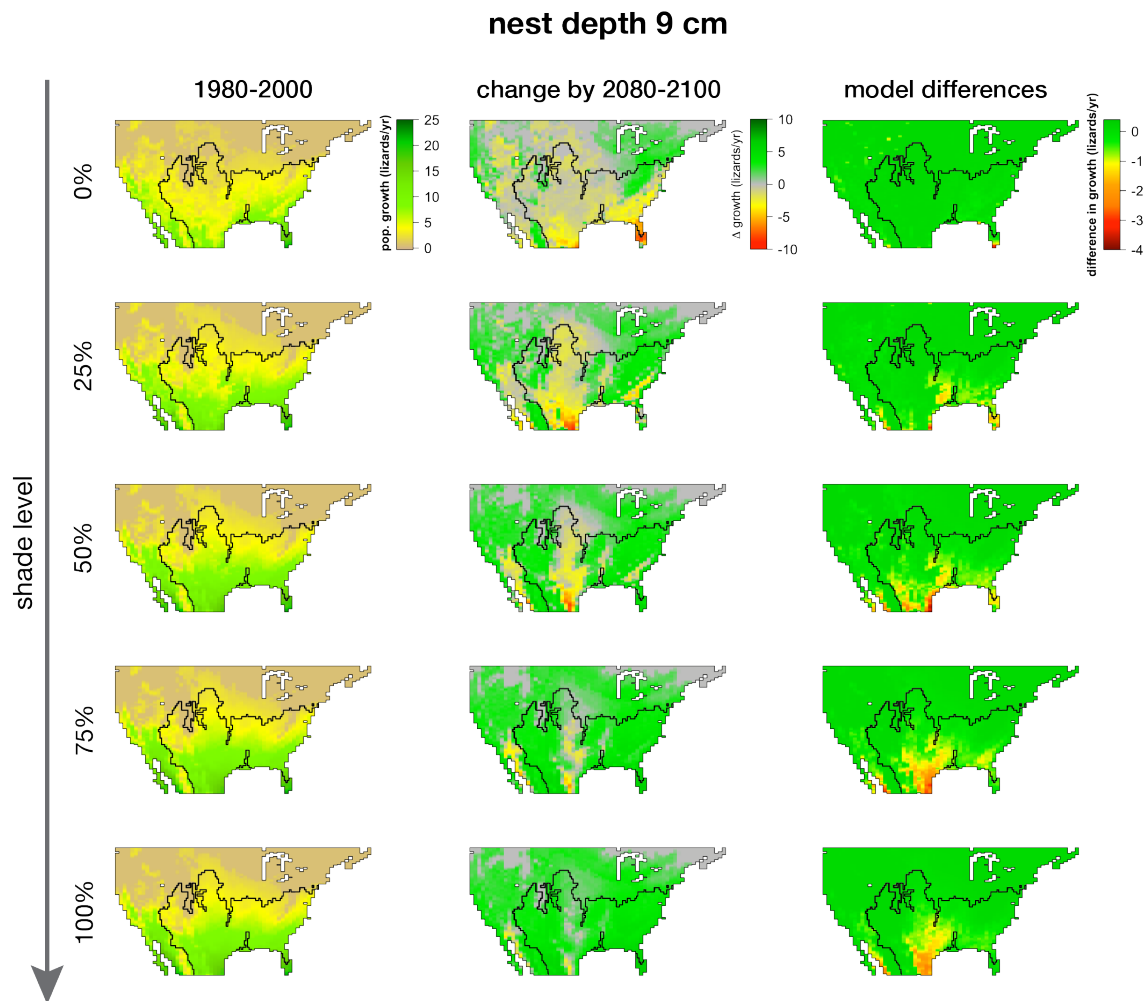


**Figure B44** Predictions of population growth rates in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models if females dig nests at 6 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of population growth rates by the lethal model. Plots of population growth and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S43-S46 to enable visual comparison between different nesting behavior (nest depth and shade above the nest site).

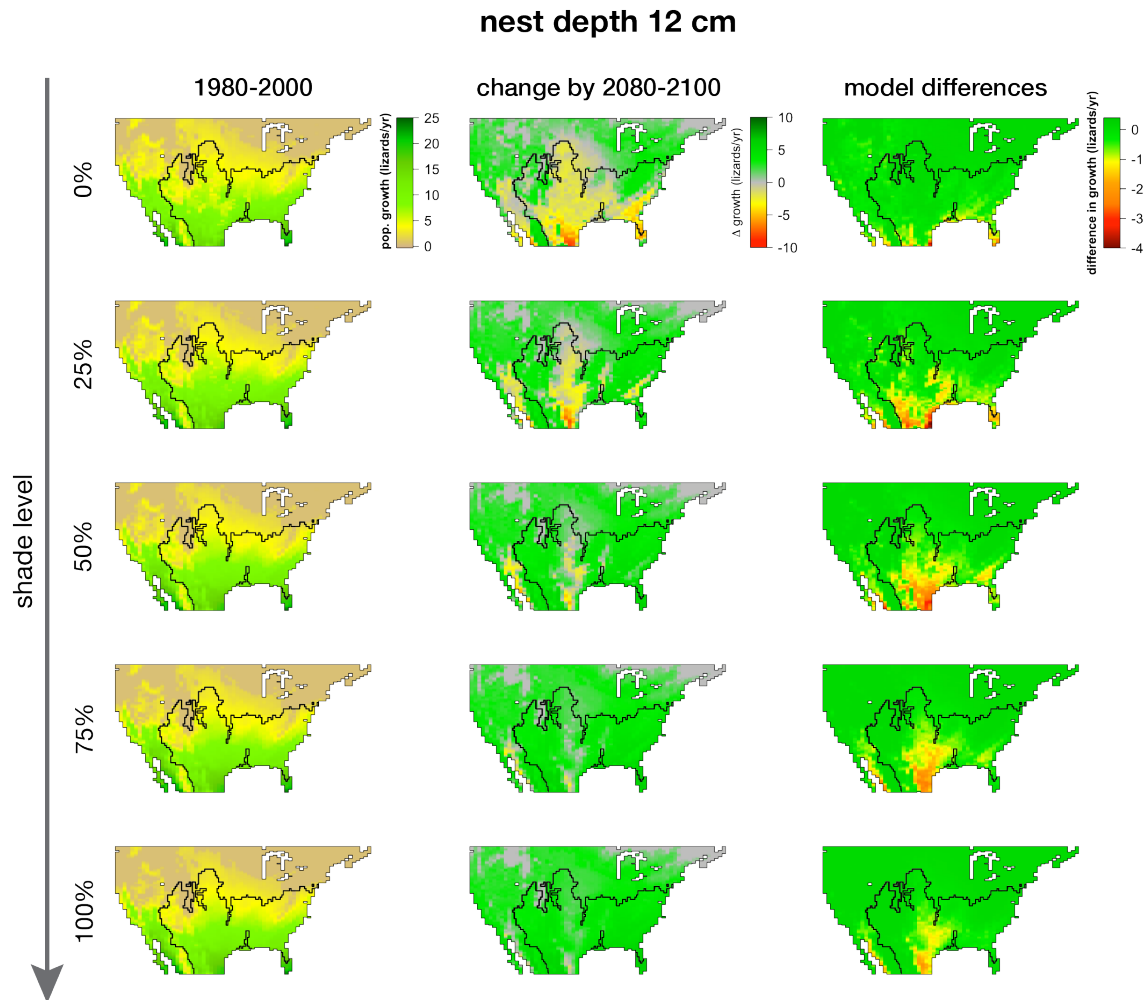




**Figure B45** Predictions of population growth rates in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models if females dig nests at 9 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of population growth rates by the lethal model. Plots of population growth and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S43-S46 to enable visual comparison between different nesting behavior (nest depth and shade above the nest site).

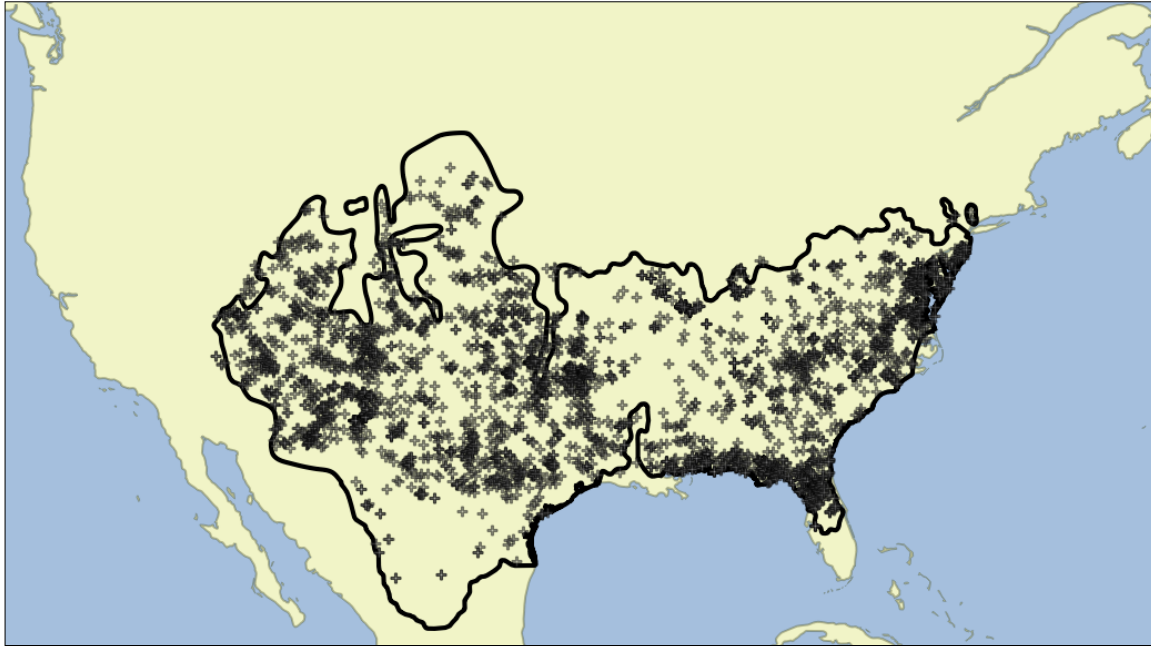


**Figure B46** Predictions of population growth rates in the period 1980-2000, predicted change in the future (2080-2100), and differences between predictions generated by the sublethal and lethal models if females dig nests at 12 cm depth. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017). Negative differences indicate overestimation of population growth rates by the lethal model. Plots of population growth and change by 2080-2100 were generated based on data from the sublethal model. Predictions are shown for a scale of shade conditions above the nest. Color scales are the same for figures S43-S46 to enable visual comparison between different nesting behavior (nest depth and shade above the nest site).



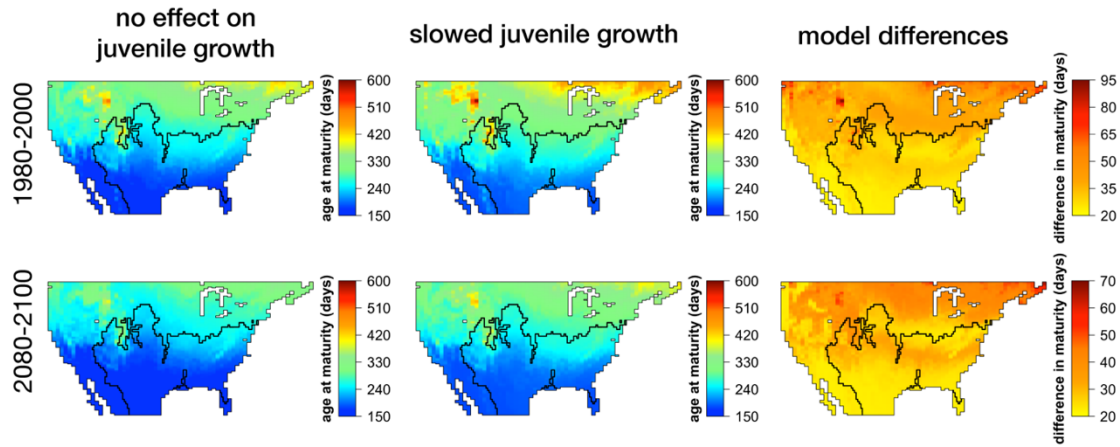
*Supplementary figure for occurrence data used in calculating sensitivity indices*

**Figure B47** Occurrence data downloaded from 11,676 records (VertNet 2016; H.E.R.P. 2017; HerpMapper 2017), which we narrowed down to 4,060 occurrences with location descriptions and with coordinates to at least  $1 \times 10^{-3}$  decimal degrees that matched the described locations. The occurrences are marked on the map with translucent grey crosses, and the black outlines within the map indicate the extant *S. undulatus* range (IUCN 2017).



*Supplementary figure for predicted age at maturity*

**Figure B48** Predictions of time to maturity (days) in the period 1980-2000 and predicted change in the future (2080-2100) with and without the effects of smaller hatchling sizes and slowed juvenile growth, as well as differences between predictions generated by the sublethal and lethal models. Black outlines within maps indicate the extant *S. undulatus* range (IUCN 2017).



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