RESEARCH ARTICLE

Effects of a Novel Climate on Stress Response and Immune Function in Painted Turtles (*Chrysemys picta*)

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JEANINE M. REFSNIDER*, MARIA G. PALACIOS, DAWN M. REDING, AND ANNE M. BRONIKOWSKI
Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa

ABSTRACT

Climate change may subject animals to increasingly stressful environmental conditions, which could have negative physiological consequences if stress levels are elevated for long periods. We conducted a manipulative experiment to determine the effects of a novel climate on stress levels and immune function in a model reptile species, the painted turtle. We collected turtles from four populations across the species' geographic range and housed them in a common-garden in one population's local climate. We measured levels of the stress hormone corticosterone and tested two aspects of innate immune function, bactericidal capacity and natural antibody agglutination, at the time of capture (baseline) and three additional time points over 1 year. The four populations did not differ in corticosterone levels over the course of 1 year, and corticosterone levels were also similar at each sampling period except that post-hibernation corticosterone levels were significantly lower than the previous three time points. Furthermore, we found no evidence that elevated corticosterone depressed immune function in the painted turtle. Our study suggests that turtles exposed to novel climatic conditions did not display a detectable stress response, nor did the novel climate depress immune function in the transplanted populations. Therefore, in terms of innate immune function, turtles may be relatively resilient to at least small changes in climatic conditions. *J. Exp. Zool. 323A:160–168, 2015.* © 2015 Wiley Periodicals, Inc.

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Numerous effects of climate change on natural systems have been documented, including range shifts, changes in phenology, altered species interactions, and shifts in community structure (reviewed in Parmesan, 2006). Climate change also affects the health of individuals and populations through increased exposure to novel diseases and pathogens (e.g., Kutz et al., 2005; Brooks and Hoberg, 2007; Lu et al., 2011) and prolonged periods of stressful environmental conditions (Romero and Wikelski, 2001), which can depress immune function (Burek et al., 2008; Martin et al., 2010) and alter metabolic rates (Dillon et al., 2010). In light of this knowledge, it is critical to quantify the physiological consequences of climate change to determine whether populations can acclimate to particular levels of environmental disturbance, and whether certain populations or

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Maria G. Palacios's current address: Consejo Nacional de Investigaciones Cientificas y Tecnicas Chubut, Argentina.

Dawn M. Reding's current address: Department of Biology, Luther College, Decorah, Iowa.

*Correspondence to: Jeanine M. Refsnider, Department of Environmental Science, Policy, and Management, University of California, Berkeley, 130 Mulford Hall, Berkeley 94720-3114, CA.
E-mail: refsnider@berkeley.edu

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species differ in their tolerance of environmental disturbance (Walker et al., 2005; Wikelski and Cooke, 2006).

The animal endocrine system is one physiological system that may be particularly susceptible to negative effects of climate change. In vertebrates, steroid hormones, such as glucocorticoids, are released in response to sudden stressors, such as capture by a predator (e.g., Moore et al., '91; Cash et al., '97), or changes in temperature (Lutterschmidt and Mason, 2009). The rate and magnitude of increase in plasma glucocorticoid concentration are indicators of the sensitivity of the hypothalamic-hypophyseal-adrenal axis and can be used to measure an individual's sensitivity to a stressor. The rapid elevation of glucocorticoid level is advantageous in directing energy towards activities essential for survival (i.e., the fight-or-flight response). However, glucocorticoids can also have harmful effects, known as allostatic overload, when levels are elevated for prolonged periods (McEwen and Wingfield, 2003).

Extreme weather events, such as severe El Niño events, floods, and droughts, can cause prolonged elevation of glucocorticoids (e.g., Romero and Wikelski, 2001), and increased glucocorticoids are often correlated with depressed immune function (Millet et al., 2007). Thus, populations exposed to prolonged periods of environmental disturbance, such as higher temperatures and increased frequency of extreme weather events associated with global warming (IPCC, 2007), are likely to experience physiological stress in the form of increased glucocorticoid levels. Elevated glucocorticoid levels may, in turn, lead to health declines in general and depressed immune function in particular, and could increase mortality in vulnerable populations (Jessop et al., 2013). Alternatively, the endocrine system, and plasticity within it, may buffer animals against some degree of climate change. Instead of acting as stress hormones, glucocorticoids may instead be "anti-stress hormones" such that increases in glucocorticoid levels following environmental perturbations might function to avoid chronic stress through flexibility in the suite of physiological and life-history responses to a stochastic environment (Wingfield and Kitaysky, 2002).

In reptiles, the main glucocorticoid hormone is corticosterone. Elevated levels of plasma corticosterone in reptiles are associated with predictable life-history events, such as nesting (e.g., tuatara: Cree and Tyrrell, 2001), giving birth (e.g., rattlesnakes: Taylor et al., 2004), and hibernation (e.g., garter snakes: Lutterschmidt and Mason, 2009). Less well-known, however, are the potential effects of unpredictable environmental perturbations on corticosterone levels in reptiles, how such perturbations might influence any physiological effects of increased corticosterone, and whether individuals can acclimate to increased levels of corticosterone over time (Landys et al., 2006). Because organismal responses to stressors, such as novel climatic conditions, likely vary among species and even populations (e.g., Schultner et al., 2013), it is important to take a comparative approach when examining physiological tolerance and reaction to environmental perturbations such as climate change (e.g., Moore et al., 2000).

Global climate change likely poses an imminent threat to turtles. In addition to potential sex ratio skews in turtles with temperature-dependent sex determination (Janzen, '94), temperatures increasing beyond their thermal optima are likely to cause physiological stress in turtles (Ihlow et al., 2012), which may manifest as elevated stress hormone levels or altered baseline and maximal stress response. Populations near the equatorial border of species' ranges may be particularly vulnerable to physiological impacts of climate change, as they already persist in environments near their thermal tolerance threshold (Dunlap and Wingfield, '95; Dillon et al., 2010; Mitchell and Janzen, 2010). Altered stress hormone levels, in turn, could lead to chronic depression of immune function, lowered body condition, and decreased reproductive success. For example, as climate change progresses, winters are becoming shorter (IPCC, 2007), which has already caused early spring emergence in hibernating mammals (Inouye et al., 2000) and will likely disrupt hibernation of reptiles as well. Immune function is known to be depressed during hibernation in painted turtles, possibly because low temperatures and small energy budgets during winter reduce turtles' need or ability to mount an immune response (Schwanz et al., 2011). If climate change induces early emergence from hibernation, and recovery of immune function is delayed, turtles may be at increased risk of infection (Schwanz et al., 2011). Currently, however, the potential effects of climate change on stress levels and physiology are virtually unknown in turtles.

We transplanted adult female painted turtles (*Chrysemys picta*) from four populations across a 12° latitudinal gradient to novel climatic conditions, a proxy for rapid climate change, in a common-garden environment. We collected plasma samples at four time points over 1 year, measured levels of the stress hormone corticosterone, and conducted two assays of innate immune function which have previously been used in reptiles, and which are negatively correlated with corticosterone levels in birds (Millet et al., 2007). We tested two hypotheses: 1) Populations exposed to a novel climate will exhibit higher levels of corticosterone compared to locally-adapted populations; and 2) Across all individuals, corticosterone levels are inversely correlated with immune function in turtles. Our comparative approach of quantifying the physiological effects of novel climatic conditions on multiple populations from different local climates will provide insight into the vulnerability of freshwater turtles to climate change.

MATERIALS AND METHODS

We conducted this study using the western painted turtle, *Chrysemys picta bellii*. Painted turtles occur in a wide variety of aquatic habitats across much of the United States and southern Canada; the western subspecies occurs primarily west of the

Mississippi River. Females emerge from wetland habitats in May and June to nest in open areas such as beaches and lawns. Nesting frequency is population-specific, with individual females nesting from one to three times per year. The length of hibernation is also population-dependent, with turtles from northern (i.e., poleward) populations inactive for up to 5 months per year, whereas turtles at the southernmost (i.e., equatorial) end of the species' range may only be dormant for a month (Ernst and Lovich, 2009). All research was conducted in accordance with Institutional Animal Care and Use Committee protocol #1–09-6677-J (Iowa State University).

Animal Collection and Husbandry

We collected 11–15 adult, female *C. picta bellii* between 26 April and 22 May 2009, before the onset of nesting, from each of four populations throughout the subspecies' U.S. range: Pierce National Wildlife Refuge, Skamania Co., WA, USA (45° 37′42″N, 122°00′52″W); Story Co., IA, USA (42°03′45″N, 93° 37′49″W); Thomson Causeway Recreation Area, Carroll Co., IL, USA (41°57′23″N, 90°07′49″W); and Bosque del Apache National Wildlife Refuge, Socorro Co., NM, USA (33°46′15″N, 106° 54′07″W (Fig. 1). Because these populations span a latitudinal gradient of 33°–45°N, they experience a range of climatic conditions to which they are locally adapted (Fig. 2). All turtles were individually marked by filing a unique combination of notches into the marginal scutes. In addition, a 0.5 mL blood sample was collected from the caudal vein of each turtle using a 28-gauge heparinized syringe within 3 min of capture. Blood samples were centrifuged for 2 min at 6000 rpm, and plasma was removed and stored on dry ice. Following transport to Iowa State University, plasma samples were stored at −80°C. Plasma samples collected at the time of capture represented "baseline" corticosterone and immune levels in each population.

Turtles from all four populations were housed in a series of identical, semi-natural artificial ponds at Iowa State University's outdoor Aquatic Research Facility (Story Co., IA, USA; see Refsnider and Janzen, 2012 for details on husbandry). Each population was housed in a separate replicate 15 × 40 m pond, which was surrounded by a 0.5 m-high drift fence, set back 7 m from the pond edge, to prevent turtles from travelling to adjacent ponds. Pond depths graded from 2 to 3 m from one end to the other, and were filled with lake water 3 weeks before introducing the experimental turtles to allow sufficient time for colonization by local aquatic plants, invertebrates, and anurans, all of which are food items for painted turtles. All ponds contained two identical basking logs and had equivalent area along the pond edges for basking. The perimeters of all ponds were patrolled hourly from 27 May to 3 July 2009 during the hours of 0600–1000 hr and 1500–2100 hr, the times of peak painted turtle nesting activity. Turtles observed nesting were monitored from a distance to prevent nest abandonment due to disturbance. Upon completion of nesting, females were briefly captured for identification and collection of a plasma sample as described above; females were then released back into their pond. These plasma samples represented the "nesting" time point.

In October 2009, the experimental ponds were drained and turtles were retrieved. Plasma samples were collected from all individuals following removal from the artificial ponds, and constituted the "pre-hibernation" sample. All turtles were then overwintered at 4°C in a controlled environmental chamber at

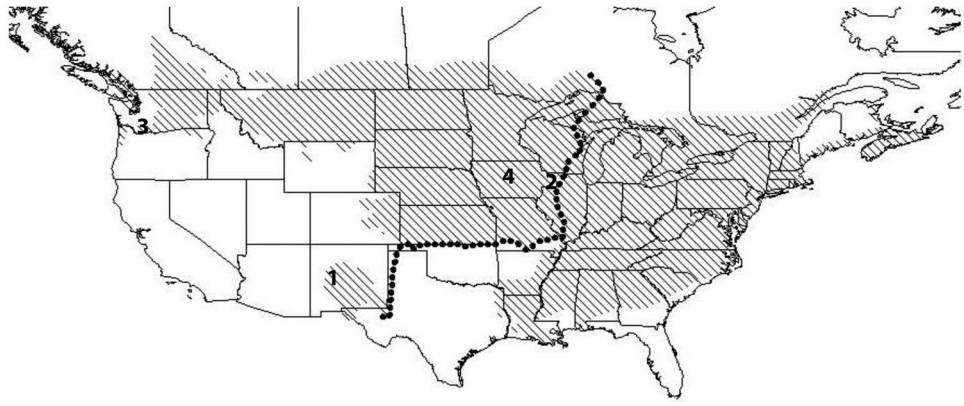

Figure 1. Geographic range of the painted turtle, *Chrysemys picta* (shading). The western subspecies (*C. p. bellii*) occurs west of the dotted line. Numbers indicate the sites from which females were collected: Bosque del Apache National Wildlife Refuge, Socorro County, New Mexico (1); Thomson Causeway Recreation Area, Carroll County, Illinois (2); and Pierce National Wildlife Refuge, Skamania County, Washington (3). Turtles were also collected in Ames, Story County, Iowa (4), which was the location of the semi-natural common-garden environment.

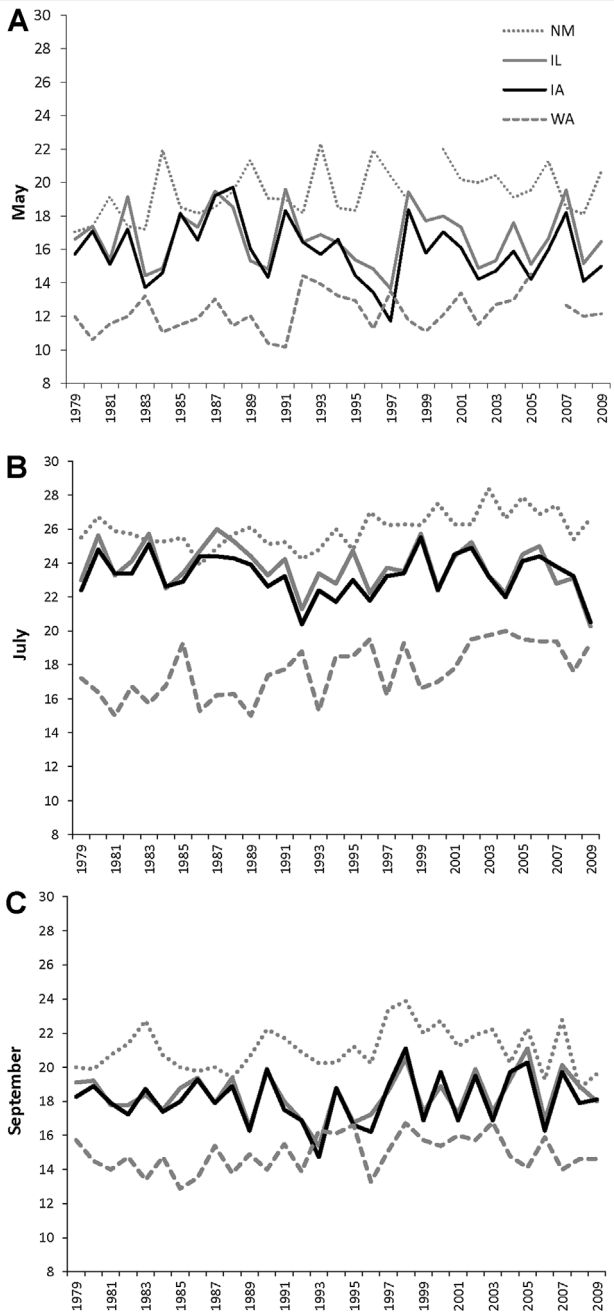

Figure 2. Mean air temperature in May (A), July (B), and September (C) from 1979–2009 at each population's site of collection, and the common-garden location in Iowa (solid black line). Climate data are from the National Climate Data Center (www.ncdc.noaa.gov) and were collected at Bosque del Apache (NM site); Clinton, IA (IL site); Ames 8 WSW (IA site); and Skamania Fish Hatchery (WA site). Data were unavailable for some sites in some years.

Iowa State University. Following emergence from hibernation in April 2010, a final plasma sample was collected from all individuals, and represented the "post-hibernation" time point.

Corticosterone Assay

We measured corticosterone (CORT hereafter) concentration in turtle plasma from each of the four sample time points using a double-antibody radioimmunoassay kit (MP Biomedical, Catalog # 07–102103, Orangeburg, NY, USA). We followed the protocol for this kit that has previously been validated in our laboratory for use in *Thamnophis* snakes (Robert et al., 2009), except that plasma samples were diluted 1:20 (i.e., 4 μl of plasma in 76 μl diluent). All samples were assayed in duplicate within a 48 hr period, with samples from each population included in each of four runs of the assay. We included a standard curve in each run, and we conducted a serial dilution (1:5, 1:10, 1:20; 1:40; 1:80) of a sample pool of six individuals to ensure that displacement curves were parallel to the standard corticosterone curve. To calculate average intra-assay variation, we averaged the percent coefficient of variation of the CORT concentrations for the duplicates in each of the four runs, and then took the average of those four values (excluding four samples with largely disparate values that were re-run). To calculate inter-assay variation, we averaged the four values of the kit-provided, low-concentration control in each run, and calculated the percent coefficient of variation on those four values. Average intra-assay variation was 14.2%, and inter-assay variation was 17.1%.

Bacteria-Killing Assay

We quantified innate immune function (i.e., non-specific, rapid-response) of turtles from each sample time point over a one-year period using two different assays. The first, the bacteria-killing assay (BK hereafter), measures the bactericidal capacity of plasma to kill *Eschericha coli*, and is a test of innate immunity (Tieleman et al., 2005). In this assay, diluted plasma samples are applied to cultures of *E. coli* and given time for bactericidal activity in the plasma to kill the bacteria. The proportion of the *E. coli* inoculum killed compared to the number of *E. coli* colonies present in control samples represents the bactericidal capacity of an individual at the time of plasma collection.

We followed the methods described in Millet et al. (2007) and Palacios et al. (2011) with a few alterations for use in painted turtles. A pellet of lyophilized *E. coli* (Microbiologics, ATCC#8739, St. Cloud, MN, USA) was reconstituted using 40 mL phosphate-buffered saline (PBS), and a working solution was prepared by further diluting a fraction of reconstituted *E. coli* to 1:60. This concentration of *E. coli* contained approximately 50 colony-forming bacteria per 10 μl. Turtle plasma samples were diluted 1:10 with PBS. We prepared sample reactions by adding 10 μl of bacterial working solution to 100 μl of the diluted plasma samples. Sample reactions were then incubated for 20 min at 28°C (based on the mean air temperature at which basking painted turtles

maintained optimum body temperatures; Ernst, '72) to allow bacteria-killing to occur. We also conducted three replicate control reactions by adding 10 μl of bacterial working solution to 100 μl PBS, and incubated them as described above for the plasma samples. We plated two replicates of all sample and controls reactions using 50 μl aliquots on 4% trypic soy agar. Plates were incubated at room temperature for 36 hr. Following incubation, we counted the number of bacterial colonies on each plate. The number of colonies in each plate was divided by the mean number of colonies in the control plates, and this value was subtracted from 1 to obtain the proportion of bacteria killed. For each sample, we used the mean proportion of bacteria killed from the two replicates as our measure of each individual's bactericidal capacity.

Natural Antibody Agglutination Assay

Natural antibodies (NAbs hereafter) are produced constitutively and function by agglutinating and mediating the lysis of foreign cells. Our second measure of immune function assessed individual turtles' constitutive innate immunity in terms of ability to agglutinate foreign red blood cells (RBCs).

We followed the protocol described by Matson et al. (2005) with several modifications for use in painted turtles (Schwanz et al., 2011). To each well in a 96-well plate, we added 15 μl PBS. We mixed 5 μl of plasma samples (rows 2–11) or positive control (row 1; rabbit anti-sheep RBC serum, MP Biomedical 55800) with the PBS in column 1 for an initial plasma dilution of 1:4. We serially diluted samples 1:2 for columns 2–8. Row 12 served as a negative control and contained only PBS. We then added 10 μl of 5% sheep RBCs in Alsever's anticoagulant (Hemostat Laboratories, Dixon, CA, USA) to all wells. Thus, the final plasma dilution in column 1 was 1:8. We incubated plates at 28°C for 90 min. Agglutination titers were then scored as $-\log_2(1/D)$, where D is the final dilution of plasma where agglutination occurred. Because the final plasma dilution in column 1 was 1:8, column 1 had a titer of 3, and column 8 had a titer of 10.

Statistical Analyzes

Data for CORT levels were log10 transformed for normality, BK was normally distributed, and NAbs agglutination was categorical. We first tested for correlations among dependent variables (CORT, BK, and NAbs). No significant correlations were found, thus, we proceeded to analyze each variable independently. We tested for overall differences among populations, sampling time points, and their interaction in CORT levels and BK capacity using repeated measures analysis of variance, and NAbs using logistic analysis for categorical integer variables. We tested for correlations between CORT and immune function at the individual level by regressing BK or NAbs against CORT levels. All statistical analyzes were conducted using SAS 9.3 (Proc Mixed and Proc Logistic, SAS Institute).

RESULTS

We sampled a total of 50 female painted turtles (13 from Washington, 13 from Iowa, 15 from Illinois, and 9 from New Mexico) at four time points over 1 year. CORT differed among time points such that the post-hibernation level was lower than the levels at the three preceding time points (back-transformed LSmeans [ng/mL (SE)]: Capture = 7.1 [1.1]; Nesting = 8.5 [1.2]; Pre-hibernation = 8.3 [1.2]; Post-hibernation 4.3 [1.2]; Table 1; Fig. 3). CORT did not differ among populations at any time point ($F_{3,46} = 1.27$; $P = 0.29$). BK did not differ among populations or time points, and variation in BK was not explained by an interaction between the two (overall mean percent killed ± standard deviation = 29% ± 23, range = 0–90%; Fig. 4). NAbs titres ranged from 3 to 10 (overall median = 9). Population and sampling time point interacted significantly such that New Mexico turtles had the lowest observed NAbs titres at both capture and nesting when compared to all other populations and time points, including the next two time points for the New Mexico turtles. Thus, turtles from New Mexico started off with low titres and increased to levels similar to the other populations pre- and post-hibernation (Table 1; Fig. 5). At the individual level, CORT was not correlated with either BK ($F_{1,91} = 1.60$, $r = 0.13$, $P = 0.21$; Fig. 6A) or NAbs agglutination ($F_{1,95} = 0.22$, $r = 0.04$, $P = 0.64$; Fig. 6B). Moreover, the two measures of immune function were not correlated with each other ($F_{1,75} = 0.31$, $r = 0.06$, $P = 0.58$).

Table 1. Repeated measures analysis of corticosterone (CORT) and bactericidal capacity (BK), and logistic analysis of natural antibody agglutination (NAbs), from four populations of painted turtles housed under common-garden conditions and sampled at four time points over 1 year.

	CORT	BK	NAbs
Source of variation	F (df_1, df_2)	F (df_1, df_2)	χ^2 (df)
Population	1.27 (3, 46)	0.41 (3, 43)	4.36 (3)
Time point	2.46 (3, 58)*	1.28 (3, 36)	2.45 (3)
Population x time point	0.78 (9, 58)	1.46 (9, 36)	(9)**

*$P = 0.07$.
**$P = 0.01$.

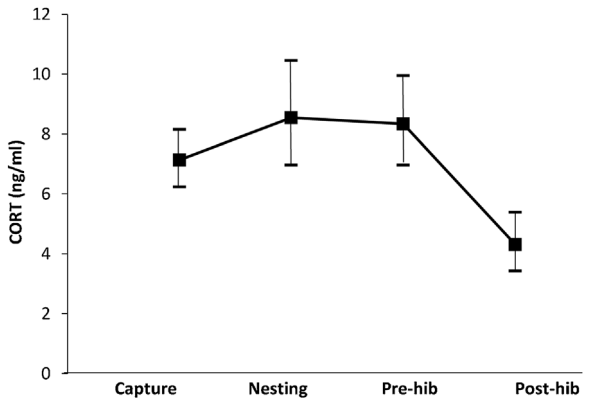

Figure 3. Back-transformed least-square means and standard error for corticosterone levels of four pooled populations of painted turtles sampled at four time points over 1 year. The post-hibernation sample was significantly lower than the earlier three time points (see Table 1).

DISCUSSION

Among the more insidious results of climate change are sub-lethal effects that may not directly cause individual mortality or extirpation of populations, but instead lead to lowered individual fitness or gradual population declines via indirect pathways. We used a common-garden design to experimentally test the effects of a novel climate on corticosterone dynamics and innate immune function in a freshwater turtle. We found that populations did not differ from each other in corticosterone levels at any of the four sampling time points, which suggest that the novel climate to which the transplanted populations were exposed did not result in a stress response (i.e., elevated corticosterone levels) in turtles from those populations. Thus, populations locally adapted to a range of climatic conditions may have the capacity to acclimatize to a limited range of novel conditions. In other reptiles, populations from the periphery of the species' range had higher corticosterone levels compared to populations from the core of the range (Dunlap and Wingfield, '95; Eikenaar et al., 2012), which has been attributed to the necessity of coping with predictably challenging conditions (Romero, 2002) such as those found near latitudinal extremes. In contrast, our results indicate that transplanted populations, including those from both margins of the species' range, did not display detectable stress in response to novel climatic conditions in the common-garden environment. The lack of difference in population-specific stress responses may indicate that painted turtles are "physiological generalists" that can quickly acclimatize to novel conditions without mounting a strong stress response. However, populations may still differ in their stress responses to sudden, acute stressors such as a predation attempt. An among-population comparison of stress response to capture (e.g., Cash et al., '97) would be necessary to determine whether population-specific differences exist in short-term stress response, in contrast to the longer term stress response studied here.

We also found that all populations had lower corticosterone levels in the spring following emergence from hibernation than for the other three time points. Immune function in painted turtles is depressed during hibernation (Schwanz et al., 2011), likely because individuals' low energy budget during hibernation reduces their ability to mount an immune response, and our study suggests that corticosterone levels are depressed during this time as well. Corticosterone levels and intra-assay variation in our study were similar to the range observed in related turtle species (Cash et al., '97; Selman et al., 2012), and inter-assay variation in our study was low compared to other studies (Selman et al., 2012).

Prolonged periods of elevated corticosterone levels can lead to negative physiological effects through allostatic overload. For example, corticosterone levels were negatively correlated with bactericidal capacity in birds (Millet et al., 2007) and body condition in snakes (Moore et al., 2000). In contrast, corticosterone levels did not explain variation in natural antibody agglutination or bactericidal capacity in snakes, which instead seemed to be shaped mainly by prevailing environmental conditions (Palacios et al., 2013). Similarly, we found no evidence that high levels of corticosterone were associated with depressed innate immune function as measured by bactericidal capacity or natural antibody agglutination in painted turtles exposed to novel climatic conditions. The nesting season and early spring after emergence from hibernation are periods during which female turtles engage in overland travel, and may be times when enhanced immune function is advantageous due to the potential

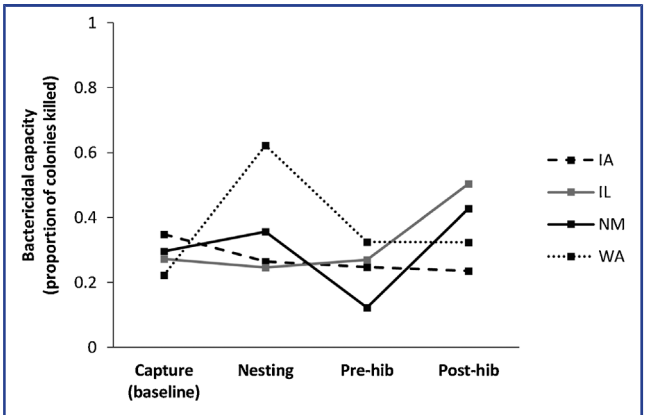

Figure 4. Mean proportion of *E. coli* colonies killed in the bacteria-killing (BK) assay for four population of painted turtles at four time point over 1 year. Bactericidal capacity did not differ among populations or sampling time points.

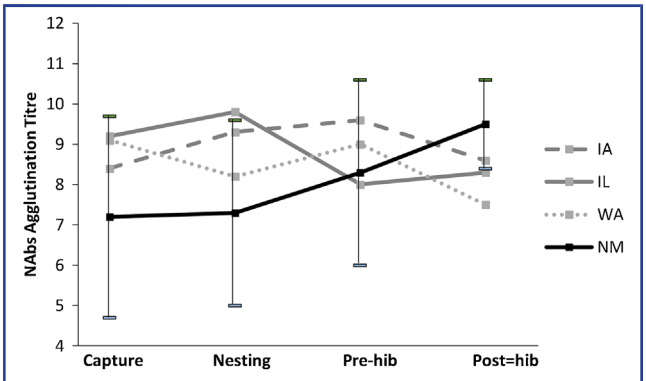

Figure 5. Mean natural antibody agglutination (NAbs) titres for four population of painted turtles at four time point over 1 year. New Mexico baseline and nesting values were significantly lower than the other three populations at those same time points, and were significantly lower than the subsequent New Mexico time points.

for increased contact with infected individuals in new environments (Zimmerman et al., 2010). Alternatively, we might expect natural antibody agglutination to display minimal seasonal variation because these recognition molecules start the complement enzyme cascade, which eventually leads to lysis of foreign cells; as part of the constitutive innate immune system natural antibodies are the first line of protection against invading microbes (Matson et al., 2005). We observed only minimal variation in natural antibody agglutination, wherein the New Mexico population had lower agglutination titres at the time of capture and during nesting than other populations or time points. At the two subsequent sampling points, agglutination titres in the New Mexico population were comparable to the other populations, which may be indicative of an acclimitization response by the New Mexico turtles. Interestingly, another study on painted turtles from the Iowa population found that natural antibody agglutination was highest in the fall, just prior to hibernation (Schwanz et al., 2011). We also observed no significant variation in bactericidal capacity over time or among populations, which supports the prediction that painted turtles should rely heavily on non-specific innate responses to recognize and initiate appropriate response to pathogens, based on their unique repertoire of toll-like receptors (Shaffer et al., 2013). Results from these and other studies suggest that components of the painted turtle immune response are regulated differentially across seasons and life stages, possibly to manage trade-offs among physiological systems, growth, reproduction, and maintenance of allostasis (e.g., Schwanz et al., 2011).

Animal populations living in stressful environments tend to have higher levels of corticosterone than those living in more benign environments (Dunlap and Wingfield, '95; Moore et al., 2000 Romero and Wilkelski, 2001; Robert et al., 2009; Eikenaar et al., 2012). Moreover, in some species, an added stressor can induce greater sensitivity in the adrenocortical responsiveness to acute stress (Berger et al., 2007), which could make species with relatively strong stress responses particularly vulnerable to climate change (Jessop et al., 2013). However, stress tolerance could also be conferred by adaptive mechanisms including phenotypic plasticity (e.g., acclimatization; Wingfield and Kitaysky, 2002; Landys et al., 2006) and evolution (e.g., via natural selection; Evans et al., 2005). Our study suggests that exposure of turtles to novel climatic conditions did not induce a detectable stress response, nor did it appear to depress immune function.

It is important to recognize that climate change will have a multitude of impacts, both within individuals' lifetimes and over the course of many generations. Fully understanding the effects of climate change and organisms' potential responses requires a whole-life cycle approach, while an evolutionary perspective is necessary to determine the degree to which selection and/or phenotypic plasticity may facilitate persistence despite a

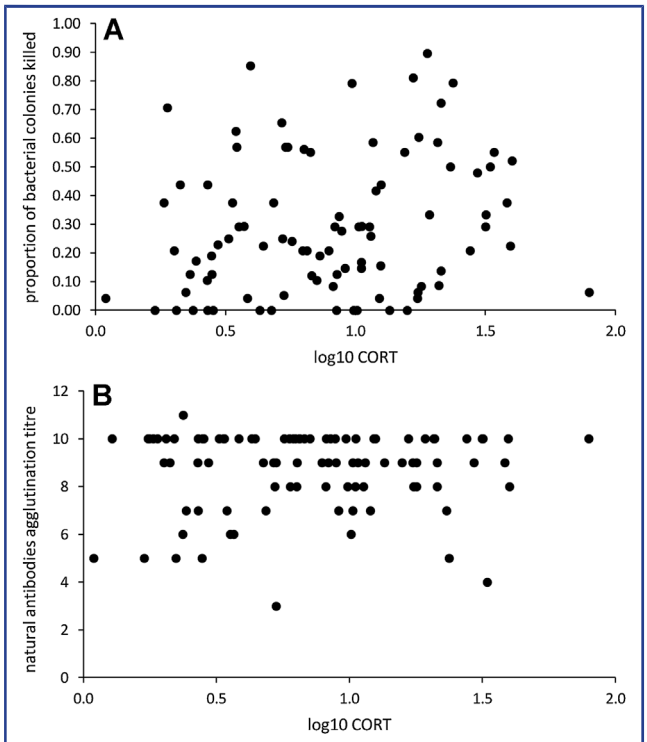

Figure 6. Across all individuals and sampling times, corticosterone level (CORT) was not inversely related to either bactericidal capacity (A; $F_{1,91} = 1.60$, $r = 0.13$, $P = 0.21$) or natural antibody agglutination (NAbs; $F_{1,95} = 0.22$, $r = 0.04$, $P = 0.64$) in four populations of painted turtles at four time points over 1 year.

rapidly-changing environment. In long-lived turtle species, survival of early life stages may decline as nest incubation temperatures become lethally-warm (Telemeco et al., 2013), while later life stages may experience sex-ratio bias as warmer incubation temperatures produce predominantly one sex as a result of the temperature-dependent sex determining system in turtles (Janzen, '94). Plasticity in nest-site choice prevents sex ratio skews in some reptiles with TSD (Morjan, 2003; Doody et al., 2006; Refsnider and Janzen, 2012), but evolutionary and ecological constraints may prevent such plasticity from being expressed (Refsnider et al., 2013). Genetic adaptation is another possible response to climate change, but evolutionary mechanisms that allowed genetic adaptation to past, gradual periods of climate change may not have time to operate under the current, rapid rate of climate change (IPCC, 2007). Indeed, in the painted turtle, low heritability of maternal nest-site choice (Morjan, 2003; McGaugh et al., 2010) suggests that this trait is unlikely to undergo sufficient genetic adaptation to keep pace with rapid climate change, and adaptation of thermal sensitivity in the sex-determining pathway may be a more realistic mechanism of responding to climate change (McGaugh et al., 2011). To the growing body of work on climate change effects on reptiles with temperature-dependent sex determination, our study adds a physiological perspective. While exposure to a novel climate may negatively impact aspects of physiology or metabolism that were not measured here, our results suggest that, in terms of innate immune function, freshwater turtles may be resilient to at least modest changes in climatic conditions.

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