

FINAL REPORT

Critical Habitat Breadth for Gopherus Tortoises: A New Paradigm for Managing Threatened and Endangered Species in a Non-stationary World

Kevin Shoemaker
Elizabeth Hunter
Marjorie Matocq
Margarete Walden
University of Nevada, Reno

Todd Esque
United States Geological Survey

Ken Nussear
Southwest Ecology, LLC

Resit Akcakaya
Stony Brook University

Lora Smith
Joseph W. Jones Ecological Research Center

Matthew Aresco
Nokuse Plantation

May 2024

This report was prepared under contract to the Department of Defense Strategic Environmental Research and Development Program (SERDP). The publication of this report does not indicate endorsement by the Department of Defense, nor should the contents be construed as reflecting the official policy or position of the Department of Defense. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the Department of Defense.

REPORT DOCUMENTATION PAGE					Form Approved OMB No. 0704-0188	
The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.						
1. REPORT DATE (DD-MM-YYYY) 04/05/2024		2. REPORT TYPE SERDP Final Report		3. DATES COVERED (From - To) 7/5/2018 - 7/5/2023		
4. TITLE AND SUBTITLE Critical Habitat Breadth for Gopherus Tortoises: A New Paradigm for Managing Threatened and Endangered Species in a Non-stationary World				5a. CONTRACT NUMBER 18-C-0018		
				5b. GRANT NUMBER		
				5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Kevin Shoemaker, Elizabeth Hunter, Marjorie Matocq and Margarete Walden: University of Nevada, Reno Todd Esque: United States Geological Survey Ken Nussear: Southwest Ecology, LLC Resit Akcakaya: Stony Brook University Lora Smith: Joseph W. Jones Ecological Research Center Matthew Aresco: Nokuse Plantation				5d. PROJECT NUMBER RC18-1103		
				5e. TASK NUMBER		
				5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Nevada, Reno Natural Resources & Environmental Science 1664 N. Virginia St. MS 186 Reno, Nevada 89557				8. PERFORMING ORGANIZATION REPORT NUMBER RC18-1103		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of the Deputy Assistant Secretary of Defense (Energy Resilience & Optimization) 3500 Defense Pentagon, RM 5C646 Washington, DC 20301-3500				10. SPONSOR/MONITOR'S ACRONYM(S) SERDP		
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) RC18-1103		
12. DISTRIBUTION/AVAILABILITY STATEMENT DISTRIBUTION STATEMENT A. Approved for public release: distribution unlimited.						
13. SUPPLEMENTARY NOTES						
14. ABSTRACT Conserving threatened and endangered species (TES) on Department of Defense installations, without sacrificing vital military objectives, is necessarily a complex balancing act – especially given the uncertainties and risks associated with climate change. This project advances the concept of critical habitat breadth as the foundation for rigorous TES conservation planning and vulnerability assessment. Gopherus tortoises - particularly Desert Tortoise (G. agassizii) and Gopher Tortoise (G. polyphemus) -- provide an excellent model system for applying the concept of critical habitat breadth because their populations have been extensively studied, and many prior translocations have been conducted which enable investigation into the inherent ability to acclimate to novel environments.						
15. SUBJECT TERMS Mojave Desert Tortoise, Gopherus agassizii, Gopher Tortoise, Gopherus polyphemus, Mojave Desert, Longleaf Pine Savanna, Nesting Ecology, Population Viability Analysis (PVA), Environmental Sex Determination, Metapopulation Modeling, Wildlife Demography, Bayesian Hierarchical Models, Critical Habitat, Climate Change Vulnerability Assessment, DOD, Department of Defense						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON	
a. REPORT	b. ABSTRACT	c. THIS PAGE			Kevin Shoemaker	
UNCLASS	UNCLASS	UNCLASS	UNCLASS	185	19b. TELEPHONE NUMBER (Include area code) 775-682-7449	

Table of Contents

List of Tables	iii
List of Figures	iv
List of Acronyms	ix
Keywords	x
Acknowledgements.....	xi
ABSTRACT.....	1
Executive Summary	2
Introduction.....	2
Objectives	2
Technical Approach.....	3
Results and Discussion	4
Implications for Future Research.....	7
Benefits	8
Objectives	9
Background.....	11
Materials and Methods.....	14
Environmental Sensitivities of Population Vital Rates (Task 1)	16
DESERT TORTOISE	17
GOPHER TORTOISE	29
Test for local adaptation (Task 2)	38
Collect field data on translocated and resident tortoises.....	39
Model local adaptation as the expected change in demographic vital rates explained by environmental differences between source and recipient sites	42
Model critical habitat breadth and forecast future population viability (Task 3)	43
Build comprehensive critical habitat breadth model (Task 3a)	45
Build and deploy a web-based conservation planning tool (Task 4)	64
Results and Discussion	64
Environmental/climatic sensitivities of population vital rates (Task 1c).....	64
DESERT TORTOISE	64
GOPHER TORTOISE	83
Evidence for local adaptation from translocation ‘experiments’ (Task 2)	98
DESERT TORTOISE	98

GOPHER TORTOISE	100
Simulated population dynamics (Task 3)	102
Analysis of simulation results (Task 3c).....	121
Conservation planning tool (Task 4).....	130
Conclusions and Implications for Future Research	132
Task 1 – Environmental sensitivities of population vital rates	134
Desert Tortoise:.....	134
Gopher Tortoise:	135
Task 2 – Test for local adaptation.....	136
Desert tortoise:	136
Gopher Tortoise:	136
Task 3 – Model critical habitat breadth	136
Desert tortoise:	137
Gopher Tortoise:	137
Task 4 – Build a conservation planning tool	138
Literature Cited	139
APPENDICES	152
Appendix A: Supporting Data	152
Appendix B: List of Scientific/Technical Publications: RC18-1103.....	152
Published papers:	152
Publications in preparation:	153
Planned publications:	153
Presentations	154
Appendix C: MITCH-Clim v0.1 User Guide	157

List of Tables

Table 1. Key demographic vital rates used in critical habitat breadth models for Mojave Desert and Gopher Tortoises.	15
Table 2. Summary of Mojave Desert Tortoise (<i>G. agassizii</i>) nesting data from fieldwork at 6 sites.	23
Table 3. —Monte Carlo simulations for identifying sex of hatchling Mojave Desert Tortoise (<i>Gopherus agassizii</i>) using threshold values of testosterone concentrations such that values lower than the threshold are more likely to be female (F) with the indicated level of certainty, while values above the threshold are more likely to be male (M) with the indicated certainty.	28
Table 4. Summary of <i>Gopherus polyphemus</i> nest and hatchling data collection in two field seasons.	34
Table 5. Random forest model results for predicting sex with morphology and testosterone.	37
Table 6. Environmental variables used in models of environmental distance effects on Gopher Tortoise nest site selection and nesting outcomes Nokuse Plantation in Walton County, FL, during years 2018-2020, and the correlated climate variables they represent. Vitellogenesis (September – January) Nesting (May – July) and Active (April – October) seasons are the average climate for biologically relevant time window that may impact reproduction. Collinear variables have Pearson correlation coefficient of $ r > 0.60$	41
Table 7. Summary of climate-sensitive components for the Desert Tortoise population simulation model.	50
Table 8. Description of binary variables (“on-off switches”) used for constructing scenario tests for Desert Tortoise.	55
Table 9. Summary of climate-sensitive components for the Gopher Tortoise population simulation model.	57
Table 10. Description of binary variables (“on-off switches”) used for constructing scenario tests for Gopher Tortoise.	63
Table 11. Mojave Desert Tortoise survival and abundance were modeled as a function of several site-level environmental and climatic variables.	65
Table 12. Climate and individual-level covariates used to model reproductive probability (RP) and egg production (EP) for Agassiz’s Desert Tortoise <i>Gopherus agassizii</i>	75
Table 13. Parameter estimates for the reproductive probability (RP) and egg production (EP) processes, with corresponding Bayesian credible intervals (95% highest posterior density).	76
Table 14. Logistic regression reveals a nest temperature effect on sex ratio from 17 naturally incubated Mojave Desert Tortoise nests. Estimates and errors are on the logit scale.	80
Table 15. Logistic regression models predicting hatching success from mean daily maximum nest temperature and mean nest temperature from 23 Mojave Desert Tortoise nests. Estimates and errors are on the logit scale.	82
Table 16. Logistic regression reveals nest temperature and precipitation effects on sex ratio from 68 naturally incubated Gopher Tortoise nests. Estimates and errors are on the logit scale.	97

List of Figures

Figure 1. Schematic diagram illustrating how critical habitat breadth is defined by the relationships between population vital rates and by the potentially opposing functional relationships between population vital rates and environmental variables (compensatory modulation).....	15
Figure 2. Schematic diagram of our approach for modeling critical habitat breadth for <i>Gopherus</i> tortoises.	16
Figure 3. Locations of the ‘permanent study plots’ used in this study to estimate Desert Tortoise survival rates as a function of climatic and environmental gradients.	18
Figure 4. Locations of previous data collection efforts for <i>Gopherus agassizii</i>	19
Figure 5. Map of sites at which surveys of reproductive output (using X-ray) were conducted from 1997 to 2002.....	20
Figure 6. - a.) The distribution of the annual number of eggs produced by Mojave Desert Tortoise.	21
Figure 7. X-radiograph image of a female Desert Tortoise with two eggs on the left and a female tortoise with five eggs on the right.	21
Figure 8. Monthly time series for one downscaled RCP model	22
Figure 9. Locations of field data collection efforts for <i>Gopherus agassizii</i>	24
Figure 10. Fitted probability density functions for plasma testosterone concentrations of captive hatchling (0–3 mo old) Mojave Desert Tortoises	27
Figure 11. Sites at Fort Stewart Army Reserve (FSAR), Georgia, USA, surveyed from 1994–2020 to investigate the response of Gopher Tortoise population dynamics to prescribed burning.	30
Figure 12. Predicted change in maximum temperature and precipitation across 33 General Circulation Models (GCMs) and two Representative Concentration Pathways for the center of the Gopher Tortoise range.	32
Figure 13. Map of study sites within the Gopher Tortoise (<i>Gopherus polyphemus</i>) range (gray area).....	33
Figure 14. Sex is only weakly predicted by naive (A) or post-FSH challenged (B) circulating testosterone concentration in neonate Gopher Tortoise.....	36
Figure 15. FSH challenge increases testosterone in both male and female neonates.	36
Figure 16. Translocation recipient site (Nokuse Plantation, yellow circle) and a sample of primary donor sites (red “x”s) throughout Florida across a range of both geographic and environmental (here, annual precipitation totals) distance from the recipient site.	39
Figure 17. Florida counties that have donated Gopher Tortoises for translocation to Nokuse Plantation (yellow star).	40
Figure 18. To model the spatial and temporal abundance dynamics of the two focal tortoise species, we used a lattice, or grid-based, simulation modeling framework. This enabled us to integrate geographic (e.g., climate) information into our simulation framework as spatial rasters. Our models for both species use a 5 km grid cell resolution, with each pixel on the landscape representing a potential tortoise population. The matrix generator function was called separately for each grid cell in each simulation year, integrating all the climate-vital rate relationships described under Task 1 of this report. These matrices were then used to update the abundance in each grid cell for each year via matrix multiplication.	47
Figure 19. The generic modeling workflow starts off with multiple replicates drawn from parameter distributions describing the climate-vital rate relationships described under Task 1.	

Based on the specific scenario, we incorporate all relevant environmental information and habitat information to initialize and simulate population abundance. The matrix generator function is called for each grid cell each year, enabling population dynamics to respond to changing environmental conditions. The simulation model archives age-structured abundance in each grid cell each year as multi-band geo-Tiffs, which serve as the raw materials for visualizing and analyzing model results.....	49
Figure 20. Time series for three derived climate variables (June-August [summer] temperature [left], November-April [winter] precipitation [middle], and 30-year normals of winter precipitation [right]) important for Mojave Desert Tortoise demography.....	51
Figure 21. Time series for two derived climate variables (annual temperature [left] and June precipitation [right]) important for Gopher Tortoise demography.	58
Figure 22. Relationship between Gopher Tortoise age-at-maturity (MA) and mean annual temperature across the species range.	60
Figure 23. Trends in temperature, precipitation, and Palmer drought index during the years for which the resurveys of the ‘permanent study plots’ occurred	66
Figure 24. Site-level survival and other demographic variables as a function of environmental and climatic covariates.....	67
Figure 25. Our models of adult survival indicated substantial variation in survival among years, with several years showing reduced survival compared with “normal” years.	67
Figure 26. Mojave Desert Tortoise density and trend estimates at a subset of the available ‘permanent study plots’	68
Figure 27. The permanent study plot data tend to indicate a general decline in capture rates for individuals smaller than around 180mm in carapace length -- which is the generally accepted juvenile cutoff for this species.	68
Figure 28. (Left) Temperature-size rule showing trade-off between growth rate and mean asymptotic body size in a turtle under cooler (blue) versus warmer (red) temperatures. (Right) Pathways for the direct and indirect effects of climate on growth rate and mean asymptotic body size	69
Figure 29. Median and 95% credible intervals of the estimates for climate, sex, and translocation status coefficients on (A) growth rate (k) and (B) mean asymptotic body size (a) for Mojave Desert Tortoise (<i>Gopherus agassizii</i>) using the von Bertalanffy growth model. Values are on the log scale.	71
Figure 30. Predicted change in growth rate k and mean asymptotic body size a across the range of Mojave Desert Tortoise (<i>Gopherus agassizii</i>) using Bias Corrected Constructed Analogs version 2 (BCCAv2) Daily Climate Projections.....	72
Figure 31. Predicted von Bertalanffy size-at-age curves using mean historical (1990 – 2019) and future (1950 – 1979) climate projections across the five Recovery Units of Mojave Desert Tortoise	73
Figure 32. Partial dependence plots illustrating fitted relationships between reproductive output of Agassiz’s Desert Tortoise <i>Gopherus agassizii</i> and covariates representing climate and individual-level attributes.	77
Figure 33. Range-wide spatiotemporal projections of annual egg production for Agassiz’s Desert Tortoise	79
Figure 34. Mean nest temperature during the critical period strongly predicts hatchling sex ratio in wild nests of the Mojave Desert Tortoise.	80

Figure 35. Hatching success declines with mean nest temperature (A) and mean daily maximum nest temperature (B) across 23 nests of the Mojave Desert Tortoise.	82
Figure 36. Gopher Tortoise habitat component comparisons. A) Percent tree cover in 2000 and 2019 (Hansen et al. 2013), B) percent sand in soil (Hengl et al. 2017), and C) years since burn among 3 sites (F zones: red; E zones: blue; Zone E12: gray) at Fort Stewart Army Reserve (FSAR), Georgia, USA.	83
Figure 37. Gopher Tortoise population parameters (emigration and survival [A, B], immigration and recruitment [C, D], and adult abundance [E, F]) in response to prescribed burning (mean years since burn for a site) at 2 sites (F zones [A, C, E] and E zones [B, D, F]) at Fort Stewart Army Reserve (FSAR), Georgia, USA, 1994–2020.	85
Figure 38. Reproductive timeline for female Gopher Tortoises <i>Gopherus polyphemus</i> at Fort Stewart Army Reserve, Georgia, USA.	87
Figure 39. Effects on female Gopher Tortoise <i>Gopherus polyphemus</i> (A–C) probability of reproduction and (D,E) clutch size at Fort Stewart Army Reserve, Georgia, USA	89
Figure 40. Change in Gopher Tortoise <i>Gopherus polyphemus</i> (A–C) reproduction and body size and (D–F) climate variables over time at Fort Stewart Army Reserve, Georgia, USA	90
Figure 41. Structural equation model of Gopher Tortoise hatching success at four sites.	94
Figure 42. Effect of mean nest temperature on Gopher Tortoise hatching success for 5 sites that span the species’ latitudinal range	95
Figure 43. Mean nest temperature (A) and mean daily precipitation (B) during the critical period influence clutch sex ratio in wild nests of the Gopher Tortoise.	97
Figure 44. Predicted growth rate k (left) and asymptotic body size a (right) in Mojave Desert Tortoise (<i>Gopherus agassizii</i>) along T_{max} , PPT, and GDD gradients by translocation status. ...	99
Figure 45. Effect of the difference in warm season temperatures (WST) between a Gopher Tortoise female’s original location (the donor county) and the recipient site on nest distance from burrow entrance.	101
Figure 46. Effects of environmental distance on hatching success for Gopher Tortoises.	102
Figure 47. Visualization of lambda across climate space. Here, we used a factor analysis to identify two main axes of climate variation for Desert Tortoise, which can be interpreted as temperature (x axis) and precipitation (y axis). Pixel locations in this figure represent different environmental conditions on these two axes, and pixel color represents population growth rate. Green pixels represent environmental conditions expected to exhibit positive growth, yellow pixels are expected to remain relatively stable over time, and orange and red pixels represent environments associated with negative population growth. Grey pixels are regions of climate space that don’t occur at any time or place in this simulation. For these simulations, all ‘climate-effect activators’ (on-off switches) were turned on to reflect the estimated effects of climate change on all key parameters. Axis 2 (precipitation) is a much stronger driver of variation in population growth for Desert Tortoise than temperature on the x axis, with higher precipitation resulting in higher expected growth rates overall.	103
Figure 48. Climate-driven changes in vital rates over time for Desert Tortoise for a single selected pixel for a single simulation replicate. Each panel in this figure displays a different Desert Tortoise vital rate for a single grid cell in a single replicate of a high carbon-emissions scenario- with time on the x axis and vital rate on the y axis. For these simulations, all ‘climate-effect activators’ (on-off switches) were turned on to reflect the estimated effects of climate change on all key parameters. Here we see some negative effects of climate change- most notably, a decline in hatching success over time. However, this negative impact is offset by a	

positive effect of climate change on reproductive output and hatchling sex ratio, both of which are expected to increase population growth. In the end, population growth (Λ), represented in red in the lower righthand corner, remains relatively stable in this grid cell.	104
Figure 49. Desert Tortoise simulated abundance trajectories from 2011 to 2099 (sum across all age classes) for each emission reduction scenario (RCP2.6, RCP4.5, RCP6.0, and RCP8.5). ..	105
Figure 50. Desert tortoise simulated abundance trajectories from 2011 to 2099 (sum across all age classes) across all emission reduction scenarios (RCP2.6, RCP4.5, RCP6.0, and RCP8.5). In A, all ‘climate-effect activators’ were turned on to reflect the estimated effects of climate change on all key parameters. In the subsequent panels, various climate effects are ‘turned off’ to reflect their overall impact on simulation results. Dark lines with gray ribbons denote mean abundance across all stages. Dark lines with beige ribbons denote mean adult abundance. Ribbons denote one standard deviation above and below the mean.....	106
Figure 51. Projected range-wide spatial patterns in final Desert Tortoise abundance (averaged final abundance for 2079-2099 to account for random fluctuations at the end of the simulation), averaged across the three most plausible carbon-emissions scenarios: RCP4.5, RCP6.0, and RCP8.5.	107
Figure 52. Average projected range-wide spatial patterns in final Desert Tortoise abundance (averaged final abundance for 2089-2099 to account for random fluctuations at the end of the simulation) across four plausible carbon-emissions scenarios	108
Figure 53. Map of critical habitat for Desert Tortoise (defined as areas that are expected to sustain Desert Tortoise populations over time in the face of climate change) across four plausible carbon emissions scenarios	109
Figure 54. Map of critical habitat for Desert Tortoise (defined as areas that are expected to sustain Desert Tortoise populations over time in the face of climate change).	110
Figure 55. Map of critical habitat for Desert Tortoise (defined as areas that are expected to sustain Desert Tortoise populations over time in the face of climate change).	111
Figure 56. Map comparing range-wide effect of climate change on final simulated abundances	112
Figure 57. Map comparing range-wide effect of climate change on final simulated abundances	113
Figure 58. Gopher Tortoise simulated abundance trajectories (sum across all age classes) for a single climate model (CESM1-BGC 8.5) across all unique burning scenarios.....	114
Figure 59. Gopher Tortoise simulated abundance trajectories (sum across all age classes) under the worst case burn scenario	115
Figure 60. Gopher Tortoise simulated abundance trajectories (sum across all age classes) under the best-case burn scenario	116
Figure 61. Gopher Tortoise simulated abundance trajectories (sum across all age classes) illustrating the effects of climate change by comparing models where vital rates are affected by a changing climate (“Climate effects”) to models where no vital rates are affected by climate (“No climate effects”).	117
Figure 62. Spatial patterns of final abundance (year 2099) for Gopher Tortoise simulations with full-magnitude burn effect scenarios, comparing (A) burn frequency decreasing with warmer climate and (B) burn interval remaining constant over time at an average of one burn every four years.	118
Figure 63. Spatial patterns of final abundance (year 2099) for Gopher Tortoise simulations with burn frequency decreasing with warmer climate, comparing (A) full-magnitude effect of fire	

history on survival rates and (B) the survival effects of fire history reduced to half the magnitude	118
Figure 64. Spatial patterns of final abundance (year 2099) for Gopher Tortoise simulations, comparing (A) a “worst-case” scenario in which juvenile and adult survival rates are affected by burn history (Hunter and Rostal 2021), and with burn frequency decreasing with climate change and (B) a “best-case” scenario in which juvenile and adult survival rates are unaffected by site-level burn history.	119
Figure 65. Map of critical habitat for Gopher Tortoise (defined as areas that are expected to sustain Gopher Tortoise populations over time in the face of climate change), assuming juvenile and adult survival decline with increasing time since burn (climate scenario CESM1-BGC 4.5).	120
Figure 66. Map of critical habitat for Gopher Tortoise with DoD site overlay	121
Figure 67. Conditional inference tree showing the impact of climate effects on particular vital rates as determinants of simulated Desert Tortoise final abundance in our population model.	123
Figure 68. Relative importance of key simulation parameters in determining final simulated Desert Tortoise abundance.	124
Figure 69. Univariate partial dependence plots illustrating the four most important determinants of simulated final abundance for Desert Tortoises.	125
Figure 70. Bivariate partial dependence plots illustrating the four most important bivariate interactions determining simulated final abundance for Desert Tortoises.	126
Figure 71. Conditional inference tree (similar to one of the many CART models comprising the random forest model) showing the impact of climate and burn effects on particular vital rates as determinants of simulated Gopher Tortoise final abundance.	127
Figure 72. Relative importance of key simulation parameters in determining final simulated Gopher Tortoise abundance.	128
Figure 73. Univariate partial dependence plots illustrating the four most important determinants of simulated final abundance for Gopher Tortoises.	129
Figure 74. Partial dependence plots illustrating the four most important bivariate interactions determining simulated final abundance for Gopher Tortoises.	130

List of Acronyms

BLM	United States Bureau of Land Management
CA	State of California
CDFW	State of California Department of Fish and Wildlife
CHU	Critical Habitat Unit
CMR	Capture Mark Recapture
CY	Calendar Year
DOD	United States Department of Defense
ELISA	Enzyme-linked Immunosorbent Assay
ESD	Environmental Sex Determination
FSAR	Fort Stewart Army Reserve
FWS	United States Fish and Wildlife Service
GCM	Global Climate Models
GIS	Geographic Information Systems
GSU	Georgia Southern University
IACUP	Institutional Animal Care and Use Permit
IPM	Integrated Population Model
MOU	Memorandum of Understanding.
NDOW	State of Nevada Department of Wildlife
NV	State of Nevada
PSP	Permanent Study Plots
SCR	Spatial Capture Recapture
SDM	Species Distribution Model
TES	Threatened and Endangered Species
UNR	University of Nevada, Reno
US	United States
USGS	United States Geological Survey

Keywords

Mojave Desert Tortoise, *Gopherus agassizii*, Gopher Tortoise, *Gopherus polyphemus*, Mojave Desert, Longleaf Pine Savanna, Nesting Ecology, Population Viability Analysis (PVA), Environmental Sex Determination, Metapopulation Modeling, Wildlife Demography, Bayesian Hierarchical Models, Critical Habitat, Climate Change Vulnerability Assessment, DOD, Department of Defense

Acknowledgements

This final report was compiled by Dr. Kevin T. Shoemaker with significant contributions from Drs. Elizabeth A. Hunter and Kevin Loope. Drs. Margarete Walden, Kenneth Nussear, Todd Esque, David Rostal, Nicole DeSha, Jeff Lovich, and Matt Aresco contributed valuable data on Gopher Tortoise and Mojave Desert Tortoise nesting ecology and demography. Michael Lam contributed to report writing, app development and generation of maps and figures for this report. This research is supported by the Strategic Environmental Research and Development Program (grant award #RC18-1103). Research was authorized under the following permits: UNR IACUC #00703, USFWS Recovery Permits TE-030659 and TE-50049D, NV-NDOW permit S36421, and GSU IACUC #19007, GA DNR permit 1000838720, FL FWC permit LSSC-18-00023B. Field work was conducted by Conservation Southeast, Dr. Elizabeth Hunter, Dr. Kevin Loope, Dr. Lora Smith, Dr. Margarete Walden and Nicole DeSha, and technicians Jack Christie, Sarah Drake, Abigail Dwire, John Floyd, Gretchen Gantz, Nathan Labieniec, Garrett Lawson, Caitlin MacMurtrie, Kristen McCarty, Nicholas Mendelsohn, Curt Vandenberg, Kenzie Wells, Dustin Haines, Mary Saethere, Kristin Murphy, Alan Green, Ron Marlow, Kathie Meyer-Wilkins, Ramona Daniels, Curt Bjurlin, Sheila Madrak, Laura Tennant, Amanda Smith, Rachel Henderson, Joshua Ennen, and Jenna Norris. Thanks to Craig Banks, Hailey Baker, Nicole DeSha, Garrett Lawson and Quinton Johnson for help with GT tortoise care and morphological measurements, and Jack Christie, Abbie Dwire and Curt Vandenberg for assistance in finding GT nests. Thanks to Roy King, Larry Carlile, and Sheryl McMillan for assistance in GT site access. Thanks to A. B. Safer, V.K. Perdue, M. J. Mitchell, J. W. Entz, M. Erickson, P. Marley, A. W. Dwire, C. A. Vandenberg, J. Floyd, and J. Christie for their help in the field. Analyses and figures related to Mojave Desert Tortoise reproductive output were largely produced by graduate students L. Phillips, C. Mitchell and D. Friend as part of a graduate statistics course taught by PI Shoemaker. Thanks to R. L. King, J. N. Macy, and L. D. Carlile for support and supplying fire data. Thanks to D. N. Jones, K. K. Drake, A. B. Safer, V. K. Perdue, M. J. Mitchell, J. W. Entz, M. Erickson, P. Marley, K. Hanley, A. W. Dwire, C. A. Vandenberg, and J. Floyd. Thanks to P. Medica, M. Peadon, C. T. Moore, B. Folt, B. Rothermel, M. Legare, P. Wolff, J. Tonnies, J. Braun, W. Siniard, A. Gorow, C. Aiello, P. Emblidge, N. Lamberski, R. Averill-Murray for key conceptual and data contributions. Additional funding and logistical support was provided by Clark County Desert Conservation Program, National Fish and Wildlife Foundation, US Geological Survey, Western Ecological Research Center, San Diego Zoo, Bureau of Land Management, California Energy Commission, USFWS, Georgia Department of Natural Resources Small Grants Program, and Georgia Southern University Research Development Fund.

Suggested citation: Shoemaker, K.T. et al., 2023; RC18-1103: *Critical Habitat Breadth for Gopherus Tortoises: a New Paradigm for Managing Threatened and Endangered Species in a Non-stationary World*. Final Report for Strategic Environmental Research and Development Program. Department of Natural Resources and Environmental Science. University of Nevada, Reno. 170 pp.

ABSTRACT

Introduction and Objectives: Conserving threatened and endangered species (TES) on Department of Defense (DoD) installations, without sacrificing vital military objectives, is necessarily a complex balancing act – especially given the uncertainties and risks associated with climate change. This project advances the concept of **critical habitat breadth** as the foundation for rigorous TES conservation planning and vulnerability assessment. *Gopherus* tortoises - particularly Desert Tortoise (*G. agassizii*) and Gopher Tortoise (*G. polyphemus*) -- provide an excellent model system for applying the concept of critical habitat breadth because their populations have been extensively studied, and many prior translocations have been conducted which enable investigation into the inherent ability to acclimate to novel environments.

Technical Approach: We compiled and re-analyzed previously collected data from across the range of both species to assess how demographic vital rates (e.g., survival, fecundity, age-at-maturity) respond to spatiotemporal environmental and climatic gradients. In addition, we conducted nesting surveys in the field to investigate how hatching success and hatchling sex ratios respond to climate gradients. We integrated these statistical models into comprehensive, spatially explicit predictive models capable of forecasting annual range-wide population dynamics for both species. We used this simulation model to forecast and visualize population dynamics through the year 2099, and to predict when and where populations are likely to be self-sustaining. We used machine-learning methods to assess which environmental and climatic drivers were most influential for determining population sustainability.

Results: Our simulation results for Desert Tortoise indicated that range-wide population abundance appears to be relatively stable. This is due to compensatory effects of climate change on different demographic rates – for instance, negative effects of warming on hatching success were balanced by positive population-level effects of warming on sex ratios. However, our simulations suggest that extreme warming could lead to slight range-wide population declines within the next century, and there could be negative effects of the highly skewed sex ratios (up to 80% female) which our model does not address (such as mating failures).

Our simulations indicated that most Gopher Tortoise populations appear to be declining, but that these declines may be driven more by low habitat quality rather than climate change *per se*. In particular, the observed decline in survival rate with years since fire strongly influenced population dynamics. Climate change, which is expected to reduce the frequency of prescribed fires across much of the southeastern United States, only exacerbated this effect in our simulations. Projected local extinction events in the Gopher Tortoise model tended to be most severe in the western and northern parts of the range, underscoring concerns about the status of Gopher Tortoise populations in this region.

Benefits: The results of our data collection linking climate to growth, reproduction, hatching success and sex ratio in both species will be directly beneficial to all future efforts to model climate effects on *Gopherus* populations. Our range-wide population viability analyses provide a tool to help resource managers prioritize conservation efforts in regions with projected climate resiliency, to identify potential future translocation sites outside of DoD lands that will remain suitable as habitat for many decades into the future, and to direct resources toward ameliorating the most damaging effects of climate change, including the reduced potential for critical habitat management (e.g. prescribed fire). Finally, we have developed a decision support tool to enable more effective information transfer to land managers. However, harnessing the value of this tool for management will require further consultations with key stakeholders.

Executive Summary

Introduction

The major goal of this project was to develop a cost-effective framework for managing threatened and endangered species (TES) in a non-stationary world. In accordance with the Statement of Need (SON), our modeling framework enables identification and ranking of critical habitat resources at a landscape (range-wide) scale, while accounting for the vast challenges and uncertainties associated with climate change.

Conserving threatened and endangered species (TES) on Department of Defense (DoD) installations, without sacrificing the ability of military installations to fulfill their stated missions, is necessarily a complex balancing act. Climate change, which can result in effective loss of habitat through processes such as increased drought frequency, extreme heat, and phenological mismatches between resource needs and availability, only intensifies these challenges (Gibbons et al. 2000, Cayan et al. 2010, Araujo et al. 2004, Lawler 2009). Consequently, habitat protection and restoration initiatives for TES species on DoD installations and elsewhere must rigorously account for new risks and uncertainties (Lawler 2009, Millar et al. 2007; Dawson et al. 2011). To determine which approach is best-suited for maintaining healthy and sustainable TES populations, it will be necessary to precisely define **critical habitat breadth** – that is, to understand and predict the full range of habitat and environmental conditions capable of harboring viable populations now and in the future.

Tortoises in the genus *Gopherus*, including the Mojave Desert Tortoise (*G. agassizii*) of the southern basin-and-range physiographic region and the Gopher Tortoise (*G. polyphemus*) of the southeastern USA provide an excellent model system for developing and applying the critical habitat breadth concept to TES populations for two important reasons: (1) *Gopherus* tortoise populations have been studied extensively for decades, at great cost to DoD and others, allowing our research team to leverage a huge arsenal of demographic and movement data across diverse environments, and (2) many prior translocation “experiments” have been conducted, providing a rare opportunity for a “common garden” approach for rigorous investigation of the relative role of local adaptation vs. phenotypic plasticity in explaining variation in key vital rates and functional traits.

In this project, we evaluate and apply the critical habitat breadth concept to *Gopherus* tortoises by (1) modeling how the variance and co-variance of key components of population fitness (vital rates; Table ES-1) are influenced by an array of relevant bioclimatic variables, and (2) coupling this information with metapopulation projection models to quantify the breadth of environmental conditions under which populations and metapopulations are capable of exhibiting stable or positive growth (critical habitat breadth). Ultimately, this information will assist resource managers in identifying mitigation and conservation strategies capable of sustaining viable metapopulations of Gopher Tortoise and Desert Tortoise under changing and uncertain future conditions.

Objectives

Our primary research objectives were to (1) understand and model the complex pathways through which environmental conditions influence population vital rates (and thereby population growth rates), specifically for two *Gopherus* tortoise species (Mojave Desert Tortoise, *G.*

agassizii and Gopher Tortoise, *G. polyphemus*) that pose operational challenges at multiple military installations, (2) use these models to rigorously define critical habitat breadth for these species (i.e., the range of environments capable of supporting viable populations and metapopulations), and (3) develop a conservation planning tool to aid DoD installation managers in developing cost-effective strategies for protecting metapopulations in the face of extensive uncertainty about the future.

Technical Approach

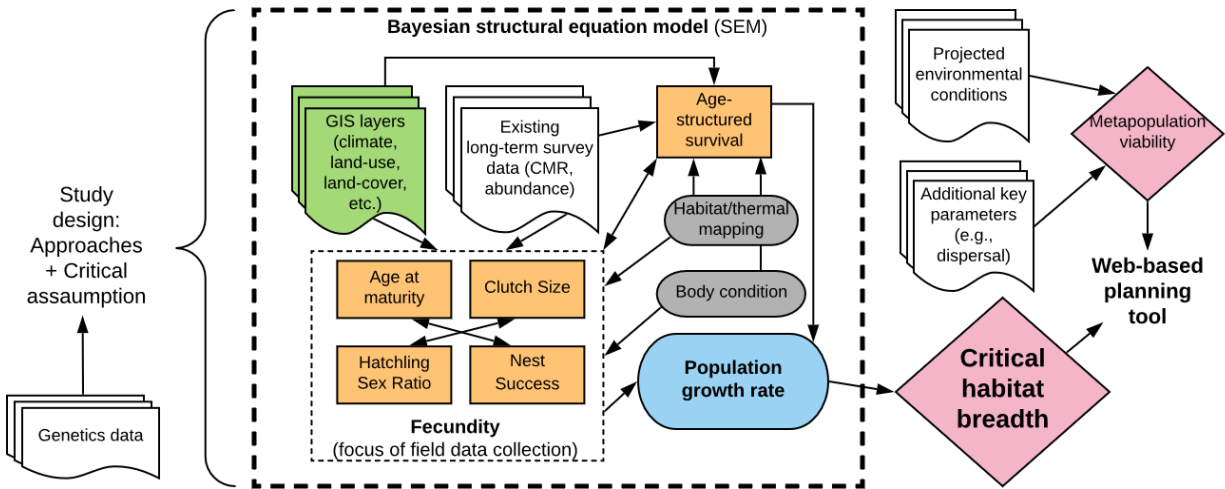


Figure ES-1. Schematic of our framework for determining population resilience and critical habitat for Desert and Gopher Tortoises.

To address our research objectives and hypotheses, we built spatially explicit, range-wide population simulation models for both focal species (Desert Tortoise and Gopher Tortoise). Each population vital rate (e.g., survival, reproductive output; Table ES-1) was modeled as a function of exogenous factors (e.g., land cover, climate) and endogenous factors (e.g., age, body size). To gather the information required for estimating the environmental sensitivities of each vital rate, we made extensive use of existing data sources. Our field data-collection efforts focused on measuring vital rates related to offspring production (fecundity) for which linkages with environmental drivers were poorly understood – including age-at-maturity, hatchling sex ratio, and nest success. To assess the potential role of local adaptation in preventing rapid *in situ* adaptation to changing environmental conditions, we conducted field studies at several translocation sites to test for differences in population vital rates and resource selection between translocated and resident individuals. Field data collection occurred both on and off DoD installations to sample across the broadest possible range of environmental conditions. To model critical habitat breadth for both focal species, we combined rigorous statistical models (whereby each population vital rate was modeled as a function of changing environmental and climatic conditions) with a suite of spatiotemporal climate projections and habitat rasters to generate a single modeling framework capable of forecasting the abundance of each species across space and time. Finally, we used these range-wide, spatially explicit population simulation models to produce projections of population growth rates and critical habitat over the next century, and to assess metapopulation viability under multiple plausible future climate scenarios.

Table ES-1. List of population vital rates (*F*=fecundity, *S*=survival) modeled as a function of climatic and environmental gradients.

	Vital rate	Data for measuring across an environmental gradient*
F	Age & size at maturity	Diagnostic imaging technology, morphometrics
	Probability of reproduction	Diagnostic imaging technology, telemetry
	Egg production (clutch size)	Diagnostic imaging technology, telemetry
	Nest success	Published literature
	Hatching success	Nest-site monitoring
	Mean hatchling sex ratio	Nest-site monitoring, hormone assays
	Hatchling survival	Published literature
S	Juvenile & adult survival	Existing capture-mark-recapture and telemetry data, published literature

* Whenever possible, field data collection was supplemented with literature review and/or re-analysis of existing data sources for modeling each vital rate.

Results and Discussion

For both species, we were able to fit statistical models of key vital rates as a function of climate drivers, enabling us to forecast how these vital rates are expected to shift across broad spatiotemporal climatic gradients. Our statistical models indicated that some vital rates are expected to become less favorable for population growth under climate change; for example, we showed that hatching success for Desert Tortoises is expected to decrease with increasing temperatures, suggesting that fewer eggs will be viable as nest temperatures increase (in contrast, temperature effects on hatching success for Gopher Tortoises were more complex and site-specific). Other climate-induced changes were expected to benefit populations in the future; for example, our nest-site monitoring data suggest that hatchling sex ratios for Desert Tortoise are expected to become more female-biased as temperatures warm (due to temperature-dependent sex determination), likely increasing population-level reproductive potential. For Gopher Tortoises, our field data suggests that warmer conditions have also increased reproductive output for some populations. In addition, we showed that warmer temperatures can hasten somatic growth for Desert Tortoise, resulting in earlier age-at-maturity, which is expected to increase lifetime reproductive potential for this species under climate change. Although many key vital rates for both species were positively dependent on precipitation (presumably due to increased production of the plants on which these species forage), the future trends in precipitation are far less clear than for temperatures (which are expected to rise substantially over the coming decades). While testing the role of local adaptation in “masking” apparent climate resilience proved more difficult than expected, the evidence we uncovered tended to indicate that local adaptations are unlikely to play a major role in preventing climate adaptation in either species.

Table ES-2. List of key vital rates (*F*=fecundity, *S*=survival) and climate sensitivities identified in this project

	Vital rate	Desert Tortoise	Gopher Tortoise
F	Age & size at maturity	Function of somatic growth, which was modeled as a function of temperature and precipitation	Modeled directly as a function of mean temperature
	Probability of reproduction	Function of temperature, precipitation, and body size	Function of temperature
	Egg production (clutch size)	Function of temperature, precipitation, and body size	Function of temperature
	Nest success	No climate drivers measured	No climate drivers measured
	Hatching success	Function of temperature	Function of temperature and precipitation
	Mean hatchling sex ratio	Function of temperature	Function of temperature and precipitation
	Hatchling survival	No climate drivers measured	No climate drivers measured
S	Juvenile survival	No climate drivers measured	Both juvenile and adult survival modeled as a function of years-since-burn, which tended to increase with climate change
	Adult survival	Function of precipitation	

Our simulation models indicated that Desert Tortoise populations are more likely to be sustainable through 2099 than Gopher Tortoise populations. In our models, large areas of the current Desert Tortoise range are estimated to be resilient across multiple climate change scenarios (Figure ES-2). For the Gopher Tortoise, few populations across the range were forecasted to be sustainable in our simulation models. This lack of sustainability was driven by low estimated baseline vital rates and a severe effect of burn history on survival. Climate change is expected to reduce the frequency of prescribed fire throughout the southeastern USA, and our models show that this effect can exacerbate the decline of Gopher Tortoise populations this century (Figure ES-3).

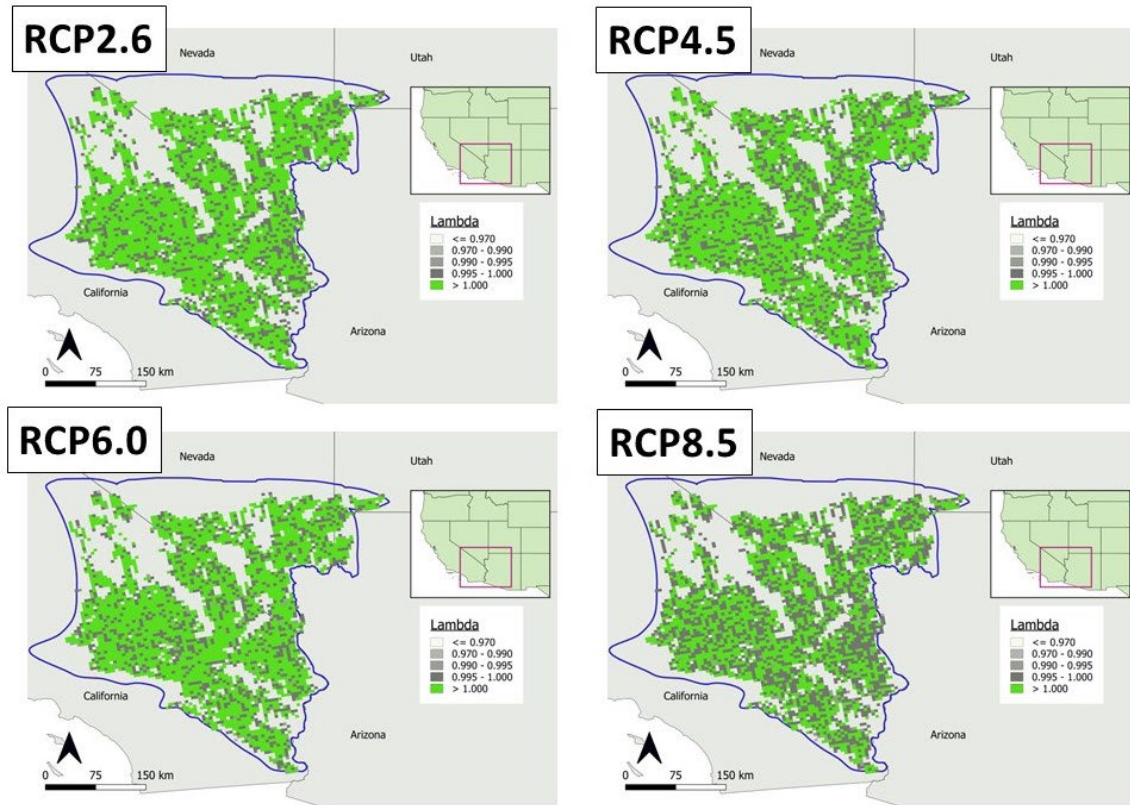


Figure ES-2. Map of critical habitat for Desert Tortoise (defined as areas that are expected to sustain Desert Tortoise populations over time in the face of climate change) across four plausible carbon emissions scenarios: RCP2.6, RCP4.5, RCP6.0, and RCP8.5. Green pixels indicate areas likely to support stable or growing Desert Tortoise populations ($\text{Lambda} \geq 1$). Shades of grey indicate that a population decline is expected to occur in a pixel, with darker shades indicating weaker declines (Lambda approaching 1) and lighter shades indicating that a substantial climate-related decline is expected to occur by the year 2099 ($\text{Lambda} < 1$). Note that our models focus on threats from climate change, and do not incorporate other

anthropogenic threats to Desert Tortoise populations, including habitat fragmentation and expanding raven populations.

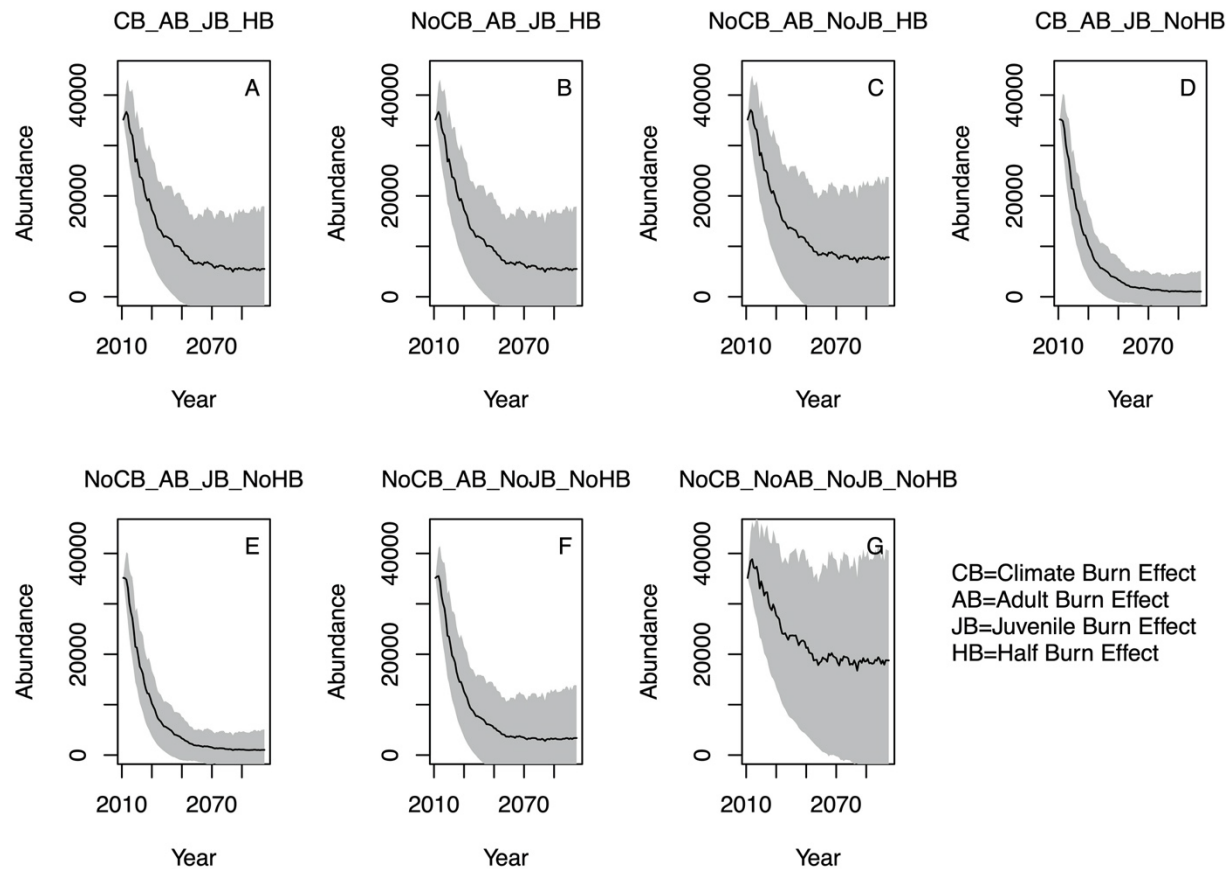


Figure ES-3. Simulated Gopher Tortoise abundance trajectories (sum across all age classes) for a single climate model (CESM1-BGC 8.5) across all unique burning scenarios. Dark lines denote mean abundance across scenarios, while gray polygons reflect one standard deviation above and below the mean. Plot titles reflect burn scenarios as a combination of binary activation switches: CB vs noCB reflects whether or not the climate effect on burn frequency was activated, AB vs noAB reflects whether or not burn frequency affected adult survival, JB vs noJB reflects whether or not burn frequency affected juvenile survival, and HB vs noHB reflects whether the effect of burn frequency was reduced by 50%.

Implications for Future Research

Although our primary focus in this project has been on quantifying the climate resilience of Desert and Gopher Tortoise populations across their respective ranges, both species face a number of threats in addition to climate change. Most importantly, human development activities threaten to remove or degrade many critical habitat areas. Future versions of our simulation models could incorporate expected land-use change scenarios, resulting in estimates of the relative impacts of land-use vs climate change for both species, and enabling identification of key habitat areas that should be conserved to maintain viable metapopulations. Because our simulation models are built to handle spatially explicit information in the form of multi-band

raster images (one band per simulation year), it will be straightforward to incorporate additional raster time-series information to these models. In addition, our current models use a relatively simplistic dispersal process by which individuals can move from one subpopulation to another. With concerns about connectivity playing a key role in management decisions for both species, it will be valuable to incorporate a more detailed, evidence-based model of connectivity in this simulation framework.

Benefits

We have built a conservation planning tool (“*MITCH-Clim v0.1*: Model-based Insights into Tortoise Critical Habitat Under Climate Change”; https://kevintshoemaker.shinyapps.io/Mitch-clim_v01/) that allows users to visualize outputs from our simulation model for a selected region of interest. This application, as well as the publications and reports emerging from this project, will help resource managers as they make critical land use decisions and species-level management decisions, such as planning potential future translocation sites that will sustain healthy populations for many decades into the future.

Objectives

Our primary research objectives were to (1) understand and model the complex pathways through which environmental conditions influence population vital rates (and thereby population growth rates), specifically for two *Gopherus* tortoise species (Mojave Desert Tortoise, *G. agassizii* and Gopher Tortoise, *G. polyphemus*) that pose operational challenges at multiple military installations, (2) use these models to rigorously define critical habitat breadth (range of environmental conditions capable of supporting viable populations and metapopulations) for these species, and (3) develop a web-based conservation planning tool to aid DoD installation managers in developing cost-effective strategies for protecting metapopulations in the face of extensive uncertainty about the future (**Figure 1**).

We posed two main research questions: (Q1) can *Gopherus* tortoise populations remain viable across a range of different environmental regimes? (Q2) can apparent tolerance of different environments be attributed to inherent resilience rather than local adaptation? We used a set of complementary approaches to address these two fundamental questions: we addressed the first question by assessing population growth and other key vital rates (survival, fecundity, age at maturity) across multiple populations as a function of environmental and climatic drivers; we addressed the second question by assessing the degree to which tortoises translocated from different environments exhibited vital rates less favorable to population growth. If the answers to both questions were affirmative, it would indicate that these *Gopherus* species have high resilience to environmental and climatic change.

Our research hypotheses were as follows:

H1: Mojave Desert and Gopher Tortoises will exhibit vital rates favorable to population growth across a wide range of environmental and climatic conditions

- a. Individual vital rates remain largely unchanged (and favorable) across the range of relevant environmental and climatic conditions
- b. Individual vital rates vary across a relevant range of environmental and climatic conditions, but in opposing directions (compensatory modulation; **Figure 2**)
- c. *Gopherus* vital rates are expected to remain favorable to population growth under plausible climate change scenarios across much of their current range

H2: Mojave Desert and Gopher Tortoise vital rates will exhibit minimal evidence for local adaptation

- a. Demographic vital rates of translocated tortoises will remain largely unchanged (and favorable) across a wide range of environmental and climatic differences from their source environments

H3: Mojave Desert and Gopher Tortoise populations are resilient to climate change

- a. *Gopherus* vital rates are expected to remain favorable to population growth under plausible climate change scenarios across much of their current range
- b. Most (>80%) currently occupied populations (in our models, treated as gridded regions at a resolution of 5 km) are expected to remain viable under plausible climate change scenarios

- c. The total fraction of the current known-occupied range that transitions from favorable to unfavorable due to climate change will be offset by an equal area of the range that is expected to transition from unfavorable to favorable.

H4: Climate change is unlikely to disrupt metapopulation viability for Mojave Desert and Gopher Tortoises

- a. Currently viable *Gopherus* metapopulations will remain viable under plausible climate change scenarios
- b. In the absence of land-use change and other anthropogenic threats to tortoise populations, current critical connectivity areas will remain connected (following currently accepted operational definitions) under plausible climate change scenarios.

Throughout this report, we have organized our performance tasks as follows:

- Task 1. **Assess environmental sensitivities** of *Gopherus* population vital rates
 - a. Compile existing data sources related to our research questions
 - b. Carry out additional field data collection and lab-based research efforts to address gaps in existing knowledge
 - c. Build statistical models of population vital rates as a function of environmental gradients (H1)
- Task 2. **Test for local adaptation** in population vital rates
 - a. Collect field data and compile existing data sources documenting the performance of translocated vs resident tortoises
 - b. Model local adaptation as the expected change in demographic vital rates explained by environmental and geographic differences between source and recipient sites (H2; using genetic assignment tests to identify source populations where necessary)
- Task 3. **Model critical habitat breadth**, effectively combining tasks 1&2
 - a. Build a comprehensive critical habitat breadth model, incorporating mean responses and sampling uncertainties
 - b. Forecast population and metapopulation growth across space and time (including multiple plausible climate change scenarios) using the critical habitat breadth model from Task 3a (H3)
 - c. Analyze simulation results to better understand how climate and environmental factors affect critical habitat breadth for both species
- Task 4. **Build conservation planning tool** using the results from Task 3
 - a. Use the R-Shiny reactive programming environment to enable stakeholders to visualize the expected impacts of climate change on Desert Tortoise populations and metapopulations under climate change for selected regions of interest.

Background

This project is responsive to the objectives articulated in RCSON18-C1. Specifically, the major goal of this project was to develop a cost-effective framework for assisting land managers tasked with managing threatened and endangered species (TES) in a non-stationary world. In accordance with the SON, our framework enables identification and ranking of critical habitat resources at a landscape scale, while accounting for the vast challenges and uncertainties associated with climate change.

Conserving threatened and endangered species (TES) on Department of Defense (DoD) installations, without sacrificing the ability of military installations to fulfill their stated missions, is necessarily a complex balancing act. Climate change, which can result in effective loss of habitat through processes such as increased drought frequency, extreme heat, and phenological mismatch in the timing of critical food resource availability, only intensifies these challenges (Gibbons et al. 2000, Cayan et al. 2010, Araujo et al. 2004, Lawler 2009). Consequently, habitat protection and restoration initiatives for TES species on DoD installations and elsewhere must rigorously account for new risks and uncertainties (Lawler 2009, Millar et al. 2007; Dawson et al. 2011).

Many researchers have attempted to measure the nature and magnitude of anticipated global change processes on specific habitat areas (e.g., military installations) and across species' geographic ranges (Pearson et al. 2014, Hunter et al. 2015, Gardali et al. 2012). However, measuring exposure to environmental change is only one piece of the puzzle – it is equally critical and much less common to measure the capacity of populations to weather those changes (Reed et al. 2010, Valladares et al. 2014, Dawson 2011, Beever 2016). Understanding TES' resilience to environmental change is essential for determining whether conservation and recovery objectives are achievable with low-cost management solutions (e.g., maintaining existing habitat on-site) or whether more resource-intensive solutions (e.g., large-scale habitat restoration, intranslocation efforts, establishing interconnected conservation reserve networks across jurisdictional boundaries) may be required to ensure persistence and/or recovery of TES metapopulations.

Species can persist in a non-stationary world via one of three primary lines of defense: first, individual organisms can adjust behaviors (e.g., to reduce thermal stress or accommodate diet changes) or acclimate physiologically (e.g., increased tolerance of hotter or drier conditions (Stillman 2003, Calosi et al. 2008)). This **functional-trait plasticity**, where applicable, provides the most immediate and effective safeguard against uncertain and changing environmental conditions because it confers populations with a latent ability to persist and even thrive under altered environmental conditions (Dawson 2011, Charmantier et al. 2008, Fuller et al. 2010, Richter et al. 2012). Secondly, organisms inhabiting degraded, formerly suitable habitat can disperse to more suitable habitat, possibly colonizing previously unsuitable sites. This **long-distance dispersal** can be considered a secondary line of defense, as it is associated with high mortality risk for most wildlife and may not be available to many species due to natural mobility limitations, movement barriers, or anthropogenic encroachment (Fordham et al. 2012, Schloss et al. 2012). Finally, populations can adapt *in situ* to changing environments via **rapid evolution**, as more favorable genes (e.g., enabling increased drought tolerance) become enriched in populations over several generations (Chevin et al. 2010, Hoffman and Sgro, 2011). Although these strategies are not mutually exclusive, those listed first can obviate the need for subsequent

lines of defense; for example, behavioral acclimation or long-distance dispersal can eliminate the need for rapid evolutionary change.

Similarly, conservation of TES populations in a non-stationary world can take several forms: (1) protect and/or restore existing habitat (e.g., on DoD installations and/or surrounding lands), (2) protect and/or restore existing habitat and adjacent lands expected to become suitable in the future, or (3) protect and/or restore non-adjacent habitats and movement corridors, likely involving extensive inter-agency coordination to establish connected habitat networks across jurisdictional boundaries. To determine which approach is best suited to maintaining TES metapopulation viability, it will be necessary to precisely define and empirically measure **critical habitat breadth** – that is, to understand and predict the full range of habitat and environmental conditions capable of harboring viable populations now and in the future. Unfortunately, standard habitat models – e.g., expert-driven habitat suitability models (HSM) and correlative statistical models (often called “species distribution models”, or SDM (Elith and Leathwick 2009, Pearson and Dawson 2003)) – are not well-suited for this task. Although these approaches generally perform well for identifying present-day habitat (Elith and Leathwick 2009, Elith et al. 2006), their ability to project species’ responses to climate change is unclear and hotly debated (Wisz et al. 2013, Davis et al. 1998, McMahon et al. 2011, Van de Putten et al. 2010). In fact, reliance on standard habitat models for performing climate risk assessments may frequently overestimate risk from climate change because most vulnerability assessments focus more on the magnitude of exposure to change (i.e., the degree to which local habitat and environmental conditions are likely to change) rather than the natural resilience or adaptive capacity that populations may possess (Beever et al. 2016, Nicotra et al. 2015, Williams et al. 2008, Moritz and Agudo 2013). Notably, rapid climate change in the late Pleistocene – which recent evidence suggests would have outpaced dispersal potential for many species – failed to result in mass extinction events, suggesting that many species may be more resilient to climate change than current habitat models predict (Dawson et al. 2011, Moritz and Agudo 2013, Hof et al. 2011).

More generally, climate change presents major existential challenges for biological conservation, and may demand an overhaul of best practices for wildlife protection. For example, habitat management and conservation planning efforts for TES often rely upon a strong notion of optimal or benchmark habitat conditions (e.g., via HSMs or SDMs), a paradigm that often culminates in the manipulation of large parcels to match idealized conditions more closely (Millsom et al. 2000, James et al. 2001). However, the effects of climate change on wildlife habitat are highly uncertain and difficult to predict, and it is often unclear whether habitat conditions that currently qualify as “optimal” will remain so under climate change (Davis et al. 1998, Hiers et al. 2016). Therefore, a more flexible, bet-hedging strategy that embraces the vast uncertainties associated with environmental change is likely to protect against a wider range of contingencies in a non-stationary world (Hiers et al. 2016). Rather than focus on protecting large parcels of “optimal” habitat, such an approach would prioritize protection of a broad range of suitable habitats and climates (including sink habitats expected to become suitable in the future). To effectively implement a formal bet-hedging strategy (e.g., for preparing Integrated Natural Resource Management Plans [INRMP]), it will be necessary to shift focus from the quantification of critical (or optimal) habitat to the quantification of critical habitat breadth.

Tortoises in the genus *Gopherus*, including the Mojave Desert Tortoise (*G. agassizii*) of the southern basin and range physiographic region and the Gopher Tortoise (*G. polyphemus*) in the southeastern USA provide an excellent model system for developing and applying the critical

habitat breadth concept to TES populations for two important reasons: (1) *Gopherus* tortoise populations have been studied extensively for decades, at great cost to DoD and others, allowing our research team to leverage a huge arsenal of demographic and movement data across diverse environments, and (2) many prior translocation “experiments” have been conducted, providing a rare and exciting opportunity for a “common garden” approach (Kawecki and Ebert 2004, Alexander et al. 2015) for rigorous investigation of the relative role of local adaptation vs. phenotypic plasticity in explaining variation in key vital rates and functional traits (**Table 1**). Recent modeling efforts have suggested that *G. agassizii* habitat and favorable conditions will be greatly reduced by climate change (Barrows et al. 2016, Barrows 2011, Lovich et al. 2014); however, these models do not account for key resilience mechanisms. In contrast, paleoclimate research coupled with fossil records indicates that *G. agassizii* has occupied much of its current range for several million years despite large climatic fluctuations (Morafka and Berry 2002). Here, we use existing data and newly collected data to define critical habitat breadth for *G. polyphemus* and *G. agassizii*, to apply this information to assess metapopulation viability for these species and to determine whether or not costly management options (e.g., option 3 above) are required to conserve these species in a non-stationary world. We hypothesize that *Gopherus* tortoise populations are more resilient to anticipated environmental change than previous research suggests.

In this project, we evaluate and apply the critical habitat breadth concept to *Gopherus* tortoises by (1) modeling how the variance and co-variance of key components of population fitness (vital rates; **Table 1**) are influenced by an array of relevant bioclimatic variables, and (2) coupling this information with metapopulation projection models to quantify the breadth of environmental conditions under which populations and metapopulations are capable of exhibiting stable or positive growth (critical habitat breadth). Finally, we developed a web-based conservation planning tool that applies the critical habitat breadth concept to assess climate-driven changes in habitat suitability and population dynamics for Desert Tortoise metapopulations across this species range. This tool allows users to select focal regions of interest (e.g., military installations and adjacent lands), and will assist land managers and other stakeholders in identifying mitigation and conservation strategies capable of sustaining viable metapopulations under changing and uncertain future conditions.

Materials and Methods

In this project, we applied the critical habitat breadth concept to *Gopherus* tortoises by modeling how key components of population fitness (vital rates; **Table 1**) are influenced by an array of spatiotemporally variable environmental and bioclimatic variables, and then coupling this information with metapopulation projection models to quantify the breadth of climate conditions under which populations and metapopulations are capable of exhibiting stable or positive growth (critical habitat breadth). By accounting for the discrete mechanisms by which climate may affect demographic rates, and the mechanisms by which these climate-driven effects may amplify or counter-balance each other, these models represent a sophisticated tool for assessing the potential role of climate change in altering habitat suitability and driving population dynamics for *Gopherus* metapopulations (**Figure 1**). These models should assist land managers in identifying mitigation and conservation strategies that account for changing and uncertain climatic conditions.

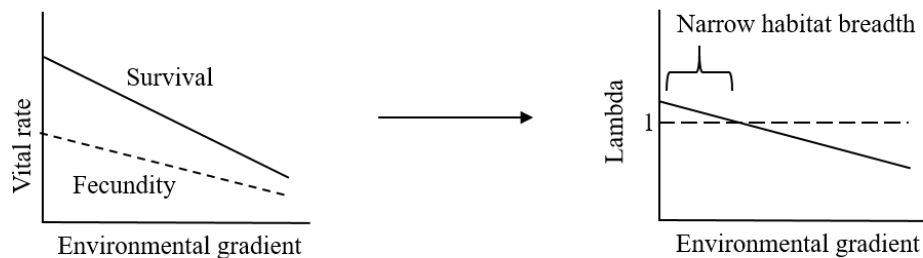
The first step in this project was to gather the data required for assessing the environmental sensitivities of demographic vital rates. We were able to secure permission to use several existing long-term survey datasets as part of this project. In addition, we conducted intensive field data collection efforts over multiple field seasons across the range of both focal species. Field data-collection efforts focused on measuring vital rates for which linkages with environmental drivers were poorly understood – including offspring production, age-at-maturity, hatchling sex ratio, and nest success. Field data collection occurred both on and off DoD installations to sample across the broadest possible range of environmental conditions. In addition, we conducted field studies at prior translocation sites to investigate the potential for local adaptation acting as a barrier to climate resilience. We then compiled a suite of climate and habitat data (GIS layers) and used statistical models to assess relationships between climate conditions and vital rates. These climate-vital rate relationships comprised the key components of our simulation models for characterizing critical habitat breadth for both *Gopherus* species (**Figure 1**), whereby population growth rate was modeled explicitly as a function of multiple pathways involving inter-correlated vital rates and spatiotemporally varying environmental conditions. Fitted population models were used to produce spatiotemporal projections of population growth rates and critical habitat, and to assess metapopulation viability under multiple plausible future scenarios (**Figure 2**). Finally, we integrated the results from these analyses into a web-based conservation planning tool. The remainder of the Materials and Methods section is organized into the four major project tasks:

- Task 1. Assessing environmental sensitivities of population vital rates,
- Task 2. Testing for local adaptation
- Task 3. Modeling and forecasting critical habitat breadth
- Task 4. Developing a web-based conservation planning tool

Table 1. Key demographic vital rates used in critical habitat breadth models for Mojave Desert and Gopher Tortoises.

	VITAL RATE
SURVIVAL	Juvenile survival
	Adult survival
FECUNDITY & RECRUITMENT	Recruitment
	Age-at-maturity
	Clutch size
	Nest success
	Hatchling sex ratio
	Hatchling survival

1. Vital rate phenotypic plasticity



2. Vital rate phenotypic plasticity with compensatory modulation

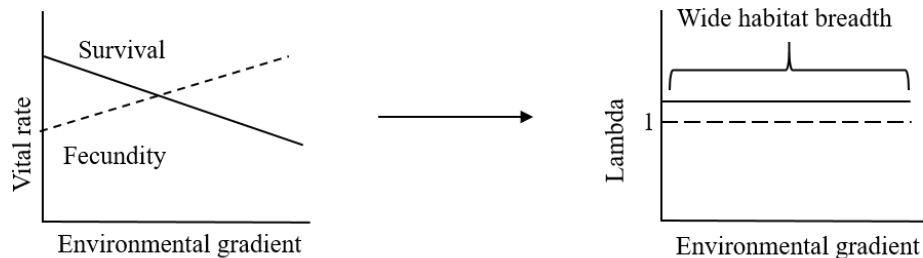


Figure 1. Schematic diagram illustrating how critical habitat breadth is defined by the relationships between population vital rates and by the potentially opposing functional relationships between population vital rates and environmental variables (compensatory modulation). Habitat breadth is defined as the range of environmental and climatic conditions under which populations can remain viable; populations with mean $\Lambda < 1$ are not self-sustaining. In this project, we focus on climatic gradients, but other types of environmental gradients could be incorporated for a more complete characterization of critical habitat breadth.

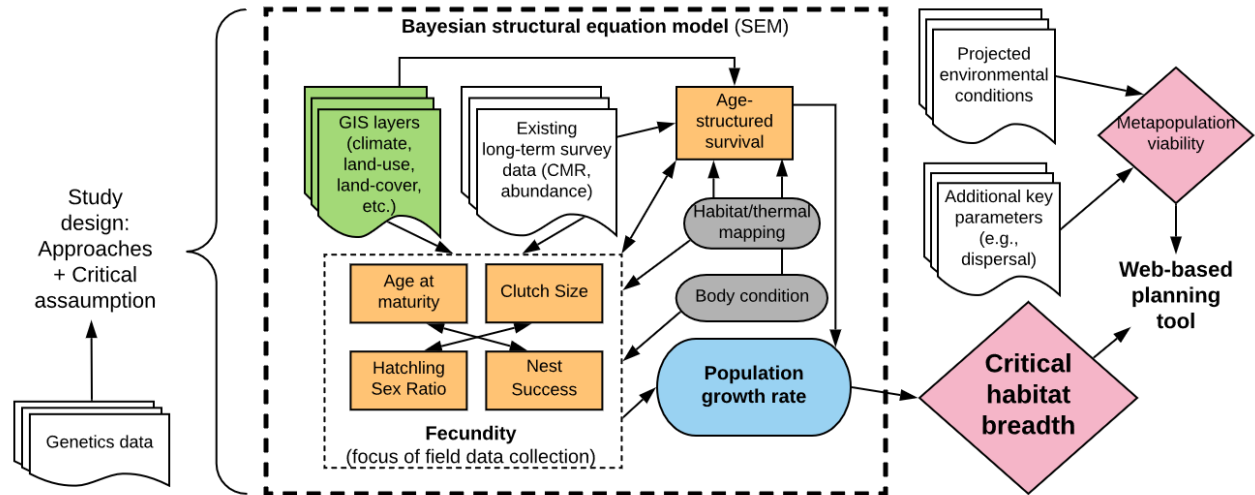


Figure 2. Schematic diagram of our approach for modeling critical habitat breadth for *Gopherus tortoises*.

Environmental Sensitivities of Population Vital Rates (Task 1)

The main objective of Task 1 was to develop a more comprehensive understanding of how Mojave Desert and Gopher Tortoise demographic vital rates (**Table 1**) vary throughout the range of these species as a function of spatiotemporal climatic gradients. To this end, we have used original and previously collected data to fit a series of models that together describe (1) which climate gradients are most important in determining key population vital rates, (2) how each vital rate varies across key environmental and climatic gradients, and (3) any positive or negative correlations among these climate-vital rate relationships that may exacerbate or reduce the population-level impacts of climate change on the two target species.

Our project involves two focal species in the genus *Gopherus*: one (Gopher Tortoise; *G. polyphemus*) occurs in the southeastern USA and the other (Mojave Desert Tortoise; *G. agassizii*) occurs in the Mojave Desert of the southwestern USA. Although extensive data sets are available for estimating key demographic parameters and their climate sensitivities for both species, including survival, somatic growth and reproductive output, we still lack sufficient information for estimating potential climate effects on hatching success and hatchling sex ratio. (**Table 1**). Therefore, the majority of our field efforts focused on identifying and monitoring nest sites for both species. Progress on these elements is provided in the sections below, organized by species (Desert Tortoise followed by Gopher Tortoise).

DESERT TORTOISE

Compile existing data sources (Task 1a)

For Desert Tortoise, we were able to compile several demographic data sources, including long-term capture-mark-recapture data, VHF telemetry data, and x-ray data on reproductive output (number of shelled eggs). We also compiled numerous range-wide GIS layers representing environmental conditions.

Demographic data

Mark-recapture data --

Capture-mark-recapture (CMR) data is among the most widely used data sources for estimating demographic parameters from wild populations (Thomson et al. 2008). CMR data enables robust estimation of survival, recruitment, abundance, population trends, and somatic growth (Cooch 2008). For Mojave Desert Tortoise, we have access to many long-term CMR datasets from across the range, many of which are known as the ‘permanent study plots’ (PSP; $n = 36$; Tracy et al., 2004).

The PSPs represent 36 plots (2.6 km²; 1 mi²) established across the species range between 1971 and 1994 by BLM, USFWS and others (**Figure 3**; Tracy et al. 2004). In survey years (plots were surveyed intermittently, not every year), at least 2 complete passes were conducted in the spring (ca 30 person-days each). Although data from the PSP sites were originally used to estimate abundance and trends in the context of assessing recovery goals (DT Recovery plan assessment report; Tracy et al. 2004), as available methods for analyzing CMR data become more powerful, these data are increasingly the subject of re-analysis. For example, Campbell et al. (2018) conducted a spatial population viability analysis (PVA) using PSPs for the Sonoran Desert Tortoise (*Gopherus morafkai*). Other efforts to re-analyze PSP data for Mojave and Sonoran Desert Tortoises have indicated that Desert Tortoise survival may be sensitive to extended drought (Longshore et al. 2003; Zylstra et al. 2013; Lovich et al. 2014). We see continued potential for reanalysis of the PSP data to improve estimates of key demographic rates for Desert Tortoise.

In particular, these data are amenable to Spatial Capture Recapture (SCR) analyses, an increasingly popular approach that is capable of increasing the precision of abundance and vital rate estimates (Royle et al. 2013). However, in order to prepare these data for SCR analysis we needed to digitize the locations of each capture on the basis of hand-drawn maps provided in unpublished reports. We completed this time-consuming task of georeferencing all recorded capture locations in 2020, and proceeded to develop spatially explicit capture-mark-recapture models to estimate survival rates, abundance, and temporary immigration rates for Desert Tortoise.

In addition, we see tremendous potential to further improve demographic vital rate estimates by applying the suite of techniques known as Integrated Population Models (IPM; e.g., Schaub and Abadi 2011). We continue to work with collaborators to integrate additional telemetry data (**Figure 4**) and line-distance transect survey data to improve vital rate estimates for Desert Tortoise. Finally, we used the PSPs (among other CMR data sources) to fit tortoise growth models (**Figure 3**), which enabled us to estimate the critical demographic parameter ‘age-at-maturity’, along with relevant environmental and climatic drivers.

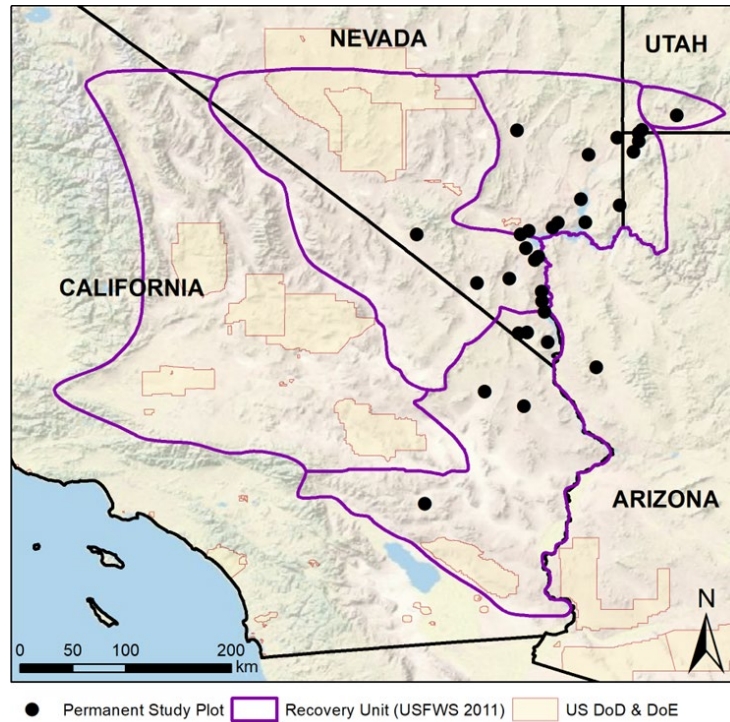


Figure 3. Locations of the ‘permanent study plots’ used in this study to estimate Desert Tortoise survival rates as a function of climatic and environmental gradients.

VHF telemetry data –

We are also fortunate to have access to long-term data from VHF telemetry studies that have been conducted across the range of the Mojave Desert Tortoise in recent decades (e.g., Drake et al. 2015). These data can help to estimate true (versus ‘apparent’) survival for Mojave Desert Tortoise (Murray 2006), to refine estimates of routine movements required for our SCR models (by combining VHF telemetry data with CMR data from the permanent study plots), and to estimate longer distance dispersal rates. Although our current model for adult survival is based only on the permanent study plot data, we are currently working with collaborators at Conservation Science Partners (CSP) to incorporate available telemetry data sources in our analyses. We hope to use the results of these analyses to refine our estimates of adult and juvenile survival as we move towards publishing our critical habitat breadth model for Desert Tortoise.

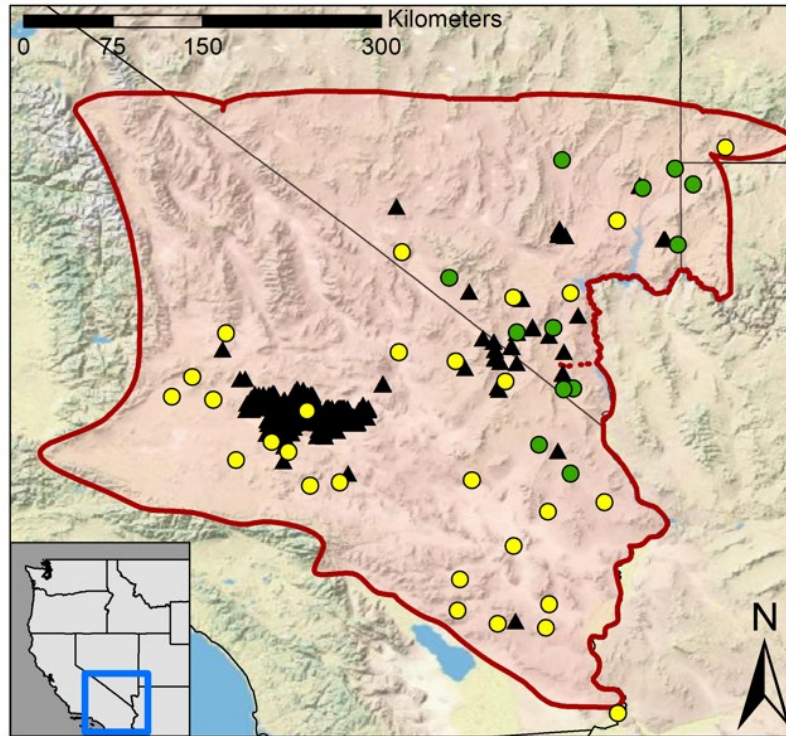


Figure 4. Locations of previous data collection efforts for *Gopherus agassizii*. Triangles represent long-term telemetry sites from which we acquired data as part of this project.

Reproductive output data (X-ray) –

We were able to acquire and re-analyze Mojave Desert Tortoise reproductive data that were collected by collaborators at 10 study sites that were distributed across the full range of the Mojave Desert Tortoise (see **Figure 5**). Data were collected from 1997 to 2002, although data were not collected at all sites for every year. The study sites were located within four of five recovery units for the Mojave Desert Tortoise: Upper Virgin River, Northeastern Mojave, Eastern Mojave, and Colorado Desert (USFWS 2011). Because this suite of sites is representative of the full range of this species, we were able to characterize the relationship between environmental drivers and reproductive output across the species range. Overall, reproduction data were collected for a total of 215 unique females (**Figure 6**). Egg production was assessed from X-radiographs of female tortoises taken biweekly between April and August of each year (**Figure 7**). Tortoises were X-rayed using a portable X-ray station. Nesting date was recorded if a female previously observed with eggs was observed without eggs during a subsequent X-ray session. From these data we computed the total number of eggs produced for each female in each year. For a detailed description of field methods see Nussear et al. 2012.

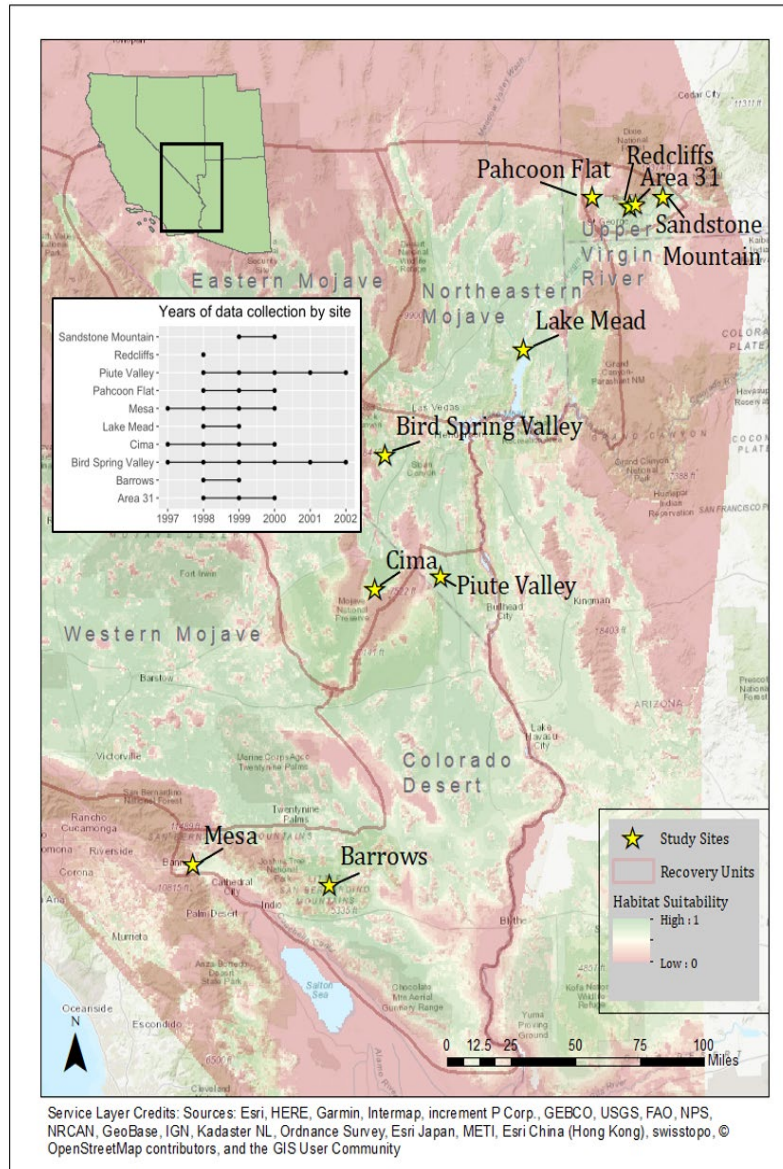


Figure 5. Map of sites at which surveys of reproductive output (using X-ray) were conducted from 1997 to 2002. The Mojave Desert Tortoise habitat suitability surface of Nussear et al. (2009) is used as a background for context. Reproduced from Mitchell et al. (2022). Inset figure indicates the number of years of data collected by site.

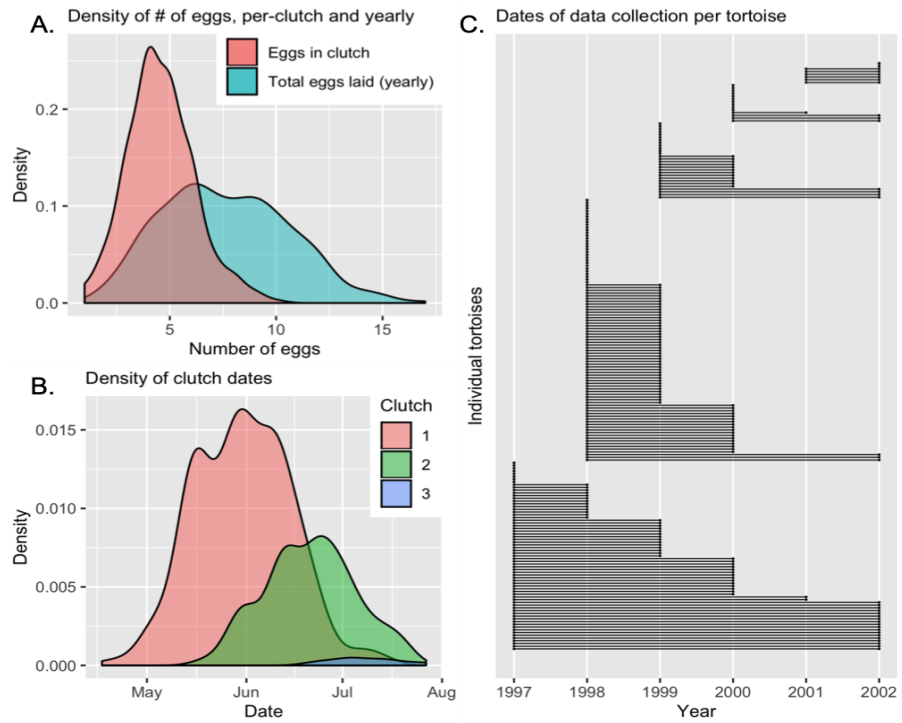


Figure 6. - a.) The distribution of the annual number of eggs produced by Mojave Desert Tortoise. The light pink curve displays the density across each individual clutch, while the blue curve displays the density of the yearly totals for each tortoise. b. The distribution of the egg-laying dates for the first, second, and third clutches. c. The years each tortoise was tracked. Each line represents a single tortoise and extends over the range of years in which data was collected for that tortoise. Single points represent tortoises that were only tracked for one year. Figure reproduced from Mitchell et al. (2022)

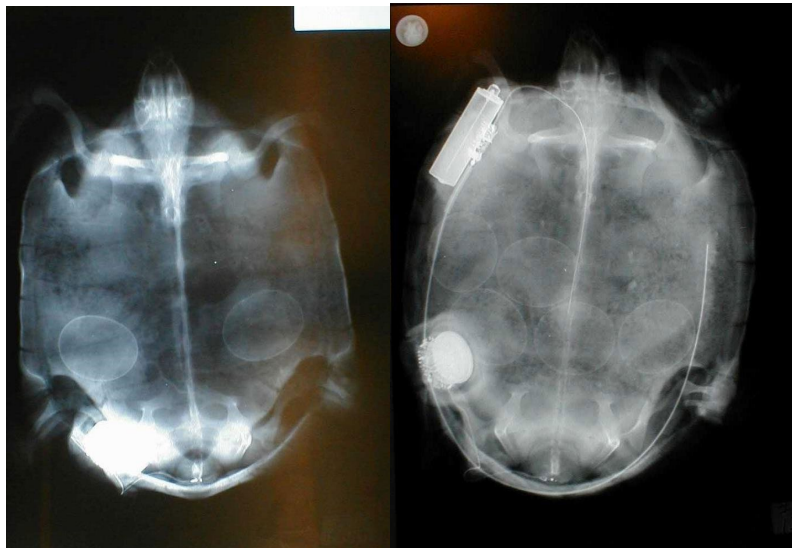


Figure 7. X-radiograph image of a female Desert Tortoise with two eggs on the left and a female tortoise with five eggs on the right. Figure reproduced from Mitchell et al. (2022).

Environmental and climate data

We worked closely with personnel from project **RC18-1207** (Heaton, Nussear et al.; *The impacts of land use and climate change on Mojave Desert tortoise metapopulation dynamics and corridor functionality*) to coordinate the development and assembly of key static and dynamic (1 month time resolution) spatial data layers (e.g., climate/weather) for the range of the Desert tortoise. **Figure 8** below represents an example of projected future climate conditions derived from a downscaled GCM for a single raster grid cell (800-m resolution) located near the center of the range of the Mojave Desert Tortoise.

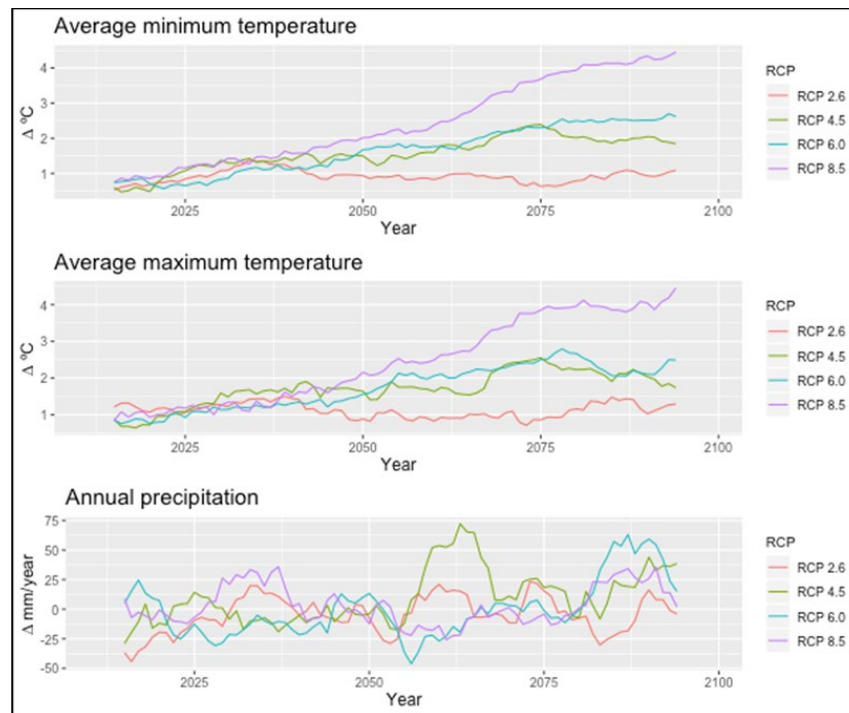


Figure 8. Monthly time series for one downscaled RCP model realization for each variable (precipitation, minimum temperature, and maximum temperature) over period CY 2011 through 2099 for a single 800m cell located near the center of the range of the Mojave Desert Tortoise. Used with permission from the principal investigators of SERDP project **RC18-1207**.

Field data collection and lab work (Task 1b)

To fill in knowledge gaps regarding climate effects on sex ratio and hatching success, we collected field data on nest sites for Mojave Desert Tortoises. To locate nests, we monitored adult females until nesting occurred. Radio transmitters were attached to females, and we performed ultrasounds approximately every 2 weeks to identify whether shelled eggs were present. When shelled eggs were detected, we attached an archival GPS unit and proceeded to measure tortoise mass daily until a sudden loss of mass indicated a nest was laid. Each nest's approximate lay date and location was determined from these repeated observations of each female. We discovered a total of 55 nests in June and July of years 2017, 2018, 2019 and 2021 at 5 sites (**Table 2**). A field season at Fort Irwin, CA in 2021 resulted in zero nests, likely due to

unprecedented drought conditions causing reproductive failure of monitored females. Upon discovery, each nest was inventoried, and eggs were returned to their original configuration. A temperature data-logger (Maxim Integrated iButton DS1921), programmed to record temperature every hour, was placed in the center of the nest chamber, and the nest was reburied and protected with hardware cloth caging on the surface. Despite this protection, 20 of the 55 discovered nests were completely depredated. Of the 35 surviving nests, we collected a total of 123 hatchlings from cages installed to protect nests from predation while allowing hatchlings to emerge naturally from the nest chamber. With a total of 173 eggs monitored, this resulted in an overall hatching success rate of $123/173 = 71\%$.

Hatchlings were collected from the cages on the morning of hatching, transported to our field office for processing and blood sample collection using the FSH injection method developed for Gopher Tortoises (see section on Gopher Tortoise field data collection), and returned and released at the nest location. We continued to collect blood samples for genetics and body condition and measurements for growth analyses from incidental tortoises encountered at all field sites. Temperature dataloggers were retrieved after all nests had hatched.

In addition, we collected growth measurements on 50 translocated juveniles at our 2018 field site, Coyote Springs, NV, so that we could compare pre- and post-translocation body size and growth in relation to the thermal environment (individuals were released in either burned or unburned habitat which exhibited $> 1^{\circ}\text{C}$ difference in average daily temperatures).

Table 2. Summary of Mojave Desert Tortoise (*G. agassizii*) nesting data from fieldwork at 6 sites.

Site	Nests	Nests depredated	Eggs after predation	# Hatchlings	Hatch. success	Year(s)	Nests with sex ratio & temperature
Bird Spring Valley, NV	7	1	35	24	0.69	2019	2
Boulder City Conservation Easement, NV	2	0	7	4	0.57	2021	1
Coyote Springs Valley, NV	23	16	41	32	0.78	2017 - 2018	3
Fort Irwin, CA*	0	-	-	-	-	2021	-
Large Scale Translocation Site, NV	11	1	49	30	0.61	2019	4
Trout Canyon, NV	12	2	41	33	0.80	2018	7
Global sum or mean	55	19	173	123	0.71	2017-2021	17

*Despite extensive fieldwork at Fort Irwin in 2021, no nests were discovered.

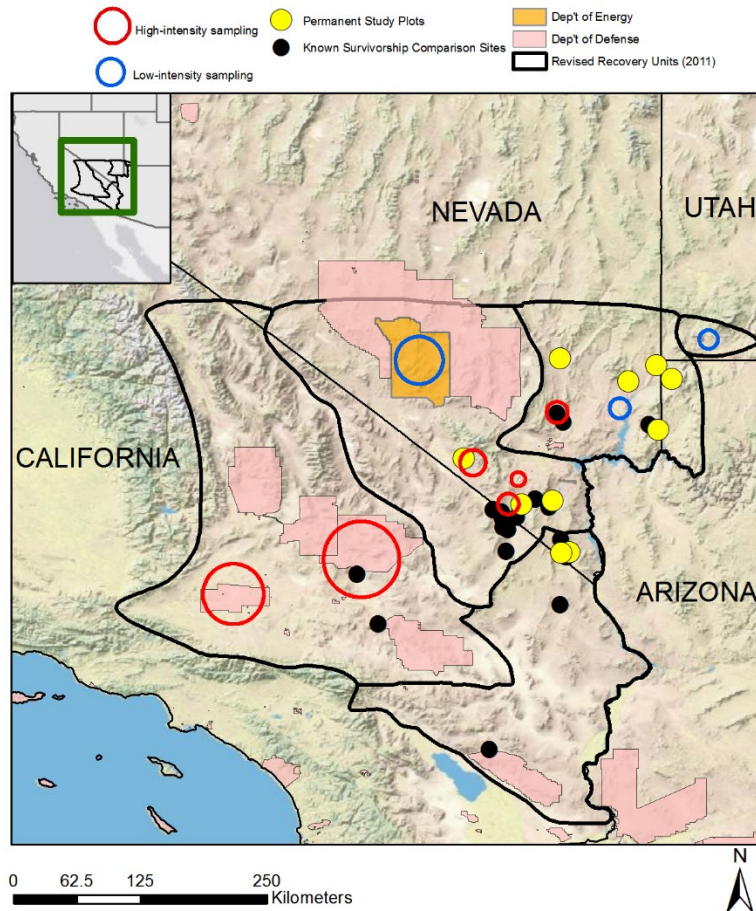


Figure 9. Locations of field data collection efforts for *Gopherus agassizii*. Red circles are high-intensity nest-monitoring sites. Blue circles and yellow dots are low-intensity demographic monitoring sites. Polygons are the currently established recovery units. Note that although we conducted an entire season of fieldwork at Fort Irwin, none of our radio-tracked females nested. In addition, planned fieldwork at Fort Edwards was abandoned due to complications during the COVID-19 pandemic.

Genetic samples

We collected genetic samples from all tortoises handled as part of this project, and worked with our collaborators in SERDP project **RC18-1207** to curate and archive these data. All Mojave Desert Tortoise samples we collected currently reside in a -20 °C freezer at the College of Agriculture, Biotechnology and Natural Resources at University of Nevada, Reno, and will be transferred to a long-term storage facility shortly after project completion. During our study, we have collected blood samples from all captured adult and juvenile Mojave Desert Tortoises in addition to 112 hatchling tortoises. In addition, we have collected bone/carcass samples and egg samples from incidentally encountered carcasses or nest remains- these samples have been preserved for future research.

Sex determination methods for neonate Gopher tortoises

We developed novel methods to determine sex of both *G. polyphemus* and *G. agassizii* neonates using circulating testosterone. We provide a summary of methods and results here; for more details, see Loope et al. (2022) and Walden et al. (in review). In addition to methods for neonates, Loope et al. (2022) describes effective sexing protocols for hatchling (6-8mo) and juvenile (>3yo) Gopher Tortoises based on testosterone concentration. Together, these methods are a critical advancement, in that they allow sex determination of field- or lab-hatched neonates within days of emergence, without captive rearing of hatchlings for several months to the size when laparoscopic surgery, the previous standard, is feasible (Rostal et al. 1994).

Hatchling Sex Determination

Candidate captive juvenile *G. agassizii* (< 100 mm straight midline carapace length) under the care of the U.S. Geological Survey, Western Ecological Research Center (Henderson, NV, USA) were evaluated for apparent age. We selected individuals that we estimated were 0–3 mo old between August–November 2019. These captive individuals originated from a broader pool of juveniles that were voluntarily surrendered from households in the Las Vegas valley (NV, USA) during this time period. We attempted to collect both a naïve and FSH-challenged sample from these candidates. All blood samples in this study were collected, processed, and stored in the manner described above for Gopher Tortoises. In addition to the captive animals, wild hatchling *G. agassizii* were sourced from wild nests with known dates of hatching in 2017–2021 (see below for field methods). Once hatched, we collected a blood sample from every wild live individual for innate testosterone in 2017–2021, except for 2019 animals which experienced FSH-challenge before blood sampling, as described in (Loope et al., 2022) and below in Gopher Tortoise section.

We quantified testosterone for each blood sample using the methods described for Gopher Tortoises (Loope et al., 2022 and below). When the captive hatchlings that we challenged with FSH were approximately 1.3–1.6 yr old, we definitively sexed them ($n = 33$) using endoscopic surgery, i.e., visual inspection of the gonads from within the coelom (Rostal et al. 1994; Hernandez-Divers et al. 2009). Wild animals were not sexed via surgery, and were released back to their collection location.

We used a probabilistic approach to estimate the relative probability of being male or female for a given value of testosterone by integrating over the density curves of fitted male and female lognormal distributions at the sample testosterone concentration using a 0.1 pg/mL interval. We then assigned sex as ‘male’, ‘female’, or ‘unknown’ based on where the sample concentration fell in relation to the threshold. We used four certainty thresholds: 50%, 80%, 95%, and 99.99% thresholds. We then used a Monte Carlo simulation approach to estimate misidentification rates in which we sampled testosterone concentrations for hypothetical known-sex hatchlings on the basis of the fitted lognormal distributions. For each replicate, we first randomly sampled parameter values for the appropriate lognormal distribution (representing male or female testosterone concentration) from a uniform distribution with upper and lower limits defined by the 95% bootstrapped confidence intervals. We then randomly drew a single testosterone concentration from this lognormal distribution and assigned a sex using the method described above. We ran a total of 10,000 replicates for each sex and computed the misidentification rate.

We collected naïve and challenged blood samples from 36 captive hatchlings in 2019, of which 33 survived for validation of sex by coelioscopic surgery in 2021. We *a priori* excluded naïve samples from six individuals due to known vial identification errors made in the laboratory. Naïve testosterone means and ranges differed substantially between sexes (**Figure 10**). Females and males were separated by 104.6 pg/mL using naïve testosterone concentrations, but overlapped between 114.8–139.0 pg/mL using challenged testosterone concentrations (**Figure 10**).

Among the wild hatchlings of unknown sex, naïve samples ($n = 48$) had 7.1–1351.7 pg/mL range while FSH-challenged samples ($n = 29$) had 13.6–2092.1 pg/mL range (**Figure 10**), with both types of samples including individuals within the range of concentrations separating both naïve and FSH-challenged captive males and females.

Our model for the effect of sex and FSH challenge on paired pre- and post-challenge samples from 23 individuals failed to show an interaction effect between sex and challenge status (repeated-measures ANOVA; $F = 0.37$, P -value = 0.55). Both females and males increased testosterone concentration in challenged samples over the naïve samples with few exceptions.

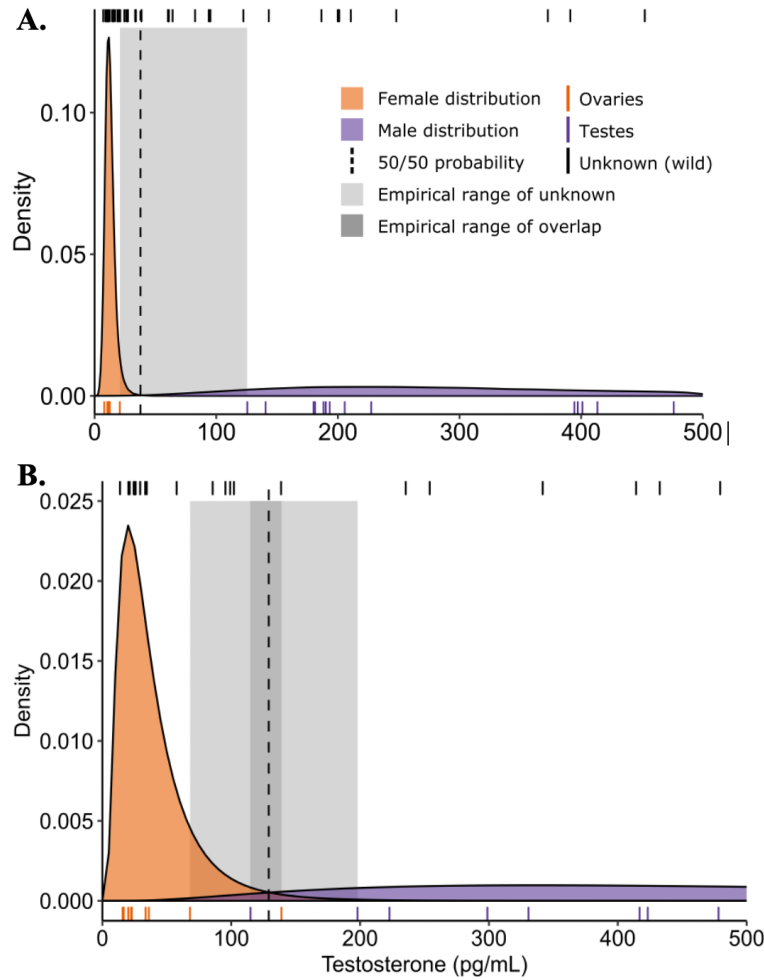


Figure 10. Fitted probability density functions for plasma testosterone concentrations of captive hatchling (0–3 mo old) Mojave Desert Tortoises (*Gopherus agassizii*) collected in Clark Co., Nevada, USA in 2019. Sex confirmed by coelioscopic surgery in 2021. Observed concentrations shown as colored bands beneath the density plot. Observed concentrations from wild hatchlings (0 dy old) of unknown sex collected 2017–2021 shown as black bands above density plots. (A) Naïve samples, (B) Follicle-stimulating hormone challenged samples. Figure reproduced from Walden *et al.* (in review)

We determined that our sample datasets with known sex followed the lognormal distribution in all cases. The probabilistic method of sex identification with 50% certainty level for the naïve samples had a very low rate of misidentification ($\sim 0.01\%$). The probabilistic method applied to the challenged samples performed poorly compared with the naïve samples, showing much higher proportions of the simulations assigning unknown sex or with higher misidentification rates (**Table 3**). Unsurprisingly, as the certainty level increased for the threshold method, the misidentification rates decreased and the number of assigned ‘unknown’ sex increased (**Table 3**).

Table 3.—Monte Carlo simulations for identifying sex of hatchling Mojave Desert Tortoise (*Gopherus agassizii*) using threshold values of testosterone concentrations such that values lower than the threshold are more likely to be female (F) with the indicated level of certainty, while values above the threshold are more likely to be male (M) with the indicated certainty.

FSH challenge	Level of certainty	Threshold (Testosterone pg/mL)	Number of individuals assigned each sex (total = 20,000)			Percent (%) misidentified	
			F	M	U	F	M
naïve	≥ 99.99%	$F \leq 23.3 < U < 56.5 \leq M$	9,788	9,982	230	0.00%	0.00%
	≥ 95.00%	$F \leq 32.7 < U < 43.2 \leq M$	9,981	9,998	21	0.01%	0.00%
	≥ 80.00%	$F \leq 35.3 < U < 40.3 \leq M$	9,991	10,000	9	0.02%	0.01%
	> 50.00%	$F < 37.7 < M$	9,993	10,007	·	0.04%	0.01%
challenged	≥ 99.99%	$F \leq 17.5 < U < 423.2 \leq M$	1,869	7,268	10,863	0.03%	0.00%
	≥ 95.00%	$F \leq 73.2 < U < 219.8 \leq M$	8,920	9,185	1,895	0.35%	0.37%
	≥ 80.00%	$F \leq 99.7 < U < 167.1 \leq M$	9,587	9,655	758	0.77%	0.73%
	> 50.00%	$F < 129.7 < M$	9,975	10,025	·	1.39%	1.26%

We identified the simple threshold of 37.7 pg/mL innate testosterone that can be used to identify the sex of hatchling *G. agassizii* with very low error, obviating the need for the more invasive and less accurate FSH challenge approach (Walden et al., *submitted*). Although we observed a large gap between the confirmed female with the highest testosterone and confirmed male with the lowest testosterone, our model did indicate some small area of overlap of the distributions of the testosterone concentrations for males and females. Indeed, we found that wild hatchling testosterone concentrations fell within that gap observed between the captive male and female hatchlings. It may be that even the short time in captivity from hatching to sampling experienced by the captive hatchlings may have been sufficient to further differentiate testosterone production in males from females. Some of the wild hatchlings that fall within this gap may not be as easily differentiable based on ELISA of innate testosterone; however, our

probabilistic approach allows for the calculation of the probability of misidentification of sex even for samples within that gap (Walden et al., *submitted*). We conclude that a single blood sampling collection occasion is appropriate and effective to identify sex of catch-and-release wild hatchlings using innate testosterone quantification.

GOPHER TORTOISE

Compile existing data sources (Task 1a)

For Gopher Tortoise, we were able to compile demographic data sources, including long-term capture-mark-recapture data, and ultrasound and x-ray data on reproductive output (presence of follicles and number of shelled eggs). We were also able to compile numerous rangewide GIS layers representing environmental conditions.

Demographic data

Mark-recapture data --

Capture-mark-recapture (CMR) data is among the most widely used data sources for estimating demographic parameters from wild populations (Thomson et al. 2008). CMR data enables robust estimation of survival, recruitment, abundance, population trend, and somatic growth (Cooch 2008). For Gopher Tortoise, we have access to a very long-term CMR dataset collected continuously since 1994 at three sites within Fort Stewart Army Reserve (FSAR; Rostal and Jones 2002; **Figure 11**). From 1994-2021, systematic road cruising transects were conducted at FSAR nearly every year. These surveys have yielded >1200 Gopher Tortoise captures over 24 annual re-surveys at FSAR. Although this dataset lacks the range-wide spatial coverage available for Mojave Desert Tortoise, the Gopher Tortoise CMR data was collected for a longer time frame at a finer temporal resolution, and was extremely valuable for estimating impacts of climate variation on key demographic vital rates. One unique and particularly valuable aspect of this dataset is that not only is it a long-term CMR dataset, but fecundity data (in the form of ultrasonography and x-radiography to determine reproduction and clutch sizes, as well as nest data) were collected on some of the same individuals. This allowed us to explicitly examine effects of climate on multiple demographic rates simultaneously.

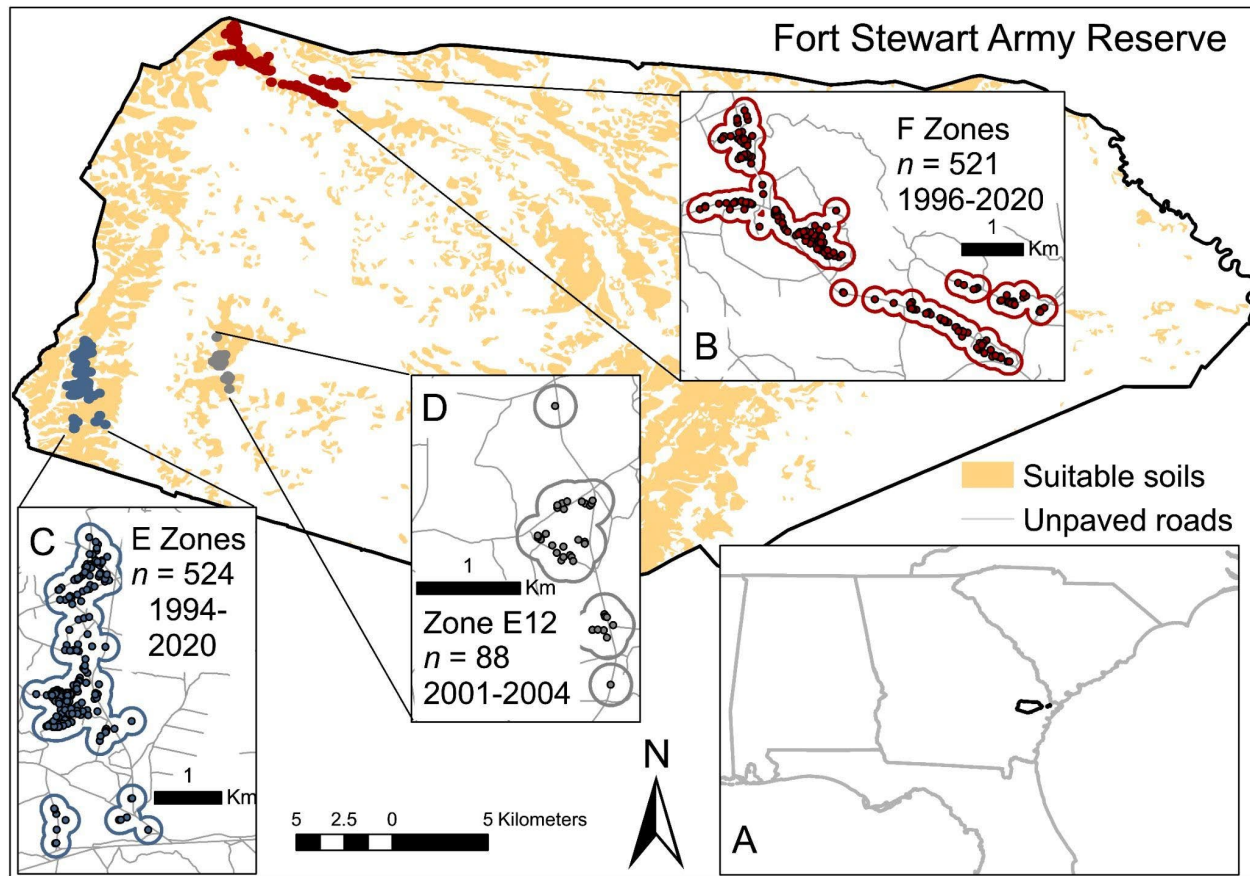


Figure 11. Sites at Fort Stewart Army Reserve (FSAR), Georgia, USA, surveyed from 1994–2020 to investigate the response of Gopher Tortoise population dynamics to prescribed burning. A) location of FSAR within the southeastern United States. Tan areas in the main map are areas of suitable soils for Gopher Tortoises (upland sandhills). Each site (F Zones [B, red, comprising FSAR zones F11 and F12], E zones [C, blue, comprising FSAR zones E17, E20, E21, and E22], Zone E12 [D, gray]) is delineated by a 100-mbuffer surrounding all capture locations (insets indicate number of tortoise captures and survey duration at each site).

Reproductive output data (ultrasound, X-ray, nests) –

For Gopher Tortoise, we used a long-term record of reproductive output derived from ultrasounds and X-ray images taken as part of the long-term CMR study at FSAR (**Figure 11**). Overall, we used reproductive data for 221 female Gopher Tortoises, many of which had reproductive data over multiple years and even decades. Although this dataset lacked spatial coverage (unlike the data available for Mojave Desert Tortoise), the Gopher Tortoise data were collected for a longer time frame at a finer temporal resolution. In order to determine the role of nest thermal environment on Gopher Tortoise hatching sex ratios, we used a historical dataset of thermal data and hatchling sex outcomes from 36 nests (from 1997, 1998, 2002, 2005, 2006, 2008) at FSAR.

Environmental and climate data

We used several environmental and climate datasets in our analyses for Gopher Tortoises. In analyses where daily information on climate variables were required, we used the Daymet climate dataset (<https://daymet.ornl.gov/>, Thornton et al. 2021). For some earlier analyses, we used Daymet version 3 (e.g., in Hunter et al. 2021); for the Gopher Tortoise PVA, we used Daymet version 4. For analyses in which longer-term climate trends were needed, we used the PRISM climate dataset (<https://prism.oregonstate.edu/>). Prescribed burning is an important management tool for Gopher Tortoise habitat, and we used spatial records of prescribed burning at Fort Stewart collected from 1991-2018 to create a spatially averaged metric of years-since-burned (Hunter and Rostal 2021).

For projection modeling, we used the NASA NEX-DCP30 dataset, which is comprised of downscaled (to 1km² grid cells) climate scenarios that are derived from the General Circulation Model (GCM) runs conducted under the Coupled Model Intercomparison Project Phase 5 (CMIP5, see Taylor et al. 2012). We selected 4 GCMs for 2 Representative Concentration Pathways (RCPs) that provided a range of possible futures for the Gopher Tortoise range. We chose RCP 4.5 (intermediate emissions scenario) and RCP 8.5 (high emissions scenario) to align with a study on how climate change will affect prescribed burning frequency that uses those two RCPs (Kupfer et al. 2020). To select the 4 GCMs, we first examined the average predicted change from 2006-2099 in maximum temperature and precipitation for the center of the Gopher Tortoise range across all 33 GCMs and the 2 selected RCPs in the NASA NEX-DCP30 dataset (**Figure 12**). We chose 4 GCMs: a wet scenario (MRI-CGCM3), a dry scenario (Inmcm4), a hot scenario (HadGEM2-CC), and a middle scenario (CESM1-BGC). This method of selecting projection models follows the “individual projection” method described by Lawrence et al. (2021). We then downloaded raster data for each month of the selected GCMs and RCPs from 2006-2099 and used monthly data to create aggregate variables that aligned with important climate effects for Gopher Tortoise demographic variables.

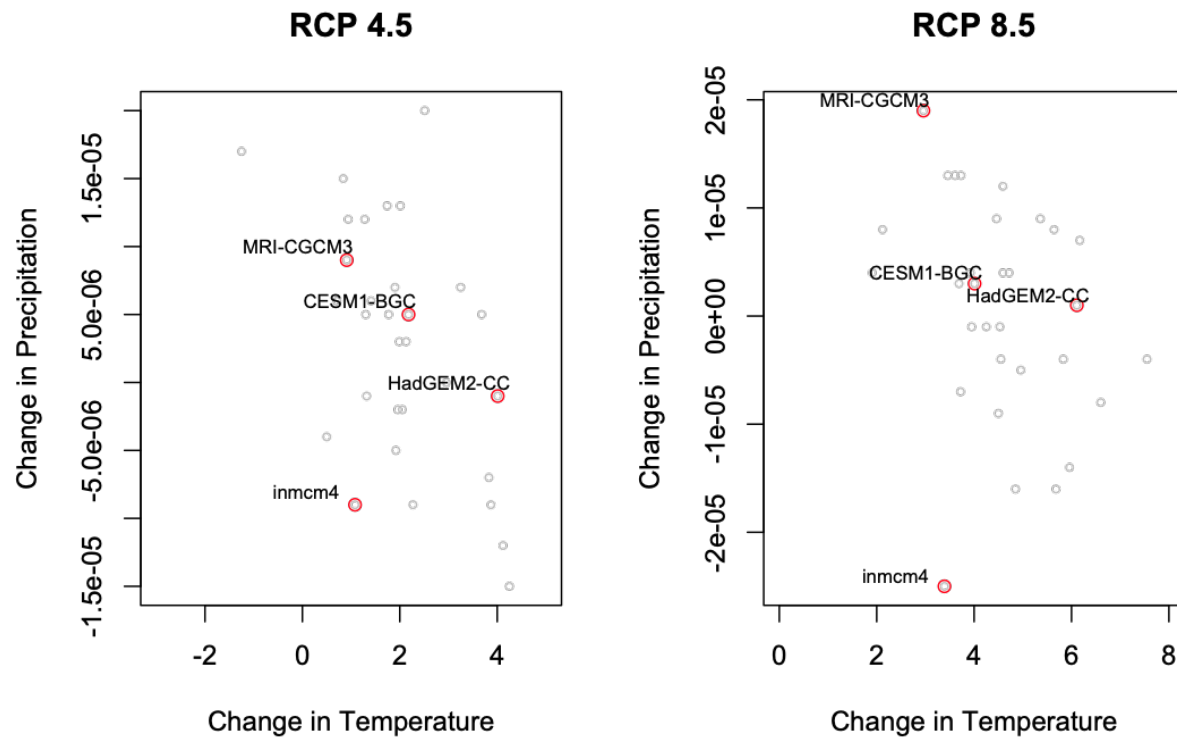


Figure 12. Predicted change in maximum temperature and precipitation across 33 General Circulation Models (GCMs) and two Representative Concentration Pathways for the center of the Gopher Tortoise range. Selected GCMs for further analysis are circled in red.

Because of the importance of prescribed burning for Gopher Tortoise habitat (and survival, see below), we also used projections of changes in prescribed burning frequency with climate change estimated by Kupfer et al. (2020). The Kupfer projections aligned with the same projection models and gave monthly projections of suitable burn windows relative to a baseline year of 2006.

Field data collection and lab work (Task 1b)

We captured Gopher Tortoise adults at two sites (Nokuse Plantation, FL, n=197; Fort Stewart Army Reserve (FSAR), GA, n=86) for ongoing mark-recapture studies and to ultrasound and x-ray females to assess reproductive status and clutch size. We found and caged 213 Gopher Tortoise nests at six sites across the species' range from 2018-2020 (**Table 4, Figure 13**). The vast majority of nests were located through hand searching of burrows and aprons, but at Nokuse Plantation (our translocation site for Gopher Tortoises), we additionally located nests through radio telemetry combined with fluorescent powder tracking. Also at Nokuse Plantation, we worked with an independent contractor (Conservation Southeast) to conduct habitat surveys of nest sites and female burrows to provide additional information about local adaptation vs. plasticity of nest site selection. Temperature data loggers were placed in all nests to collect data on nest temperatures and nest site selection, and were retrieved after nests had hatched. Nest site

characteristics were measured for all nests (nest depth, distance of nest from the burrow mouth onto the burrow apron, and canopy cover).

In 2018, nests were allowed to hatch *in situ* at Nokuse Plantation. This method allowed for the most accurate assessment of hatching success; however, at least 15% of hatchlings were depredated by fire ants immediately upon hatching and before emergence from the nest, which meant we were not able to collect data on those hatchlings. Therefore, in 2019 and 2020, we switched protocols to excavate nests in late July and August (depending on the latitude of the site), and bring eggs into captivity to hatch in incubators. The date of excavation was determined to be as close to hatching as possible to ensure that the critical period for sex determination had passed. This process still allowed for assessment of hatching success, as hatching success rates were similar at Nokuse Plantation in 2018 and 2019-20 under both methods. Nests produced a total of 1,111 hatchlings from which we collected body condition and blood samples for sex determination and genetics. Most hatchlings were released at nest sites immediately following processing, but a subset was maintained in a captive animal facility at Georgia Southern University until release at 1 year of age. We performed laparoscopic surgery on those older hatchlings to validate sex determination assays (see below).

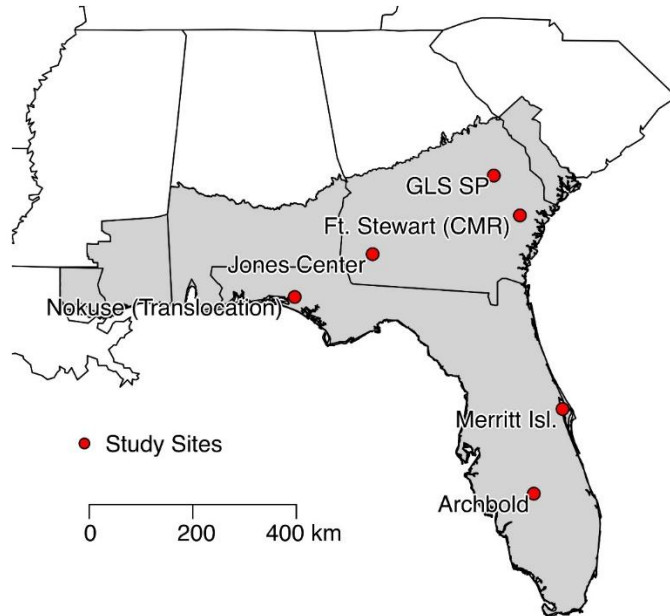


Figure 13. Map of study sites within the Gopher Tortoise (*Gopherus polyphemus*) range (gray area). From north to south: George L. Smith State Park (GLS SP), Fort Stewart Army Reserve (capture-mark-recapture [CMR] site), the Joseph Jones Ecological Research Center, Nokuse Plantation (translocation site), Merritt Island National Wildlife Refuge, and Archbold Biological Station.

Table 4. Summary of *Gopherus polyphemus* nest and hatchling data collection in two field seasons.

Site	Nests found	Hatchlings processed
GLS SP	16 (2018: 3; 2019: 8; 2020: 5)	64 (2018: 13; 2019: 29; 2020: 22)
Ft. Stewart	28 (2018: 7; 2019: 6; 2020: 15)	148 (2018: 39; 2019: 23; 2020: 86)
Jones Center	38 (2019: 15; 2020: 23)	166 (2019: 60; 2020: 106)
Nokuse	63 (2018: 14; 2019: 24; 2020: 25)	304 (2018: 75; 2019: 119; 2020: 110)
Merritt Island	38 (2019: 20; 2020: 18)	226 (2019: 108; 2020: 118)
Archbold	32 (2019: 18; 2020: 14)	203 (2019: 124; 2020: 79)
TOTAL	213	1,111

Genetic samples

For the Gopher Tortoise, although we already knew the source locations for most if not all translocated females, we were often unable to associate these individual females with the nests they deposit. The reason for this was that most of the nests we were able to identify were discovered by searching likely nest locations (skirts of burrows) rather than by tracking females (as we do with Mojave Desert Tortoise, and which was originally planned for Gopher Tortoises). Gopher Tortoises were very difficult to capture and track, and surveying likely nest sites was very effective in locating nests (**Table 4**). To determine which females deposited which nests (connecting females to nests is critical for tests of local adaptation vs. plasticity) we used genetic maternity tests to identify which adult females in the population deposited each discovered nest. To do this, we compiled a large set of genetic samples from adult females in the population, and we also collected blood samples from offspring. We archived 130 adult and 314 hatchling blood samples captured in the 2018-2022 field seasons in a -20 C freezer at Virginia Tech. We genotyped 60 male and 70 female candidate parents from two sites at Nokuse Plantation (translocation site), as 314 hatchlings from 44 nests discovered in the 2018-2020 field seasons, using 12 variable microsatellite loci (Schwartz et al. 2003; Tuberville et al. 2011; Kreiser et al. 2013). Using these data and the maximum-likelihood-based parentage reconstruction software COLONY (Jones & Wang 2010), we identified 30 females that produced 44 nests across three field seasons.

Hatchling Sex Determination

For Gopher Tortoises, we sampled 110 neonates collected from wild nests across two nesting seasons (2019 and 2020) at Ft. Stewart Army Reserve and George L. Smith State Park in Georgia. Within 7 days of hatching, blood was drawn from the subcarapacial sinus, we collected up to 0.1 mL blood from the subcarapacial cervical plexus (Hernandez-Divers et al. 2002). Once drawn, we transferred the sample to 70-mm heparinized capillary tubes that we placed on wet ice. Within 4 hours of sample collection, we centrifuged capillary tubes at 1200 rpm for 3 min, and stored plasma at -70°C until laboratory analysis. For 62 of these individuals, we assayed

only naive circulating testosterone. For 48 individuals, we instead quantified circulating testosterone from a blood draw 4 hours after an injection of 0.1 mL of porcine-Follicle-Stimulating Hormone solution (0.1 units/1 mL sterile saline; MP Biomedicals LLC, Irvine, CA, USA) for a total dose of 0.01 units p-FSH into the coelomic cavity following the method of Lance et al. (1992). In some turtle species, FSH challenge results in temporary, differential elevation of testosterone in males (Lance et al. 1992). Steroid hormones were extracted from plasma using a diethyl ether extraction. Testosterone concentration was determined using enzyme-linked immunosorbent assay kits (ELISA; Cayman Chemical) following manufacturer's protocol.

To determine each individual's sex, we directly inspected gonads using standard laparoscopic surgery (Rostal et al. 1994) performed by David Rostal on individuals reared in the lab until at least 4 months of age. For each neonate used in the testosterone experiments, we also took five morphological measurements (midline carapace length (MCL), shell height (SH), carapace width (CW), minimum and maximum plastron length (PMIN and PMAX)) in the first 1–2 weeks after hatching in 2018, 2019 and 2020. We calculated several derived variables based on these measurements that were suggested to be sex-informative in an earlier study ($VOL = MCL * SH * CW$, $MCL.V = MCL/VOL$, $SH.V = SH/VOL$, $CW.V = CW/VOL$, $PMIN.V = PMIN/VOL$, $PMAX.V = PMAX/VOL$, $CW.MCL = CW/MCL$, $CW.SH = CW/SH$; Burke et al., 1994). All raw measurements, as well as the composite measurements, were included in a random forest model (Liaw & Wiener, 2002) to see if they could be used to classify neonate sex. Because sexes were not equally represented in some datasets, we down-sampled the larger class such that class frequencies were equal. We created models using only morphological data for the full dataset, and using morphological data and testosterone data for two subsets of neonates, those with an FSH challenge (2019) and those without (2020), and compared these against models with only testosterone included (in these cases, $mtry = 1$), to see if morphological data improved sex identification by testosterone level alone.

For naive neonates, males had higher average testosterone than females (female mean $\pm$ SE in pg/mL: 17.9 ± 1.1 , $n = 44$; male: 51.7 ± 13.5 , $n = 18$; **Figure 14A**). The ranges of testosterone levels for male and for female neonates overlapped extensively, but a subset of males exhibited testosterone values well above the female range **Figure 14A**). Adopting an equal-error threshold to separate males from females (T_E ; the testosterone concentration at which a simple cutoff generates equal rates of mis-assigned males and females) would result in a substantial error rate (approximately 22% for each sex).

Neonates challenged with FSH in 2019 had higher testosterone levels than naive testosterone levels in 2020 neonates (data were not paired, and were collected in different years), suggesting that FSH elevates testosterone in neonates. Comparing the distributions of male and female neonates post-FSH challenge, we observed substantial overlap in male and female testosterone concentrations (**Figure 14B**), such that the T_E of 29.5 pg/mL resulted in ~30% errors for each sex in our dataset.

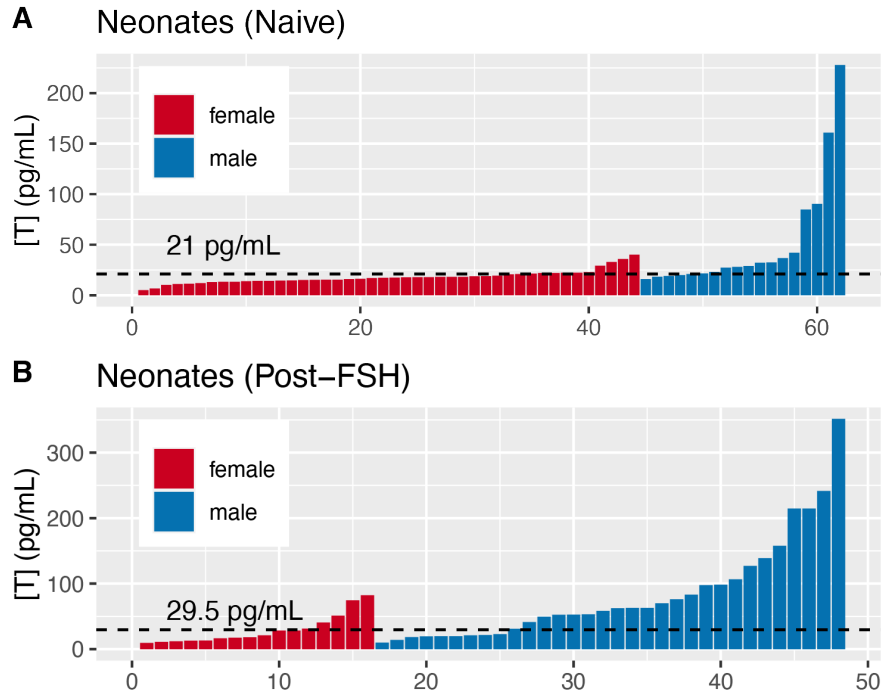


Figure 14. Sex is only weakly predicted by naive (A) or post-FSH challenged (B) circulating testosterone concentration in neonate Gopher Tortoise. Dashed lines indicate thresholds at which an equal error rate occurs for female and male assignment.

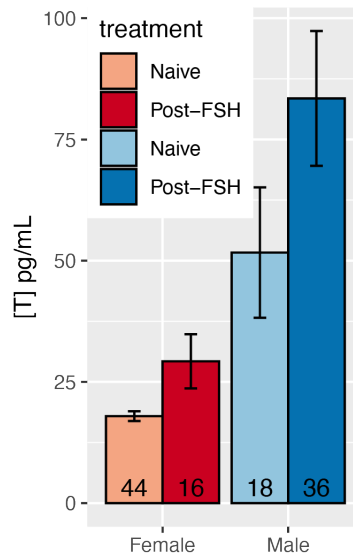


Figure 15. FSH challenge increases testosterone in both male and female neonates. Mean neonatal testosterone concentrations [T] were higher in post-FSH individuals than in naive individuals for both females and males. Data are not paired, and every individual was only measured once. Sample sizes are reported inside of each bar. Error bars depict standard errors.

The random forest model trained on morphological measurements from 174 neonates weakly predicted sex (out-of-bag (OOB) error = 33.3%, with 29.6% error for females and 38.2% error

for males; **Table 5**). Analyzing the subset of 18 male and 44 female neonates from 2020 for which we also had naive testosterone concentrations, OOB error rate was 24.2%, which was not substantially different from a random forest model trained on the testosterone data alone (OOB error = 27.4%). For post-FSH neonates (n = 16 females + 32 males from 2019), the OOB error for the model including morphological and testosterone data was 8%, substantially better than the OOB error for the testosterone-only model (25%). For this best model, testosterone, CW/SH, Pmax and SH were the four most important predictors.

Table 5. Random forest model results for predicting sex with morphology and testosterone.

<i>Model predictors</i>	<i>nfemale</i>	<i>nmale</i>	<i>OOB error</i>	<i>Female error</i>	<i>Male error</i>
Morphology	98	76	33%	30%	38%
Morphology + naive T	44	18	24%	21%	33%
naive T	44	18	34%	30%	44%
Morphology + postFSH T	16	32	8%	19%	3%
postFSH T	16	32	25%	31%	22%

Baseline and post-FSH testosterone only weakly predicted sex in neonate Gopher Tortoises. Unlike in 15-month-old snapping turtle juveniles (Ceballos and Valenzuela 2011), Arrau neonates (Lance et al. 1992), and 6–8-month-old Gopher Tortoises (Loope et al. 2022), the boost in neonatal testosterone following FSH injection was not sufficiently male-biased to completely separate males and females. However, the combination of basic morphological data and post-FSH data produced relatively accurate predictions in a random forest model (**Table 5**). Error rates in this model were higher for females than males, and thus could bias overall sex ratios if not corrected. Furthermore, this model was based on a relatively small sample size (16 males, 32 females), and thus is best interpreted as provisional. Given the substantial variability of turtle morphology across populations (Carretero et al., 2005; Djurakic & Milankov, 2020), future studies in other parts of the range may benefit from validating the morphological model before applying it.

Sexing hatchlings using differential methylation analysis.

Given the substantial error rates in the testosterone-based sexing protocol developed for neonate gopher tortoises, we were unable to apply this method to the neonate blood samples taken from natural nests at six sites, meaning we could not estimate variation in sex ratio and sex determination reaction norms across the range as planned. However, a recent novel method

using differential DNA methylation in blood genomic DNA was successful at determining sex for neonatal American alligator, another reptile with temperature-dependent sex determination (Bock et al. 2022). We are adopting this approach, with the goal of identifying differentially methylated sites that collectively distinguish males and females. To this end, we have generated a draft genome for the species from a single Oxford Nanopore flowcell, for use in a full-genome methylation study of neonate blood. Initial tests suggest a reasonably complete draft genome, with a single-copy ortholog content of 95.2% and approximately 11x coverage. We have also applied the novel Enzymatic Methylation sequencing (EM-seq; Hoppers et al. 2020) method to obtain full-genome methylation data for 32 neonatal gopher tortoise blood samples. These samples consist of 8 males and 8 females from each of two Georgia sites (Ft. Stewart Army Reserve and George L. Smith State Park), each with sex determined independently by laparoscopy. We are in the process of identifying differentially methylated sites in the genome. Once these sites are determined, we will construct a predictive model of sex using penalized binomial logistic regression with elastic net regularization. The methylation status of these informative sites will then be assessed using targeted approaches (bisulfite conversion followed by multiplex PCR amplification) to determine sex for the remaining neonatal samples.

Test for local adaptation (Task 2)

Species recovery for Desert and Gopher Tortoises is at risk from a myriad of landscape-level threats, including habitat fragmentation (Averill-Murray et al. 2013). Mitigation measures often include translocation of animals from disturbed areas to neighboring undisturbed or more distant intact habitat, either to prevent direct mortality from land use or to augment low density recipient populations (Nussey et al. 2012). However, it is unclear if Desert and Gopher Tortoise demographic vital rates have been ‘fine-tuned’ to local environmental differences by evolutionary processes, or if phenotypic plasticity enables tortoises to thrive across wide environmental and climatic gradients. Previous studies have reported little or no significant differences in vital rates between translocated and resident tortoises (e.g., Nussey et al. 2012) or differences in vital rates based on translocation distance (e.g., Scott et al. 2020, McKee et al. 2021; however, the population origin of translocated tortoises is not always known, meaning that the assumption of interpopulation differences between the two groups may be false (Averill-Murray and Hagerty 2014). By using high-resolution genotype data of individuals, we can test assumptions underlying the modeling of reaction norms describing the relationship of a vital rate to the environment from which individuals were translocated.

Fecundity measures, particularly hatching success and hatchling sex ratio, represent challenging vital rates to measure for these species because both species can lay nests well inside burrows, resulting in the need for researchers to trail individual tortoises on a daily basis to ensure that nests can be located and protected before predation can occur (M. Walden, pers. obs). While clutch size and clutch frequency have been successfully measured at multiple sites over the past half century using radio telemetry and x-radiography of adult females (e.g., Lovich et al. 2018, Turner et al. 1984), nest sites were usually not located in these studies, and even fewer studies have monitored nests *in situ* until hatching. Furthermore, we know of no studies to date that have examined hatchling sex ratio resulting from *in situ* nests in these species. Understanding the potential for plasticity of vital rates across environmental gradients is essential

for predicting population resilience to rapid anthropomorphic climate change in this long-lived species.

We have built a series of models that describe whether (and how much) demographic vital rates are expected to differ between translocated and resident tortoises as a function of the degree of difference between their environments of origin.

Collect field data on translocated and resident tortoises

We have selected sites for both Desert and Gopher Tortoise that have a significant number of translocated individuals. For both species, we located nests for many translocated individuals, in addition to measuring growth of captured adults.

Desert Tortoise Nesting - For the Mojave Desert Tortoise, we identified 19 nests of translocated individuals vs 34 nests for resident individuals, in part due to low reproduction in 2021, likely the result of extreme drought. Furthermore, as a result of high predation, only 15 and 19 such nests survived.

Desert Tortoise Growth - In the growth analysis described in the Results and Discussion section (under Task 1), there were sites at which translocated and resident tortoises were monitored with radio telemetry for several years post-translocation. The analysis included 106 translocated individuals with 196 growth periods for comparison to >1,000 resident tortoises.

Gopher Tortoise Nesting - For Gopher Tortoises, our common garden ‘experiment’ was conducted at a single site, Nokuse Plantation in the Florida panhandle (**Figure 16**), where the entire population consists of translocated individuals ($n > 5,000$) from throughout the state. Instead of comparing translocated to resident individuals, we modeled differences in vital rates as a function of geographic or environmental distance from source populations.

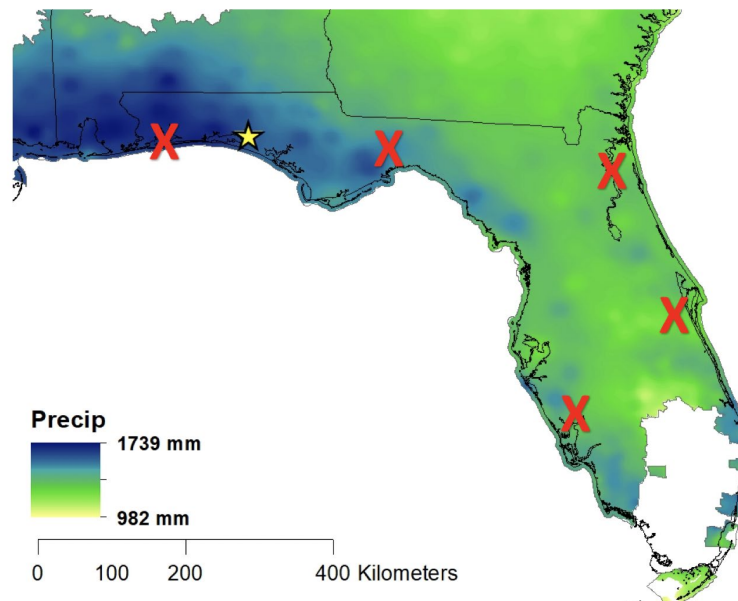


Figure 16. Translocation recipient site (Nokuse Plantation; yellow star) and a sample of primary donor sites (red “x”s) throughout Florida across a range of both geographic and environmental (here, annual precipitation totals) distance from the recipient site.

(May-July). Nests were found by carefully excavating the soil in and around the burrow entrance and apron to a depth of 30 cm. To examine how nesting behavior may differ between tortoises from different origin locations, data were collected on the following nest characteristics: depth, distance from the burrow entrance onto the apron, and canopy cover. Before hatching and after the critical period for sex determination, eggs were excavated from the nest and placed in an incubator for the remainder of the incubation period.

Climate data were obtained from the PRISM Climate Group online database (<https://prism.oregonstate.edu/normals/>). The climate data were 30-year normals that describe average monthly and annual climate conditions for each donor county's centroid point. There was limited *a priori* knowledge as to which metrics and which seasonal time windows may impact Gopher Tortoise fecundity, we examined multiple metrics across several time periods (monthly, annually, seasonally). Since many climate variables (and other important covariates of nesting behaviors and hatching success, such as female size) were correlated, we applied a Pearson correlation coefficient cutoff of $|r| < 0.60$ to select which covariates to use in models (**Table 6**). The final set of uncorrelated predictor variables included female size (MCL), annual precipitation, monthly temperature range (MTR; monthly maximum – monthly minimum temperature) for January, and mean temperature of July. Due to the high collinearity of climate variables, these selected variables represent more than the variables themselves. They also represent those variables they are correlated with (**Table 6**). To represent this collinearity, the climate variables were renamed to “precip” (as many precipitation variables were correlated with annual precipitation), “MTR” (as many MTR variables were correlated with the January MTR), and “WST” (as many warm season temperature variables were correlated with July temperature), respectively (**Table 6**). The environmental distance was calculated as the difference in a given climate variable between the original location (donor county) and the recipient site (Nokuse). For example, if a tortoise was relocated from Sarasota County (annual precipitation = 1426 mm) to Nokuse (annual precipitation = 1593 mm), the environmental distance would be -167 mm. We used the absolute value of the difference for precip and MTR to aid in data interpretation.

Table 6. *Environmental variables used in models of environmental distance effects on Gopher Tortoise nest site selection and nesting outcomes Nokuse Plantation in Walton County, FL, during years 2018-2020, and the correlated climate variables they represent. Vitellogenesis (September – January) Nesting (May – July) and Active (April – October) seasons are the*

average climate for biologically relevant time window that may impact reproduction. Collinear variables have Pearson correlation coefficient of $|r| > 0.60$.

VARIABLE NAME	Maximum Carapace Length (MCL)	Annual Precipitation (Precip)	Monthly Temperature Range (MTR)	Warm Season Temperature (WST)
ENVIRONMENTAL VARIABLE USED IN MODELS	Female Maximum Carapace Length	Annual Precipitation	Monthly Temperature Range for January	July Temperature
COLLINEAR VARIABLES	<u>Precipitation for:</u> June Vitellogenesis season <u>Temperature for:</u> January February March April May June August September October November December Annual Active season Nesting season Vitellogenesis season	<u>Precipitation for:</u> January February March April May November December Vitellogenesis season Distance (km)	<u>Precipitation for:</u> October <u>MTR for:</u> Active season Nesting season Vitellogenesis season Annual February March April May June July August September October November December	<u>Temperature for:</u> Active season Nesting season May June July August September <u>Precipitation for:</u> March May June July August November Vitellogenesis season

Model local adaptation as the expected change in demographic vital rates explained by environmental differences between source and recipient sites

Desert Tortoise Nesting - For Desert Tortoise nests, the small number of nests discovered for translocated individuals precluded rigorous statistical comparison of nest site characteristics or hatching outcomes that accounts for site and year. Instead, we simply compared hatching success between nests produced by translocated and resident tortoises using a logistic regression with each nest weighted by the number of potentially hatching eggs it contained. To reduce site effects, we only included data from the three sites that contained both resident and translocated tortoises ($n = 14$ resident and $n = 13$ translocated tortoise nests).

Desert Tortoise Growth - Much more data were available to test for differences in growth patterns between translocated and resident tortoises. To determine the effect of translocation status on growth, we first fit a von Bertalanffy growth model using long-term capture-recapture data with repeated growth measurements to fit key growth parameters (a and k from the von Bertalanffy model; more details in Results and Discussion section below) as a function of climate and environmental covariates. In addition, we also included an effect of translocation status on both growth parameters (a and k), as well as interaction effects allowing climate effects to change with translocation status. We interpreted any differences in growth parameters between translocated and resident tortoises as evidence for local adaptation (although other explanations are plausible; see below). However, we lacked sufficient data or replicates to model these differences as functions of environmental/climatic differences between translocation and release sites.

Gopher Tortoise Nesting - For Gopher Tortoises, we performed multiple linear regressions to estimate the effects of environmental distance on five different sets of response variables: probability of reproduction (whether or not a female had follicles or eggs), clutch size, egg mass, variables related to nest site selection, and hatching success. All models included female size

(MCL) as a baseline variable because female size can affect nest characteristics and fecundity (Iverson 1980).

To assess whether a female's original environmental conditions influenced her probability of reproduction, we created models of all possible linear combinations of MCL, MTR, Precip, WST, and time-since-release (to account for lack of reproduction in the first year following release). We also included a "methodological" variable of date of capture to account for the fact that a tortoise captured later in the nesting season may have already laid her eggs, meaning her reproduction would not be observed via ultrasound (technique used in Hunter et al. 2021). Models were logistic regressions with either fixed or random effects of pen (see pen effects below). We ran models both with all observations, or only the first observation for females that were observed multiple years, and found qualitatively identical results.

To assess whether a female's original environmental conditions influenced clutch size or egg mass, we created a set of linear regression models that included models of all possible linear combinations of MCL, MTR, Precip, and WST.

To assess whether a female's original environmental conditions influenced her nest site selection, we created models for three nest characteristic response variables: canopy cover, nest distance from burrow entrance, and nest bottom depth. For each nest characteristic response variable, we created a set of linear regression models that included models of all possible linear combinations of MCL, MTR, Precip, and WST.

Environmental distance could directly influence hatching success through some physiological mechanism, or it could indirectly impact hatching success by influencing the female's nest site selection. To separate the potential direct and indirect effects of environmental distance on hatching success, we created three different model sets for the response variable of hatching success. To assess the effects of environmental distance on hatching success, we created a set of binomial regression models that included models of all possible linear combinations of MCL, MTR, Precip, and WST. To assess the effects of nest site selection on hatching success, we created another set of binomial regression models that included models of all possible linear combinations of canopy cover, nest distance from burrow entrance, and nest bottom depth. Models for each response variable were compared using Akaike's Information Criterion corrected for small sample sizes (AICc). Because data were collected over three nesting seasons, some mothers had multiple nests in the dataset. For these situations (n=10 mothers), nest characteristics and hatching success were averaged across years to avoid pseudoreplication. To account for high levels of model uncertainty (i.e., there was rarely a clear "top model" in a model set), we made predictions of the response variable for each model in the model set and then averaged those predictions weighted by each model's AICc weight.

Model critical habitat breadth and forecast future population viability (Task 3)

Conservation measures to enable the persistence or recovery of species of concern rely on identifying habitat that supports stable or growing populations at present and into the future. Current approaches to identify areas appropriate for protective measures include habitat suitability models or species distribution models (Elith and Leathwick 2009). Such models may fail to address species' capacity to adapt or respond to rapidly changing environmental conditions such as climate change (Nicotra et al. 2015). The ranges of the Mojave Desert Tortoise (*Gopherus agassizii*) and Gopher Tortoise (*G. polyphemus*) cover large portions of

public lands in the United States, including military installations. A scalable, targeted approach for identification of suitable habitat that land managers could apply to specific installations or parcels would be valuable as climate change continues to affect species viability.

Accelerating climate and land-use changes leave species with three primary means of continued viability into the uncertain future: (1) rapidly evolve to changing conditions in situ, (2) make long-distance migrations to colonize suitable habitat outside of their existing range, or (3) acclimate to changing conditions in situ. In an effort to understand how and why species may be able to successfully deploy any of these defenses, correlative species distribution models are used to predict where species will persist based on past occupancy and magnitude of external environmental change, an approach that fails to account for species' intrinsic capacity for rapid evolution or plastic acclimation (Elith and Leathwick 2009). Critical habitat breadth is an operationalization of ecological niche theory that describes the full range of external conditions (habitat and climate) that support viable populations into the future.

Gopherus agassizii and *G. polyphemus* are species of high conservation concern because of declining population trends and the fact that their geographic ranges span an array of human-altered landscapes, including military installations, renewable energy facilities, and suburban sprawl (Berry and Aresco 2014). As large-bodied, long-lived tortoises, long-distance dispersal and rapid evolution are unlikely to be successful defenses against future habitat and climatic changes (Butler 2019). The decades of scientific study and extensive translocation projects render these two species excellent model organisms with which to test the critical habitat breadth concept.

This simulation task combines the results of Tasks 1 and 2, and allows us to more comprehensively analyze expected Desert and Gopher Tortoise population growth rates (Table 1) across a wide range of plausible climate change scenarios. The centerpiece of our simulation framework is our critical habitat model, which is implemented as a high-level function that takes any arbitrary set of environmental and climatic conditions as input, and uses those inputs to generate population projection matrices on the fly. This 'matrix generator' function is able to accommodate climate-driven changes in age-at-maturity, age-structured survival, and reproductive output as conditions shift over space and time. This function also enables efficient computation of population growth rate (λ) under different environmental and climatic conditions. We have also developed spatially explicit, range-wide simulation models that make use of the matrix generator functions to forecast population dynamics under a wide range of climate scenarios, and to estimate the likelihood that current populations of each focal species will remain viable under climate change. Our spatially explicit metapopulation simulation model incorporates key information about demographic processes (survival, reproduction, dispersal) and climate sensitivities in demographic vital rates, and enables us to project the conservation status over space and time, and to identify future areas of conservation priority for Mojave Desert Tortoise and Gopher Tortoise.

This task can be broken into three subtasks:

Task 3a. Build comprehensive critical habitat breadth model, incorporating mean responses and sampling uncertainties

Task 3b. Forecast population growth across space and time (including climate change scenarios) using the critical habitat breadth model (H3)

Task 3c. Visualize and analyze simulation results to better understand critical habitat breadth for both species and to project future abundance across space and time

Build comprehensive critical habitat breadth model (Task 3a)

We constructed a generic simulation modeling framework capable of forecasting metapopulation viability for both Desert and Gopher Tortoises under climate change. Here we first describe the generic model structure in detail. We then discuss the specific parameterization for Desert Tortoise and Gopher Tortoise. For the Desert Tortoise model, parameters were largely drawn from our data analyses, including previously collected data in addition to data collected in the field as part of this study. However, additional information on some parameters was drawn from the published literature, including for the hatchling survival, nest success, and juvenile survival parameters. For the Gopher Tortoise, our field data collection and re-analysis of the Fort Stewart capture-mark-recapture data informed many aspects of the model, including climate effects on hatchling sex ratio and hatching success, and the effects of fire history on survival rates. Many of the remaining parameter estimates (distributions from which to draw samples for each replicate) were drawn directly from, or modified from, a recently published rangewide Gopher Tortoise population model (Folt et al. 2022) that was used by USFWS in their recent status assessment for the Gopher Tortoise.

Generic simulation modeling framework

Task 3a: Critical habitat breath model (matrix generator function)

We used a female-only, pre-breeding transition matrix (Caswell 2000) structured by age to simulate tortoise population dynamics under arbitrary environmental or climate conditions. Males and females were each modeled with 20 stages, with all but the final stage (stages 1 to 19) representing a single year of life. The final stage (stage 20) represented an adult stage, and was the only stage in which individuals could remain for multiple consecutive years. That is, the first 19 stages represented true age-classes, starting with yearlings and proceeding through age 19, while the final stage represented a stage class consisting of reproductive adults 20 years old and above. All individuals at or above the assigned *age-at-maturity* parameter were assigned a non-zero fecundity value (elements in the top row of the projection matrix), with all individuals in earlier stages assigned a fecundity value of zero. Fecundity rates for all individuals above the age-at-maturity were computed as the product of six vital rates: *annual probability of reproduction*, *per-capita annual egg production (clutch size)*, *nest success*, *hatching success*, *fraction of female hatchlings*, and *hatchling survival*, each of which was potentially a function of environmental and climatic covariates. Survival rates (matrix subdiagonal elements, representing advancement to the next age class, as well as the final matrix diagonal representing adult survival) were each assigned one of two unique values for each grid cell: a juvenile survival rate below the age-at-maturity, and an adult survival rate for individuals at or above the age-at-maturity -- each of which was potentially modeled as a function of environmental and climatic covariates.

In many cases the relationship between climate conditions and vital rates were fairly straightforward – we simply used the coefficients from a linear regression model or generalized linear model (GLM) directly from our analysis (or another published study) to model the relationship between a parameter and a climate variable. Because the data sources to which our

climate models were fitted -- representing data sources interpolated from weather station data (e.g., PRISM, Daymet, Gridmet) -- differed in some important ways from the data sources used in our simulation models (climate projections based on global circulation models; GCMs), we developed a general strategy for enabling us to account for this discrepancy in our matrix generator function without biasing simulation results. Specifically, our strategy was to (1) fit all models to treat mean climate conditions during the period to which models were fitted as “baseline” climate conditions, with any anomaly from the baseline climate conditions potentially driving changes in mean vital rate, and (2) re-compute all GCM scenarios (data used for simulations) in terms of difference relative to the mean baseline conditions under that particular climate scenario. We then used these projected differences from baseline conditions as the climate covariates for determining vital rates in our critical habitat breadth models.

Several key reproductive vital rates (in particular, hatching success and sex ratio for each nest) were modeled in our study as a function of nest temperature rather than directly as a function of mean air temperature. For these vital rates, our general strategy was to (1) model the relationship between air temperature and nest temperature for the relevant period of time from our own field data (change in nest temperature as a function of the difference from baseline air temperature), and (2) use the results from the above model (expected change in nest temperature based on a given air temperature anomaly) to generate the expected change in baseline vital rates (either hatching success or sex ratio) on the basis of our models (see section 1c of this report for more details about the models of vital rates as a function of climate). Where possible, we estimated the baseline vital rates using information from our field study combined with information from the literature.

Our matrix generator functions were designed to accommodate parameter uncertainty in all key vital rates and their relationships with climate and environmental covariates. To do this, our framework enables the creation of multiple unique replicate matrix generator functions, each of which reflects parameter values sampled from a range of plausible values. Parameter uncertainty can then be accommodated by generating multiple replicate matrix generators, and using these functions to assess population dynamics under changing environmental conditions. For parameters fitted in a Bayesian framework, we sampled parameter values directly from the joint posterior distribution. For parameters fitted in a frequentist framework (e.g., a generalized linear model; GLM) we sampled from a multivariate normal distribution defined by the model’s variance-covariance matrix. Finally, for parameters drawn from the literature we sampled from a distribution defined by the entire set of relevant published studies (more details in the model parameterization section below).

Task 3b: spatially explicit metapopulation simulation models

We used a lattice (grid-based) framework to model spatial demographic structure (e.g., Pilowsky et al. 2022), whereby each 5 x 5 km grid cell was treated as a unique subpopulation. We chose a 5 km resolution for our simulation model to optimize the inherent trade-off between simulation speed and the ability to incorporate useful spatiotemporal variation in climate and habitat suitability. We modeled the effects of climate change by enabling population vital rates (used for determining matrix elements above) to change over space and time as a function of local climate conditions, using matrix generator functions as described above. To enable efficient simulation, we pre-calculated all key climate covariates (climate factors used for modeling all vital rates)

from monthly global circulation model (GCM) projections of temperature and precipitation from approximately the present day to 2099 (with slightly different methods and dates used for Gopher and Desert Tortoise modeling protocols; see below). All covariate rasters (including dynamic climate layers and static environmental variables) were resampled to the same 5 X 5 km grid as the lattice population model described above, allowing seamless integration of covariate information into the simulation model. Although our model focuses on climate change, future versions of this model can easily incorporate additional raster layers, including representations of land-use change and other threats to population viability.

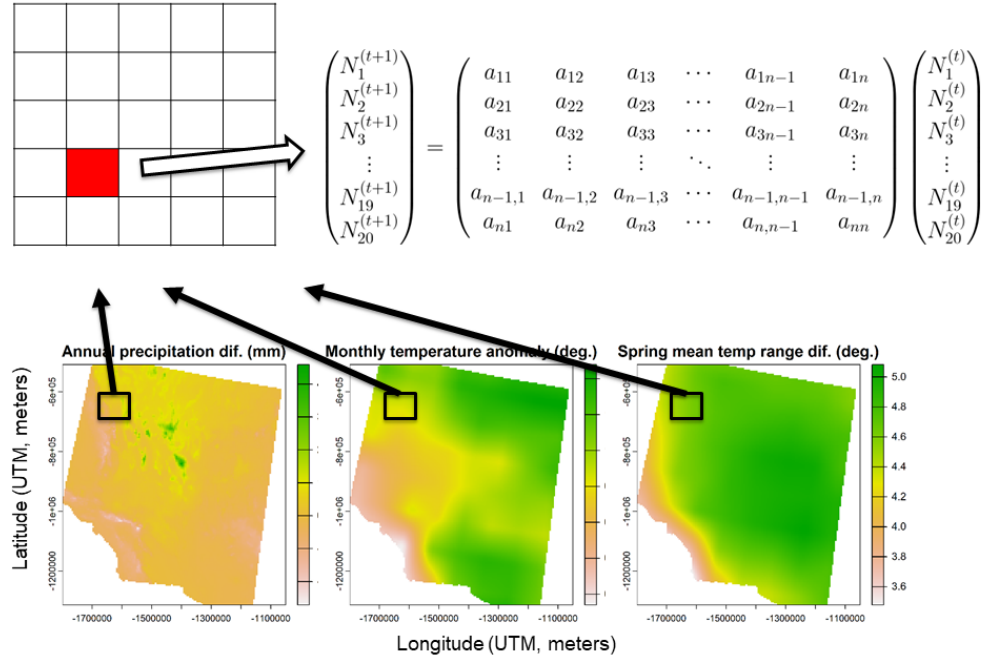


Figure 18. To model the spatial and temporal abundance dynamics of the two focal tortoise species, we used a lattice, or grid-based, simulation modeling framework. This enabled us to integrate geographic (e.g., climate) information into our simulation framework as spatial rasters. Our models for both species use a 5 km grid cell resolution, with each pixel on the landscape representing a potential tortoise population. The matrix generator function was called separately for each grid cell in each simulation year, integrating all the climate-vital rate relationships described under Task 1 of this report. These matrices were then used to update the abundance in each grid cell for each year via matrix multiplication.

We used a ceiling density-dependence process to ensure that abundance in each grid cell remained at realistic levels constrained by estimated habitat suitability within each pixel. Specifically, when the abundance in a grid cell exceeded its pre-defined maximum value, the transition matrix in that cell was replaced with a transition matrix with below-replacement growth (all elements in the baseline matrix multiplied by 0.75, which resulted in a $\lambda \ll 1$).

To accommodate metapopulation dynamics, we implemented a flexible dispersal model that spreads individuals across neighboring pixels according to a negative exponential dispersal kernel:

$$w(d) = \begin{cases} \gamma \cdot \exp\left(-\frac{d}{\sigma}\right) & \text{if } d \leq d_{max} \\ 0 & \text{if } d > d_{max} \end{cases}$$

Currently, our model only enables movement to adjacent grid cells, but the system is easily modified to enable dispersal across multiple (potentially smaller) grid cells. The model also enables relative dispersal probability to increase for higher quality grid cells. Stochasticity in dispersal rates is introduced using a multinomial random number generator.

Scenario tests

Because one of our primary objectives was to estimate population resilience to climate change, we ran all climate change models for each unique set of parameter values (that is, each replicate comprised a full run of the simulation model across all years for each climate scenario), enabling increased power for detecting differences in simulation results among climate scenarios. We used Poisson distributions to simulate demographic stochasticity in total annual births in each grid cell (per-capita production of age 1 individuals at year $t+1$ by individuals in any given stage at time t) and Binomial distributions to simulate stochastic variation in survival rate and sex ratio. Due to data limitations, and to better enable us to model climate resilience and to reduce unnecessary model complexity, we did not include temporal process variation (environmental stochasticity) in most model parameters (aside from climate-driven effects). However, we were able to model spatial and temporal variance in adult survival rate for Desert Tortoise, and this process variation was incorporated into our simulation models (see below).

Aside from climate change scenarios, which were incorporated into each simulation replicate (all climate scenarios were run for each unique set of parameter values drawn from their respective uncertainty distributions), other scenarios were defined by binary variables (true/false, or “on-off switches”) that allowed us to turn each climate effect on or off. For example, one “switch” allowed us to enable or disable the effect of temperature on hatchling survival. We tested all possible combinations of these binary variables, with each unique combination being considered a single “scenario”.

To accommodate parameter uncertainty, we ran multiple simulation replicates for each scenario. Parameter uncertainty was accounted for by generating multiple replicate matrix generator functions (described above), and using each replicate matrix generator to run complete simulations (forecasting range-wide abundance from present day through 2099) under each defined scenario.

Our modeling framework was developed entirely in the R language for statistical computing, and relies heavily on existing high-level R functions for spatial raster manipulation in the packages ‘raster’ and ‘terra’ (Hijmans 2022, 2023). All simulation code can be accessed on GitHub: <https://github.com/kevintshoemaker/GopherusPVA>.

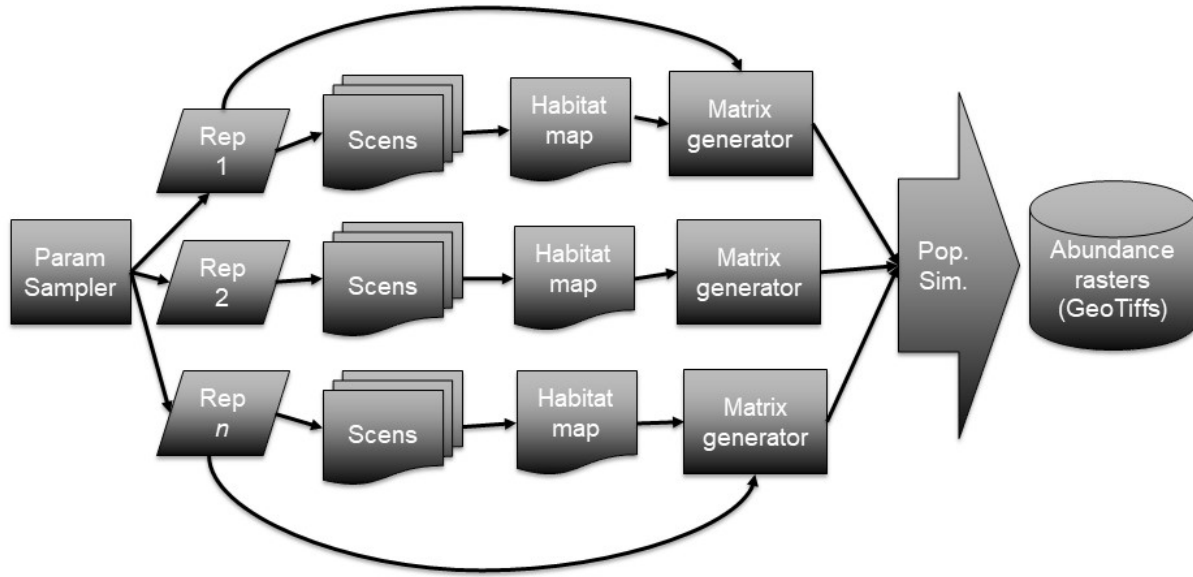


Figure 19. The generic modeling workflow starts off with multiple replicates drawn from parameter distributions describing the climate-vital rate relationships described under Task 1. Based on the specific scenario, we incorporate all relevant environmental information and habitat information to initialize and simulate population abundance. The matrix generator function is called for each grid cell each year, enabling population dynamics to respond to changing environmental conditions. The simulation model archives age-structured abundance in each grid cell each year as multi-band geo-Tiffs, which serve as the raw materials for visualizing and analyzing model results.

Task 3c. Analyzing and visualizing simulation results

The simulations described above (Task 3b) result in a database of geo-Tiffs representing spatially-explicit, age-structured abundance for each scenario, replicate, and simulation year. We used these raw materials to visualize abundance across space and time for each scenario. We also recorded key summary statistics for each replicate (e.g., final rangewide abundance), and used machine learning methods (Random Forest; Cutler et al 2007) to model the relationships between these summary statistics and replicate-level features such as the climate scenario and attributes of the relevant matrix generator function (e.g., mean adult survival, strength of the relationship between climate and hatching success).

Parameterization: Desert Tortoise

Climate change scenarios

We used downscaled monthly temperature and precipitation rasters for the entire range of the Desert Tortoise that were developed as part of **RC18-1207** to derive dynamic yearly rasters representing climate covariates for determining key population vital rates in our Desert Tortoise

simulations. These included four different climate change scenarios, reflecting different responses to climate change and regulations of carbon emissions: RCP2.6, RCP4.5, RCP6.0 and RCP8.5. More details on the methods for deriving these downscaled climate rasters can be found in the RC18-1207 final report. The table below provides a summary of key climate relationships that are incorporated into the Desert Tortoise simulation model:

Table 7. Summary of climate-sensitive components for the Desert Tortoise population simulation model.

Model component	Climate variable name	Climate variable description	Estimate source
Growth/age-at-maturity (MA)	feb_may_tmax	Maximum air temperature, mean across Feb-May	This project (Walden 2022)
Growth/age-at-maturity (MA)	ann_tmin	Mean annual minimum monthly temperature	This project (Walden 2022)
Growth/age-at-maturity (MA)	nov_apr_pr	Total precipitation, November-April	This project (Walden 2022)
Adult survival rate (PHI_A)	nov_apr_pr_norm	30-year mean precipitation, November-April	This project (<i>in prep.</i>)
Clutch size (CS)	mar_mar_pr	Total prior-year precipitation, March-March	This project (Mitchell et al. 2021)
Clutch size (CS)	spr_tmean	Mean air temperature, March-May	This project (<i>in prep.</i>)
Hatching success	jun_jul_aug_tmean	Mean air temperature, June-August	This project (<i>in prep.</i>)
Hatchling sex ratio	july_tmean	Mean air temperature, July	This project (<i>in prep.</i>)
Probability of reproducing (PR)	mar_apr_mtr	Monthly air temperature range, mean across March-April	This project (Mitchell et al. 2021)
Probability of reproducing (PR)	mar_mar_pr	Total prior-year precipitation, March-March	This project (Mitchell et al. 2021)

The figure below provides a visualization of how some of these summarized climate variables (averaged across the Desert Tortoise range) are expected to change over time under the different climate-change scenarios (**Figure 20**).

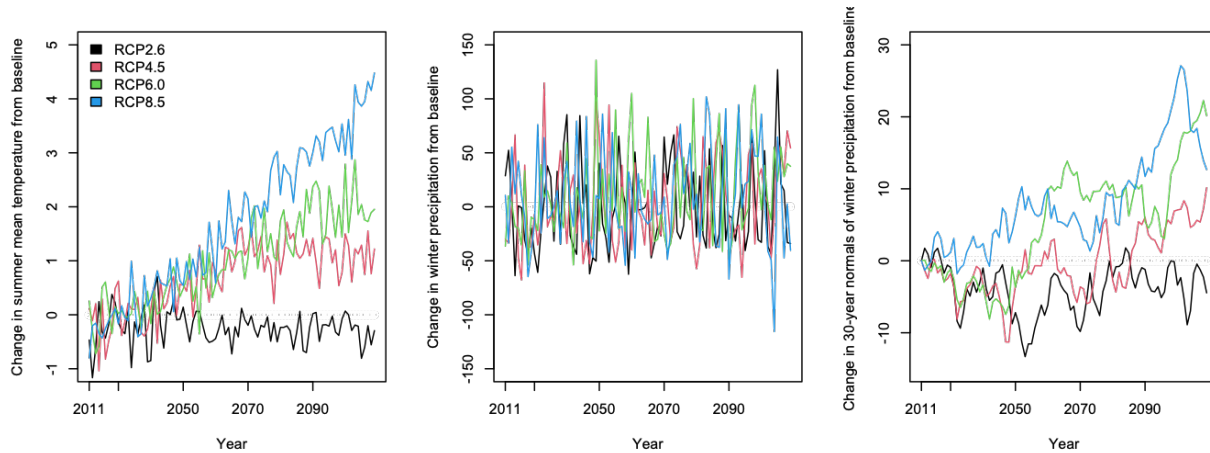


Figure 20. Time series for three derived climate variables (June-August [summer] temperature [left], November-April [winter] precipitation [middle], and 30-year normals of winter precipitation [right]) important for Mojave Desert Tortoise demography. Y-axes represent mean projected values across the range, shown here compared to baseline (present-day) values. Time series are shown for all RCPs from 2011-2099. Data used with permission from the principal investigators of SERDP project **RC18-1207**.

Initial abundance and habitat suitability

We obtained a raster map of habitat suitability for the Mojave Desert Tortoise, which was fitted on the basis of range-wide occurrence data as a function of numerous environmental and climatic features by the RC18-1207 team (PIs Nussear and Heaton; see their final report for details about how this layer was generated). We then resampled this habitat map at a 5 X 5 km spatial resolution, summarizing mean habitat suitability within each pixel, and rescaled the map to sum to 1 across all pixels. We then multiplied the value in each of the resulting pixels by the most recent estimate of total rangewide female abundance for this species (106,171 females, from Allison and McLuckie 2018) to derive the initial total female abundance for each grid cell (effectively ‘spreading’ the total abundance throughout the landscape in accordance with the habitat suitability map). We distributed the abundance within each grid cell across all 20 stages according (approximately) to the stable stage distribution for this population. Maximum (ceiling) abundance in each subpopulation (5 X 5 km grid cell) was set to twice the initial value in each pixel (allowing all subpopulations some room for expansion but allowing maximum abundance in each cell to still reflect the estimated relative habitat suitability). We then ran a ‘burn-in’ in which we simulated abundance dynamics for 50 years under current climate conditions (sampling randomly from the first 10 years of climate projections for each of the four climate scenarios). This resulted in a unique initial abundance for each of the four climate scenarios (RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5). By using this ‘burn-in’ approach, we were able to ensure that all population simulations were initiated in equilibrium with the present-day conditions to the best extent possible, ensuring that any drastic changes in abundance over time were likely due to climate change.

Age-at-maturity

We used our model of Desert Tortoise somatic growth (see below) to estimate age-at-maturity as a function of climate for each year and for each subpopulation (grid cell). For each simulation replicate, we first sampled a single value from a uniform distribution (189-212 mm) representing size-at-maturity values from the literature (Lovich et al., 2018; Mueller et al., 1998; Turner et al., 1986). We also sampled (once per simulation replicate) a single draw from the joint posterior distribution using the Bayesian model of Desert Tortoise growth to represent the baseline somatic growth parameters and the effect of climate variables on Desert Tortoise growth. For each simulation year and for each subpopulation (pixel), we used the von Bertalanffy growth equation to determine the age at which females would be expected to reach the size-at-maturity, and used this age to represent age-at-maturity. Therefore, age-at-maturity was allowed to vary across replicates, subpopulations, and years to capture parameter uncertainty in addition to spatiotemporal climate variation.

Adult Survival

The annual survival rate for all reproductive adults (individuals above the age-at-maturity) was modeled as a function of prior-year precipitation (derived from analysis of the ‘permanent study plot’ data; see above). For each simulation, we used the parameters from a single random draw from the posterior distribution (see section on Desert Tortoise survival analysis, above) to represent uncertainty about baseline adult survival rate, the magnitude of spatial and temporal variation in adult survival (process variance), and the precipitation coefficient. We incorporated spatial process variation for each simulation replicate by drawing an independent replicate from the standard normal distribution (mean=0, sd=1) for each subpopulation (pixel), multiplying this value by the magnitude of spatial process variance to determine the degree to which (logit-transformed) survival probability differed across pixels. This spatial process variation remained fixed for all years throughout the simulation. Similarly, we modeled temporal process variation in adult survival by sampling a single replicate from the standard normal distribution for each year of each simulation, multiplying this value by the magnitude of temporal process variance to determine the degree to which (logit-transformed) survival probability differed across years. This temporal process variation term remained fixed across all pixels (assuming temporal environmental stochasticity was perfectly correlated across the range of this species). Since the fitted survival model included a single random effect term for both spatial and temporal process variance, we assumed that process variance was split evenly between the spatial and temporal components in our simulation framework. We hope to be able to fit separate spatial and temporal random effects in future versions of this model as additional data sources are incorporated into our models of Desert Tortoise adult survival.

Juvenile survival

Juvenile survival (for all stages below age-at-maturity) was not modeled as a function of climate, but instead was set at a fixed value for each simulation replicate, drawing from a uniform distribution (0.76-0.85) based on values from the published literature (Reed et al. 2009, Doak et al. 1994, Turner 1987).

Fecundity

Desert tortoise fecundity (per-capita number of new age-1 individuals in the population at year $t+1$ produced by individuals in a given stage at year t) was modeled as the product of six vital rates: *annual probability of reproduction* (PR), *per-capita annual egg production* (clutch size; CS), *nest success* (NS), *hatching success* (HS), *fraction of female hatchlings* (PF), and *hatchling survival* (PHI_H). Of these six vital rates, four were modeled as functions of climate (PR, CS, HS and PF).

Probability of reproduction (PR)

Probability of reproduction (PR) was drawn directly from our range-wide analysis of Desert Tortoise reproductive output (see above; also published as Mitchell et al. 2021). Since this analysis was fit in a Bayesian framework, we sampled randomly from the joint posterior distribution for each replicate to account for parameter uncertainty. Because both egg production (clutch size; CS) and PR parameters were drawn from this model, each unique draw from the joint posterior distribution for this model was used to parameterize both the PR and the CS model. Reproductive probability was determined as a logit-linear function of two climate variables -- maximum air temperature range (March-April; higher temperature range corresponding to lower probability of reproduction) and cumulative prior-year precipitation (higher reproductive rates with higher values for precipitation) -- in addition to an intercept term (representing baseline probability of reproduction), a coefficient for mean body size (higher probability of reproduction for higher body sizes; see below), and a term reflecting the average prior-year reproductive status (individuals reproducing in year $t-1$ were more likely to reproduce in year t ; for this model, we assumed that 80% of females were reproductive in all years across all populations, reflecting the observed average level in our study). Because body size was an important covariate in this model, we used our somatic growth model to estimate mean body size in each subpopulation (see above; also dependent on local climate conditions) and used the resulting mean body size estimate in the function for determining probability of reproduction.

Per-capita egg production (clutch size; CS)

Total annual per-capita egg production (clutch size; CS) was drawn directly from our range-wide analysis of Desert Tortoise reproductive output (see above; also published as Mitchell et al. 2021). Since this analysis was fit in a Bayesian framework, we sampled randomly from the joint posterior distribution for each replicate to account for parameter uncertainty. Because both egg production (clutch size; CS) and PR parameters were drawn from this model, each unique draw from the joint posterior distribution for this model was used to parameterize both the PR and the CS model. Annual egg production was determined as a log-linear function of two climate variables -- spring mean temperature (in the original Mitchell et al. [2021] parameterization, this was a daily temperature threshold model, which we could not accommodate in climate projection models at a monthly scale, March-May temperature was closely correlated with this variable; higher temperature corresponded to higher egg production, likely due to ability to produce more than one clutch in a year) and cumulative prior-year precipitation (higher egg production with higher values for precipitation) -- in addition to an intercept term (representing baseline egg production) and a term reflecting the role of body size in influencing total egg production (larger individuals tend to produce larger clutches). Because body size was an important covariate in

this model, we used our somatic growth model to estimate mean body size in each subpopulation (see above; also dependent on local climate conditions) and used the resulting mean body size estimate in the function for determining total egg production.

Nest success (NS)

The nest success parameter represents one minus the probability of nest predation (Desert Tortoise nests are frequently destroyed by a number of mammalian and avian predators). Although we had some depredated nests from our own study, we did not use our data because we attempted to protect all nests from predation, thus artificially decreasing predation rates. Instead, we used values drawn from the published literature to define a uniform distribution from which to draw a value for NS for each simulation replicate: specifically, we used a normal distribution with mean of 0.45 and standard deviation of 0.13, based on the mean and standard error of five field studies (Bjurlin & Bisonette 2004, Ennen et al. 2012, Karl 1998, Roberson et al. 1985, McLuckie and Fridell 2002).

Hatching success (HS)

The hatching success parameter reflects the fraction of eggs within each nest that hatch successfully, assuming the nest does not fall victim to predation (see NS parameter above). Our field study represents the richest source of data for estimating this parameter- and the only source for estimating the effects of climate conditions on HS. We modeled HS as a function of nest temperature, where temperatures greater than the baseline conditions tended to result in lower hatching success (see description for Task 1c, above). We used the results from our study, supplemented with results from other published studies, to establish an estimate for baseline hatching success (hatching success under baseline climate conditions). Specifically, we used a uniform distribution to draw a value for baseline HS for each replicate, with lower bound of 0.71 and upper bound of 0.84 based on four published field studies in addition to our own data (Bjurlin & Bisonette 2004, Ennen et al. 2012, Nafus et al. 2015, **Table 15** in Task 1b.). We drew another value (one draw per replicate) from a normal distribution to describe the logit-scale relationship between HS and nest temperature, with mean of -0.41 and standard deviation of 0.17, based on our analysis from Task 1c.

Proportion of female hatchlings (PF)

The sex ratio parameter (proportion of hatchlings born female; PF) reflects the fraction of successful hatchlings that are female. Since this species has temperature-dependent sex determination, this parameter was modeled as a function of temperature. Our field study represents the only source of data for estimating this parameter for natural nests. We modeled PF as a function of nest temperature, where temperatures greater than baseline conditions tended to result in a greater proportion of female hatchlings. We assumed that the baseline value for the fraction of female hatchlings was exactly 0.5 (even 1:1 sex ratio), with deviations from that baseline driven by expected local temperature changes. We decided to use a baseline of 0.5 instead of the baseline value from our data (0.58) because our data were drawn from a small sample of sites and years, and may not accurately reflect the range-wide baseline sex ratios.

Hatchling survival (PHI_H).

Little is known about hatchling survival in Desert Tortoises; however, we were able to locate several studies documenting survival over the first year of life. Two older studies interpolated hatchling survival rates based on life table analyses and estimated hatchling survival rates between 55-72% (Turner 1987, Doak et al. 1994). More recent studies directly estimated hatchling survival rates through radiotelemetry, but hatchlings were raised in captivity for varying periods of time. However, those hatchlings were exposed to raven predation (a condition that is prevalent across the species' range), and therefore are more likely a better representation of true present-day hatchling survival rates. Estimates ranged from 37-48% (Daly et al. 2019, Tuberville et al. 2019). We used the full range of these published estimates, drawing parameter values from a uniform distribution between 37 and 72%.

Dispersal

To model the dispersal process, we assumed that only adult individuals (stage 20) engaged in permanent long-distance dispersal (although this assumption could be relaxed in future versions). For now, we assumed that a fixed fraction (1%) of adult females dispersed from each subpopulation (5 X 5 km grid cell) each year, with the rest remaining in their original subpopulation. For each subpopulation, dispersing females were distributed across neighboring grid cells (with movement to the 4 directly adjacent cells occurring more often than movement to corner-adjacent cells) according to a negative exponential dispersal kernel (see above), with movement to cells with higher quality habitat favored over lower-quality habitat. In addition, we will continue coordinating with the RC18-1207 team after the project end date to incorporate insights from their efforts concerning local and regional genetic connectivity to better inform the dispersal rates used in this model.

Simulation scenarios

In addition to the four climate change scenarios (RCP2.6, RCP4.5, RCP6.0 and RCP8.5), we included binary “on-off switches” enabling us to activate various climate effects and better understand how climate change may affect Desert Tortoise populations in the future. We ran all unique possible combinations of these binary switches and climate change scenarios, with each unique combination considered as a “scenario”. We ran 256 unique scenarios in total for the Desert Tortoise model (with 120 replicates per scenario).

Table 8. *Description of binary variables (“on-off switches”) used for constructing scenario tests for Desert Tortoise.*

Binary variable	Description
phi.a_clim	Does adult survival depend directly on climate (precipitation)?
cs_clim	Does clutch size change with climate change?
pr_clim	Does reproductive probability depend directly on climate?
hs_clim	Does hatching success depend directly on climate?

pf_clim	Does sex ratio depend directly on climate?
growth_clim	Does somatic growth and age-at-maturity depend directly on climate?

All simulation routines and statistical analyses were performed using R v4.0.3 (R Core Team, 2021). All spatial representations of climate or demographic processes were simulated in R using the ‘raster’ and ‘terra’ packages (Hijmans 2022, 2023). The R code for running these analyses can be downloaded from GitHub: <https://github.com/kevintshoemaker/GopherusPVA>.

Parameterization: Gopher Tortoise

As noted above, parameter estimates (distributions from which to draw samples for each replicate) were drawn from two main sources: data analysis and field data conducted as part of this study, and from a recently published range-wide Gopher Tortoise population model (Folt et al. 2022) that was used by USFWS in their recent status assessment for the Gopher Tortoise. Major differences between our model and Folt et al. (2022) include: (1) Folt et al. (2022) used a spatially implicit metapopulation modeling framework (network with nodes as populations and edges to represent connectivity) whereas our model treated space explicitly using a lattice (or grid-based) population model, (2) a change in how the dispersal process works given the switch to a spatially explicit model, and (3) the Folt et al. (2022) model used a stage-based modeling framework with two stages (juvenile and adult) where the juvenile-to-adult transition rate was specified as the inverse of the number of years spent in the juvenile stage; see Kendall et al 2019), whereas our model used an age-structured transition matrix to more accurately account for years spent in juvenile stages (while also enabling age at maturity to vary across space and time).

Climate change scenarios

We used downscaled monthly temperature and precipitation rasters for the entire range of the Gopher Tortoise (see Gopher Tortoise Task 1a above) to derive dynamic yearly rasters representing climate covariates for determining key population vital rates in our Gopher Tortoise simulations. The table below provides a summary of key climate relationships that are incorporated into the Gopher Tortoise simulation model. **Figure 21** (below) provides a visualization of how some of these summarized climate variables used in the Gopher Tortoise population model (and averaged across the Gopher Tortoise range) are expected to change over time under the different climate-change scenarios.

Table 9. Summary of climate-sensitive components for the Gopher Tortoise population simulation model.

Model component	Climate variable name	Climate variable description	Estimate source
Age-at-maturity (MA)	<i>mn_ann_temp</i>	Mean annual temperature	Folt et al. 2022
Juvenile survival (PHI-J)	<i>YSB*</i>	Years since burn	This project (Hunter and Rostal 2021)
Adult survival (PHI-J)	<i>YSB*</i>	Years since burn	This project (Hunter and Rostal 2021)
Clutch size (CS)	<i>june</i>	Maximum air temperature, June	This project (Hunter et al. 2021)
Hatching success (HS)	<i>jun_jul_diff</i>	Maximum air temperature, mean of June-July	This project (<i>in prep.</i>)
Hatching success (HS)	<i>jun_jul_pr_diff</i>	Total precipitation, June-July	This project (<i>in prep.</i>)
Hatchling sex ratio (PF)	<i>jun_diff</i>	Mean air temperature, June	This project (<i>in prep.</i>)
Hatchling sex ratio (PF)	<i>jun_pr_diff</i>	Total precipitation, June	This project (<i>in prep.</i>)
Probability of reproducing (PR)	<i>apr_may</i>	Maximum temperature, mean of April-May	This project (Hunter et al. 2021)

* Note: *years since burn* is included here as a ‘climate variable’ only because burn frequency may decrease under climate change, as acceptable time windows shrink for conducting safe prescribed burns in the southeastern USA (Kupfer et al. 2020).

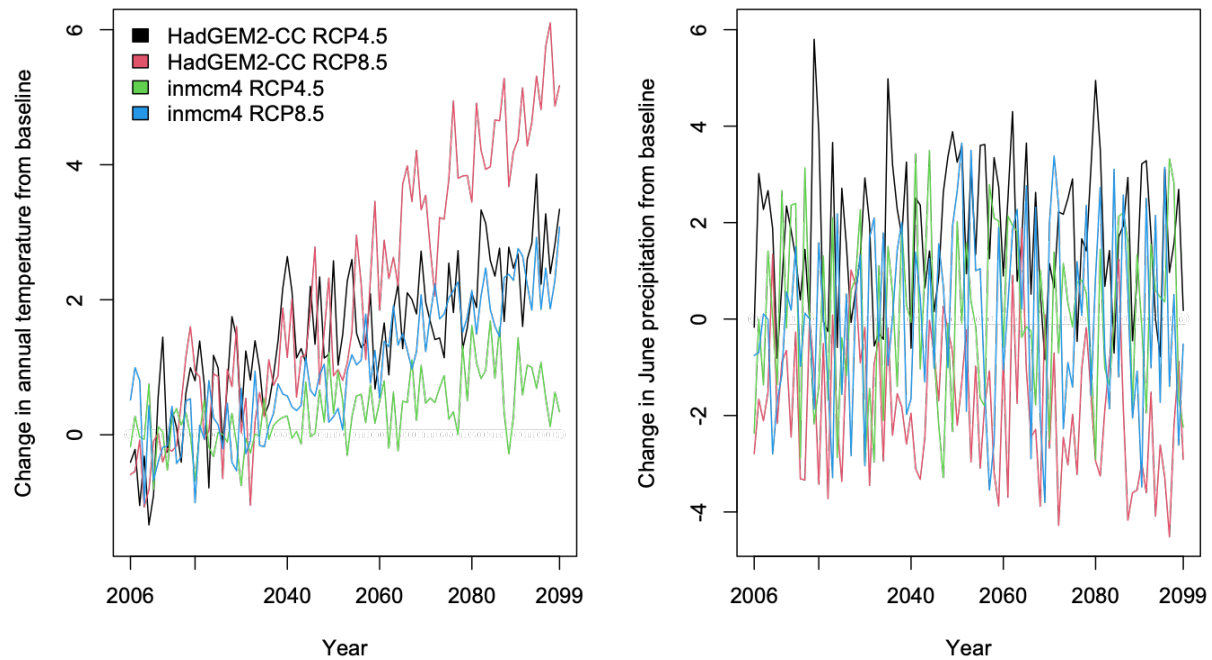


Figure 21. Time series for two derived climate variables (annual temperature [left] and June precipitation [right]) important for Gopher Tortoise demography. Y-axes show change in the projected values compared to a baseline year of 2018. Time series are shown for both RCPs and two GCMs: a dry scenario (inmcm4) and a hot scenario (HadGEM2-CC).

Because the model we use for the PR and CS parameters for Gopher Tortoise relies on data from Fort Stewart, which is near the northern edge of the Gopher Tortoise range, we also ran scenarios in which the observed climate effects become weaker at lower latitudes and higher at higher latitudes (see additional details below).

Initial abundance and habitat suitability

To initialize abundance for each 5 X 5 km grid cell, we used (1) the initial abundances for each subpopulation in the Folt et al. (2022) population viability analysis for Gopher Tortoises, and (2) a recently developed habitat suitability map developed by Crawford et al. (2020) and re-sampled to the same 5 X 5 km grid resolution as the population model. We then used a “moving window” algorithm to distribute the total abundance throughout the area corresponding to each subpopulation in Folt et al. (2022), with higher abundances corresponding to cells with higher habitat suitability according to the Crawford et al. (2020) model. Maximum (ceiling) abundance in each subpopulation (5 X 5 km grid cell) was set to twice the initial value in each pixel (allowing all subpopulations some room for expansion but allowing maximum abundance in each cell to still reflect the estimated relative habitat suitability). In the Gopher Tortoise simulation model, only cells currently occupied by Gopher Tortoises were considered as viable habitat, with all other grid cells within the range assumed to be incapable of supporting a Gopher Tortoise population (this assumption could be relaxed in a future version). All pixels

(populations) were initialized approximately at stable age distribution. Finally, we distributed the initial abundance within each grid cell across all 20 stages according (approximately) to the stable stage distribution for this population. Because Gopher Tortoise simulations tended to exhibit a steep decline over time, we chose not to implement a ‘burn-in’ (as we did for Desert Tortoise) because this would have resulted in initial abundances much reduced relative to the best available empirical estimates.

Prescribed fire and burn frequency

Because we found that fire -- in particular, the years since the most recent burn -- is an important driver of survival rates for Gopher Tortoises (Hunter and Rostal 2021), we incorporated a simple fire forecasting model within our simulation framework for Gopher Tortoises. First of all, we assumed a baseline average fire return interval of approximately 4 years across the southeastern United States, based on Folt et al. (2022). In addition, Kupfer et al. (2020) found a relative change in duration of burning windows (from baseline year 2006) – as climate warms, the number of days in which prescribed burning could occur decreases, particularly in the growing season. We assumed that as the number of burning days decreases, the fire return interval would increase as less land overall can be burned in a single year. Kupfer et al. (2020) provided estimates of how the number of burn days changes across our climate change scenarios (4 GCMs and 2 RCPs).

To simulate the change in fire return interval and subsequent effects on tortoise survival, we assigned all grid cells a $\frac{1}{4}$ chance of being burned each year under baseline climate conditions. Each year of each simulation, the burn probability is modified using the Kupfer et al. (2020) model: for a given climate change scenario, we assume the number of burning days in 2006 results in a 4 year fire return interval. We then multiply the fire return interval (inverse of probability that a grid cell is burned in a year) for each subsequent year by the proportional change in number of burning days. Within the simulation model, we used a random binomial draw for each grid cell to determine whether or not a fire occurred (with burn probability assigned as above). For each grid cell, we monitored the number of years since the most recent burn event. When a burn event occurred, this value was reset to zero, otherwise, the value was incremented by one each year. This “years since burn (YSB)” raster was then used as a predictor variable for determining adult and juvenile survival rates within our Gopher Tortoise simulation model. The effect of YSB on survival was drawn from Hunter and Rostal (2021), in which the maximum observed YSB value was 4 years. We therefore cap the effect of YSB on survival at 4 years (i.e., the linear effect is not extrapolated past 4 YSB). In addition, Hunter and Rostal (2021) only estimated the burning effect on adult survival, but it is likely that burning also influences juvenile survival in similar ways, so we included scenarios with a burning effect on juvenile survival. The effect of YSB on survival may also be an overestimate – Hunter and Rostal (2021) estimated “apparent survival” (mortality and emigration is somewhat confounded), and so we also tested scenarios in which the effect of YSB on survival is cut in half.

Age-at-maturity (MA)

For the Gopher Tortoise, we did not have sufficient data to build an explicit growth model as we did for the Desert Tortoise model (see Desert Tortoise model parameterization). For Gopher Tortoises, we modeled age-at-maturity following the approach used by Folt et al. (2022), in

which 10 previously published studies documenting age-at-maturity for Gopher Tortoises across the species range was used to link age-at-maturity to mean annual temperature.

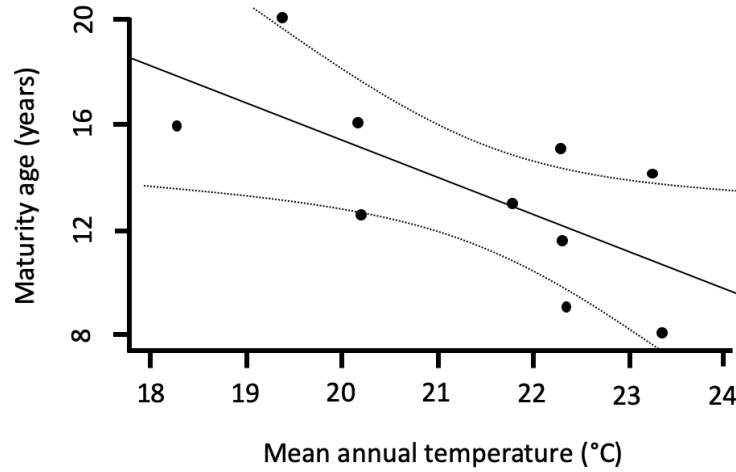


Figure 22. Relationship between Gopher Tortoise age-at-maturity (MA) and mean annual temperature across the species range. Figure based on data and analysis results from Folt et al. (2022).

Adult and juvenile survival (PHI_A and PHI_J)

The annual survival rate for all reproductive adults (individuals at or above the age-at-maturity) and juveniles (individuals below the age-at-maturity) were both modeled as a function of years-since-burn (see above). Baseline adult survival rate (PHI_A_base) was sampled from a normal distribution (one sample per replicate) based on Folt et al. (2022) and Hunter and Rostal (2021), with a mean of 0.96 and a standard deviation of 0.03. Baseline juvenile survival rate (PHI_J_base) was also sampled from a normal distribution (one sample per replicate), this time with a mean of 0.77 and a standard deviation of 0.07 (modified from Folt et al. 2022). To represent the coefficient relating burn frequency to adult and juvenile survival ('burn.eff'), we drew another random sample from a normal distribution, this time with a mean of -0.027 and standard deviation of 0.003, based on Hunter and Rostal (2021). For each pixel and for each year, we then computed adult and juvenile survival rate simply as:

$$\text{PHI_A} = \text{PHI_A_base} + \text{burn.eff} * \text{YSB}$$

$$\text{PHI_J} = \text{PHI_J_base} + \text{burn.eff} * \text{YSB}$$

where YSB represents the number of years since the most recent burn (see above). As part of our scenario tests (see below) we were able to turn the burn effect off and on for juveniles and adults separately (when the burn effect was turned off, survival was always held constant at baseline values), control whether or not the burn frequency changed in response to climate change, and determine whether or not the effect of YSB on survival is cut in half to account for the possibility of dispersal instead of mortality in the Hunter and Rostal (2021) study.

Fecundity

Gopher Tortoise fecundity (per-capita number of new age-1 individuals in the population at year $t+1$ produced by individuals in a given stage at year t) was modeled as the product of six vital rates: *annual probability of reproduction* (PR), *per-capita annual egg production* (clutch size; CS), *nest success* (NS), *hatching success* (HS), *fraction of female hatchlings* (PF), and *hatchling survival* (PHI_H). Of these six vital rates, four were modeled as functions of climate (PR, CS, HS and PF).

Probability of reproduction (PR)

Probability of reproduction (PR) was drawn directly from our analysis of Gopher Tortoise reproductive output at Fort Stewart (Hunter et al. 2021). Since this analysis was fit in a Bayesian framework, we sampled randomly from the joint posterior distribution for each replicate to account for parameter uncertainty. Because both egg production (clutch size; CS) and PR parameters were drawn from this model, each unique draw from the joint posterior distribution for this model was used to parameterize both the PR and the CS model. Reproductive probability was determined as a logit-linear function of one climate variable -- April-May maximum temperature, with higher probability of reproducing corresponding to higher temperatures -- in addition to an intercept term (representing baseline probability of reproduction). Because this model was fit to data from Fort Stewart, in the northern part of the species range (with lower mean annual temperatures), it was unclear if this positive effect of temperature would also be observed in the warmer, more southern latitudes. Therefore, we also included an option (activated in some scenarios; see below) whereby this temperature effect was reduced (coefficient moved closer to zero) for populations nearer to the southern edge of the range.

Per-capita egg production (clutch size; CS)

Total annual per-capita egg production (clutch size; CS) was from our analysis of Gopher Tortoise reproductive output at Fort Stewart (Hunter et al. 2021). Since this analysis was fit in a Bayesian framework, we sampled randomly from the joint posterior distribution for each replicate to account for parameter uncertainty. Because both egg production (clutch size; CS) and PR parameters were drawn from this model, each unique draw from the joint posterior distribution for this model was used to parameterize both the PR and the CS model. Annual egg production was determined as a linear function of one climate variable -- June maximum temperature, where higher temperatures corresponded to higher egg production -- in addition to an intercept term (representing baseline egg production). Because this model was fit to data from Fort Stewart, in the northern part of the species range (with lower mean annual temperatures), it was unclear if this positive effect of temperature would also be observed in the warmer, more southern latitudes. Therefore, we also included an option (activated in some scenarios; see below) whereby this temperature effect was reduced (coefficient moved closer to zero) for populations nearer to the southern edge of the range.

Nest success (NS)

The nest success parameter essentially represents one minus the probability of nest predation (Gopher Tortoise nests are frequently destroyed by a number of mammalian and invertebrate

predators). We used values drawn from the published literature to define a uniform distribution from which to draw a value for NS; specifically, we used a lower bound of 0.15 and an upper bound of 0.55, based on Folt et al. (2021), but with expanded uncertainty as the central tendency estimate is based on a single study.

Hatching success (HS)

The hatching success parameter reflects the fraction of eggs within each nest that hatch successfully, assuming the nest does not fall victim to predation (see NS parameter above). Our Gopher Tortoise field study represents a rich source of data for estimating this parameter- and the only source for estimating the effects of climate conditions on HS from wild nests. However, given that we did not detect clear declines in hatching success at higher temperatures (unlike what was observed in our Desert Tortoise data), we incorporated data from a laboratory study (Demuth 2001) to complement our results from wild nests. None of our nests experienced mean temperatures above 32°C (**Figure 42** [task 1c]). In Demuth's study, experimental clutches incubated at a constant 32°C experienced a sharp drop-off in hatching success relative to cooler temperatures, and hatching success dropped to virtually zero at 34°C. Thus, we chose to implement a simple threshold effect of temperature: nests experiencing mean temperatures >32°C fail completely. We used the empirical distribution of nest temperatures from our field study of 150 nests from 5 sites across the range, and adjusted these nest temperatures using a model that predicted the change in mean nest temperature from the change in mean temperature and precipitation in June and July (the core of nesting season), relative to baseline. For each year and grid cell in the simulation, we adjusted the observed nest temperatures from our empirical dataset of mean nest temperatures by the offset predicted by the climate effect model, then computed the fraction of those nest temperatures with adjusted temperatures below the threshold of 32°C. This fraction represented the hatching success adjustment due to climate change, and was multiplied by a baseline hatching success parameter to estimate overall hatching success. We used the results from our study, supplemented with results from other published studies (see Folt et al. 2022), to establish an estimate for baseline hatching success (hatching success under baseline climate conditions). Specifically, we used a uniform distribution to draw a value for HS for each replicate, with lower bound of 0.66 and upper bound of 0.97.

Proportion of female hatchlings (PF)

The sex ratio parameter (proportion of hatchlings born female; PF) reflects the fraction of successful hatchlings from each nest that are female. Since this species has temperature-dependent sex determination, this parameter was modeled as a function of temperature. Our field study represents the only source of data for estimating this parameter from natural nests. We modeled PF as a function of nest temperature and precipitation during the critical period. To link climate change to nest conditions, we assumed that the relationship between ambient temperature and nest temperature in the month of June represents the link between ambient and nest temperatures during the typical nest's critical period (the entirety of which typically occurs in June in our dataset). As for other parameters, we model the effect of ambient temperature and precipitation on nest temperature in the month of June using our dataset, and then apply that model to estimate the change in nest temperature that results from a given grid cell's change from baseline June temperature and precipitation. We then add that change in nest temperature to the pivotal nest temperature (29°C) that corresponds to a 50:50 sex ratio in our logistic

regression model (**Figure 43**), and use the model to predict sex ratio from the grid cell's estimated nest temperature and precipitation. We decided to use a baseline sex ratio of 50:50 instead of the baseline value from our field data because our data were drawn from a small sample of sites and years, and may not accurately reflect the range-wide baseline sex ratios.

Hatchling survival (PHI_H).

Little is known about hatchling survival in Gopher Tortoises; however, we were able to locate several studies documenting survival over the first year of life. Based on these studies, we used a uniform distribution to sample PHI_H for each replicate, with a minimum of 0 and maximum of 0.4 based on minimum and maximum values in a meta-analysis of Gopher Tortoise hatchling survival (Perez-Heydrich et al. 2012).

Dispersal

To model the dispersal process, we assumed that only adult individuals (stage 20) engaged in permanent long-distance dispersal (as assumed in Folt et al. 2022). For now, we assumed that a fixed fraction (1%) of adult females dispersed from each subpopulation (5 X 5 km grid cell) each year, with the rest remaining in their original subpopulation. For each subpopulation, dispersing females were distributed across neighboring grid cells (with movement to the 4 directly adjacent cells occurring more often than movement to corner-adjacent cells) according to a negative exponential dispersal kernel, with movement to cells with higher quality habitat favored over lower-quality habitat. For this analysis, we assumed no dispersal of juvenile individuals, although this assumption could be relaxed in a future version of this model. In addition, Gopher Tortoises were not allowed to disperse into or colonize any grid cells representing non-habitat (i.e., any cells with no Gopher Tortoises in the initial abundance raster).

Simulation scenarios

In addition to the 8 climate change models (4 GCMs and 2 RCPs, see above), we included binary “on-off switches” enabling us to run multiple scenarios turning various climate (and years-since-burn) effects on and off in order to better understand how climate change and fire may affect Gopher Tortoise populations in the future. We ran 3584 unique combinations of these binary switches and the 8 climate change models, with each unique combination considered as a “scenario”. Thus, there were 3584 scenarios in total for the Gopher Tortoise model. Each scenario was simulated using the same 120 replicate draws of parameter values from the distributions described above.

Table 10. Description of binary variables (“on-off switches”) used for constructing scenario tests for Gopher Tortoise.

Binary variable	Description
burn_clim	Is burning frequency dependent on climate?
phi.a_burn	Does adult survival depend on burn frequency?
phi.j_burn	Does juvenile survival depend on burn frequency?

cs_temp	Does clutch size change with increasing temperatures?
pr_temp	Does the probability of reproduction change with increasing temperatures?
hs_clim	Does hatching success change as a function of climate conditions?
pf_clim	Does sex ratio change as a function of climate conditions?
ma_temp	Does age-at-maturity depend on temperature?
phi_burn_half	Should the slope for the effect of years-since-burn on adult and juvenile mortality be cut in half?

All simulation routines and statistical analyses were performed using R v4.0.3 (R Core Team, 2021). All spatial representations of climate or demographic processes were simulated in R using the ‘raster’ package (Hijmans 2022). R code for performing all simulations and analyses can be downloaded from GitHub: <https://github.com/kevintshoemaker/GopherusPVA>.

Build and deploy a web-based conservation planning tool (Task 4)

We used the ‘R-shiny’ (Chang et al. 2022) reactive programming platform to build and deploy a web-based conservation planning application that enables users to visualize Desert Tortoise simulation results for a selected region of interest (ROI). The application is hosted on the web using ‘shinyapps.io’, a fee-based service provided by Posit, Inc. (Boston, MA). Due to limitations in download speeds and data processing, only 20 replicates (randomly selected from out of 120 replicates that were run per scenario) are currently available for visualization through the application. Detailed instructions on the web application (*MITCH-Clim 0.1*) and how to use it can be found in Appendix C.

Results and Discussion

Environmental/climatic sensitivities of population vital rates (Task 1c)

DESERT TORTOISE

Adult survival –

We analyzed long-term Mojave Desert Tortoise CMR data sets (‘permanent study plots’) from Arizona, California, Nevada, and Utah that we have compiled and digitized. These capture records are derived from resurveys of the one-square-mile permanent study plots, most surveys of which occurred from around 1980 to 2000. These plots were resurveyed every few years, and each survey consisted of two complete passes occurring within the same season- generally in spring. The permanent study plots that are available for reanalysis span a large portion of the range of the species, allowing us to make broad inference about the role of environmental factors in driving demographic rates.

Specifically, we used a modified Cormack-Jolly-Seber (CJS) formulation to estimate adult survival rate, and its sensitivity to spatial and temporal environmental and climatic gradients. We used Pollock’s ‘robust design’ to improve parameter estimation by increasing the precision of the capture efficiency estimates (Bailey et al. 2004). In addition, our parameter estimation is improved by incorporating a spatially-explicit capture-mark-recapture model (SCR) component in our model (Royle et al. 2013). All analyses were implemented in R using JAGS software (Plummer 2015; R Core Team 2022). We ran three parallel chains for 25,000 iterations each after a burn-in sample of 2000, and stored every 10th run for a total of 7500 joint posterior samples. We tested for convergence using visual inspection of traceplots and using Gelman and Rubin’s convergence diagnostic (values <1.1 indicate convergence).

Table 11. Mojave Desert Tortoise survival and abundance were modeled as a function of several site-level environmental and climatic variables (see below), with all models fitted using the ‘permanent study plot’ data.

Variable	Description	Source
Blkdens	Soil bulk density	STATSGO
Dpth2bdrk	Depth to bedrock	STATSGO
Pctcov	Perennial plant cover	Wallace et al. (2008)
Annprox	Annual green-up potential	Derived from NDVI (MODIS)
Rufavg	Average surface roughness	DEM
Precip_dry	Precipitation, May-Oct, 30-year normal	PRISM
Precip_wet	Precipitation, Nov-Apr, 30-year normal	PRISM
Temp_dry	Temperature, May-Oct, 30-year normal	PRISM
Temp_wet	Temperature, Nov-Apr, 30-year normal	PRISM
Meandens	Mean population density, estimated	PSP re-analysis

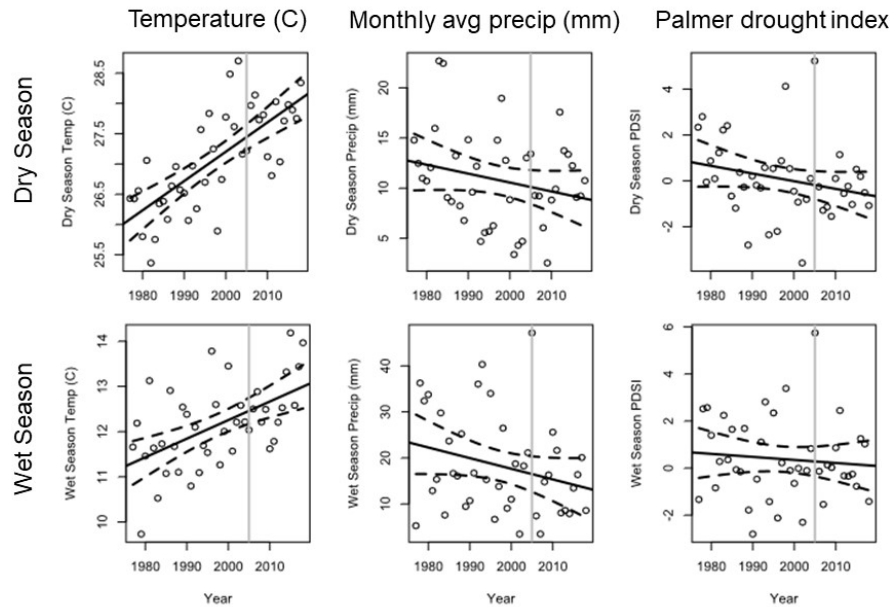


Figure 23. Trends in temperature, precipitation, and Palmer drought index during the years for which the resurveys of the ‘permanent study plots’ occurred, illustrating substantial trends from the start of the study in 1977 to present. The upward trend in temperature- particularly dry season temperature - is strong, while declines in precipitation and drought indices tended to exhibit weaker trends. The time series extend to the present, but the gray lines indicate the final year for which these long-term capture-mark-recapture data are available.

Results from these models indicate that variation in observed adult survival rates and abundance trends appear to be driven in part by wet-season precipitation at the site level, averaged across the 30 years prior to each survey (**Figure 24**). Other site-level covariates that influenced adult survival rates in our model included perennial vegetation cover (**Figure 24**), which tended to be positively correlated with adult survival. In addition, adult survival appears to exhibit substantial variation among years (**Figure 25**). Furthermore, much of the among-year variation in adult survival rate cannot be explained by any of the climate variables we used in our models, including multi-year summaries of prior precipitation and drought indices. Abundance estimates from our analyses (derived using a Horvitz-Thompson estimator) are generally consistent with previous estimates using the same data (**Figure 26**). We also noticed a decline in the capture rate of tortoises smaller than around 180mm in carapace length -- which is the generally accepted juvenile cutoff for this species. A logistic regression analysis suggested that the probability of encountering juveniles declined substantially over the study period. A similar decline in juvenile captures was noted more recently by Allison and Mcluckie (2018) on the basis of the range-wide distance sampling surveys conducted since the early 2000s.

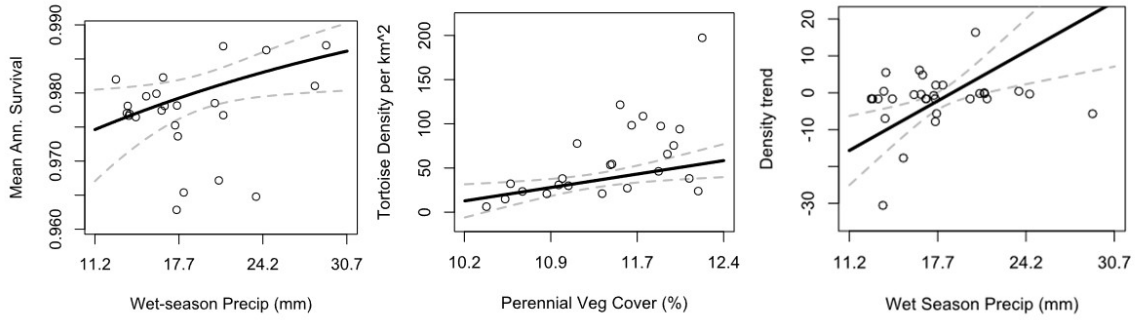


Figure 24. Site-level survival and other demographic variables as a function of environmental and climatic covariates. In these figures, each open circle represents a study plot with three or more surveys. Notably, wet-season precipitation (averaged throughout the study period) was positively correlated with adult survival rates and with positive abundance trends. This result makes some sense, since wet-season precipitation is generally associated with higher forage availability. Other patterns detected at the site level pertained to site-level soil and vegetation characteristics. For example, the mean density (averaged across the study period) at each site was correlated with a remote-sensing metric designed to estimate perennial vegetation cover.

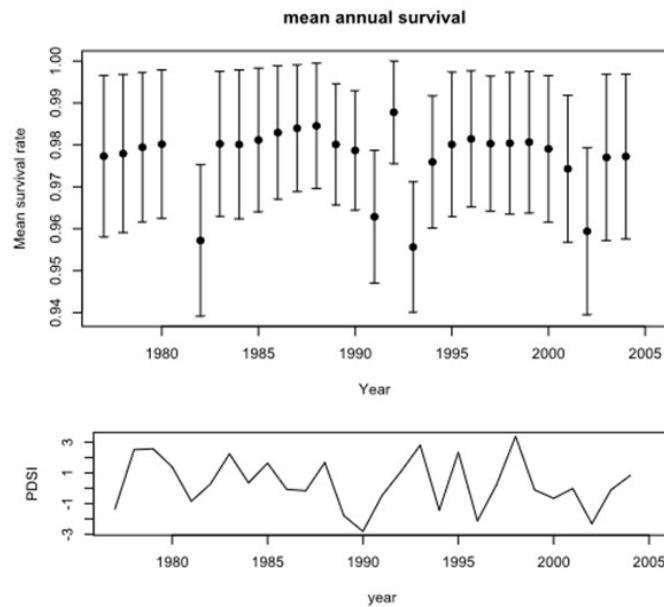


Figure 25. Our models of adult survival indicated substantial variation in survival among years, with several years showing reduced survival compared with “normal” years. However, when this variation was not correlated with any temperature, precipitation, or drought severity variable we tested in our models. The upper panel in this figure represents the annual adult survival rate for Desert Tortoises, based on the “permanent study plot” data. The lower panel in this figure represents the Palmer drought severity index (PDSI).

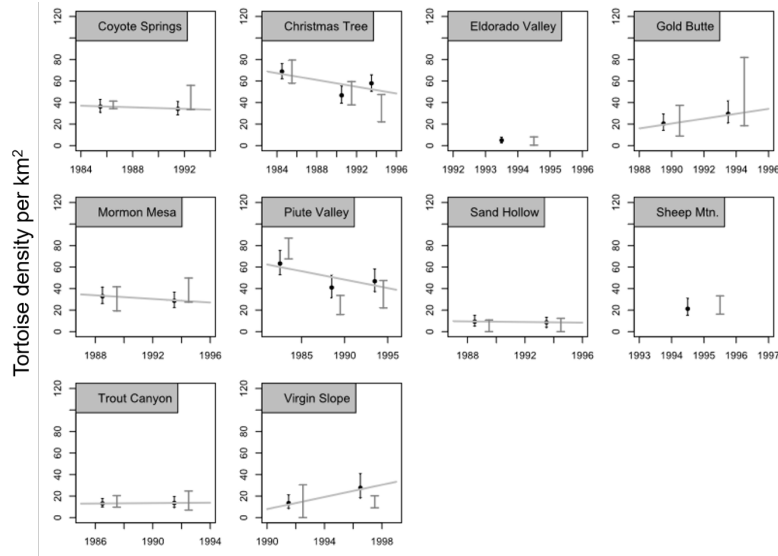


Figure 26. Mojave Desert Tortoise density and trend estimates at a subset of the available 'permanent study plots'. Data from additional sites have recently been made available and this figure will be expanded in the near future. Abundance estimates from these models tend to be consistent with previous reported estimates using the same data, although our estimates tend to be more precise. In this figure, gray bars represent previously reported estimates, while black circles and error bars represent estimates from this study.

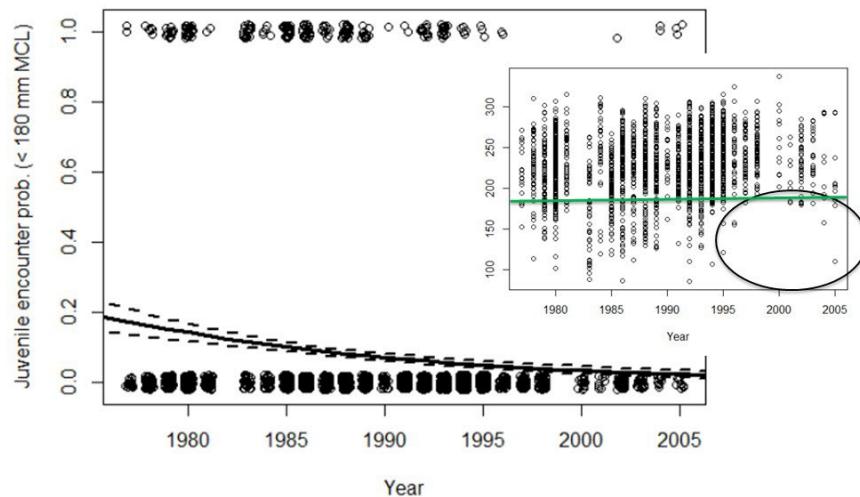


Figure 27. The permanent study plot data tend to indicate a general decline in capture rates for individuals smaller than around 180mm in carapace length -- which is the generally accepted juvenile cutoff for this species. A logistic regression suggested that the probability of encountering juveniles declined substantially over the study period. A similar decline in juvenile captures was noted by Allison and Mchuckie (2018) documenting results of the rangewide distance sampling surveys.

Growth and age/size at maturity –

Mean body size in ectotherms is generally predicted to decrease with increased temperature, a prediction termed the “temperature-size rule” (Angilletta and Dunham 2003) although contrary patterns of body size and temperature in both inter- and intra-specific systems have been reported (Cabanita and Atkinson 2006). In long-lived, large bodied terrestrial vertebrate ectotherms, age-at-maturity and female body size can have significant effects on fecundity, while size-specific predation and resource limitations can affect mean body size in a population, all of which can lead to concerns for population viability under different climate change scenarios (**Figure 28**).

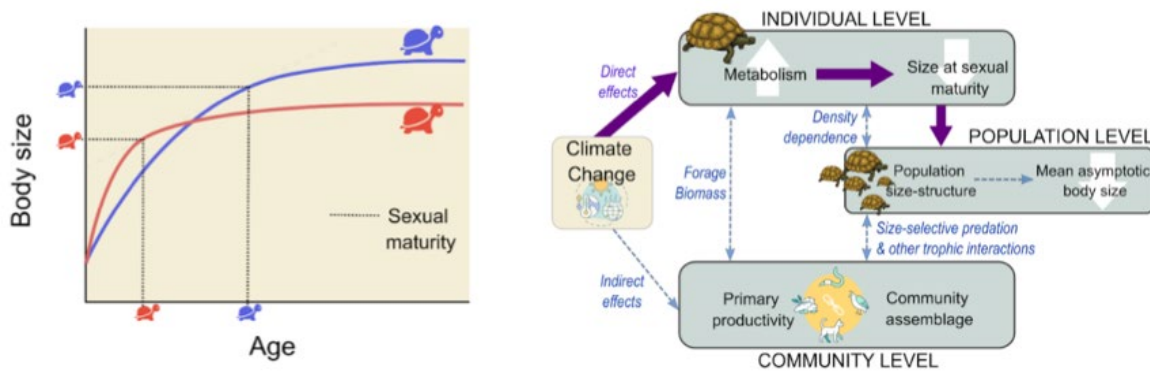


Figure 28. (Left) Temperature-size rule showing trade-off between growth rate and mean asymptotic body size in a turtle under cooler (blue) versus warmer (red) temperatures. (Right) Pathways for the direct and indirect effects of climate on growth rate and mean asymptotic body size in a turtle assuming the temperature-size rule. Modified from Ohlberger 2013.

We leveraged long-term longitudinal capture-recapture and telemetry studies of *G. agassizii* to (1) determine if this species follows the temperature-size rule, *i.e.*, we predict that growth rate will increase and body size will decrease as temperature increases (Ashton & Feldman, 2003), and (2) predict how growth rate and body size will respond to predicted changes in climate in future decades.

We incorporated four data sources of mark-recapture measurements of straight midline carapace length (MCL) of individual tortoises. These data include (1) plot surveys performed annually or several years apart, (2) telemetry data for adults in which animals were regularly relocated and assessed as needed at different times of the year, (3) sites at which translocated and resident tortoises were monitored with radio telemetry for several years post-translocation, and a known-age set of tortoises followed from yearling to death. Combined, our full dataset consisted of 1,416 individuals with 5,021 growth periods where captures were at least one calendar year apart. Of these individuals, 18 were known-age with 518 growth periods, 106 were translocated individuals with 196 growth periods (see Task 2), and the remainder were 1,292 resident individuals with 4,307 growth periods.

To model somatic growth in tortoises, we used the Von Bertalanffy growth model parameterized by Armstrong and Brooks (2013). The equation for individuals of known age can be expressed as:

$$L = a \left(1 - e^{-k/a(t-t_0)} \right)$$

where L = length of an individual at capture, a = asymptotic length, k = growth rate, t = age at capture, and t_0 = theoretical age at which length is zero (Armstrong & Brooks, 2014). For individuals of unknown age, the equation can be expressed as:

$$L_r = a(a - L_c) \left(1 - e^{-k/a(t_r-t_c)} \right)$$

where L_r = length of an individual at recapture, L_c = length of an individual at initial capture, $t_r - t_c$ = duration of time between capture and recapture, and a and k are as described in the previous equation (Armstrong & Brooks, 2014).

We identified three environmental variables hypothesized to influence tortoise growth: (1) total winter-spring precipitation summed across Nov–Apr (PPT), (2) annual total of growing degree days defined as the minimum temperature above 10°C for the 24-hr period (GDD), and (3) mean maximum daily spring temperature averaged by month across Feb–May (T_{max}). These variables were selected based on previously published reports of correlated climate metrics and growth in *G. agassizii* (Medica et al., 2012) and were associated with pairwise correlation coefficient < 0.42. These variables were downloaded using package ‘prism’ from the PRISM dataset of monthly (PPT and T_{max}) or daily (> 1981 minimum temperature for GDD) data at 4 km² resolution (Hart & Bell, 2015; PRISM Climate Group, 2021).

We created logit-linear models of a and k that included effects of the three climate variables, and we also included site (defined area within which surveys were performed or telemetered animals were regularly monitored) as a random effect on both a and k to account for the differences in number of individuals at the site, measurement methods between studies, and for aspects of habitat or geography not otherwise captured in other explanatory variables. At the individual level, we included an effect of sex on a , but not on k , as previous studies indicated different growth trajectories but only after sexual maturity (Medica et al., 2012).

We used the resulting model to predict spatiotemporal variation in growth rate k and maximum body size a across all U.S. Fish and Wildlife Service Recovery Units (U.S. Fish and Wildlife Service, U.S. Department of the Interior, 2011). For climate for the past (1990–2019) and future (2060–2089), we used Bias Corrected Constructed Analogs version 2 (BCCAv2) Daily Climate Projections for two of the World Climate Research Programme’s Coupled Model

Intercomparison Project phase 5 scenarios: (1) moderate-risk RCP4.5, and (2) high-risk RCP8.5 scenarios. We chose two ensembles from the ‘CCSM4’ model by the National Center for Atmospheric Research (NCAR) modeling center. We used the ensemble mean for calculations. For the past and future 30-yr periods separately, we performed 1000 simulations per raster grid cell and year (drawing randomly from the joint posterior for parameter values for each simulation) to estimate growth rate k and asymptotic size a . We then subtracted the historical mean estimates for growth rate k and asymptotic size a from the predicted mean estimates for each climate scenario and Recovery Unit to identify how growth rate k and asymptotic size a would change in the future. Using these predicted historical and future estimates for growth rate

k and asymptotic size a , we then plotted the growth curves for each Recovery Unit and climate scenario. We could then identify the age at which a female would reach the reported threshold for size-at-maturity (18.9–21.2 mm MCL; Lovich et al., 2018; Mueller et al., 1998; Turner et al., 1986).

Growth rate k increased with Tmax and PPT but decreased with GDD, and did not appear to be affected by translocation status (**Figure 29**). Asymptotic size a increased for males and translocated individuals and with both PPT and GDD, while it decreased with increasing Tmax (**Figure 29**).

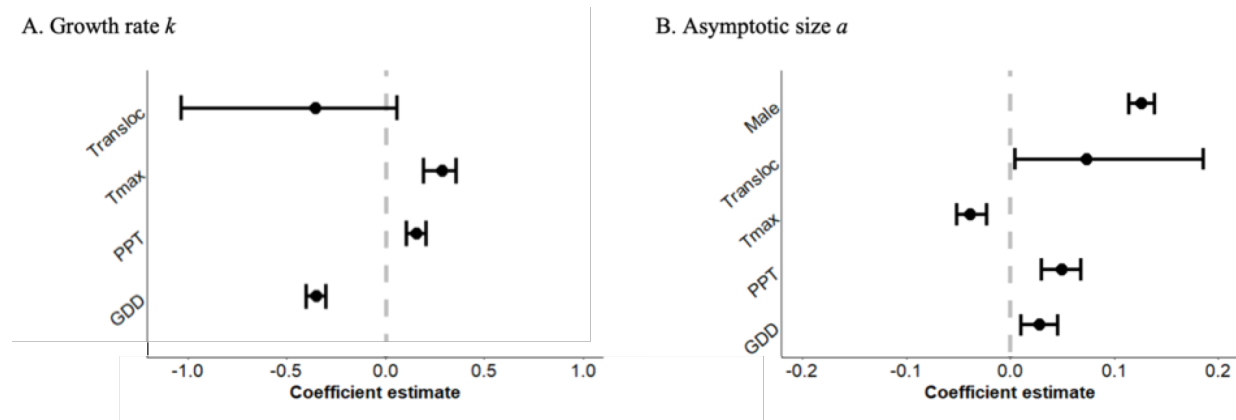


Figure 29. Median and 95% credible intervals of the estimates for climate, sex, and translocation status coefficients on (A) growth rate (k) and (B) mean asymptotic body size (a) for Mojave Desert Tortoise (*Gopherus agassizii*) using the von Bertalanffy growth model. Values are on the log scale.

Under the moderate-risk climate change scenario, asymptotic body size a is predicted to decrease across three of the five Recovery Units, increasing in the Upper Virgin River and the Western Mojave, while growth rate will increase across all Recovery Units (**Figure 30**). Under the high-risk climate change scenario, asymptotic body size a is predicted to increase across all five Recovery Units, while growth rate will also increase (**Figure 30**). On balance, a female is predicted to reach size-at-maturity approximately one year earlier under the moderate-risk scenario (0.8–1.4 yr earlier, **Figure 31**), and one to two years earlier under the high-risk scenario (0.9–2.2 yrs earlier, **Figure 31**).

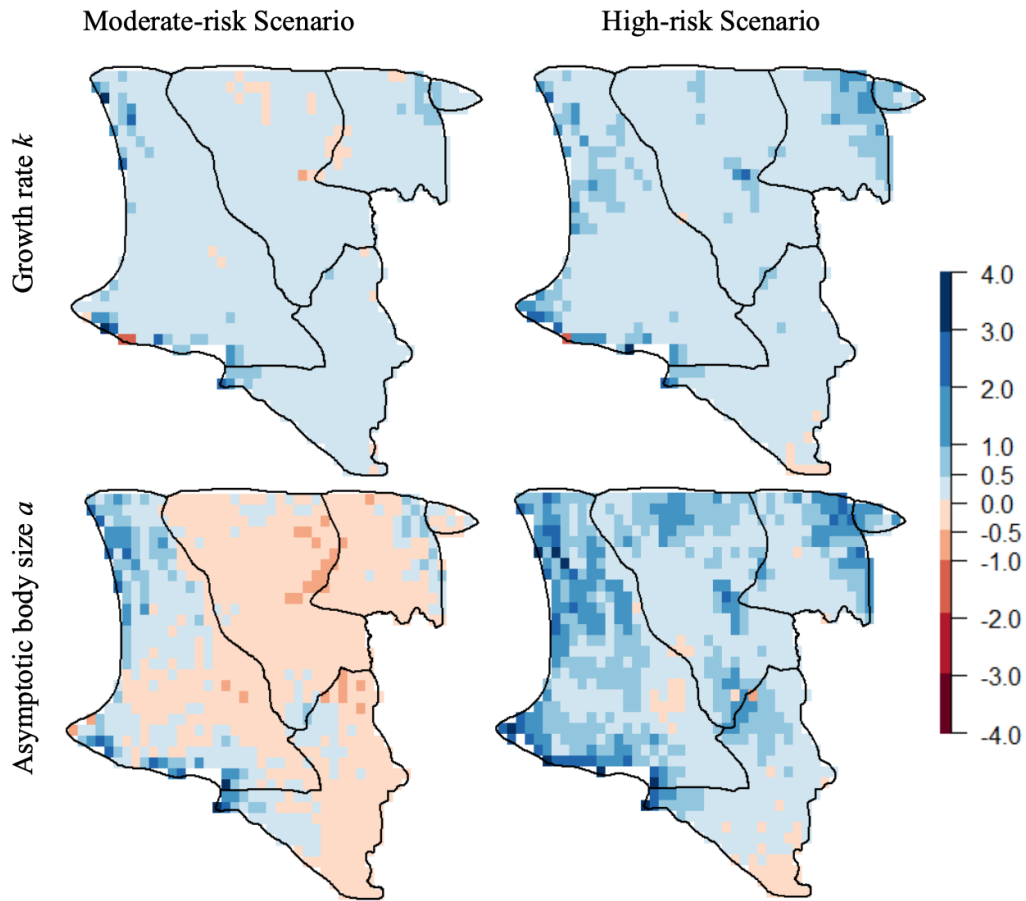


Figure 30. Predicted change in growth rate k and mean asymptotic body size a across the range of Mojave Desert Tortoise (*Gopherus agassizii*) using Bias Corrected Constructed Analogs version 2 (BCCAv2) Daily Climate Projections for two of the World Climate Research Programme's Coupled Model Intercomparison Project phase 5 scenarios: (1) moderate-risk RCP4.5, and (2) high-risk RCP8.5 scenarios from 2050–79 compared with 1990–2019. Growth is modeled using the von Bertalanffy equation.

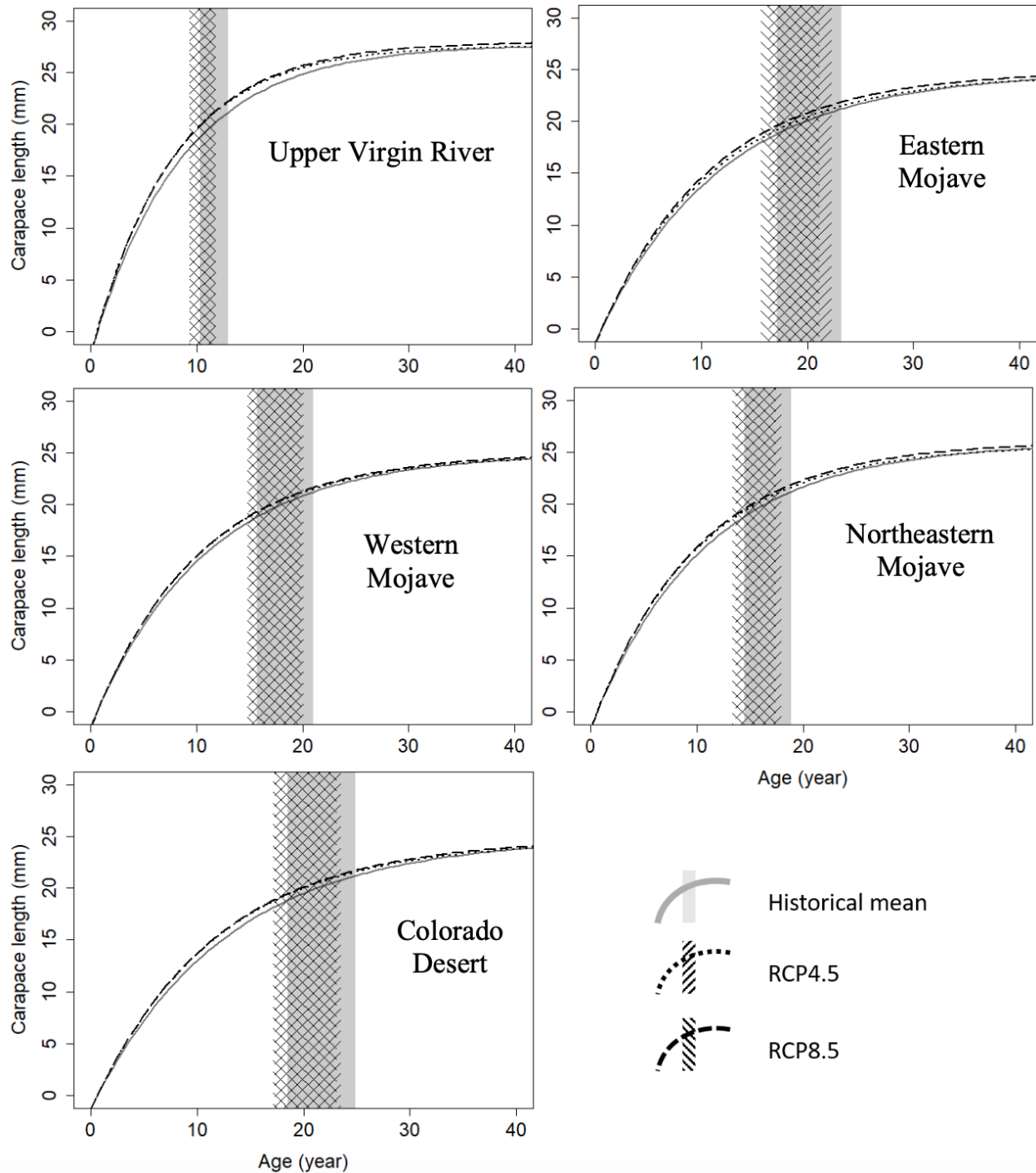


Figure 31. Predicted von Bertalanffy size-at-age curves using mean historical (1990 – 2019) and future (1950 – 1979) climate projections across the five Recovery Units of Mojave Desert Tortoise (*Gopherus agassizii*) using Bias Corrected Constructed Analogs version 2 (BCCAv2) Daily Climate Projections for two of the World Climate Research Programme’s Coupled Model Intercomparison Project phase 5 scenarios: (1) moderate-risk RCP4.5, and (2) high-risk RCP8.5. Vertical shading indicates the age at which an individual female would be likely to reach the minimum size for reproduction (18.9 – 21.2 mm MCL).

Reproductive output –

As is the case for many other reptiles, reproduction in the Desert Tortoise is seasonal, and larger females tend to produce more eggs than females with smaller body size (Turner et al. 1986, Mueller et al. 1998). However, body size typically only explains a small proportion of variation in reproductive output, and thus other exogenous factors, such as climate, also drive reproduction for this species (Wallis et al. 1999). More details of our analysis of the effects of climate and individual attributes on Desert Tortoise reproduction can be found in Mitchell et al. (2021).

Monthly climate data of mean daily maximum temperature (Tmax), mean daily minimum temperature (Tmin), and total precipitation (mm), were obtained from the PRISM database (PRISM Climate Group 2021; 800 m resolution). The monthly temperature range (MTR) was derived by subtracting Tmin from Tmax. Daily temperatures were obtained from Daymet (Thornton et al. 2021; 1km² resolution). We used Daymet data to determine the initiation of the spring activity period (activity– temperature threshold), which we defined as the first calendar day on which the maximum daily temperature reached or exceeded 25°C for 5 consecutive days (based on Zimmerman et al. 1994, which indicated minimal tortoise activity below 25°C).

We used moving window analyses to determine optimal time windows for modeling egg production (‘climate windows’; implemented in the ‘climwin’ package for R; Bailey & van de Pol 2016, van de Pol et al. 2016). For each climate variable (Tmax, MTR, and precipitation) and for each reproductive process (reproductive probability [RP], egg production [EP]; see below), we assessed all possible climate windows in the 18 mo period leading up to the 1 May initiation of the nesting period in 1 mo increments (570 models: 3 climate variables × 190 climate windows). We used logistic regression to model RP and Poisson regression to model EP (using total annual egg counts for all females that produced ≥1 clutch). The ‘baseline’ models for both reproductive processes (upon which climate variables were subsequently added) included female body size (MCL). We compared all models to the baseline (MCL-only) model using Akaike’s information criterion corrected for small sample size (AICc). To guard against inflated Type I error due to multiple comparisons, we used a permutation test (100 randomizations) implemented in ‘climwin’ that iteratively re-ran the climate window analysis with randomized dates (Bailey & van de Pol 2016, van de Pol et al. 2016). The top model (‘optimal’ climate window) was then compared to the null distribution of ΔAICc scores to calculate the likelihood that its ΔAICc score occurred by chance ($P\Delta\text{AICc}$). We only interpreted climate covariates with $P\Delta\text{AICc} < 0.05$.

We fit our final egg production model using a Bayesian hierarchical model consisting of an RP process model and an EP process model (**Table 12**). In this framework, individuals either reproduce or not each year (with probability determined on the basis of the RP model), and the total number of eggs produced each year by each reproductive tortoise is determined on the basis of the EP model. We modeled RP as a Bernoulli process and EP as a Poisson process, analogous to a ‘zero inflated Poisson’ model (Zuur et al. 2009). We rescaled all continuous covariates (MCL, climate variables; see **Table 12**). to have a mean of 0 and standard deviation of 1.

The expected probability of reproducing for each individual each year was modeled as a logit-linear function of the observed reproductive status of individuals in the year prior, MCL, cumulative prior-year precipitation, and mean MTR from March–April of the current year. Mean

egg production for each tortoise in each year (conditional upon being reproductive) was modeled as a log-linear function of MCL, cumulative precipitation, and the activity–temperature threshold.

To assess model predictive performance, we ran a 10-fold cross-validation procedure in which one-tenth of individual tortoises were held out from the model fitting process in turn and the observed number of eggs was compared with the predicted number of eggs for all held-out observations. We used area under the curve (AUC; for the RP process) and R2 (for the EP process) metrics to summarize model predictive performance across all holdout samples. Predictive performance for the EP process was only evaluated for observations with ≥ 1 egg produced. Predictive maps of annual egg production potential were calculated using an 800×800 m grid matching the PRISM data grid over the range of Desert Tortoise habitat (Carter et al. 2020) using parameters from the best model and raster products of all input data spanning the study area.

Table 12. Climate and individual-level covariates used to model reproductive probability (RP) and egg production (EP) for Agassiz’s Desert Tortoise *Gopherus agassizii*.

Covariate	Description	Source
RP process		
Reproductive status (rs_{ij-1} , latent variable)	Yes/No nesting observed in year prior; recorded annually for each tortoise ^a	See Table 1
Cumulative precipitation (cp_{ij})	Cumulative precipitation over the 13 mo (March–March) prior to the nesting season	PRISM Climate Group (2019); 800 m
Midline carapace length (mcl_{ij})	The length of the carapace measured annually by caliper for each tortoise (mm)	See Table 1
Monthly temperature range (mtr_{ij})	The average monthly temperature range (mean daily maximum – mean daily minimum) of the 2 mo leading to the nesting season (March–April)	PRISM Climate Group (2019); 800 m
EP process		
Cumulative precipitation (cp_{ij})	Cumulative precipitation over the 13 mo (March–March) prior to the nesting season	PRISM Climate Group (2019); 800 m
Midline carapace length (mcl_{ij})	The length of the carapace measured annually by caliper for each tortoise (mm)	See Table 1
Activity-temperature threshold (att_{ij})	Calendar day of first 5 d period where the maximum temperature is above 25°C	Daymet: Thornton et al. (2018); 1 km
^a Instances of unknown prior-year reproductive status were estimated as latent parameters in our model		

Both cumulative precipitation and MTR had meaningful effects on the RP and EP processes, while Tmax had no meaningful effect on either. However, the coefficient on MTR in the EP model was not significant and was therefore excluded from our final Bayesian predictive model in favor of the Daymet activity–temperature threshold variable. The optimal climate windows which were incorporated into our final model were cumulative precipitation from 14–2 mo before 1 May (13 mo; March–March) of the nesting year and monthly temperature range from 2–1 mo before 1 May (March–April) of the nesting year (**Table 12**).

Table 13. Parameter estimates for the reproductive probability (RP) and egg production (EP) processes, with corresponding Bayesian credible intervals (95% highest posterior density).

Parameters	Mean	Lower CI	Upper CI
RP process			
<i>bpR</i> : Baseline probability of reproducing	0.66	0.56	0.77
β_1 : Reproductive status (year prior)	1.46	0.67	2.24
β_2 : Cumulative precipitation	0.62	0.24	1.05
β_3 : Midline carapace length	0.34	0.07	0.64
β_4 : Monthly temperature range	-0.41	-0.80	-0.02
EP process			
<i>BE</i> : Baseline number of eggs	6.50	6.24	6.74
β_5 : Cumulative precipitation	0.15	0.11	0.19
β_6 : Midline carapace length	0.12	0.08	0.15
β_7 : Activity-temperature threshold	-0.07	-0.12	-0.03

The fitted Bayesian hierarchical model confirmed the importance of all factors included in the linear model, as none of the 95% credible intervals for regression coefficients overlapped zero (**Table 13**). The coefficient estimates indicated positive effects for cumulative precipitation and MCL on both the RP and EP processes, a negative effect of MTR on the RP process, and a negative effect of the calendar day of first activity (activity-temperature threshold) on the EP process (**Table 13**).

The intercept of the RP model was estimated at 66% and interpreted as the baseline probability of reproducing for an average female in an average year. Cumulative precipitation had the highest magnitude coefficient value in the RP process ($\beta = 0.62$), resulting in the expected probability of reproducing increasing from 0.76–0.99 as cumulative precipitation increased from 30.2–637.1 mm (**Figure 32**). March–April MTR ($\beta = -0.41$) also had a significant effect on the RP process, resulting in expected probability of reproduction decreasing from 0.89–0.66 as temperature range increased from 9.6–19.2°C. For the EP process, the baseline number of eggs was estimated as 6.5 eggs per average female in an average year. Cumulative precipitation ($\beta = 0.15$) had the strongest influence on the EP process, with the mean number of eggs expected to increase from 5–13 eggs with an increase from 30.2–637.1 mm precipitation. The activity-temperature threshold ($\beta = -0.07$) had a weak negative effect on the EP process, with egg production expected to decrease from 8–5 eggs as the date of reaching the activity-temperature threshold shifts from late January to mid-June (**Figure 32**).

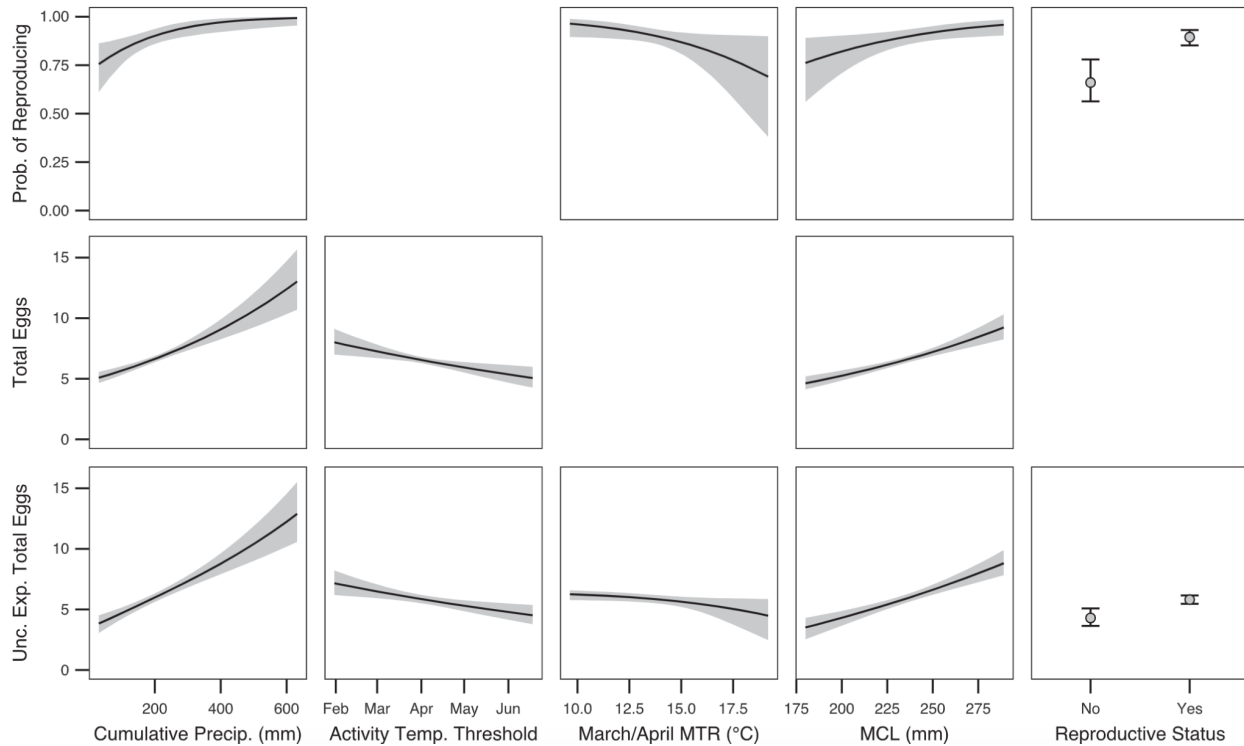


Figure 32. Partial dependence plots illustrating fitted relationships between reproductive output of Agassiz's Desert Tortoise *Gopherus agassizii* and covariates representing climate and individual-level attributes. Top row: expected probability of reproducing; middle row: total egg production conditional on reproducing; bottom row: expected unconditional egg production (accounting for both of the above processes). Predicted means (solid lines or circles) and 95% credible intervals (gray regions or error bars) were derived from a fitted Bayesian model of annual egg production. MTR: monthly temperature range; MCL: midline carapace length.

From 10-fold cross-validation (leaving out entire individuals and thereby eliminating any information about prior reproductive status), we showed that model performance was generally weak for predicting reproductive status (AUC = 0.67; 95% CI: 0.65–0.7). When trained to the full data set, AUC increased to 0.75 (95% CI: 0.72–0.79). For reproductive individuals, our model explained 30% of the observed variance in egg production for withheld individuals (R^2 ; 95% CI: 0.28–0.31).

Egg potential maps indicated that egg production ranges from low values (e.g. 2–5 eggs female⁻¹ yr⁻¹) in years with unfavorable climate conditions for reproductive processes to average egg production values of 6–12 eggs female⁻¹ yr⁻¹ in favorable conditions. These values differed among the 5 RUs, and among years, and some RUs benefited more from improved conditions. For example, increases in egg production relative to the lowest year were highest for the Northeastern Mojave and Upper Virgin River RUs in both moderate and high years, while increases in the more southern RUs were more modest (**Figure 33**). Further, there were spatial differences in egg production potential that remained lower even in the highest years (e.g. the southernmost portion of the distribution, as well as areas near Death Valley and the Owens Valley of California) (**Figure 33**—High). Annual variation in egg production potential indicates a generally declining trend over time ($p = 0.02$), with the notable exception of a single

year (2004) in which reproductive potential was higher in all RUs (**Figure 33**). Annual climate-driven variation in expected egg production was highly correlated among RUs, with pairwise correlation ranging from $|r| = 0.91$ (Western Mojave vs. Upper Virgin River) to $|r| = 0.97$ (Eastern Mojave vs. Western Mojave).

We found that precipitation and temperature at several different temporal scales influence reproductive potential for Agassiz's Desert Tortoise, both in the ability to produce eggs in a given year and in the total number of eggs laid. Our results underscore the combined influence of body size (Wallis et al. 1999) and local environmental conditions on reproductive output in this species. This information may be increasingly important to management for this species when accounting for future climate change, as arid areas — and in particular the desert southwest — are expected to be highly influenced by climate change and to show a greater rate of temperature increase than other regions (Seager et al. 2007, Archer & Predick 2008). Precipitation will likely be influenced as well. In the last century, some areas of the Mojave Desert have already seen a 20% reduction in precipitation (Guida et al. 2019); however, some future climate projections disagree about whether precipitation will increase or decrease for this region (Bachelet et al. 2016). Indeed, increasing aridity may already be affecting Desert Tortoises; over the modeled timeframe (1990–2018) our models predict a gradual but significant decline in range-wide egg production potential.

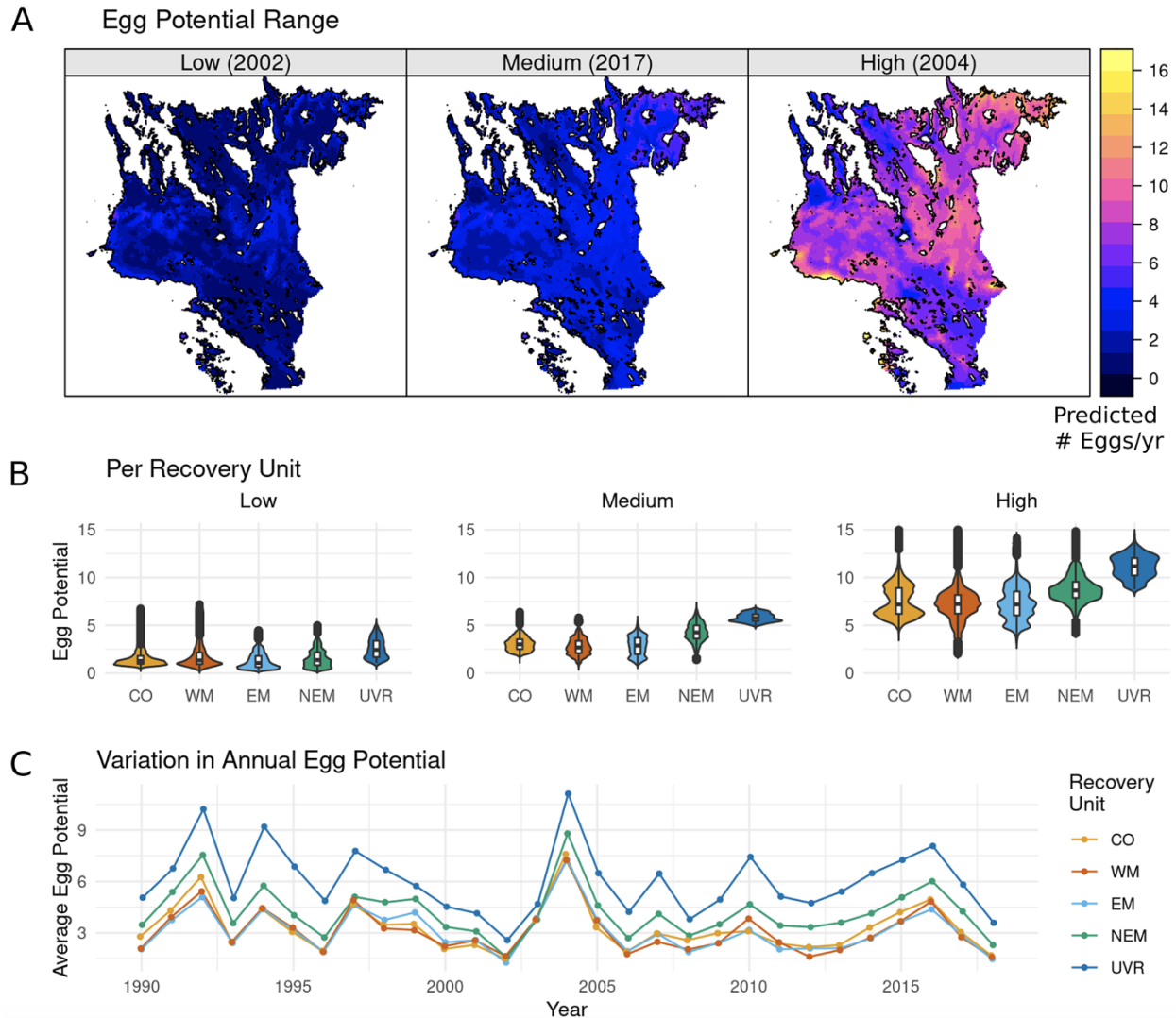


Figure 33. Range-wide spatiotemporal projections of annual egg production for Agassiz's Desert Tortoise *Gopherus agassizii*. (A) Maps of expected egg potential for the years with the lowest, average ('medium'), and highest mean annual predicted egg potential (range-wide) for 1990–2018. (B) Mean annual predicted egg potential by Recovery Unit (CO: Colorado Desert; WM: Western Mojave; EM: Eastern Mojave; NEM: Northeastern Mojave; UVR: Upper Virgin River) for the same years depicted in (A), where the 25th, 50th, and 75th quartiles are given by the white bar within the violin plot representing the range of the data. (C) Averaged annual egg potential plotted by year for the 5 Recovery Units. Reproduced from Mitchell et al. (2021)

Sex ratios –

For 103 hatchlings from 25 field nests, we determined sex using the method described above based on blood samples drawn from the subcarapacial sinus. For 17 of these nests with sex ratio data, we obtained complete temperature datasets during the critical period, estimated as the time between 25% and 50% of the total incubation duration (i.e., the time between estimated lay date and first hatchling emergence). For *Gopherus* tortoises, this second quartile of development is

thought to be the period when embryos are sensitive to temperature; warmer temperatures during this period result in a greater proportion of hatchlings that are female (Rostal and Wibbels 2014). We calculated the mean nest temperature during the critical period for the 17 nests with sex ratio and temperature data available.

In order to link nest thermal environment to hatchling sex ratio, we used a logistic regression with probability female as the response variable and mean nest temperature during the critical period as the predictor variable. Nests were weighted by the number of hatchlings sexed to account for the fact that lower sample sizes have increased error in the estimate of the clutch's sex ratio.

We determined sex for a mean of 4.2 hatchlings per nest, resulting in a mean percent female of $58 \pm 36\%$ (SD) across the 25 nests with available data (range: 0 - 100% female). The logistic regression predicting sex indicated a strong effect of mean nest temperature during the critical period (**Table 14; Figure 34**).

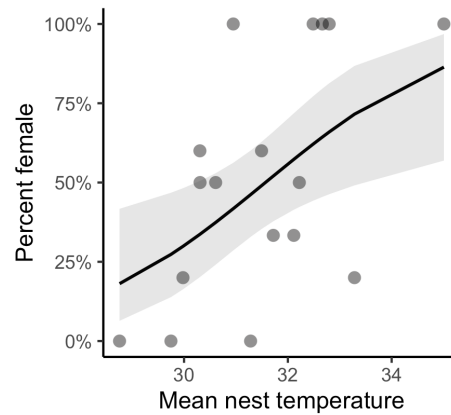


Figure 34. Mean nest temperature during the critical period strongly predicts hatchling sex ratio in wild nests of the Mojave Desert Tortoise. This effect is based on weighted logistic regression predicting the sex ratios of 17 nests from 5 sites. The critical period is defined as the second quartile (25-50%) of the incubation period (i.e. the time from the lay date to the first hatchling emergence). Nest temperature is measured in degrees C.

Table 14. Logistic regression reveals a nest temperature effect on sex ratio from 17 naturally incubated Mojave Desert Tortoise nests. Estimates and errors are on the logit scale.

Predictors	Estimate	Std. Error	95% CI	z	p
Intercept	-16.96	6.40	-30.75 – -5.33	-2.65	0.008
Temperature	0.54	0.20	0.17 – 0.98	2.63	0.008

We recover a logit-linear relationship between mean nest temperature and observed hatchling sex ratio, despite a sample size of only 17 nests. From our model, the estimated mean critical period nest temperature producing a 50:50 sex ratio is 31.6 degrees, which matches closely with the pivotal temperature estimate of 31.8 derived from constant-temperature experiments in the lab (Spotila et al. 1994). These results suggest that we can predict sex ratio in field nests with reasonable accuracy in this species; previous studies have found it difficult to accurately estimate sex ratios from field nests of a variety of turtles based on temperature profiles (Schwartzkopf and Brooks 1985; Georges 1989; Weisrock and Janzen 1999; Baxter et al. 2008). More complicated models of the thermal environment have yielded greater success at predicting sex from naturally varying nest temperatures (e.g. Valenzuela et al. 2019; Monsinjon et al. 2022), but these models require much more detailed information about the thermal environment and the developmental biology of the organism, and are more challenging to link to climate change projections. A simple and strong effect of mean nest temperature can be easily linked to predictive models of climate change by describing the relationship between mean ambient temperature and mean nest temperature, as we do in Task 3.

Hatching success –

Beyond the effect of nest temperature on hatchling sex ratios, climate change can influence turtle reproduction by reducing hatching success. Nests incubated at temperatures above a lethal threshold may produce few offspring (Valverde et al. 2010; Laloe et al. 2017), resulting in reduced recruitment. To determine the effect of nest temperature on mortality in wild nests of the Mojave Desert Tortoise, we calculated mean nest temperature and mean daily maximum nest temperature for the 23 observed nests with available temperature time series across the entire period of development (**Figure 35**; see above for methods on temperature data and nest incubation). We calculated the hatching success of each nest as the proportion of eggs escaping predation that later hatched (**Table 15**). We used logistic regression with temperature as the predictor variable and hatching success as the response, weighting nests by the number of potentially hatching eggs they contained. We compared the fit of the two predictors by comparing the AICc values of the two models.

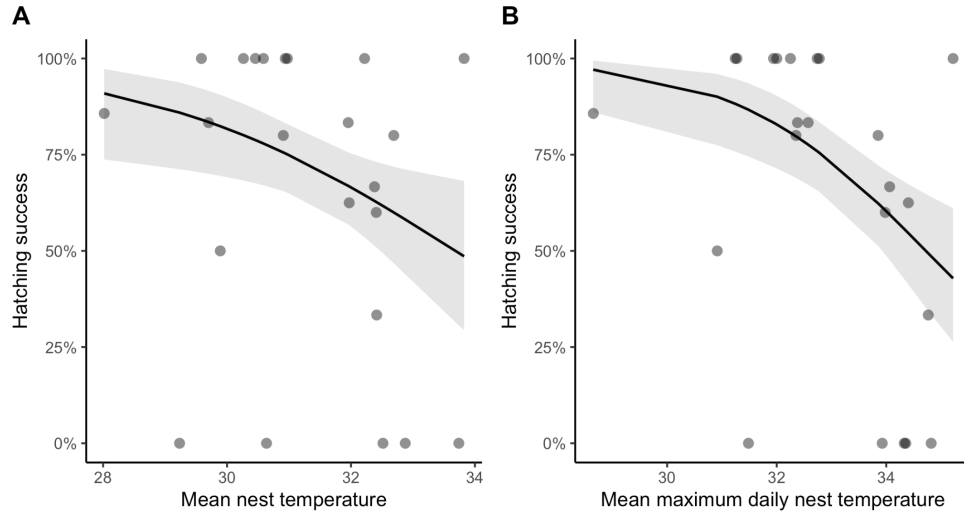


Figure 35. Hatching success declines with mean nest temperature (A) and mean daily maximum nest temperature (B) across 23 nests of the Mojave Desert Tortoise.

Table 15. Logistic regression models predicting hatching success from mean daily maximum nest temperature and mean nest temperature from 23 Mojave Desert Tortoise nests. Estimates and errors are on the logit scale.

Predictors	Estimate	Std. Error	95% CI	z	p	$\Delta AICc$
Intercept	20.09	5.82	9.49 – 32.41	3.45	0.001	
Mean daily max nest T	-0.58	0.17	-0.95 – -0.26	-3.33	0.001	0
Intercept	13.70	5.30	3.86 – 24.82	2.58	0.010	
Mean nest T	-0.41	0.17	-0.76 – -0.10	-2.43	0.015	7.58

Both mean nest temperature and mean daily maximum nest temperature predict hatching success, with daily maximum temperature resulting in a substantially better fit, as indicated by a lower AICc score (**Table 15**). This suggests that temperature extremes, rather than elevated mean temperature, may be responsible for the reduced hatching success observed at higher temperatures. The fact that we observe these trends in wild, naturally occurring nests indicates that Desert Tortoise populations are currently experiencing nest mortality due to extreme temperatures even absent any additional temperature increase due to climate change.

GOPHER TORTOISE

Survival, recruitment and abundance --

We used a Bayesian, open population Jolly-Seber model to estimate 3 population parameters of interest from the Fort Stewart Gopher Tortoise CMR data: a combined probability of emigration and survival, a combined rate of immigration and recruitment (recruitment into the adult size class that could be captured by our survey technique), and adult abundance. More details of this model are described by Hunter and Rostal (2021).

Prescribed burning is an important habitat management tool of the pine savannas that Gopher Tortoises inhabit, and climate change is likely to make prescribed burning more challenging to implement (Kupfer et al. 2020). The effects of burning on long-term tortoise demography have never been described, so we first investigated the effects of prescribed burning on adult demographics (Hunter and Rostal 2021). Prescribed burning at FSAR occurs on a 1–5-year rotation (**Figure 36**). Burns almost never covered an entire site, so we created a spatially averaged variable (years-since-burn) to account for heterogeneity in burn age.

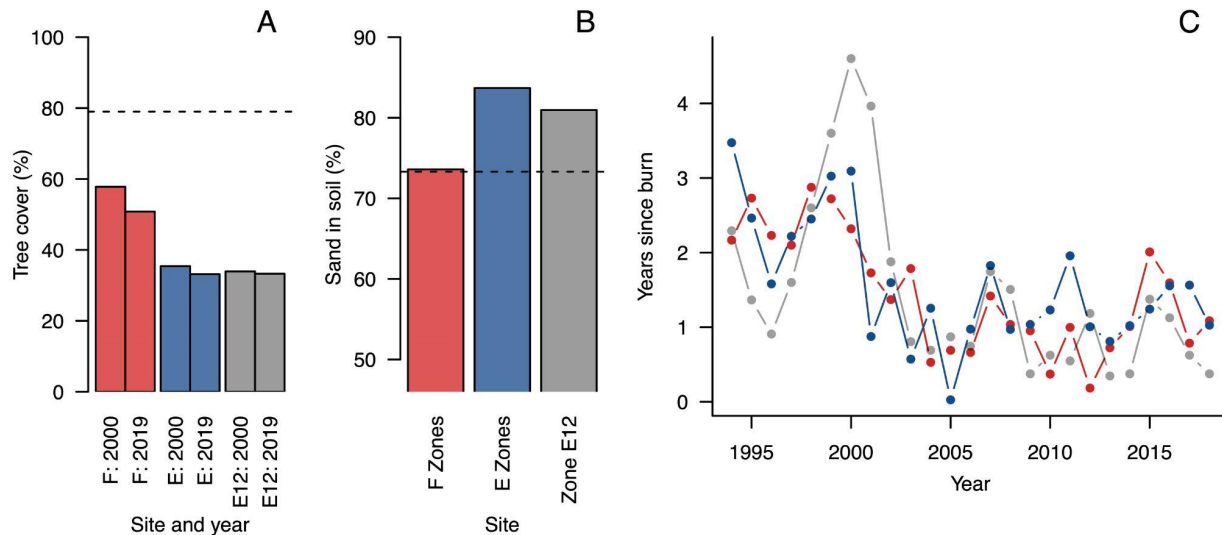


Figure 36. *Gopher Tortoise habitat component comparisons. A) Percent tree cover in 2000 and 2019 (Hansen et al. 2013), B) percent sand in soil (Hengl et al. 2017), and C) years since burn among 3 sites (F zones: red; E zones: blue; Zone E12: gray) at Fort Stewart Army Reserve (FSAR), Georgia, USA. In A and B, y-axis ranges indicate the minimum and maximum values observed at FSAR over the study period 1994–2020, and dashed horizontal lines indicate mean values for FSAR.*

We modeled emigration and survival (combined and called “probability of staying”) as a logit-linear function of sex, carapace length, and years-since-burn. We derived the population size (N) and number of immigrants and recruits for each site-year combination (Royle and Dorazio 2008). We then divided the number of immigrants and recruits by N for each site-year to produce a per capita immigration and recruitment rate (i.e., per capita inflow). We calculated the slopes and intercepts of the linear relationship of N and per capita inflow regressed on years-since-burn as additional derived parameters.

Our results demonstrate that Gopher Tortoise population dynamics have complex responses to prescribed burning. We observed opposing responses of tortoise populations to years since burn at 2 sites that are ≤ 20 km of each other and have experienced similar burning regimes in the last several decades (**Figure 36C**). Adult Gopher Tortoise abundance and ‘probability of staying’ increased within the F zones when it had been more recently burned, but abundance and probability of staying in the E zones increased when it had been longer since the site was burned (**Figure 37**). It is likely that differences in landscape features (other than burning regime) between the sites contributed to whether or not burning had an effect on tortoise population dynamics. The F zones had substantially greater tree cover and lower sand contribution to soil composition (**Figure 36B**), which are variables that other studies have reported to be influential for Gopher Tortoise demographic rates (Rostal and Jones 2002, Baskaran et al. 2006, Lamb et al. 2013, Howell et al. 2020). In essence, the conditions are likely overall worse for Gopher Tortoises in the F zones, and so more frequent prescribed burning improves conditions and increases probability of staying and overall abundance. In the E zones, where conditions are better with lower canopy cover and higher sand composition, less frequent burning results in better tortoise habitat. The vegetation and conditions in the F zones are likely more representative of Gopher Tortoise habitat throughout the species range (the intensive management that occurs at Fort Stewart and produces the good habitat in the E zones is not replicated on state or privately managed lands). Therefore, we used the relationship between probability of staying and years-since-burn for the F zones in our model of critical habitat breadth (Task 3).

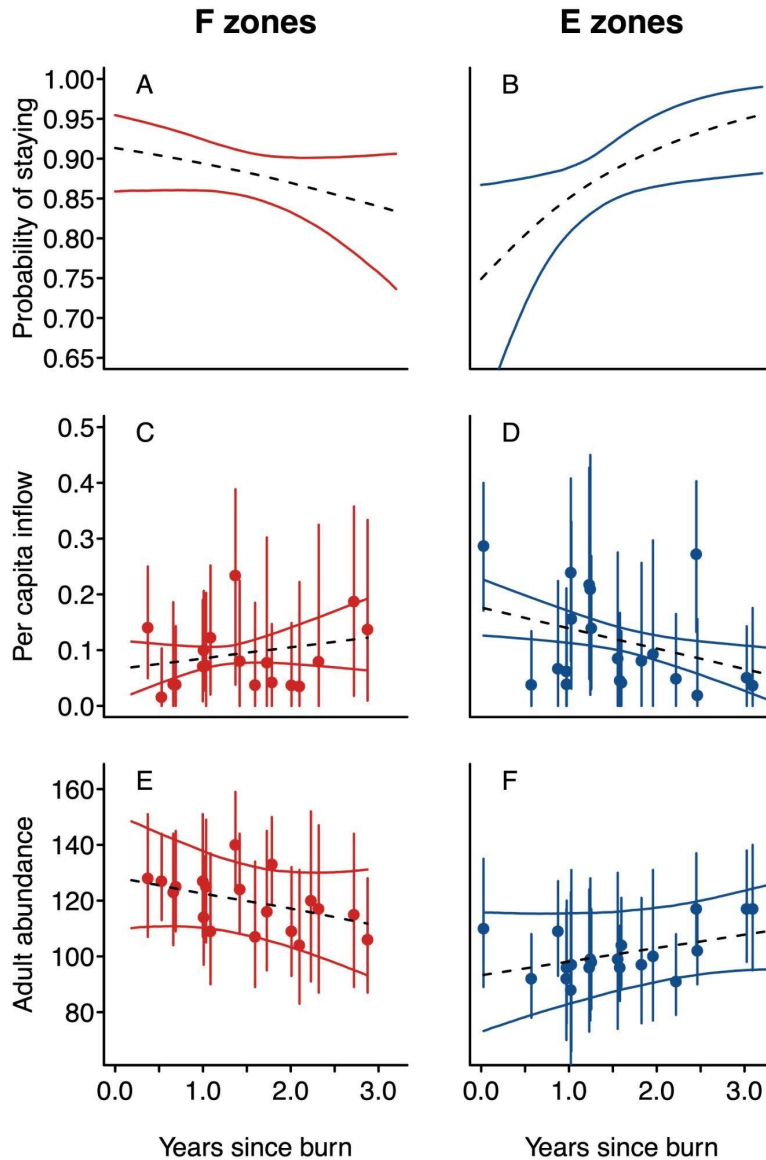


Figure 37. Gopher Tortoise population parameters (emigration and survival [A, B], immigration and recruitment [C, D], and adult abundance [E, F]) in response to prescribed burning (mean years since burn for a site) at 2 sites (F zones [A, C, E] and E zones [B, D, F]) at Fort Stewart Army Reserve (FSAR), Georgia, USA, 1994–2020. The F zones comprise FSAR zones F11 and F12, and the E zones comprise FSAR zones E17, E20, E21, and E22. We estimated effects of burning on emigration and survival (probability of staying = survival + [1–emigration]) within a Cormack-Jolly-Seber mark-recapture model, and effects of burning on immigration and recruitment (per capita inflow) and adult abundance were derived parameters. Dashed lines represent the values predicted by the regression relationship (medians of the posterior distribution) and solid lines are 95% credible intervals for the predictions. We displayed the effects of years since burning on probability of staying for average-sized females.

After accounting for the effects of prescribed burning on adult demography, we tested for direct climate effects. We hypothesized that precipitation and cold temperatures would be the most likely to influence adult survival. Dry spells may reduce forage availability, or particularly wet years may make diseases more prevalent (particularly shell fungal diseases). Cold winters could cause direct mortality. We used Daymet data to calculate these climate variables and then created linear models of the residuals of the “probability of staying” variable (after accounting for the effect of years-since-burn). We did not find any direct effects of climate on Gopher Tortoise survival.

Reproductive output –

We used ultrasound and X-ray images taken during long-term CMR surveys at FSAR to determine the probability of reproduction and clutch size for Gopher Tortoises (see Hunter et al. 2021 for additional information on these data and analysis).

We used daily climate data from the Daymet climate database (Thornton et al. 2018; 1 km² resolution): daily maximum temperature (Tmax), daily total precipitation, and daily temperature range (DTR; daily maximum minus daily minimum temperature). We did not include daily minimum temperature in the group of climate variables as it was highly correlated with maximum temperature ($r = 0.92$), and the 3 remaining variables were not correlated ($|r| < 0.4$). We averaged these daily climate variables to a monthly scale for use in analyses.

We used sliding window analyses of several climate variables (taking the mean of each variable within each climate window) to determine the optimal time windows for estimating effects on Gopher Tortoise reproduction (‘climate windows’; implemented in the ‘climwin’ package for R; Bailey & van de Pol 2016, van de Pol et al. 2016). For each climate variable (Tmax, DTR, and precipitation) and for each reproductive process (probability of reproduction, clutch size; see next paragraph), we assessed all possible climate windows (ranging in length from 1 to 24 mo) in the 24 mo period leading up to the 1 May initiation of the nesting season (**Figure 38**) in 1 mo increments (900 models: 3 climate variables $\times$ 300 climate windows). We tested for linear effects of all climate variables and windows on probability of reproduction (logistic regressions; see next paragraph) and clutch size (Poisson regressions; see below).

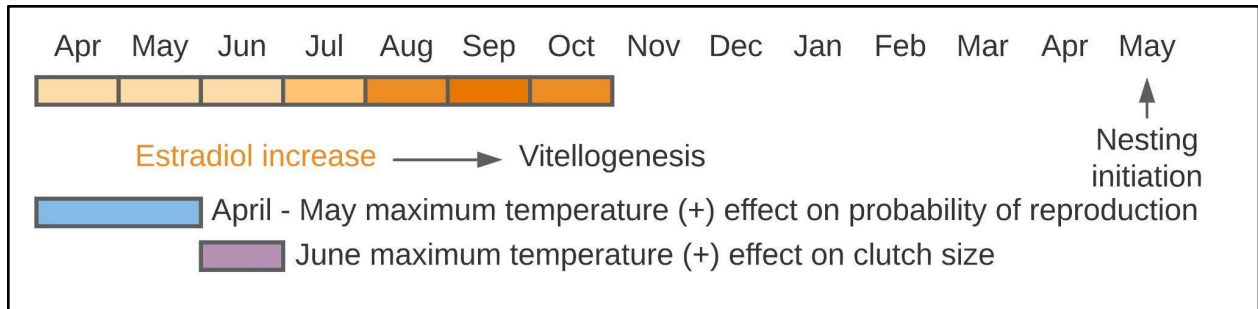


Figure 38. Reproductive timeline for female Gopher Tortoises *Gopherus polyphemus* at Fort Stewart Army Reserve, Georgia, USA. Estradiol production begins to increase in July, with a peak in September (orange; following data from Ott et al. 2000, Rostal 2014), which leads to the process of follicle development (vitellogenesis) for eggs that will be laid in the following May and June. Our results (data spanning 1994–2019) indicate that the probability of reproduction for a female is positively influenced by the maximum temperature in April–May prior to vitellogenesis (blue), and clutch size is positively influenced by the maximum temperature in June prior to vitellogenesis (purple)

We used logistic regressions to model probability of reproduction where females that were captured with either follicles (only detected with ultrasound) or eggs (detected with both ultrasound and X-ray) were designated as reproducing within a year. We used Poisson regressions to model clutch size (only for females that had shelled eggs and could be X-rayed at the time of capture). The ‘baseline’ models for both the probability of reproduction and clutch size processes (to which climate variables were subsequently added) included female body size (SCL). For the probability of reproduction, date (days since 1 January) was also included to account for the sampling design: tortoises were caught throughout the nesting season, so females that were caught late in the season could have already laid their eggs. Including the date variable accounted for this potential bias, and we predicted that date would have a negative effect on probability of reproduction.

We compared all models to the baseline (SCL and date only) model using Akaike’s information criterion corrected for small sample size (AICc). To avoid inflated type I error due to multiple comparisons, we used the built-in permutation test (100 randomizations) in ‘climwin’ that iteratively repeated the climate window analysis with randomized dates (Bailey & van de Pol 2016, van de Pol et al. 2016). The top model (‘optimal’ climate window) was then compared to the null distribution of the ΔAICc score to calculate the likelihood that its ΔAICc score occurred by chance ($P\Delta\text{AICc}$). We only interpreted climate covariates with $P\Delta\text{AICc} < 0.05$.

After identifying meaningful climate variables, we combined both reproductive processes together in a single Bayesian hierarchical model to account for the linkage between probability of reproduction and clutch size (i.e. only reproductive females can produce a clutch). Modeling the 2 reproductive processes jointly in a Bayesian framework had the benefit of accounting for missing data (e.g. when reproduction was only detected via ultrasound visualization of unshelled follicles so clutch size data was missing for that individual), and so provided more robust estimates of the effects of climate on reproduction than the separate ‘climwin’ analyses. Thus, the Bayesian hierarchical model tested whether the effects identified in the ‘climwin’ variable selection process would be verified when the structure of the data was more properly modeled.

Models were fitted in a Bayesian framework using JAGS software, which was called from the R environment. Initial analyses indicate that probability of reproduction and clutch size are not sensitive to management (prescribed burning), in contrast to rates of emigration and immigration, and so prescribed burning was not included in reproduction models.

To assess the predictive performance of the model, we ran a cross-validation procedure in which, for each of 10 iterations, one-tenth of individual tortoises were withheld from the model fitting process. Each resulting model was used to predict both the probability of reproduction and the clutch size produced by the withheld tortoises, and these predictions were compared to the observed reproduction and clutch size for those individuals. We used the area under the curve (AUC; for probability of reproduction process) and R^2 (for clutch size process) metrics to summarize model predictive performance across all withheld samples. Predictive performance for the clutch size process was only evaluated for observations with ≥ 1 egg produced.

The maximum temperature of April–May prior to the initiation of a nesting season (13–12 mo prior to 1 May) had a positive effect on probability of reproduction ($P\Delta AICc = 0.05$). Maximum temperature of the previous June (11 mo prior to 1 May) had a positive effect on clutch size ($P\Delta AICc < 0.001$). Precipitation and DTR did not influence either reproduction response variable.

In the Bayesian hierarchical model, the baseline probability of reproduction for an average-sized female caught on the earliest possible date, 10 April (i.e. with no chance of missing a clutch already laid), in a year with average climate conditions was 97.4% (95% CI: 94.7–98.8%, intercept of **Figure 39A**). The expected clutch size for an average-sized reproducing female in an average temperature year was 6.5 eggs. As predicted, probability of reproduction decreased substantially with date of capture, likely because some females, when caught later in a nesting season, had already laid their eggs. Female body size had positive effects on both probability of reproduction and clutch size, although the effect on clutch size was much greater (**Figure 39**).

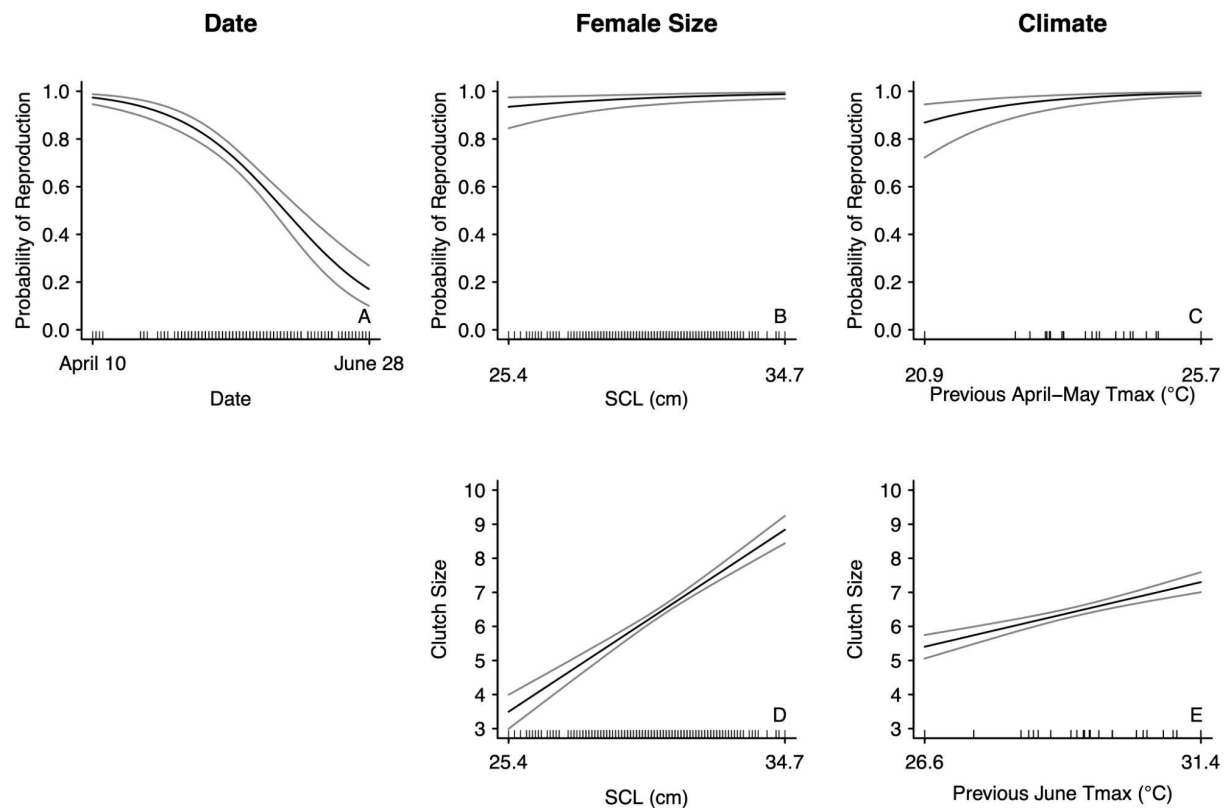


Figure 39. Effects on female Gopher Tortoise *Gopherus polyphemus* (A–C) probability of reproduction and (D,E) clutch size at Fort Stewart Army Reserve, Georgia, USA, studied from 1994 to 2019, estimated using a Bayesian hierarchical mixture model. See Section 2.4 for details on variable selection for each reproductive process. Black lines are medians and gray lines are 95% credible intervals for predictions from the model over the observed values for each variable while holding other variables at their mean value (straight carapace length [SCL] = 30.5 cm, April–May maximum temperature [Tmax] = 23.7°C, June Tmax = 29.4°C), except for date, which was held at its earliest value (10 April) to remove the confounding effect of date on detection of reproduction.

The Bayesian hierarchical model supported the inclusion of the prior April–May maximum temperature effect on the probability of reproduction and the prior June maximum temperature effect on clutch size, as these effects had 95% CIs that did not overlap zero. April–May mean daily maximum temperatures ranged between 20.9 and 25.7°C for the study period and had a positive effect on probability of reproduction, which ranged from 86.9% (95% CI: 72.5–94.5%) at the lowest temperatures to 99.2% (95% CI: 98.1–99.7%) at the highest temperatures for average-sized females (with date set to the earliest possible capture date, 10 April). June mean daily maximum temperatures had a positive effect on clutch size, although body size had a greater effect on clutch size than temperature within the observed range of variability (**Figure 39**). Average June maximum temperatures ranged between 26.6 and 31.4°C, and clutch sizes ranged from 5.4 eggs (95% CI: 5.1–5.8 eggs) at the lowest temperatures to 7.3 eggs (95% CI:

7.0–7.6 eggs) at the highest temperatures for average-sized females. Both climate effects occurred in the summer prior to a focal nesting season, which matches with the initiation of increases of estradiol in late summer (**Figure 38**). From 10-fold cross-validation, the model had a fair performance in predicting the probability of reproduction for an individual (AUC = 0.74; 95% CI: 0.73–0.75). For reproductive individuals, our model explained a high proportion of observed variance in clutch size, with $R^2 = 0.76$ (95% CI: 0.75–0.77).

Both climate variables significantly increased in magnitude over the 27 yr of the study period (**Figure 40**). April–May maximum temperatures increased by 1.6°C ($p = 0.01$), and June maximum temperatures increased by 2.5°C ($p < 0.01$). This rate of change was greater than for temperatures in other months of the year (**Figure 40F**). Observed clutch size significantly changed over the study period, increasing by 1 egg from 1994 to 2019 ($p = 0.03$, **Figure 40B**), but body size (SCL) of reproducing females did not change ($p = 0.45$, **Figure 40C**).

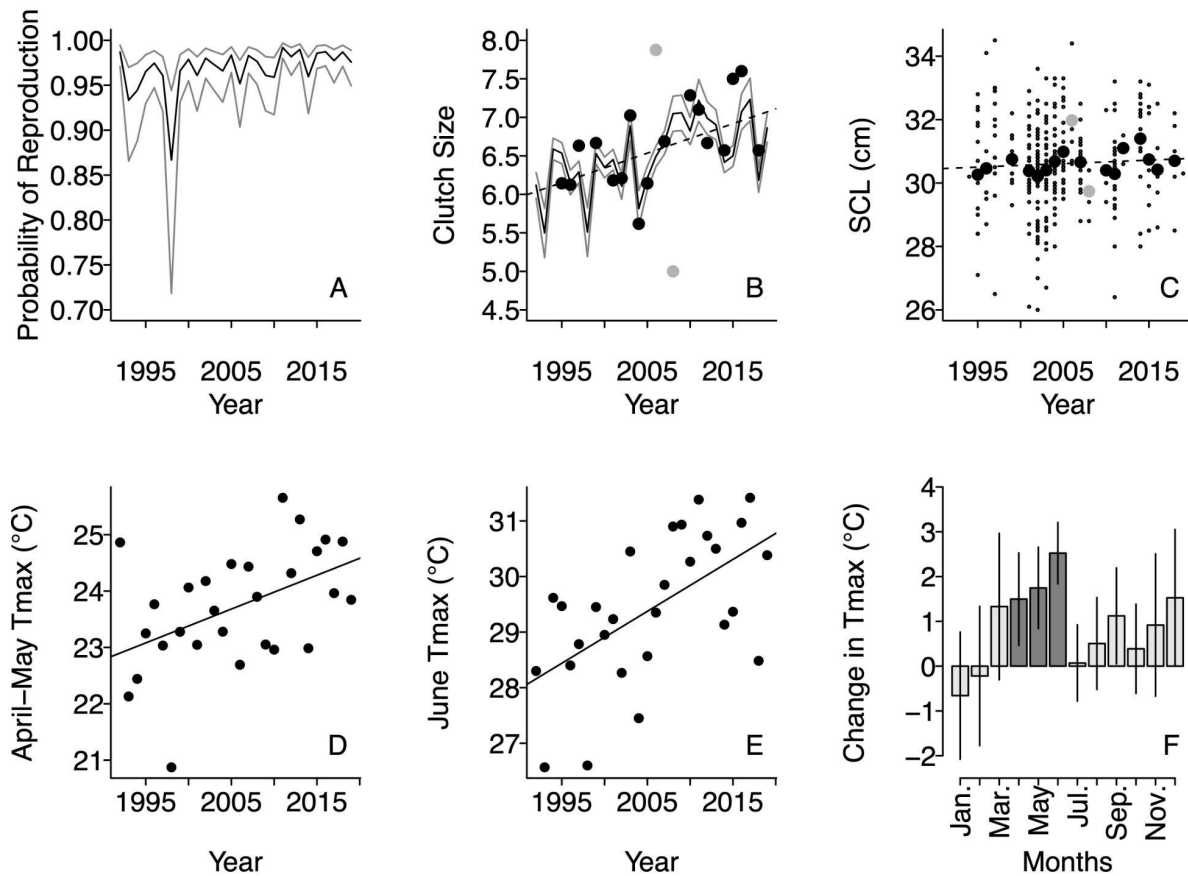


Figure 40. Change in Gopher Tortoise *Gopherus polyphemus* (A–C) reproduction and body size and (D–F) climate variables over time at Fort Stewart Army Reserve, Georgia, USA. In (A) and (B), solid black lines indicate the median prediction of a Bayesian hierarchical mixture model of reproduction, and gray solid lines are 95% credible intervals for predictions from the model over the observed values of the climate variable for each year (A: April–May maximum temperature [Tmax]; B: June Tmax) while holding straight carapace length (SCL) at its mean value (30.5 cm), and date at its earliest value (10 April) to remove the confounding effect of date on detection of reproduction. In (B) and (C), large points are means of clutch size (B) and SCL

of re-producing females (C) for each year (gray points are outliers due to small sample sizes and biased female SCL in those years; small open circles in C are raw data points of reproducing female SCLs), with dashed lines indicating the slope of linear models of clutch size or reproducing female SCL over time (an analysis of change in probability of reproduction over time could not be conducted because of the confounding effect of date of ultrasound). April–May (D) and June (E) Tmax have been significantly increasing over the study period (solid lines). Bars in (F) are estimates of the change in Tmax for each calendar month over the time period 1992–2019 (intervals are standard errors); dark gray highlighted bars are months in which temperature has an effect on Gopher Tortoise reproduction (April–June)

We found strong effects of climatic variability on the probability of reproduction and clutch size of Gopher Tortoises: the prior April–May maximum temperature positively affected probability of reproduction and the prior June maximum temperature positively affected clutch size. The timing of these temperature effects immediately precedes production of estradiol that begins to elevate in July and peaks in September, leading to vitellogenesis (**Figure 38**; Ott et al. 2000, Rostal 2014). This timeline (a temperature effect on the probability of reproduction followed by a temperature effect on clutch size, then initiation of estradiol production) suggests that temperature plays a role in the process of whether to initiate reproduction and how much to invest in it. Detection of this pattern was only possible with a long-term dataset that sampled a wide range of climatic variability at FSAR coupled with a climate window analysis (van de Pol et al. 2016) to discover the timing of effects without a priori knowledge of the exact window of the temperature effect.

Over the 25 yr study period, mean clutch sizes of reproducing females have increased by approximately 1 egg, a 15% increase based on the mean clutch size of 6.5 eggs. Body size of the population of reproducing females has not changed over the study period, indicating that the change in clutch size is likely due to the changing climate influencing reproduction directly, rather than via an effect on adult size (Fig. 4). Without the climate window analysis, we would not have been able to make this connection, as yearly average temperatures are not warming as rapidly as spring and summer temperatures (due to mostly stable fall and winter temperatures, **Figure 40F**). Warming leading to a reproductive boost has been documented in other populations at the ‘leading edge’ (or northern edge for northern hemisphere species) of species’ ranges (Gaston et al. 2005, Dangremond & Feller 2016). Gopher Tortoise clutch sizes increase linearly with decreasing latitude across populations (Ashton et al. 2007), and thus spring and early summer warming in northern populations may result in tortoises becoming more ‘southern’ in their reproductive patterns. It is likely that the northern range limit for Gopher Tortoises is determined, in part, by temperature – further north, colder conditions may not allow for sufficient incubation temperatures for the duration of nest development (Rostal & Wibbels 2014; **Figure 38**). Thus, warming conditions that increase reproduction could not only bolster northern edge populations but also allow for range movement northward.

Hatching success –

We developed structural equation models (SEMs) to estimate the direct and indirect effects of nest characteristics (including nest depth, distance from burrow mouth, and canopy cover) on Gopher Tortoise hatching success (Grace et al. 2012). The SEMs used a hierarchical approach to estimate the overall effects of nest characteristics on hatching success by accounting for all

potential pathways of effects. We hypothesized that nest temperature (both mean and variability), egg mass, and clutch size would have direct effects on hatching success, whereas nest characteristics that are consequences of female nest site selection would have indirect effects on HS (through effects on temperature). However, we also tested for direct effects of nest characteristics on hatching success in case nest characteristics affect another intermediate variable that we did not measure (such as nest moisture or soil type). (We attempted to measure nest moisture levels with humidity iButtons, but they all failed in the high moisture environment of Gopher Tortoise nests).

We developed SEMs for each site at which we collected Gopher Tortoise nest data across the latitudinal gradient of the species range (excluding the translocation site, where we modeled hatching success differently to test for local adaptation, see Task 2; we also excluded George L. Smith State Park as there were too few nests at the site to create an SEM). We created site-specific models because we wanted to determine whether hatching success responded to nest characteristics and nest temperatures similarly across the range. We also pooled all data together to build an all-site model.

For Gopher Tortoise nests at the latitudinal sites, we rarely knew the exact (or even approximate) lay date because nests were found by searching all known burrow locations (not through tracking females). Therefore, we did not have full temperature datasets for nests as iButtons were placed in nests when they were found, not when they were laid. To account for this, we clipped all temperature datasets at a site to use a common set of dates so that we were comparing differences in nest temperatures within a site. We then calculated mean nest temperature and the standard deviation of nest temperature for that time period for all nests at a site. For nests that had longer temperature time series, we compared the means for the clipped time period and the full time period, and found consistently high correlation between these values, indicating that the clipping of the temperature time series to a common set of dates had little influence on these summary statistics (i.e., hotter nests are always hotter, no matter the time period over which temperature is measured).

We built the SEM in a Bayesian framework using JAGS (Plummer 2015), run in the R version 3.5 environment (R Core Team 2022). The observed number of hatchlings from each nest was binomially distributed so that for the i th nest:

$$h_i \sim \text{Bin}(p_i, N_{\text{total}_i})$$

where h_i is the number of hatchlings from nest i , p_i is the probability of hatching success, and N_{total_i} is the total number of hatched and unhatched eggs at the end of incubation. We modeled the direct effects of mean nest temperature, variability of nest temperature, clutch size, egg mass, and nest characteristics on p_i as logit-linear effects. The temperature variables were modeled as having quadratic effects to allow for discovery of optimal temperature conditions for hatching success. For the temperature variables, we also modeled the indirect effects of nesting decisions as linear effects of nest characteristics.

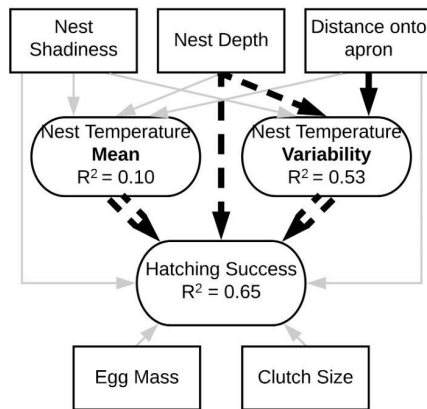
All covariates were standardized using z -score scaling so that estimated effects could be directly compared for relative importance. We summarized posterior distributions for all parameters with

the mean of all MCMC samples as a point estimate and the 2.5 and 97.5 percentiles of the MCMC samples as a 95% credible interval (Bolker 2008). We estimated the amount of variance explained by predictor variables for each response variable by computing pseudo R^2 values using linear regressions (Grace et al. 2012).

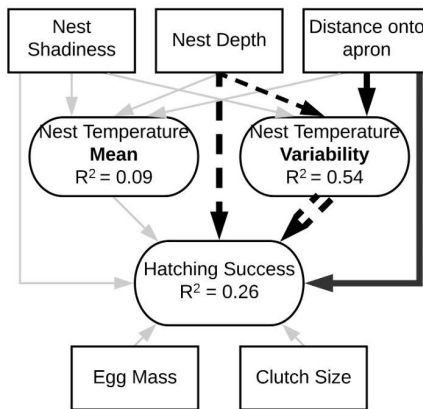
We found that there was substantial site-level variability in the capacity of the SEMs to explain variance in hatching success. Pseudo R^2 values ranged from 0.08-0.65. Which variables were important in the SEMs also differed across sites. In general, nest characteristics only explained variability in temperature variability, not mean temperature (higher R^2 values for temperature variability than temperature mean). Nests that were further away from the burrow entrance onto the burrow apron and were shallower had greater temperature variability. Across three sites, temperature variability had a quadratic effect on hatching success (with mid-range variability producing the highest hatching rates). That is the extent of similarity across the sites. Three sites had direct effects of nest characteristics on hatching success (all different effects), suggesting that there is a missing intermediate effect such as moisture or soil type. The site that had the lowest R^2 value for hatching success (Archbold Biological Station) also has the smallest population size. There is evidence that inbreeding lowers hatching success at Archbold (Yuan et al. 2019), and therefore external variables may have less influence. Our model combining data from all the sites had an extremely low R^2 of 0.01, which may be due to challenges of comparing temperature datasets across sites. However, this result combined with the different important predictors in the site-level models suggests that tortoise hatching may respond to environmental conditions differently at different sites.

Despite this evidence for site-level variability, we did observe that nests experienced very similar mean nest temperatures across the sites that spanned the temperature extremes of the species' range (**Figure 38**). Mean nest temperatures were essentially bounded between 27-31°C, which encompasses the lab-tested critical temperature for sex determination of 29.5°C (Demuth 2001). This congruence occurs even though there is substantial variation in ambient temperature at the sites (the sites span >5 degrees of latitude). It is possible that at each site, female tortoises are selecting nest sites that will fit within this temperature range that is optimal for hatching success and produces balanced sex ratios. Given differences in ambient temperatures, nest characteristics will differ at each site that produce those nest temperatures.

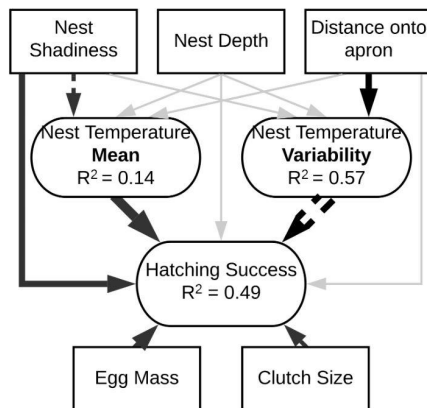
Ft. Stewart



Jones Center



Merritt Island



Archbold

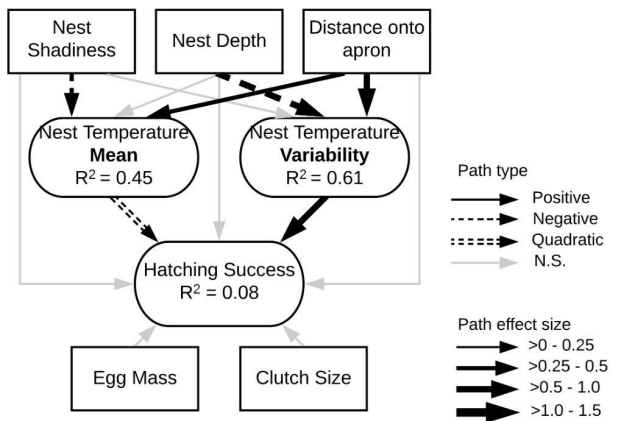


Figure 41. Structural equation model of Gopher Tortoise hatching success at four sites. Black paths are those with non-zero effects (i.e. 95% credible interval did not overlap with zero); gray paths were tested but had zero effect sizes. Arrow width indicates the absolute value of the median effect size for each standardized covariate.

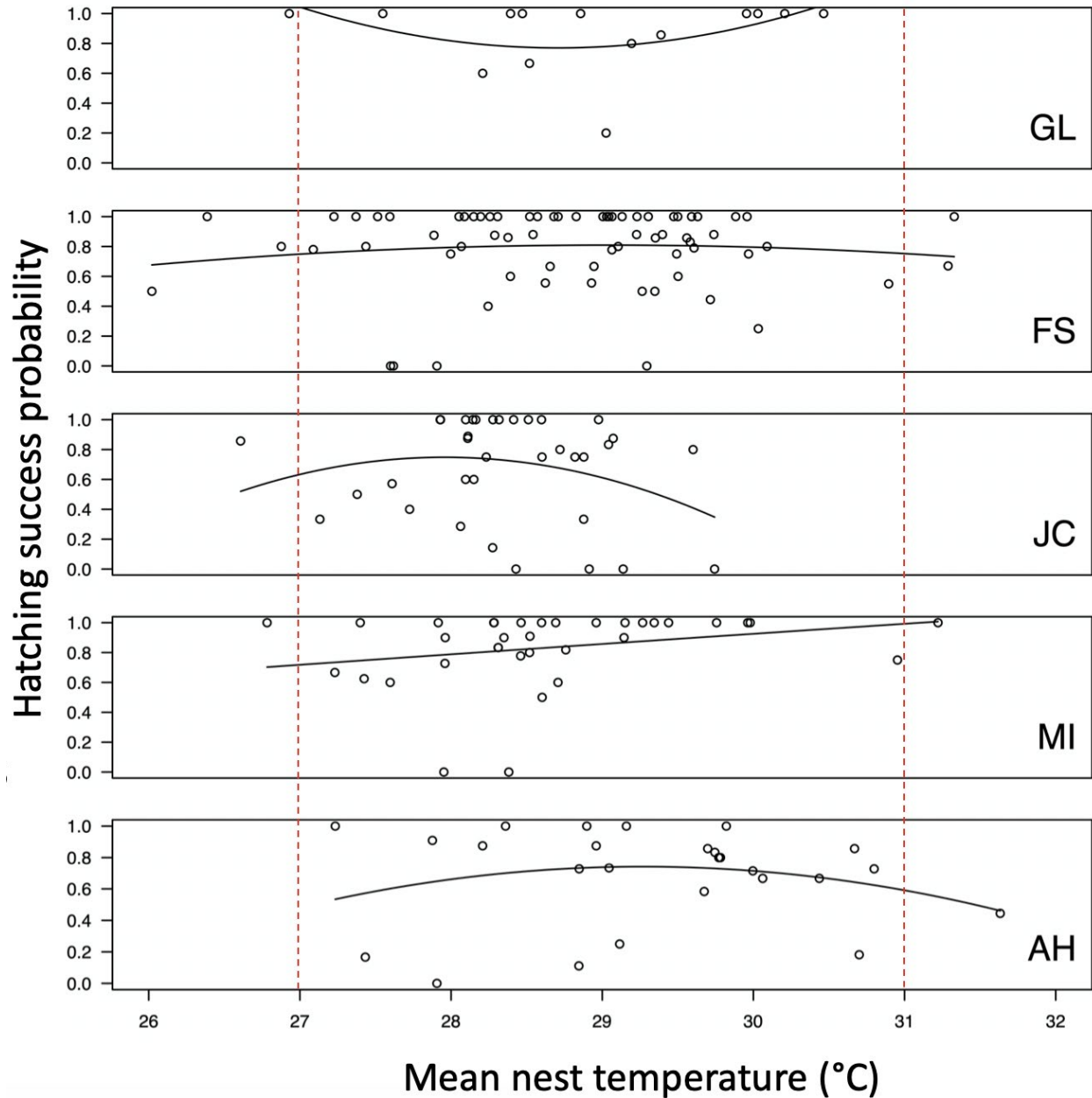


Figure 42. Effect of mean nest temperature on Gopher Tortoise hatching success for 5 sites that span the species' latitudinal range, from north to south: GL=George L. Smith State Park, FS=Fort Stewart, JC=Jones Center, MI=Merritt Island National Wildlife Refuge, AH=Archbold Biological Reserve. Mean nest temperatures almost exclusively fall within 27-31°C, and have similar average hatching success across sites (~75%).

Sex ratios –

Climate change has the potential to alter tortoise population sex ratios by influencing the proportion of hatchlings that develop at female-producing temperatures (Janzen 1994; Mitchell & Janzen 2010). To determine the role of nest thermal environment on Gopher Tortoise hatching sex ratios, we combine a historical dataset of thermal data and hatchling sex outcomes

from 36 nests (from 1997, 1998, 2002, 2005, 2006, 2008) collected by David Rostal with our own data from 32 nests observed at the same field sites (2018-2020).

Nests were discovered in May and June by excavating burrow mouths and aprons at Fort Stewart Army Reserve (all years) and George L. Smith State Park (2018-2020). Nests were carefully inventoried, eggs were returned to their exact original positions, a temperature data-logger was placed in the center of the nest (HOBO 2K by Onset Computer Corp. before 2017; iButton DS1921 by Maxim Integrated after 2017). Loggers were programmed to record nest temperature every 60-90 minutes. Nests were re-buried to their original depth and protected from predation with hardware cloth buried ~2 cm below the surface. Nests were re-excavated in the third week of August (approximately 1-6 weeks prior to hatching) and incubated at constant temperature (29.9 deg C) in the lab for the remainder of development. This excavation date is long after the critical period for sex determination, which is approximately the second quarter of development (Rostal and Wibbels 2014). After hatching, hatchlings were kept in captivity and provided ad libitum food and water until at least 8 months of age, then released at the site of their natal nest. Captive hatchlings were sexed using laparoscopic inspection of gonads after they reached at least 5 months of age (Rostal et al. 1994; Loope et al. 2022).

Gopherus tortoises, like all species with TSD, exhibit a critical period in embryonic development during which their sexual fate is sensitive to the temperature they experience in the nest. To determine the relationship between sex ratio and climate, we require nest thermal data specifically during the critical period. Our nests were found an unknown period of time after the lay date, though often they were found early in the season when they must have been freshly laid. Given that the lay date, and thus the start and end of the critical period were unknown for most nests, we first estimated the timing of the critical period for each nest by back-calculating development from the hatch date. To do this, we applied the DEVAR method (see Valenzuela et al. 2019) to fit a curve describing the relationship between developmental rate (in percent of total egg development per hour) and temperature based on literature data on developmental rates under constant temperature in laboratory incubators (Demuth 2001; Rostal and Jones 2002). Then, for each nest, we used this curve to estimate the development that occurred during each hour of incubation based on the observed temperature. We then summed the cumulative development starting with the hatch date and moving backward in time to determine the date at which 50% of development had occurred and the date at which 25% of development had occurred. This interval was used as our estimate of the critical period for that nest.

To obtain a temperature estimate for the critical period for each nest, we averaged the observed temperature data from our data loggers across all hours in the critical period. As precipitation is likely to affect nest moisture, which could influence development and sex ratio (Lolavar & Wyneken 2017; Lolavar & Wyneken 2020), we also obtained estimates of average daily precipitation during the critical period for each nest location from the PERSIANN-CSS-CDR dataset, which estimates global daily precipitation on a 4km grid based on machine learning models of remote-sensed data (Sadeghi et al 2021). To quantify the effect of mean nest temperature and mean precipitation on sex ratio, we calculated the sex ratio of each nest from the laparoscoped hatchlings, then modeled nest sex ratio (as percent female) with a logistic regression using the average temperature (in degrees C) and precipitation (in mm) as predictors. Given that the accuracy of the sex ratio estimate for each nest is related to clutch size, we weighted each nest based on the number of observed hatchlings.

The logistic regression indicated a significant effect of both critical period nest temperature and precipitation on sex ratio (**Table 16**). Warmer average nest temperatures resulted in a greater proportion of females, and greater precipitation reduced the proportion of females (**Figure 43**).

Table 16. Logistic regression reveals nest temperature and precipitation effects on sex ratio from 68 naturally incubated Gopher Tortoise nests. Estimates and errors are on the logit scale.

Predictors	Est.	Std. Error	95% CI	z	p
Intercept	-17.42	4.68	-26.85 – -8.45	-3.72	<0.001
Temperature	0.62	0.16	0.32 – 0.94	3.93	<0.001
Precipitation	-0.13	0.05	-0.23 – -0.03	-2.62	0.009

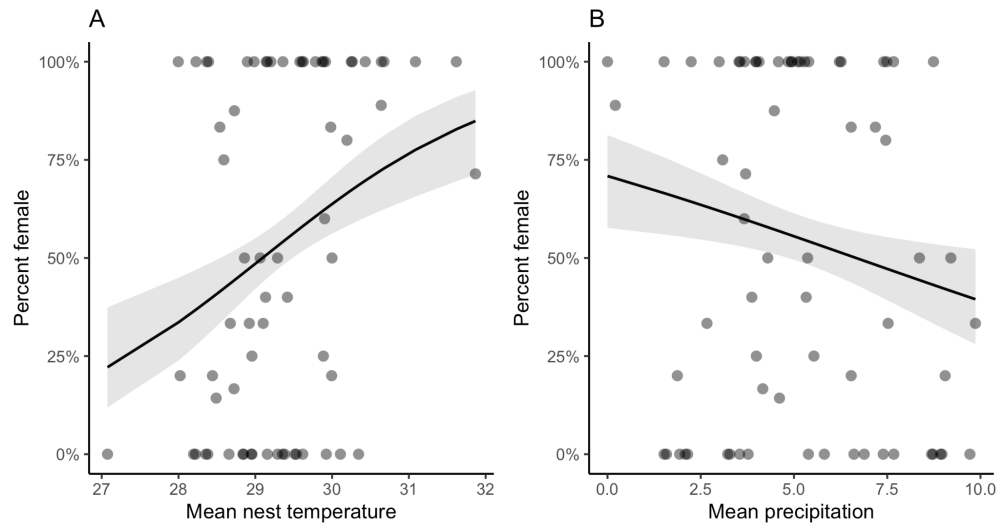


Figure 43. Mean nest temperature (A) and mean daily precipitation (B) during the critical period influence clutch sex ratio in wild nests of the Gopher Tortoise. Data come from 68 nests monitored during summers between 1997 and 2020 at FSAR and GLS. Nest temperature data were collected hourly using data loggers; mean daily precipitation was estimated using the PERSIANN-CSS-CDR dataset. Nest temperature is measured in degrees C, precipitation is in mm.

These estimates are the first to quantify how natural incubation environments influence Gopher Tortoise sex ratios in wild nests, and importantly suggest roles for both nest temperature and nest moisture (via precipitation). While nest moisture has been found to influence hatchling

sex ratio in manipulated sea turtle eggs (Lolavar & Wyneken 2017; Lolavar & Wyneken 2020), this is the first study to indicate an additive effect of precipitation beyond the effect it has on nest temperature, suggesting an unexplored avenue of future research. Furthermore, while most TSD experiments are performed on lab-incubated nests under artificial temperature regimes, ours uses wild nests incubated in situ to quantify the relationships between natural nest thermal profiles and hatching outcomes, resulting in model coefficients that are directly applicable for modeling the effects of climate change on wild populations.

Evidence for local adaptation from translocation ‘experiments’ (Task 2)

DESERT TORTOISE

Nesting – Translocated and resident tortoises had nearly identical mean hatching success across our three sites (translocated = 0.66 ± 0.43 SD; resident = 0.64 ± 0.43 SD), and logistic regression indicated no differences between the two groups (estimate = 0.06, $z = 0.161$, $p = 0.87$, $n = 27$).

Growth - Growth rate k increased with $T_{\max}$ and PPT but decreased with GDD, and was not affected by translocation status (**Figure 44**). Asymptotic size a increased for males and translocated individuals and with both PPT and GDD, while it decreased with increasing $T_{\max}$ (**Figure 44**). This provided weak evidence that a change in climate affected how tortoises grew, although it may also indicate that tortoises from different climates than the translocation site simply have larger asymptotic sizes.

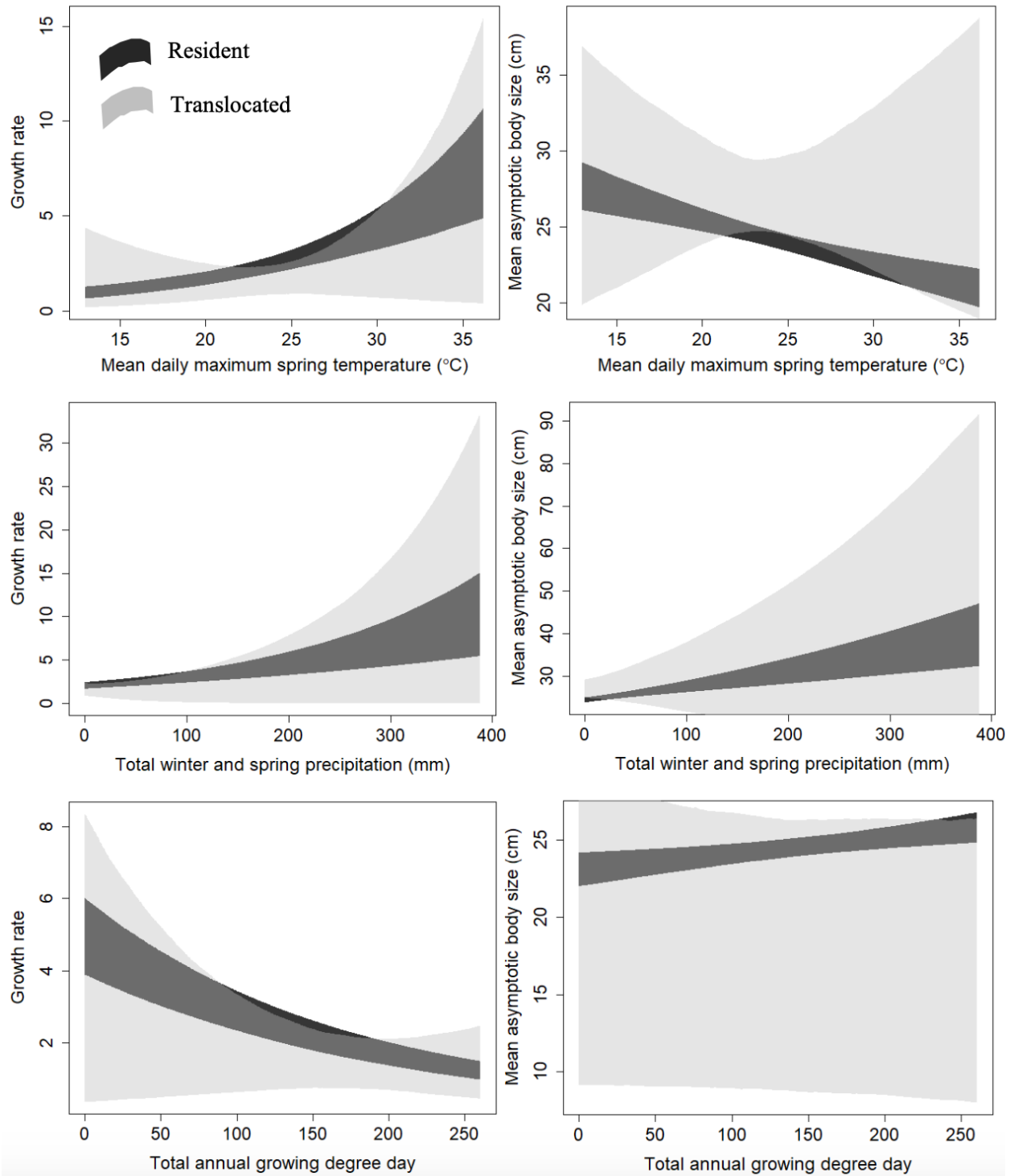


Figure 44. Predicted growth rate k (left) and asymptotic body size a (right) in Mojave Desert Tortoise (*Gopherus agassizii*) along T_{max} , PPT, and GDD gradients by translocation status. Growth is modeled using the von Bertalanffy equation. T_{max} = mean daily maximum temperature averaged by month for February–May, PPT = total winter and spring precipitation Nov–April, and GDD = total annual growing degree days. Curves show 95% credible intervals. Only females are predicted for mean asymptotic body size for clarity.

GOPHER TORTOISE

Our dataset included 102 detections and non-detections of female reproduction via ultrasonography from 2018-2022, with 75 unique females in the dataset (i.e., some individuals were ultrasounded in multiple years), of which 70 were successfully genotyped. From 2018-2020, we found 62 nests, of which we connected 44 nests to 30 unique females translocated from 16 Florida counties.

We did not see any significant effect of tortoise origin on the probability of reproduction, clutch size, or egg mass across all the different ways of measuring environmental distance between the donor county and Nokuse. However, we did see effects of environmental distance on nest site selection behaviors and hatching success. The biggest effects of environmental distance on nest site selection was for the distance of a nest onto the burrow apron (although environmental distance also influenced nest depth and nest canopy cover). Distance of a nest onto the burrow apron has substantial influence on the nest's temperature (as measured by nest temperature data loggers). Nests further away from the burrow entrance onto the burrow apron are warmer and have greater daily temperature variation, both of which can influence hatching success and sex ratios. We found that tortoises originally from warmer climates than Nokuse placed their nests closer to the burrow entrance ().

The top model (32% model weight) for predicting hatching success included all of the environmental distance measures: MTR, Precip, and WST. As the difference in MTR and WST increased, hatching success increased (). However, as the difference in Precip between site of origin and recipient site increased, hatching success decreased (). For the model examining how nest-site characteristics influenced hatching success, the null model was the top model (containing 25% of the total model weight), indicating that the nest characteristics included in our model accounted for little of the variation in hatching success, and providing greater support to the evidence that climate of origin influences hatching success.

Our results provide mixed evidence of local adaptation for reproduction in Gopher Tortoises. Changing climate (through translocation) did not affect whether or not females reproduced, but it did affect where they nested and the hatching success of their eggs in complex ways. This study is not a perfect proxy for how Gopher Tortoise nesting behaviors and outcomes will respond to climate change because tortoises were generally translocated from warmer to cooler climates – the opposite direction of temperature changes from climate change. However, it does provide some insight into how Gopher Tortoises respond to novel climates. The results from this study suggest that Gopher Tortoises may have limited plasticity to respond to climate change. However, these results do not indicate whether this lack of plasticity will result in poorer outcomes for populations. We predicted that if tortoises exhibited local adaptation, there would be a decrease in fecundity for tortoises translocated to a new environment; however, this was not the case. We found that nests laid by females that originated from environments with greater differences in temperatures (both warm season temperature mean, and monthly temperature range) actually had higher hatching success rates than nests from females that originated from locations closer in temperature to the translocation site (). The result for the difference in precipitation was the opposite: hatching success declined as the difference in precipitation increased. This suggests that the response of reproductive rates to climate change will be difficult to predict, as there are many axes of variation in climate. Many predictions of how species will be affected by climate change ignore the possibility of local adaptation (Williams et al. 2008;

Mortiz & Agudo 2013; Nicotra et al. 2015; Beever et al. 2016), but our study shows that local adaptation can have complex consequences that need to be taken into account.

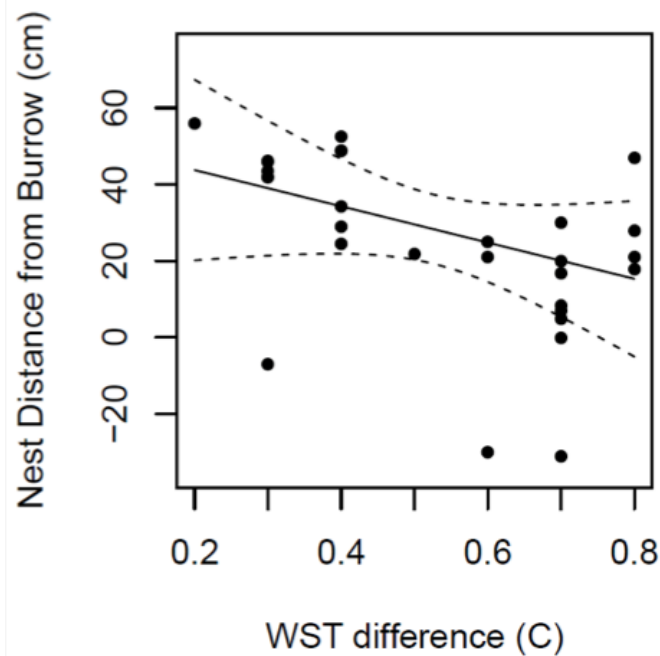


Figure 45. Effect of the difference in warm season temperatures (WST) between a Gopher Tortoise female's original location (the donor county) and the recipient site on nest distance from burrow entrance. Data was collected at Nokuse Plantation, a Gopher Tortoise recipient site located in Walton County, FL, during nesting seasons in 2018-2020. Solid lines represent the mean model prediction and dashed lines indicate 95% confidence intervals from the top model for nest distance from burrow entrance. Points are values for each nest, or averaged nests for females with multiple nests in the dataset.

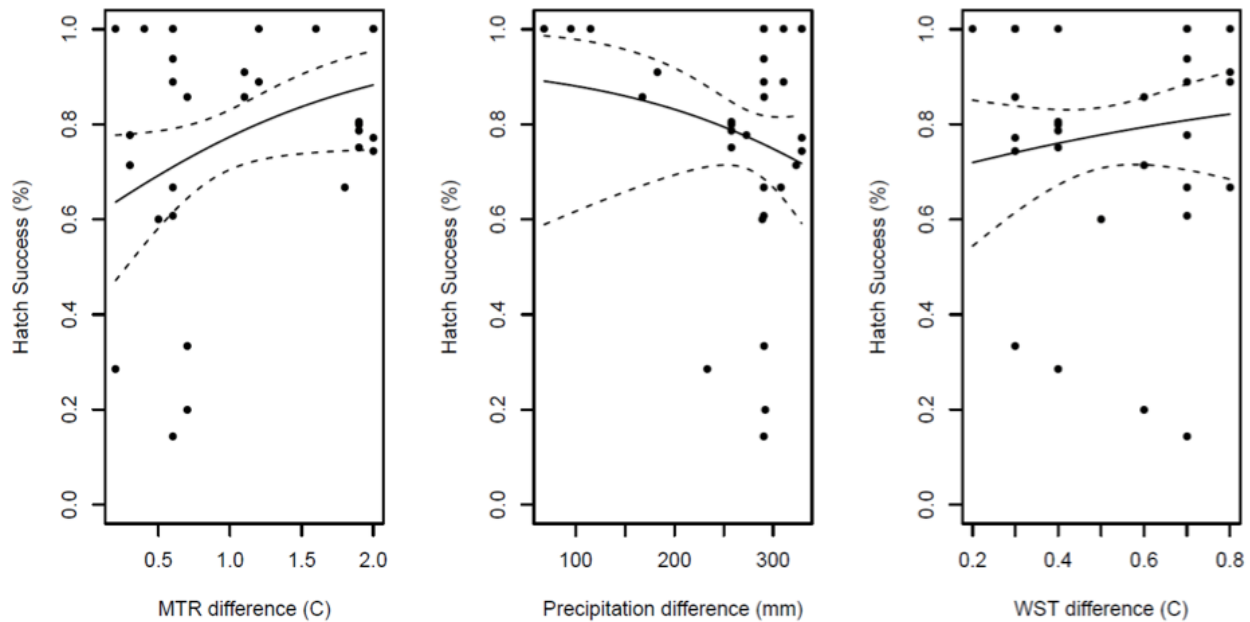


Figure 46. Effects of environmental distance on hatching success for Gopher Tortoises. Data was collected at Nokuse Plantation, a Gopher Tortoise recipient site located in Walton County, FL, during the 2018-2020 nesting season. Environmental distance variables are monthly temperature range (MTR) for January, annual precipitation, and warm season temperature (WST) for July. Solid lines represent the mean model prediction and dashed lines indicate 95% confidence intervals for the top model for hatching success. Points are values for each nest, or averaged nests for females with multiple nests in the dataset.

Simulated population dynamics (Task 3)

For both species, we ran all simulation replicates to the year 2099 (from 2011 to 2099 for Desert Tortoise, and from 2006 to 2099 for Gopher Tortoise). We ran 120 replicates per scenario for Gopher Tortoise (430,080 replicates total across 3584 scenarios) and 120 replicates per scenario for Desert Tortoise (30,720 replicates total across 256 scenarios). Although we ran multiple scenarios activating or inactivating various climate effects (and burn effects for Gopher Tortoise) in our simulations, we treated scenarios with activated climate effects as the most likely “true” scenarios for visualizing critical habitat breadth and future abundance. For the Gopher Tortoise, few populations were forecasted to be sustainable in our simulation models, although lack of sustainability had more to do with low baseline vital rates and a severe effect of burn history on survival, and was largely not driven by climate change (more details below). For Desert Tortoise, baseline populations were far more likely to be sustainable over time. In addition, significant areas of the current Desert Tortoise range were estimated to be resilient across multiple climate change scenarios.

Desert tortoise simulation results

Climate effects on vital rates

For Desert Tortoise, precipitation is a much stronger driver of variation in population growth for Desert Tortoise than temperature on the x axis, with higher precipitation resulting in higher expected growth rates overall. Because projected precipitation (as opposed to temperature) shows very little spatial or temporal trend for any of the four emissions scenarios, this tended to result in Desert Tortoise vital rates and abundance also showing minimal spatial or temporal trends.

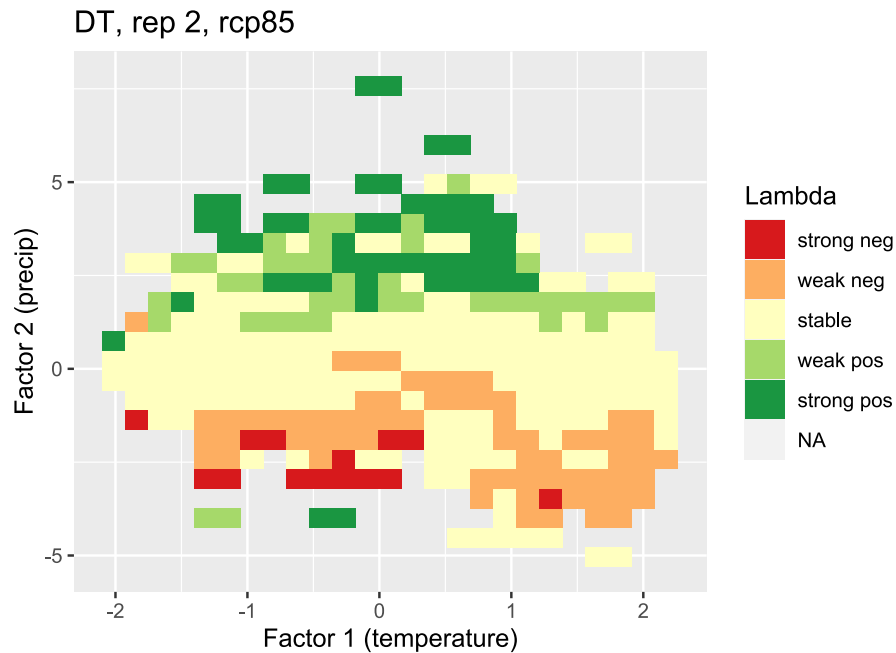


Figure 47. Visualization of lambda across climate space. Here, we used a factor analysis to identify two main axes of climate variation for Desert Tortoise, which can be interpreted as temperature (x axis) and precipitation (y axis). Pixel locations in this figure represent different environmental conditions on these two axes, and pixel color represents population growth rate. Green pixels represent environmental conditions expected to exhibit positive growth, yellow pixels are expected to remain relatively stable over time, and orange and red pixels represent environments associated with negative population growth. Grey pixels are regions of climate space that don't occur at any time or place in this simulation. For these simulations, all 'climate-effect activators' (on-off switches) were turned on to reflect the estimated effects of climate change on all key parameters. Axis 2 (precipitation) is a much stronger driver of variation in population growth for Desert Tortoise than temperature on the x axis, with higher precipitation resulting in higher expected growth rates overall.

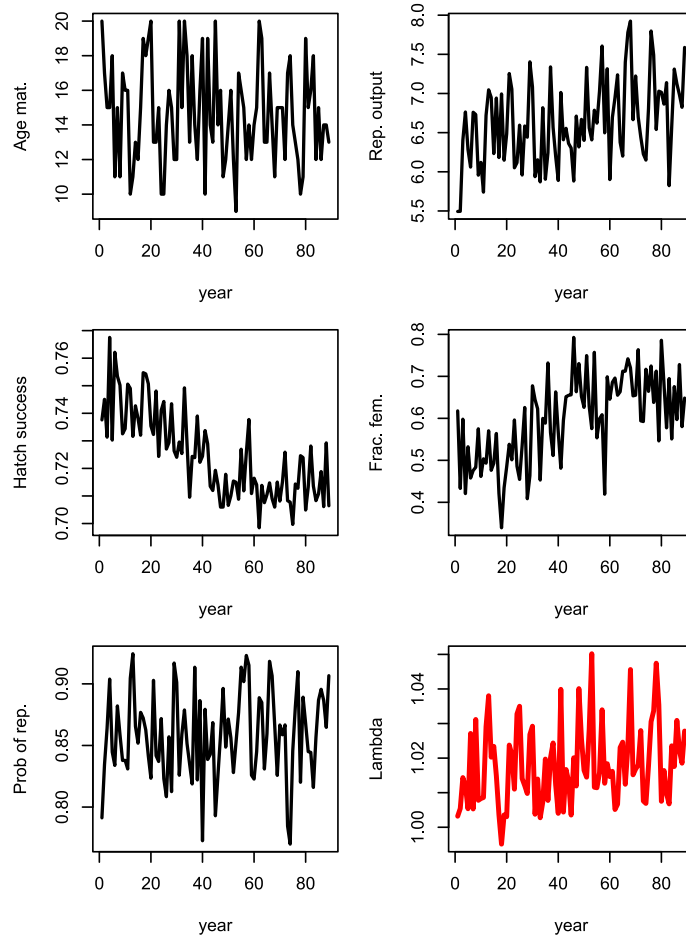


Figure 48. Climate-driven changes in vital rates over time for Desert Tortoise for a single selected pixel for a single simulation replicate. Each panel in this figure displays a different Desert Tortoise vital rate for a single grid cell in a single replicate of a high carbon-emissions scenario- with time on the x axis and vital rate on the y axis. For these simulations, all ‘climate-effect activators’ (on-off switches) were turned on to reflect the estimated effects of climate change on all key parameters. Here we see some negative effects of climate change- most notably, a decline in hatching success over time. However, this negative impact is offset by a positive effect of climate change on reproductive output and hatchling sex ratio, both of which are expected to increase population growth. In the end, population growth (Lambda), represented in red in the lower righthand corner, remains relatively stable in this grid cell.

Temporal trends in total abundance

Overall, our simulations indicated that Desert Tortoise populations are likely to be resilient to climate change. Population viability was high across all four different emissions scenarios (RCP2.6, RCP4.5, RCP6.0, and RCP8.5), although weak population declines are expected under RCP 8.5 towards the end of the century. Our results suggest climate warming is likely to increase the fraction of hatchlings born female, thereby improving population outlook. However, it is important to note that our female-only models do not currently account for any potential negative consequences of strongly biased sex ratios.

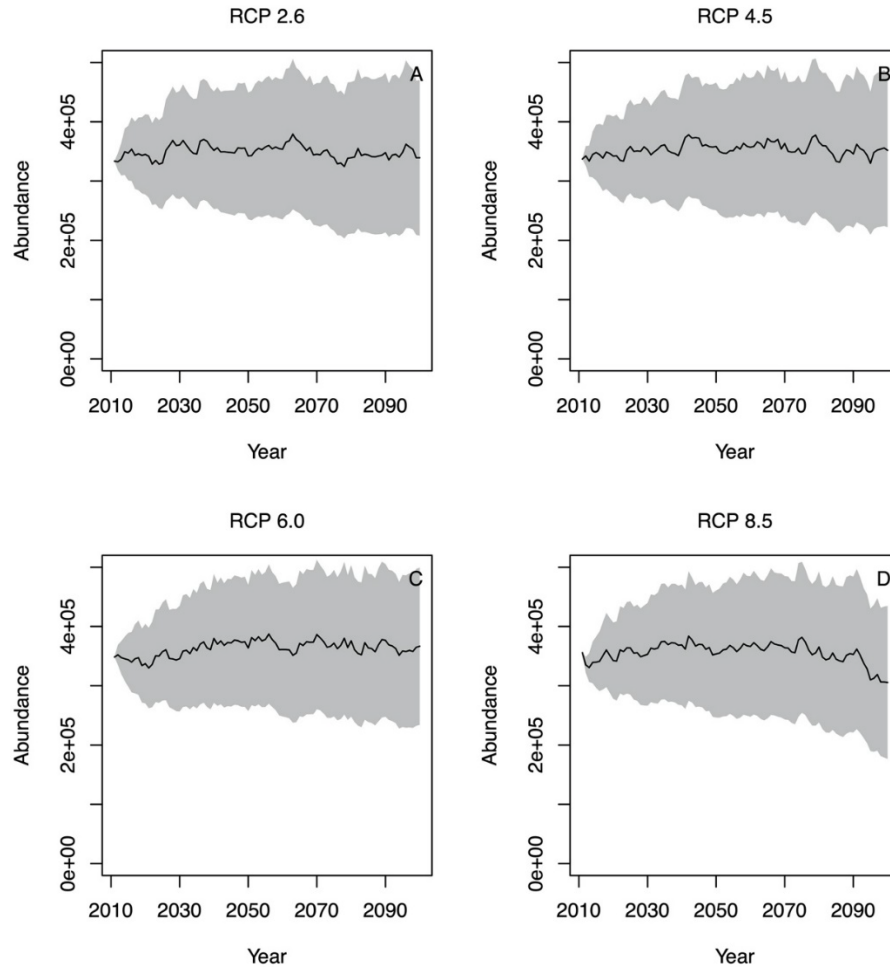


Figure 49. Desert Tortoise simulated abundance trajectories from 2011 to 2099 (sum across all age classes) for each emission reduction scenario (RCP2.6, RCP4.5, RCP6.0, and RCP8.5). For these simulations, all ‘climate-effect activators’ (on-off switches) were turned on to reflect the estimated effects of climate change on all key parameters. Dark lines denote mean abundance across scenarios, while gray polygons reflect one standard deviation above and below the mean.

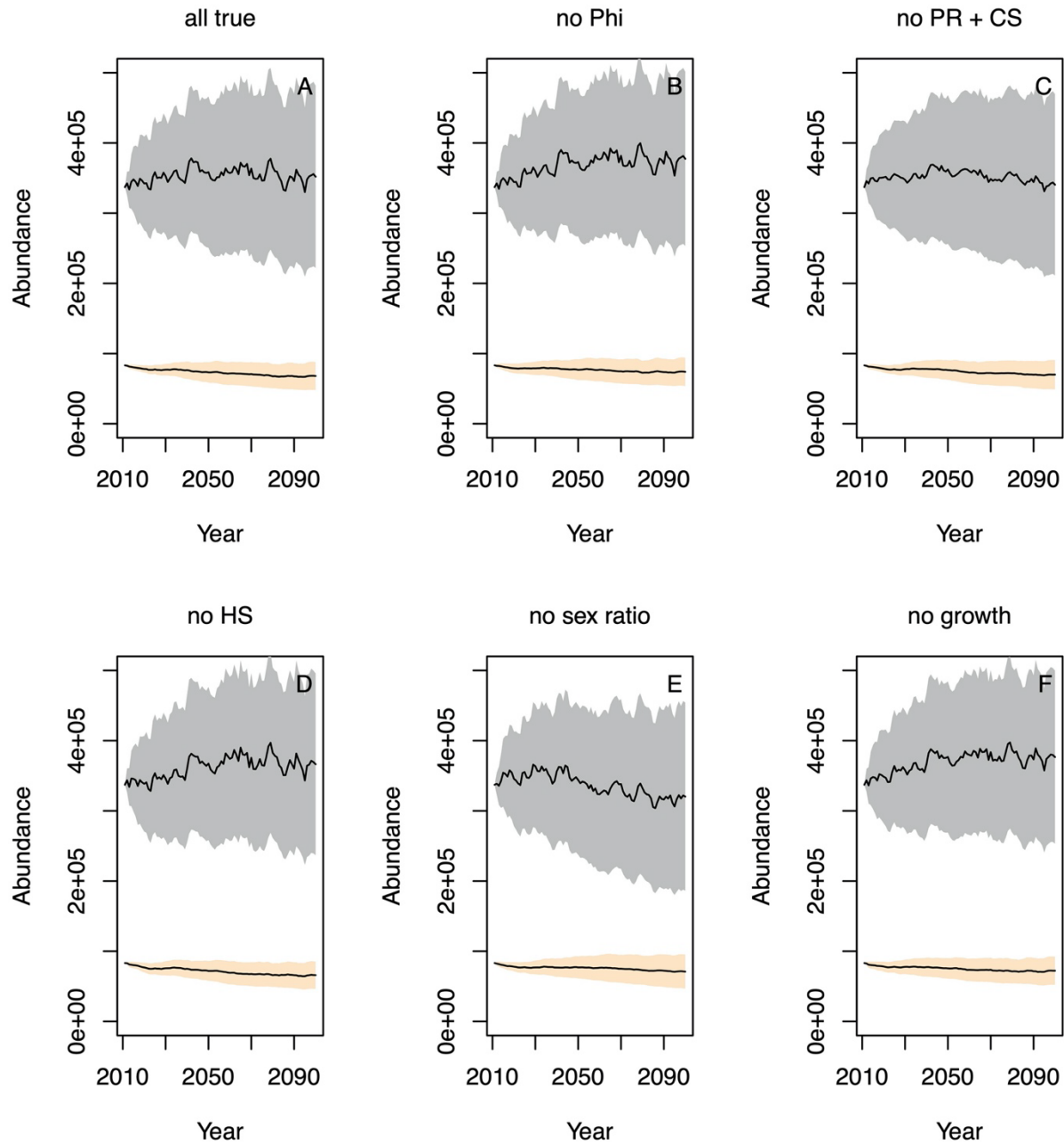


Figure 50. Desert tortoise simulated abundance trajectories from 2011 to 2099 (sum across all age classes) across all emission reduction scenarios (RCP2.6, RCP4.5, RCP6.0, and RCP8.5). In A, all ‘climate-effect activators’ were turned on to reflect the estimated effects of climate change on all key parameters. In the subsequent panels, various climate effects are ‘turned off’ to reflect their overall impact on simulation results. Dark lines with gray ribbons denote mean abundance across all stages. Dark lines with beige ribbons denote mean adult abundance. Ribbons denote one standard deviation above and below the mean.

Spatial patterns in final abundance

Spatial projections of our Desert Tortoise simulation models reflect a general pattern of stability across the range with few detectable spatial patterns in variability. There is also little detectable difference in outcome with any of the emissions reduction scenarios.

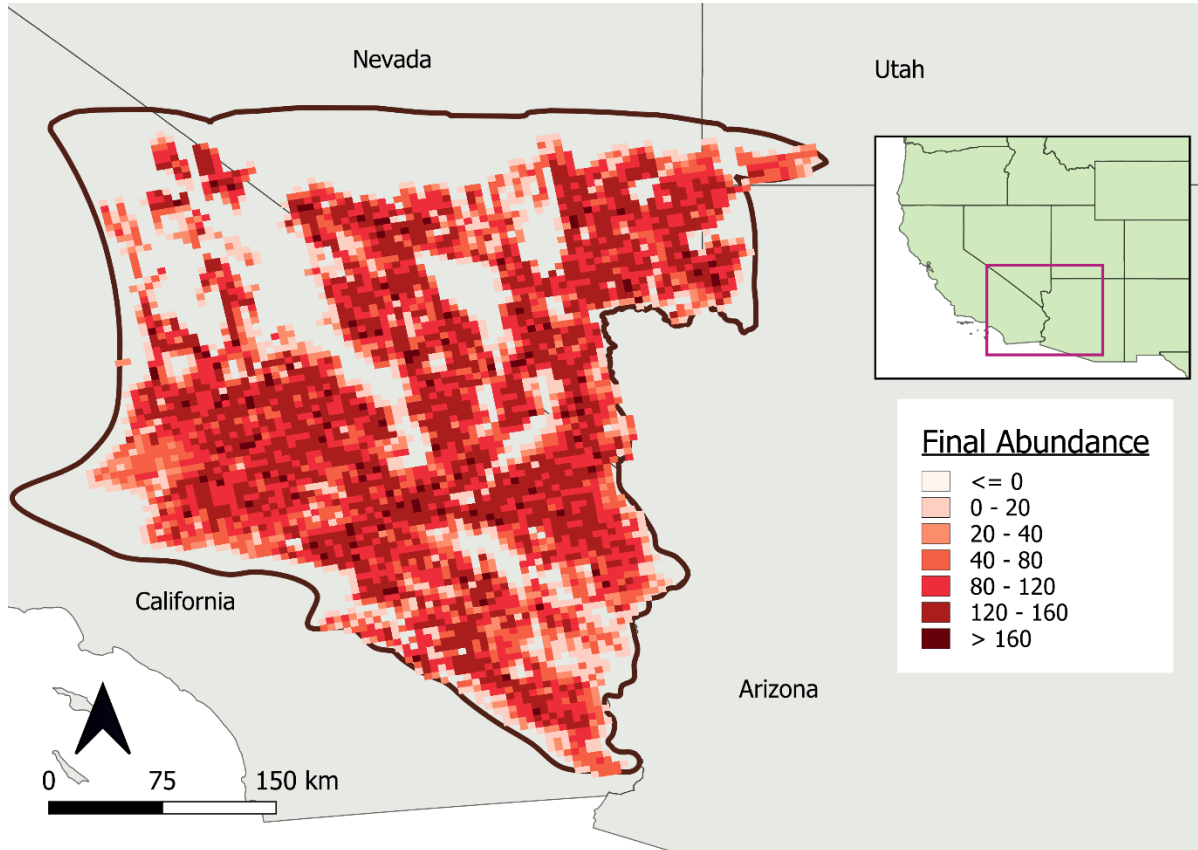


Figure 51. Projected range-wide spatial patterns in final Desert Tortoise abundance (averaged final abundance for 2079-2099 to account for random fluctuations at the end of the simulation), averaged across the three most plausible carbon-emissions scenarios: RCP4.5, RCP6.0, and RCP8.5. For these model runs, all climate effects on vital rates were activated.

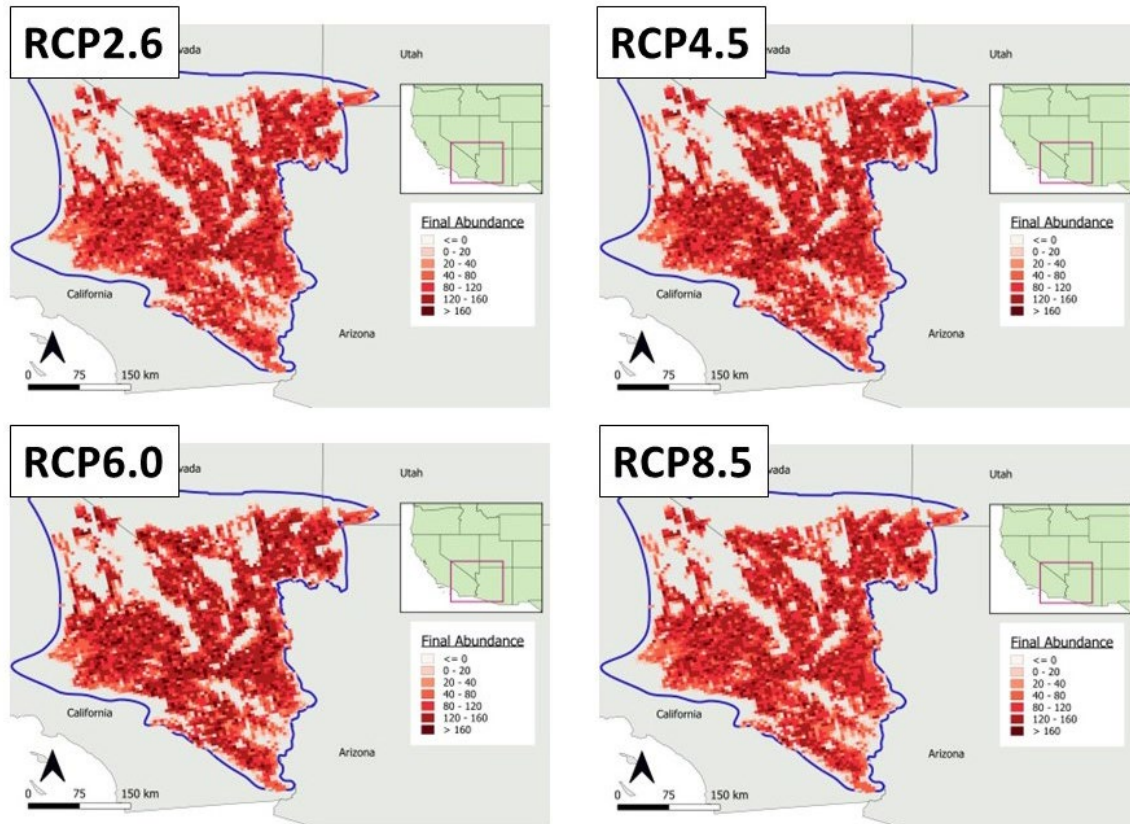


Figure 52. Average projected range-wide spatial patterns in final Desert Tortoise abundance (averaged final abundance for 2089-2099 to account for random fluctuations at the end of the simulation) across four plausible carbon-emissions scenarios: RCP2.6, RCP4.5, RCP6.0, and RCP8.5. Pixel values refer to total abundance within each 5 X 5 km pixel. For these model runs, all climate effects were activated.

Critical habitat for Desert Tortoise

In general, critical habitat (habitat capable of sustaining Desert Tortoise populations under climate change) appears to be widely available across several climate change scenarios, although extreme warming (RCP 8.5) may lead to slight decreases in population size (**Figure 49**) and habitat availability (**Figure 53**). The effect of climate change on final abundance appears to have the greatest positive effects in RCP 6.0 (**Figure 57**) – the reason for this is not clear, but it is possible that a moderate amount of warming (as experienced in RCP 6.0) produces the greatest amount of demographic benefits in terms of sex ratios, but that with greater warming (RCP 8.5), these benefits are more offset by reductions in hatching success.

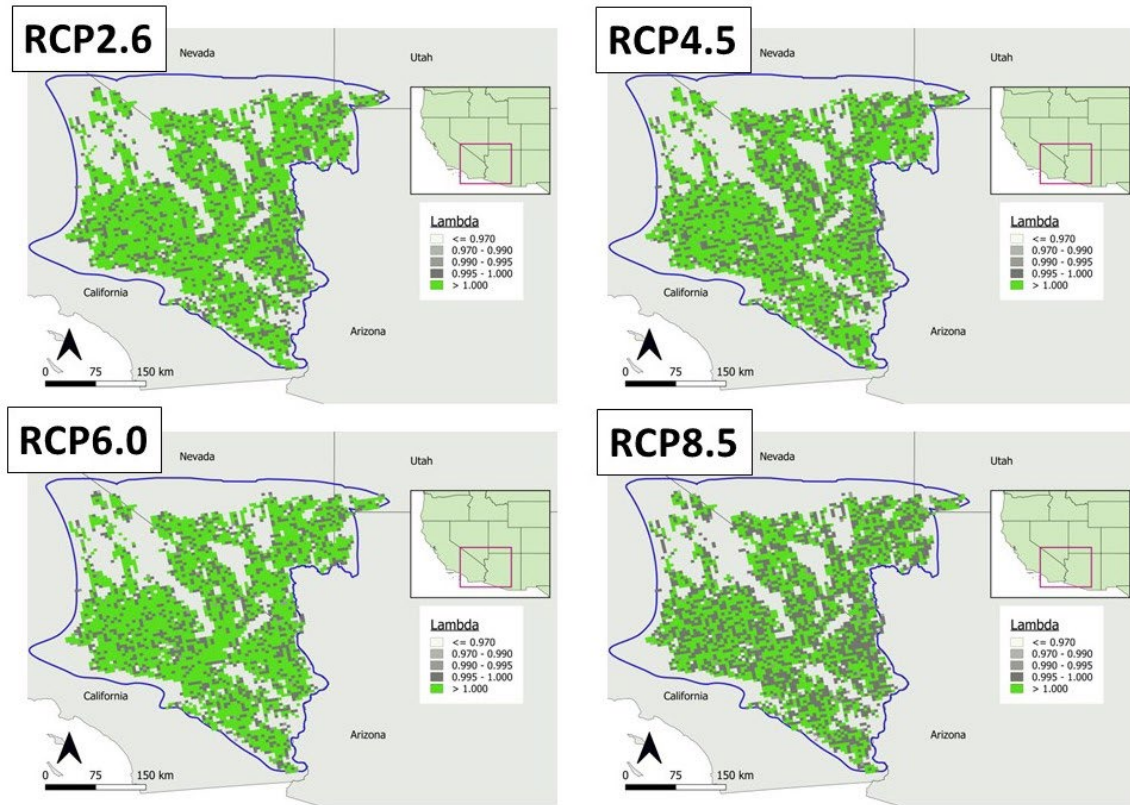


Figure 53. Map of critical habitat for Desert Tortoise (defined as areas that are expected to sustain Desert Tortoise populations over time in the face of climate change) across four plausible carbon emissions scenarios: RCP2.6, RCP4.5, RCP6.0, and RCP8.5. Green pixels indicate areas likely to support stable or growing Desert Tortoise populations ($\Lambda \geq 1$). Shades of grey indicate that a population decline is expected to occur in a pixel, with darker shades indicating weaker declines (Lambda approaching 1) and lighter shades indicating that the pixel has limited ability to support Desert Tortoises under climate change (Lambda < 1).

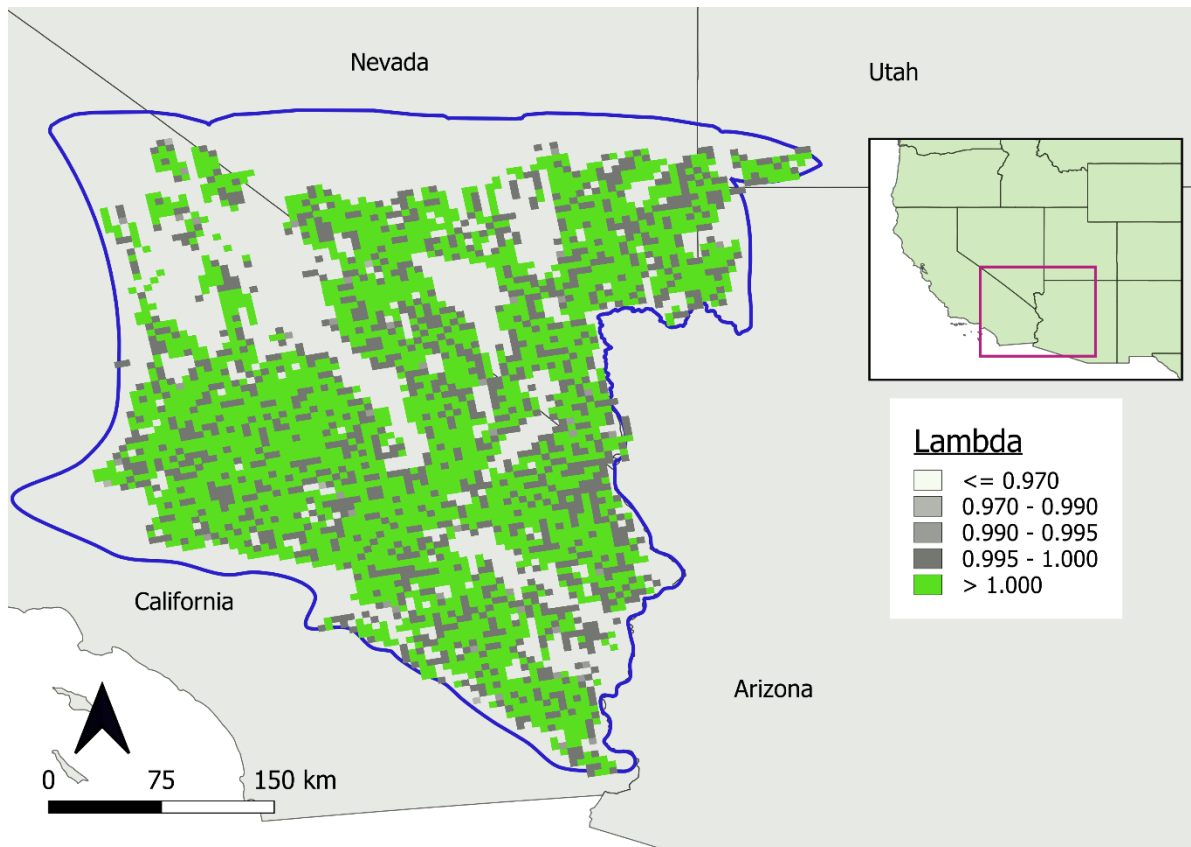


Figure 54. Map of critical habitat for Desert Tortoise (defined as areas that are expected to sustain Desert Tortoise populations over time in the face of climate change). For this figure, simulation results were averaged across the three most plausible carbon emissions scenarios: RCP4.5, RCP6.0, and RCP8.5. Green pixels indicate areas likely to support stable or growing Desert Tortoise populations ($\Lambda \geq 1$) under climate change. Shades of grey indicate that a population decline is expected to occur in a pixel, with darker shades indicating weaker declines (Λ approaching 1) and lighter shades indicating that the pixel has limited ability to support Desert Tortoises under climate change ($\Lambda \ll 1$).

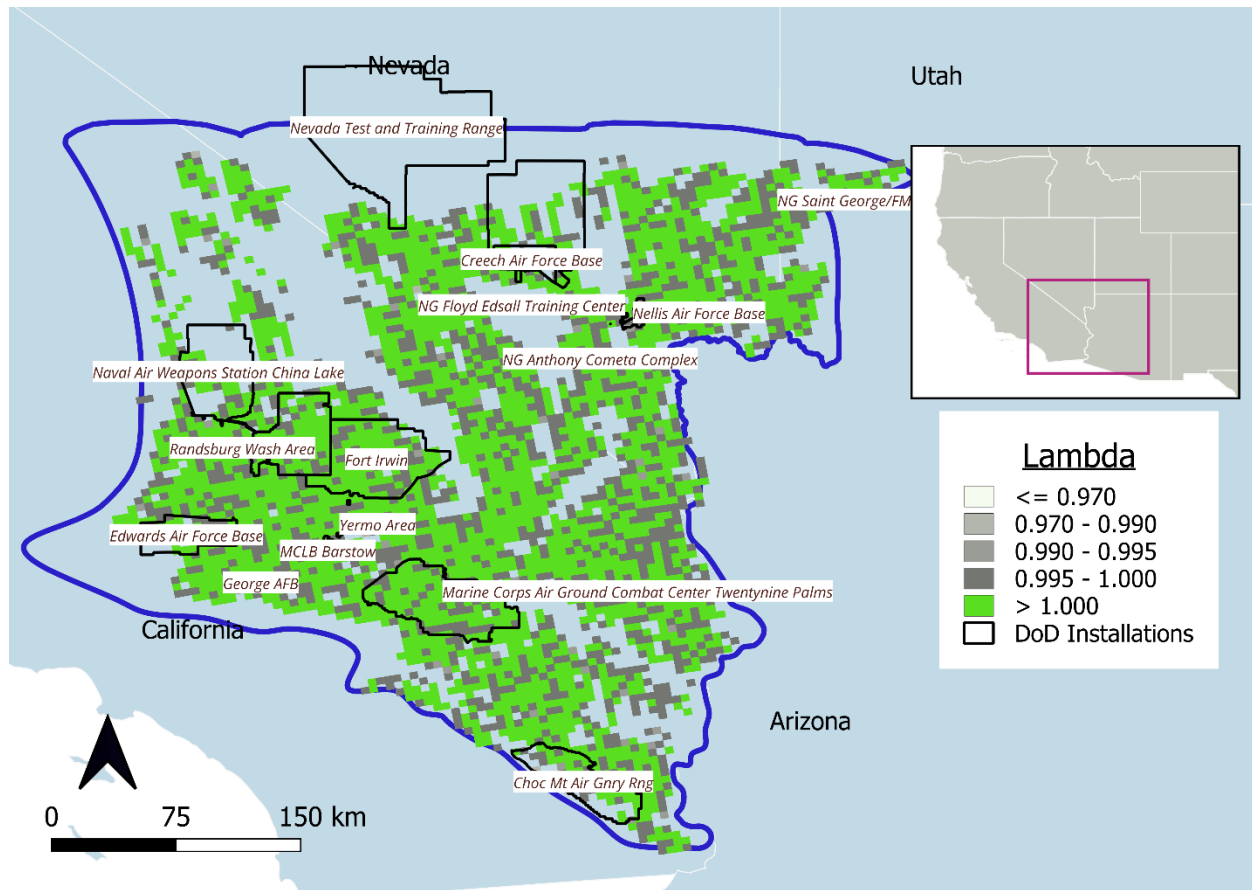


Figure 55. Map of critical habitat for Desert Tortoise (defined as areas that are expected to sustain Desert Tortoise populations over time in the face of climate change). For this figure, simulation results were averaged across all the three most plausible carbon emissions scenarios: RCP4.5, RCP6.0, and RCP8.5. Green pixels indicate areas likely to support stable or growing Desert Tortoise populations ($\text{Lambda} \geq 1$). Shades of grey indicate that a population decline is expected to occur in a pixel, with darker shades indicating weaker declines (Lambda approaching 1) and lighter shades indicating that the pixel has limited ability to support Desert Tortoises ($\text{Lambda} < 1$). Labeled black polygons indicate Department of Defense installations within the range of the Desert Tortoise (indicated as a blue outline).

Mapping effects of climate change

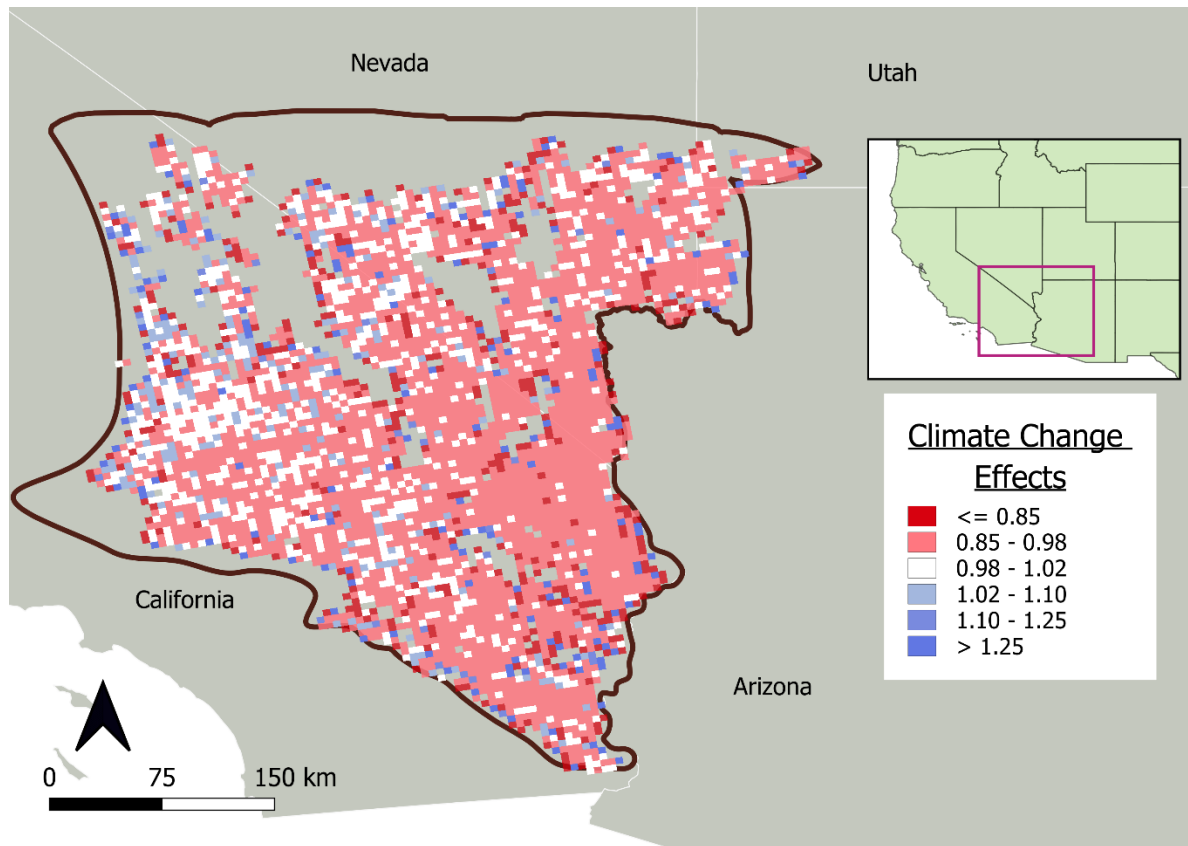


Figure 56. Map comparing range-wide effect of climate change on final simulated abundances (final abundance assuming all vital rates are affected by climate change divided by final abundance assuming no vital rates are affected by climate change). For this figure, simulation results were averaged across the three most plausible carbon emissions scenarios: RCP4.5, RCP6.0, and RCP8.5. Shades of orange and red indicate pixels for which abundance is expected to decline due to climate-driven changes in vital rates, with darker red shades corresponding to more severe negative population-level effects. Shades of blue indicate pixels for which abundance is expected to increase due to climate-driven changes in vital rates, with darker blue shades corresponding to more positive effects of climate change.

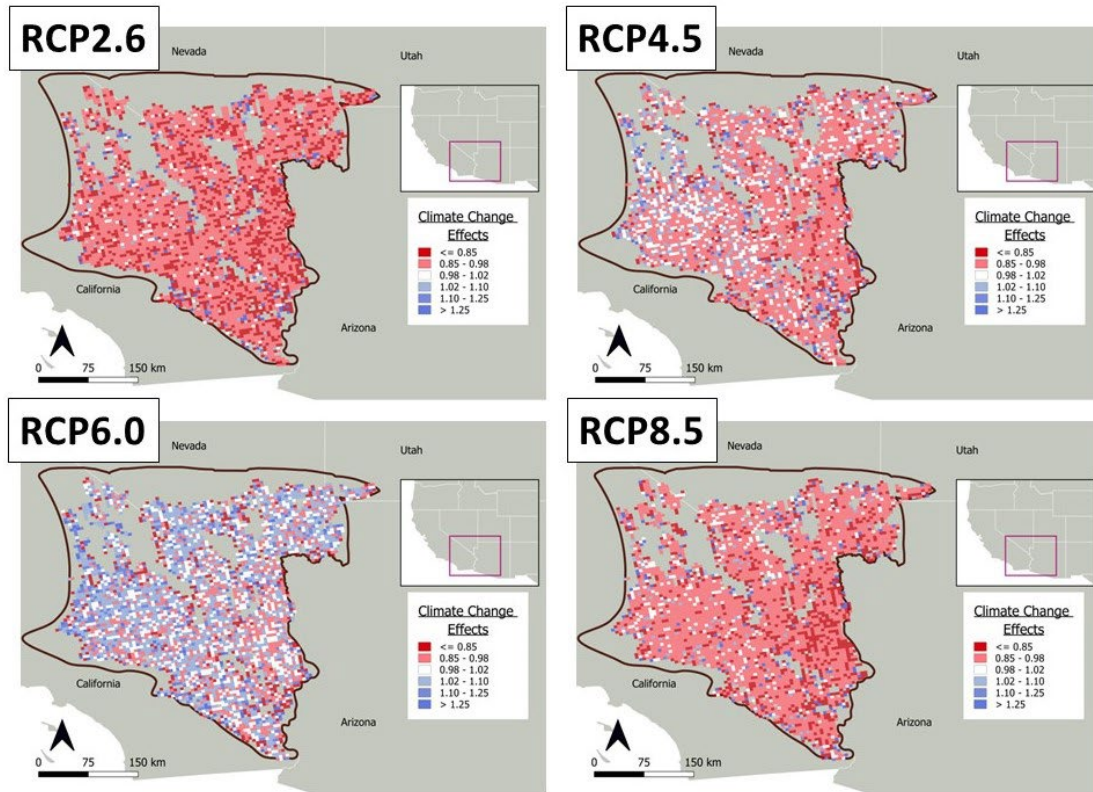


Figure 57. Map comparing range-wide effect of climate change on final simulated abundances (final abundance with all climate effects turned divided by final abundance with all climate effects turned off, under each of the four carbon emissions scenarios). The simulations were run under four plausible carbon emissions scenarios: RCP2.6, RCP4.5, RCP6.0, and RCP8.5, with RCP8.5 representing a no-action baseline, or a 'business-as-usual' scenario. Shades of orange and red indicate pixels for which abundance is reduced due to climate-driven changes in vital rates, with darker red shades corresponding to more severe negative effects of climate change. Shades of blue indicate pixels for which abundance is expected to increase due to climate-driven changes in vital rates, with darker blue shades corresponding to more positive effects of climate change. The predicted positive effects of climate change are maximized under the RCP 6.0 emissions scenario.

Gopher Tortoise simulation results

Our simulation models predicted a severe range-wide population decline for Gopher Tortoise under nearly all scenarios and replicates. Fire history had the greatest effect on Gopher Tortoise expected abundance, whereby increased burn frequencies resulted in more sustainable populations. Carbon emissions scenarios had little additional effect on abundance dynamics.

Temporal trends in total abundance

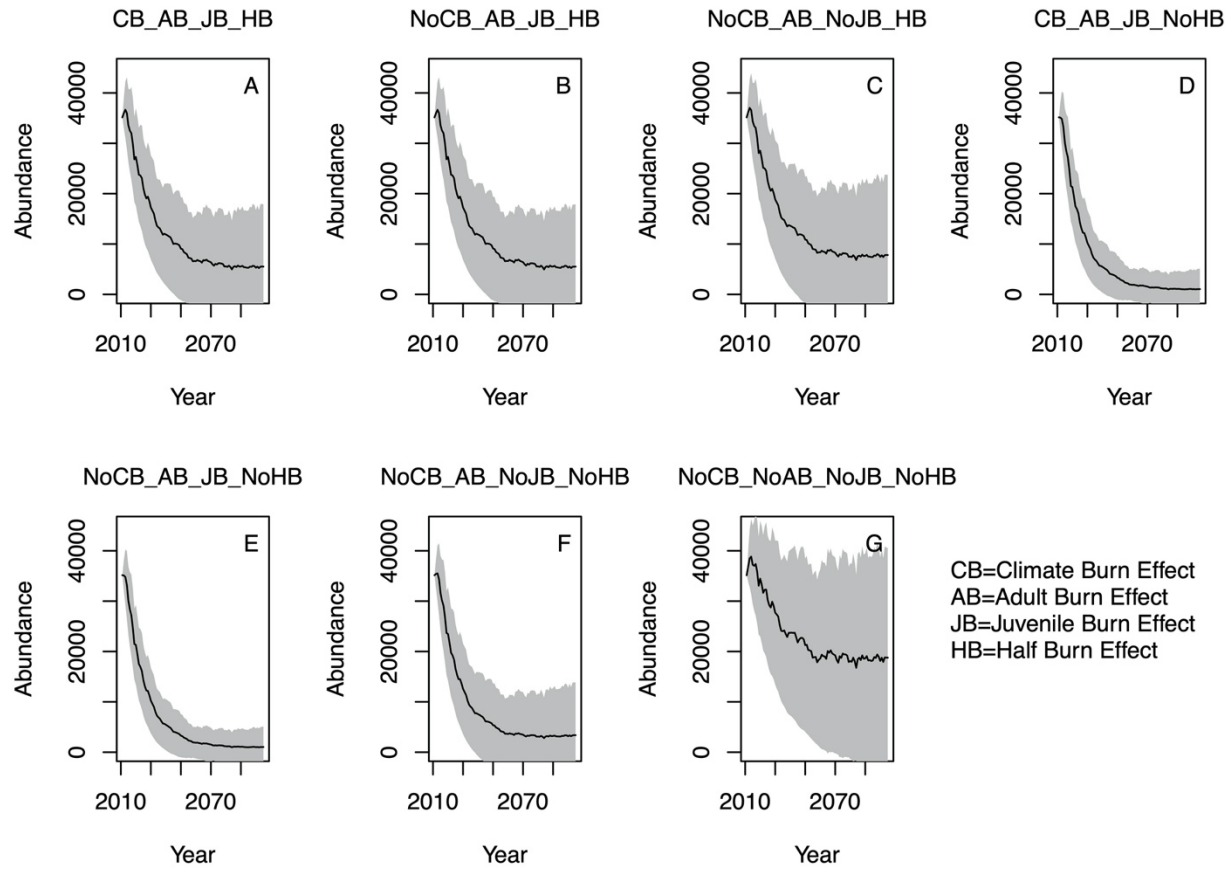


Figure 58. Gopher Tortoise simulated abundance trajectories (sum across all age classes) for a single climate model (CESM1-BGC 8.5) across all unique burning scenarios. Dark lines denote mean abundance across scenarios, while gray polygons reflect one standard deviation above and below the mean. Plot titles reflect burn scenarios as a combination of binary activation switches: CB vs noCB reflects whether or not the climate effect on burn frequency was activated, AB vs noAB reflects whether or not burn frequency affected adult survival, JB vs noJB reflects whether or not burn frequency affected juvenile survival, and HB vs noHB reflects whether the effect of burn frequency was reduced by 50%.

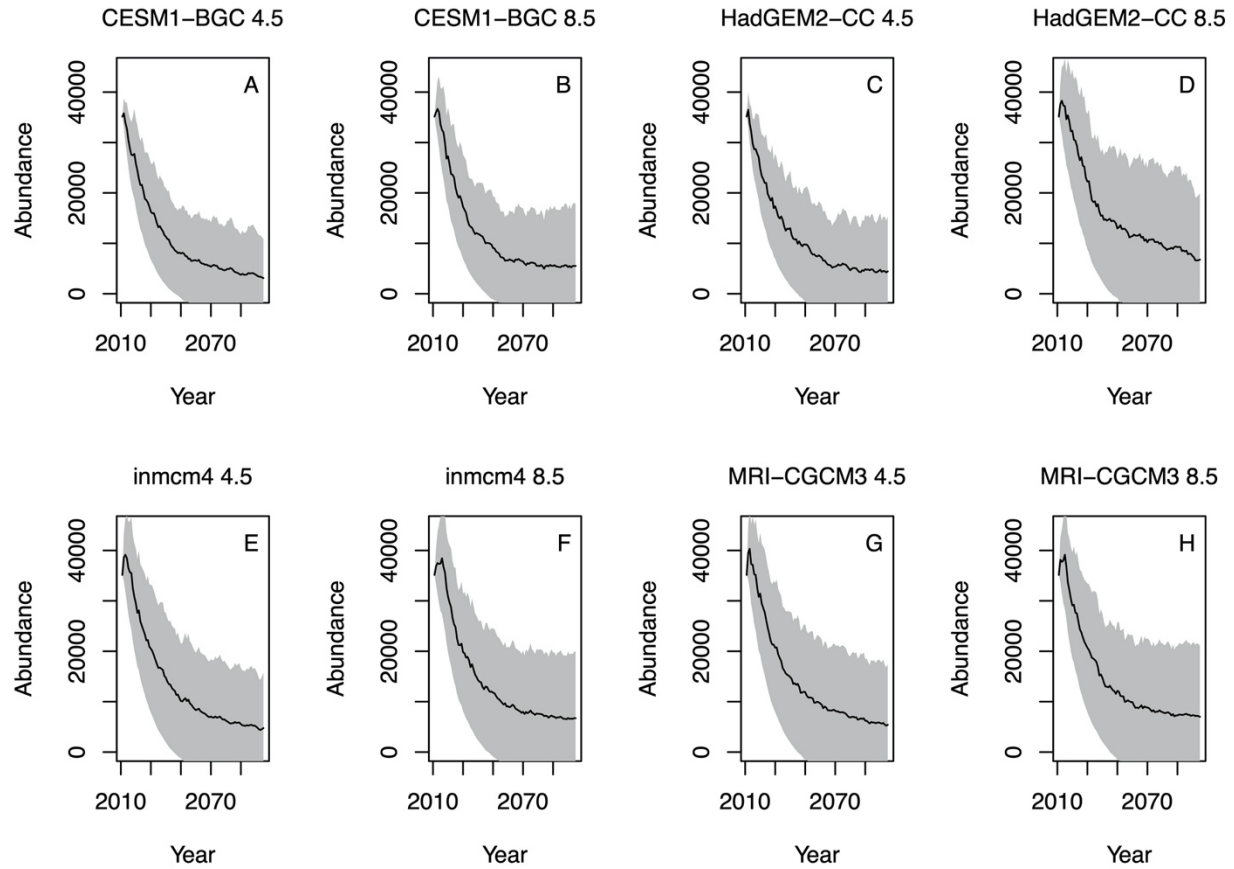


Figure 59. *Gopher Tortoise simulated abundance trajectories (sum across all age classes) under the worst case burn scenario (full-magnitude, climate-responsive burn frequency affecting both juvenile and adult survival) across all 8 climate models. Dark lines denote mean abundance across scenarios, while gray polygons reflect one standard deviation above and below the mean. Climate models reflect four different GCMs (CESM,1-BGC, HadGEM2-CC, inmcm4, and MRI-CGCM3) under two different carbon emissions scenarios (RCP4.5 and RCP8.5).*

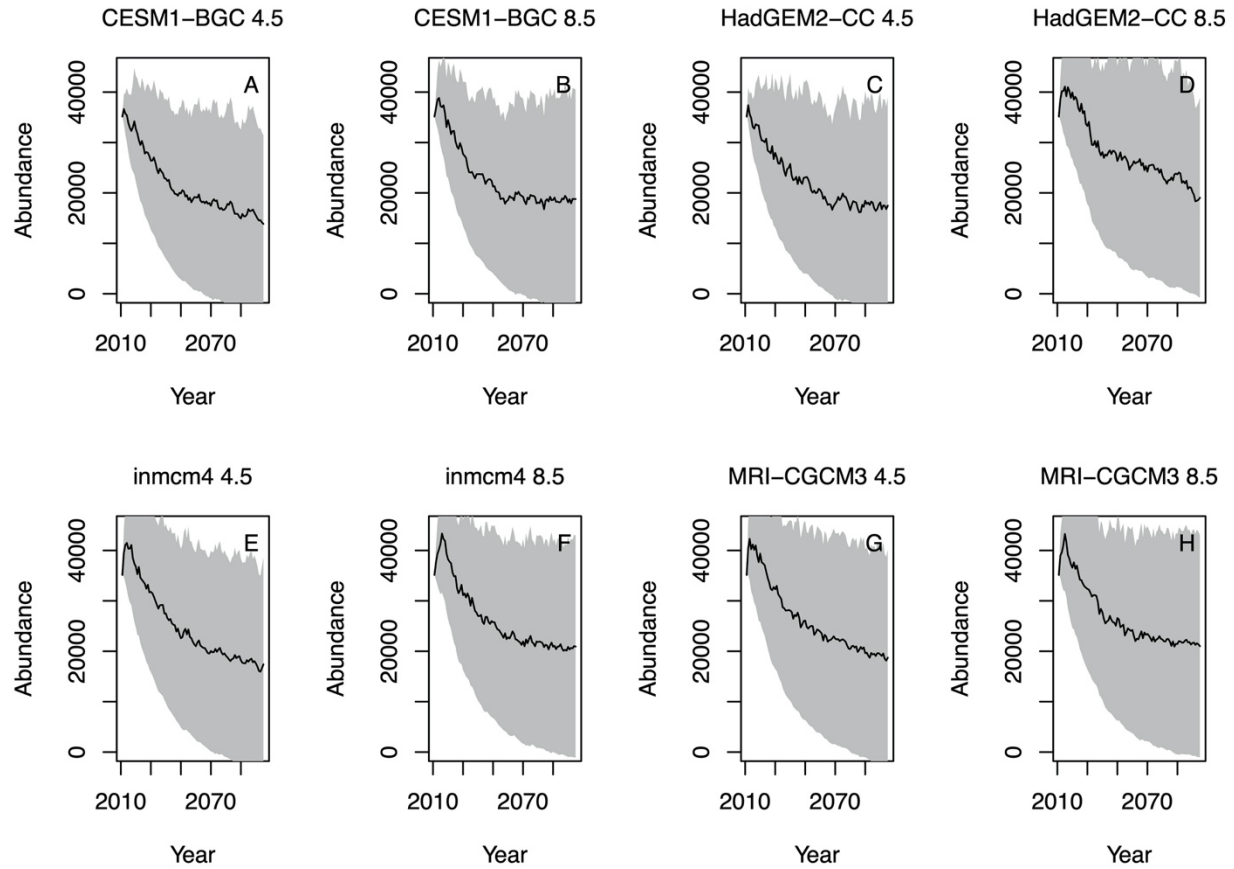


Figure 60. *Gopher Tortoise simulated abundance trajectories (sum across all age classes) under the best-case burn scenario (no effect of years-since-burn on survival) across all 8 climate models. Dark lines denote mean abundance across scenarios, while gray polygons reflect one standard deviation above and below the mean. Climate models reflect four different GCMs (CESM,1-BGC, HadGEM2-CC, inmcm4, and MRI-CGCM3) under two different carbon emissions scenarios (RCP4.5 and RCP8.5).*

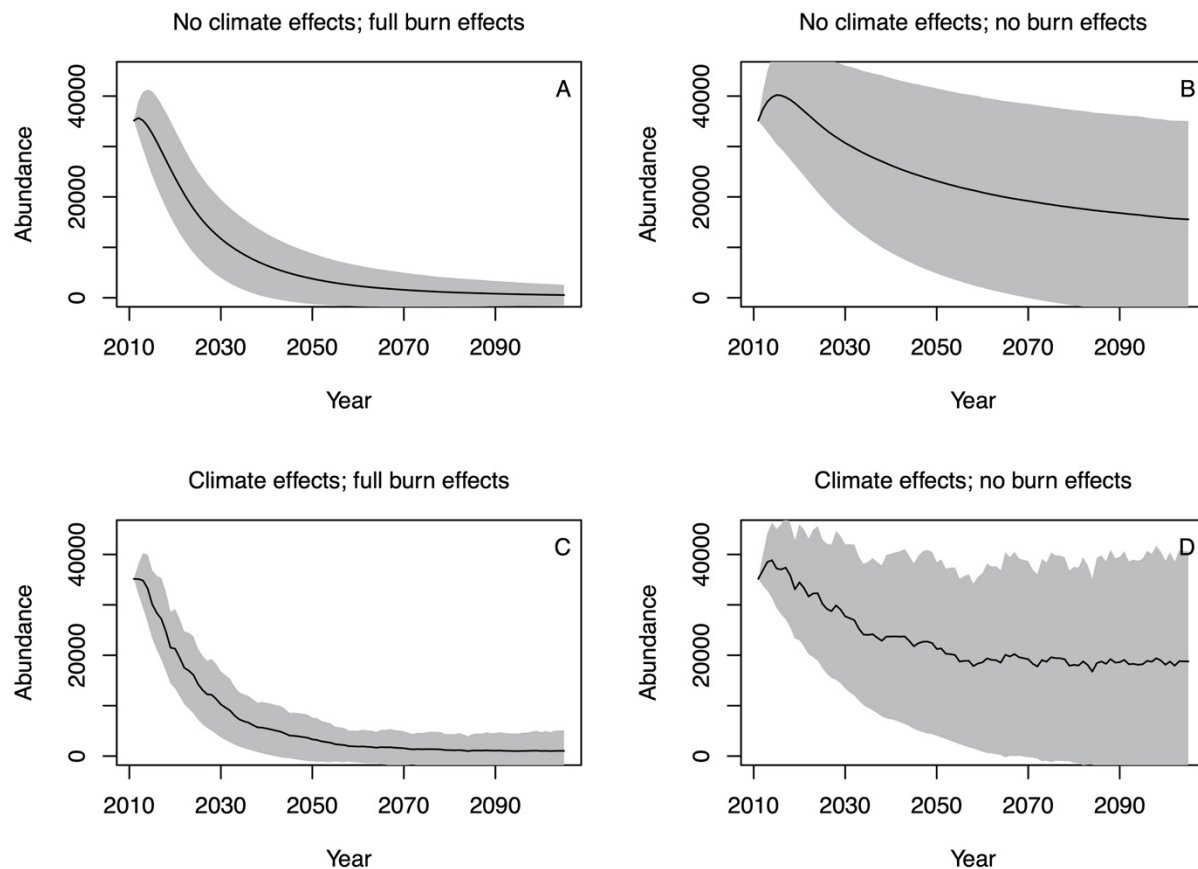


Figure 61. Gopher Tortoise simulated abundance trajectories (sum across all age classes) illustrating the effects of climate change by comparing models where vital rates are affected by a changing climate (“Climate effects”) to models where no vital rates are affected by climate (“No climate effects”). Model results are shown under the best-case burn scenario (right-hand panels; no effect of years-since-burn on survival) across all 8 climate models, as well as the worst-case burn scenario (full-magnitude, climate-responsive burn frequency affecting both juvenile and adult survival). Dark lines denote mean abundance across scenarios, while gray polygons reflect one standard deviation above and below the mean. Displayed simulations reflect a single climate model (GCM: CESM1-BGC; scenario; RCP8.5).

Spatial patterns in final abundance

As noted previously, burn history and the effect of climate change on burn frequency had the strongest impact on simulated Gopher Tortoise populations. Here we present three visual comparisons of spatial patterns in final abundance to highlight these effects, including (1) a comparison of a scenario with burn frequency increasing over time due to climate warming vs. a scenario with no climate effect on fire frequency (**Figure 61A**), (2) a comparison of a scenario with burn frequency affecting survival rates according to Hunter and Rostal (2021) vs. a scenario with the fire-effect coefficient reduced by 50%, and (3) a comparison of a “worst-case” scenario (full-magnitude, climate-responsive burn effect on juvenile and adult survival rates) with a “best-case” scenario (no effect of burn history on survival rates).

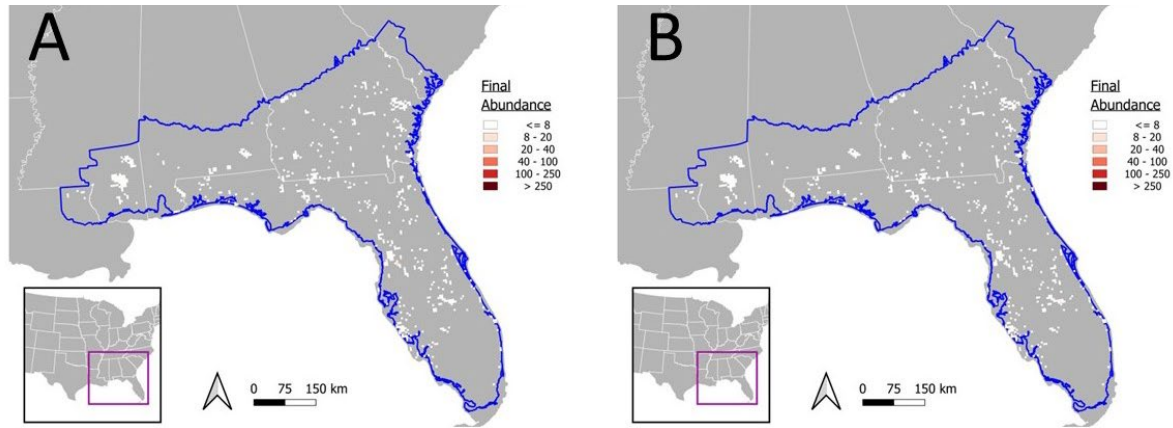


Figure 62. Spatial patterns of final abundance (year 2099) for Gopher Tortoise simulations with full-magnitude burn effect scenarios, comparing (A) burn frequency decreasing with warmer climate and (B) burn interval remaining constant over time at an average of one burn every four years. In these scenarios, burn frequency affects both adult and juvenile survival rates.

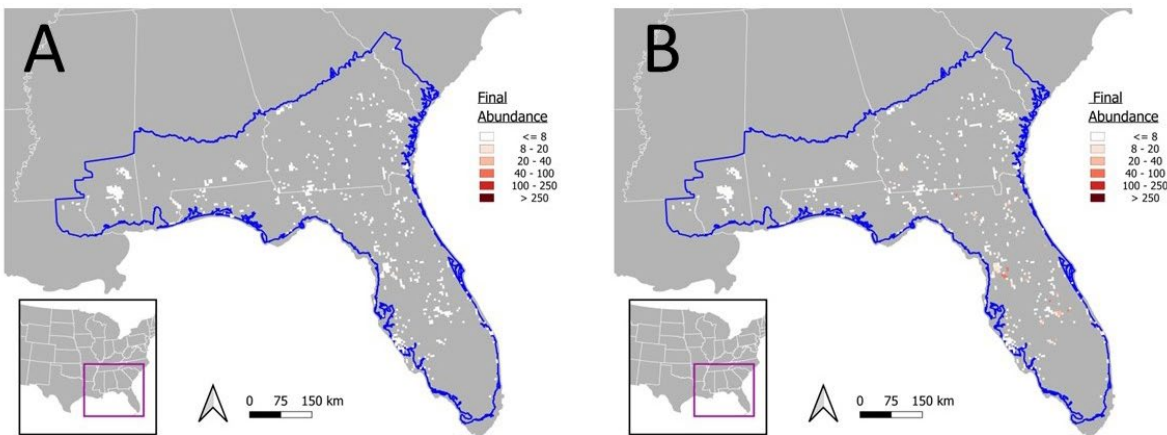


Figure 63. Spatial patterns of final abundance (year 2099) for Gopher Tortoise simulations with burn frequency decreasing with warmer climate, comparing (A) full-magnitude effect of fire history on survival rates and (B) the survival effects of fire history reduced to half the magnitude reported in Hunter and Rostal (2021). In these scenarios, burn frequency affects both adult and juvenile survival rates.

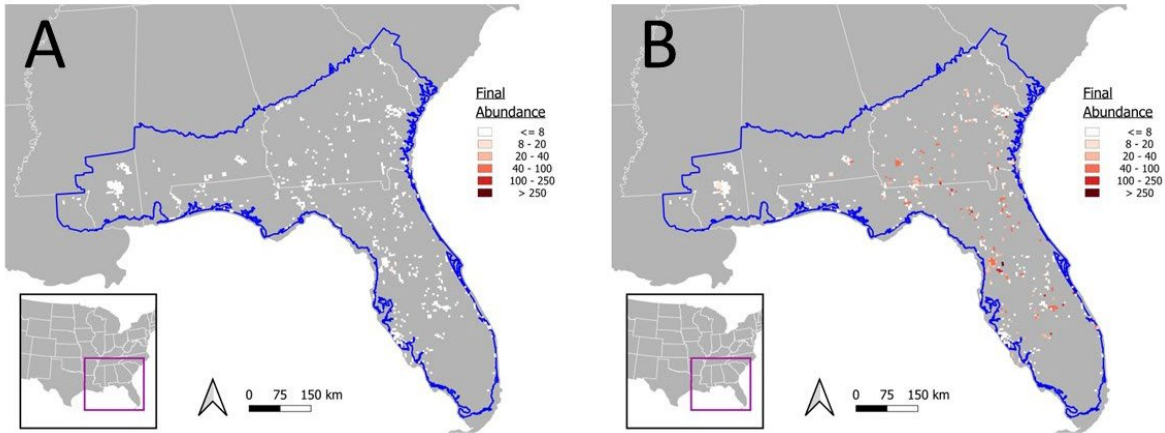


Figure 64. Spatial patterns of final abundance (year 2099) for Gopher Tortoise simulations, comparing (A) a “worst-case” scenario in which juvenile and adult survival rates are affected by burn history (Hunter and Rostal 2021), and with burn frequency decreasing with climate change and (B) a “best-case” scenario in which juvenile and adult survival rates are unaffected by site-level burn history.

Critical habitat for Gopher Tortoise

In general, our model results suggests that Gopher Tortoise populations are expected to decline substantially across much of its range, although this decline does not seem to be a direct response to climate change. Some isolated populations (pixels) throughout the range display final abundances near to initial population values over the course of the simulation, although no pixel in our model was expected to increase in abundance over the course of the simulation under scenarios in which survival was a function of burn history. Furthermore, pixels forecast to be more sustainable than others in our model likely represent areas with high quality habitat (per Crawford et al. 2020) but whose initial population was low on the basis of our “spreading” algorithm (using Folt et al. 2022 as a primary information source for population sizes). Nonetheless, these subpopulations flagged in our models as “near-sustainable” habitat areas should be investigated for their potential as translocation sites for release efforts, especially in western and northern parts of the species range, where populations appear to be particularly endangered.

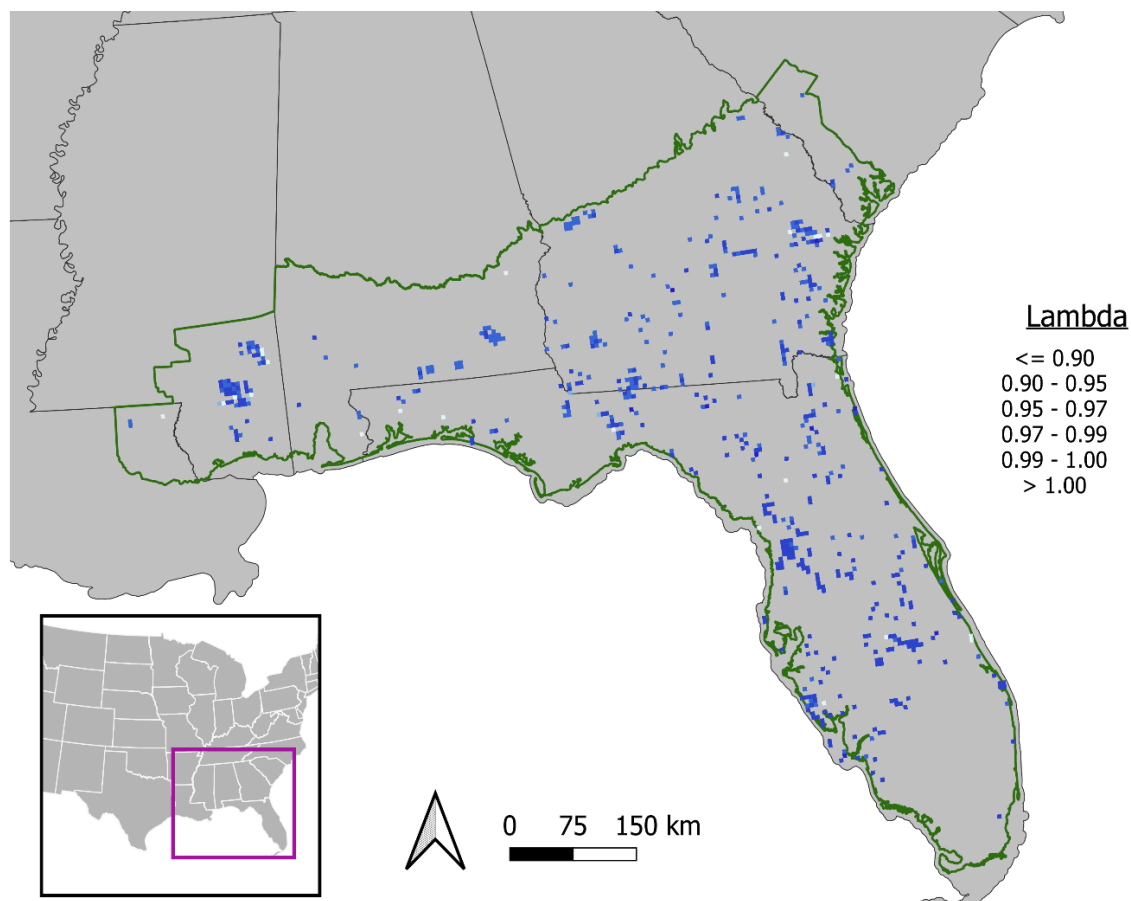


Figure 65. Map of critical habitat for Gopher Tortoise (defined as areas that are expected to sustain Gopher Tortoise populations over time in the face of climate change), assuming juvenile and adult survival decline with increasing time since burn (climate scenario CESM1-BGC 4.5). Green pixels indicate areas likely to support stable or growing Gopher Tortoise populations ($\text{Lambda} \geq 1$; note that no pixels meet this condition in this scenario). Shades of blue indicate that a population decline is expected to occur in a pixel, with darker shades indicating weaker declines (Lambda approaching 1) and lighter shades indicating that the pixel has limited ability to support Gopher Tortoises ($\text{Lambda} \ll 1$).

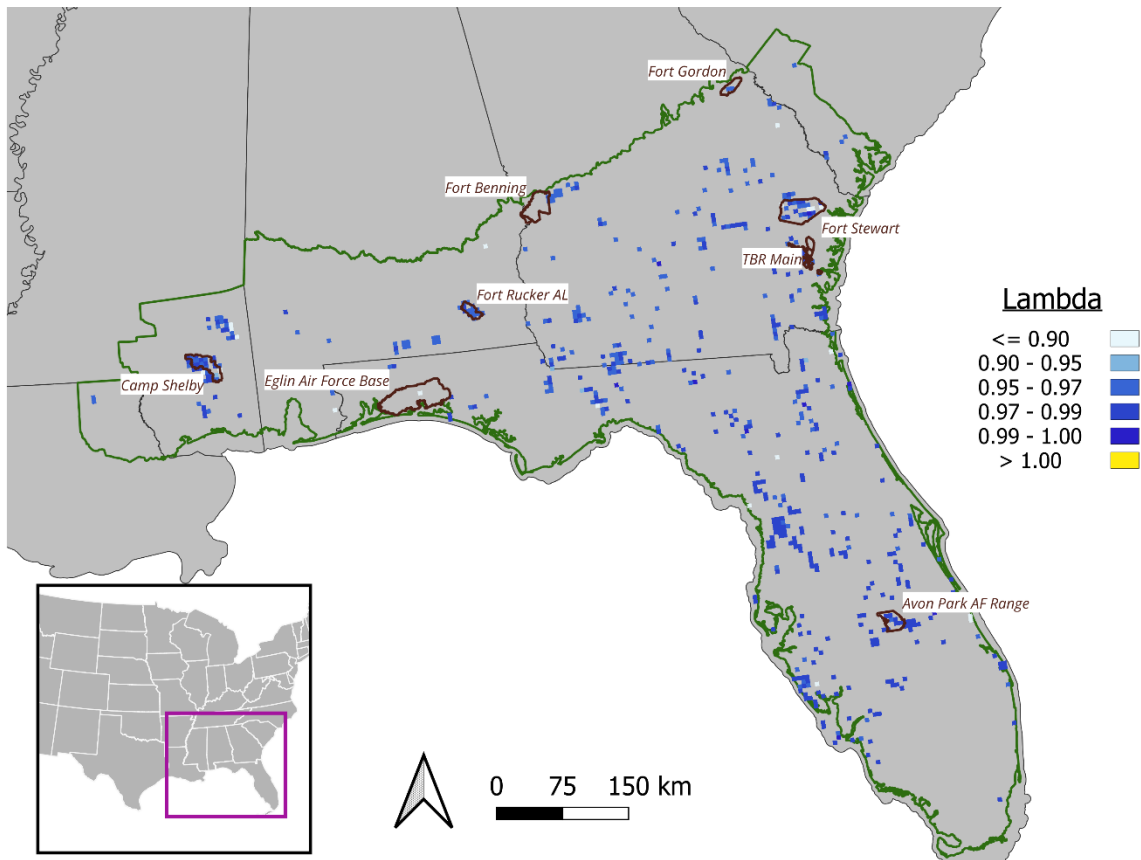


Figure 66. Map of critical habitat for Gopher Tortoise with DoD site overlay (defined as areas that are expected to sustain Gopher Tortoise populations over time in the face of climate change). Simulations depicted here assume juvenile and adult survival decline with increasing time since burn (climate scenario CESM1-BGC 4.5). Green pixels indicate areas likely to support stable or growing Gopher Tortoise populations ($\text{Lambda} \geq 1$; note that no pixels meet this condition in this scenario). Brown polygons indicate Department of Defense installations within the range of the Gopher Tortoise.

Analysis of simulation results (Task 3c)

To better understand how alternative plausible model specifications (representations of reality) affected tortoise population dynamics, we used multivariate machine learning methods to explore how these parameter settings affected final tortoise abundance at the year 2100. We first ran conditional inference trees (a class of classification and regression trees; CART) using the ‘party’ package in R (Hothorn et al. 2006) to visualize how the ‘climate effect activation’ terms (on-off switches) interacted to determine key response variables (notably, final simulated abundance) in our models. We also used conditional inference trees to visualize how the model parameters themselves interacted to determine final abundance. We then used a Random Forest algorithm implemented in the ‘ranger’ package in R (Wright and Ziegler 2017) to explore relationships between scenario settings, vital rates, and the magnitudes of climate relationships in determining response variables (e.g., final abundance, final age ratio). We assessed the relative importance of each of the predictor variables as the loss in out-of-bag predictive performance

after scrambling (permuting) the values of each predictor variable in turn (Breiman, 2001). We visualized the potentially non-linear univariate relationships between these variables and final tortoise abundance using partial dependence plots (making predictions from the model, holding all other covariates at their mean value). We then used a *post hoc* procedure described by Elith et al. (2008) to identify potentially important interaction terms: briefly, for every possible two-way interaction, we (1) used the random forest model to make predictions of final tortoise abundance across a 2-dimensional array (10x10) ranging from the lowest to highest observed values for each covariate (all other covariates held at their mean values). (2) we then fit a linear model of the predicted tortoise densities from step 2 as an additive function of the two covariates, with each variable treated as a categorical variable with 10 levels, and (3) we interpreted the residuals of the additive model fit in step 2 (degree to which the fully additive model was inadequate) as a measure of relative strength of interaction. The strongest interactions were plotted as heatmaps (for two quantitative covariates) or covariance or interaction plots when one variable was categorical (one regression line per category).

Desert tortoise results

The most important determinant of final simulated Desert Tortoise abundance was the baseline juvenile survival rate (PHI_JUV), where higher rates yielded more favorable population outcomes. Climate-change induced effects on Desert Tortoise growth rates vary from positive to negative in terms of their impact on final simulated abundance. For example, climate effects on the sex ratio are expected to have weakly positive effects on Desert Tortoise abundance, averaged across the species range, whereas climate effects on hatching success are likely to have negative effects. Many of these effects of climate change effectively cancel each other out, resulting in fairly stable abundance over time.

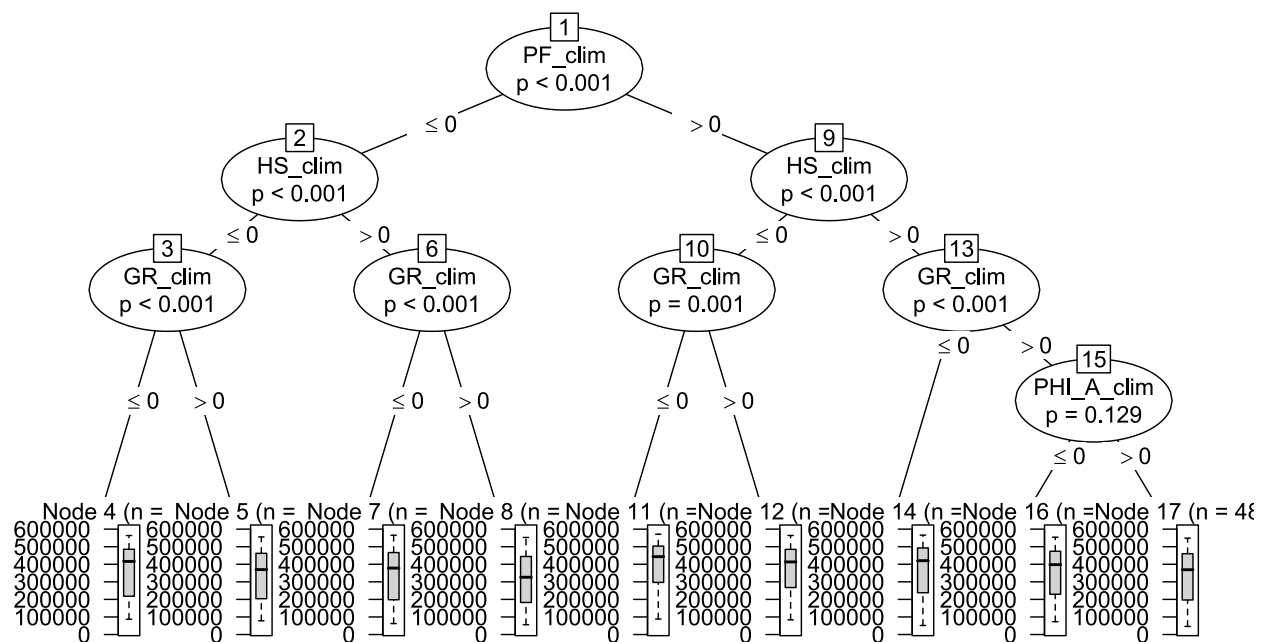


Figure 67. Conditional inference tree showing the impact of climate effects on particular vital rates as determinants of simulated Desert Tortoise final abundance in our population model.

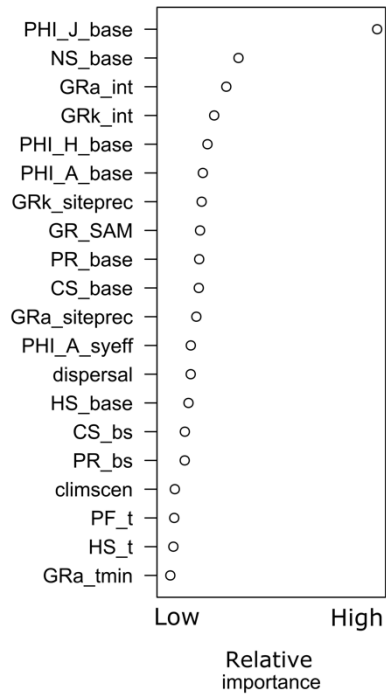


Figure 68. Relative importance of key simulation parameters in determining final simulated Desert Tortoise abundance. The top variables were baseline juvenile survival, baseline nest survival, and baseline growth parameters.

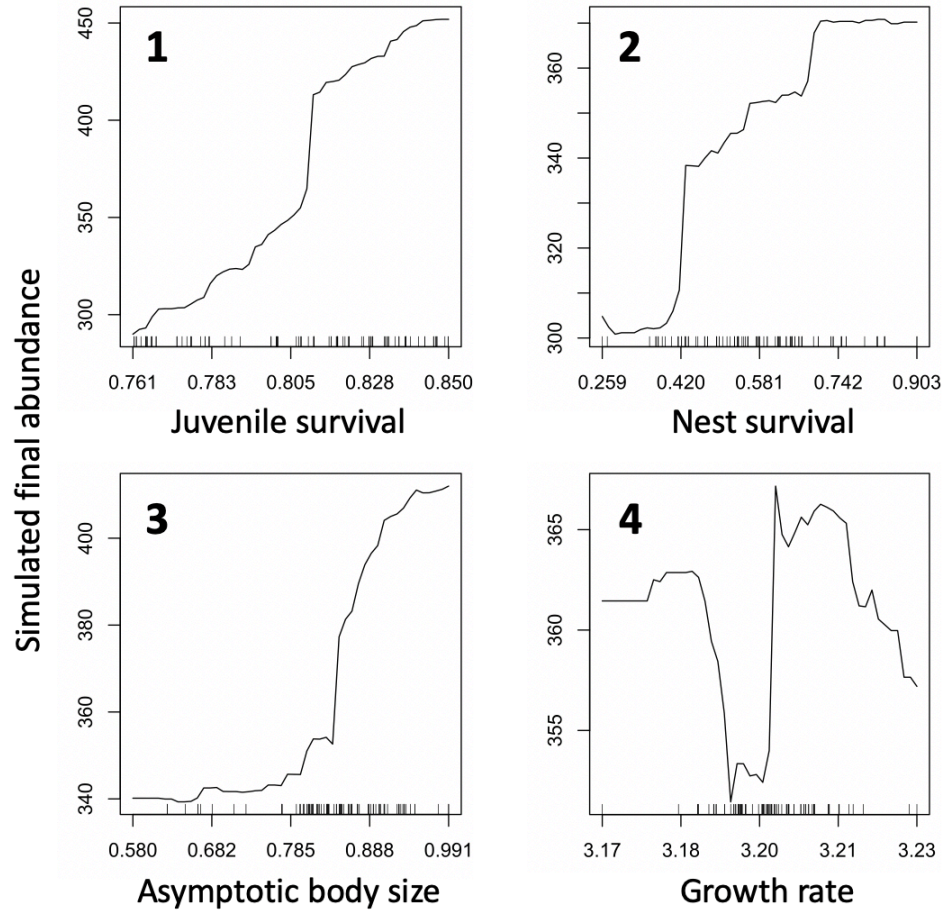


Figure 69. Univariate partial dependence plots illustrating the four most important determinants of simulated final abundance for Desert Tortoises. Plots were produced using a random forest machine-learning algorithm fit to the results from all simulation replicates. Panel numbers represent the relative importance of each parameter as a determinant of final abundance.

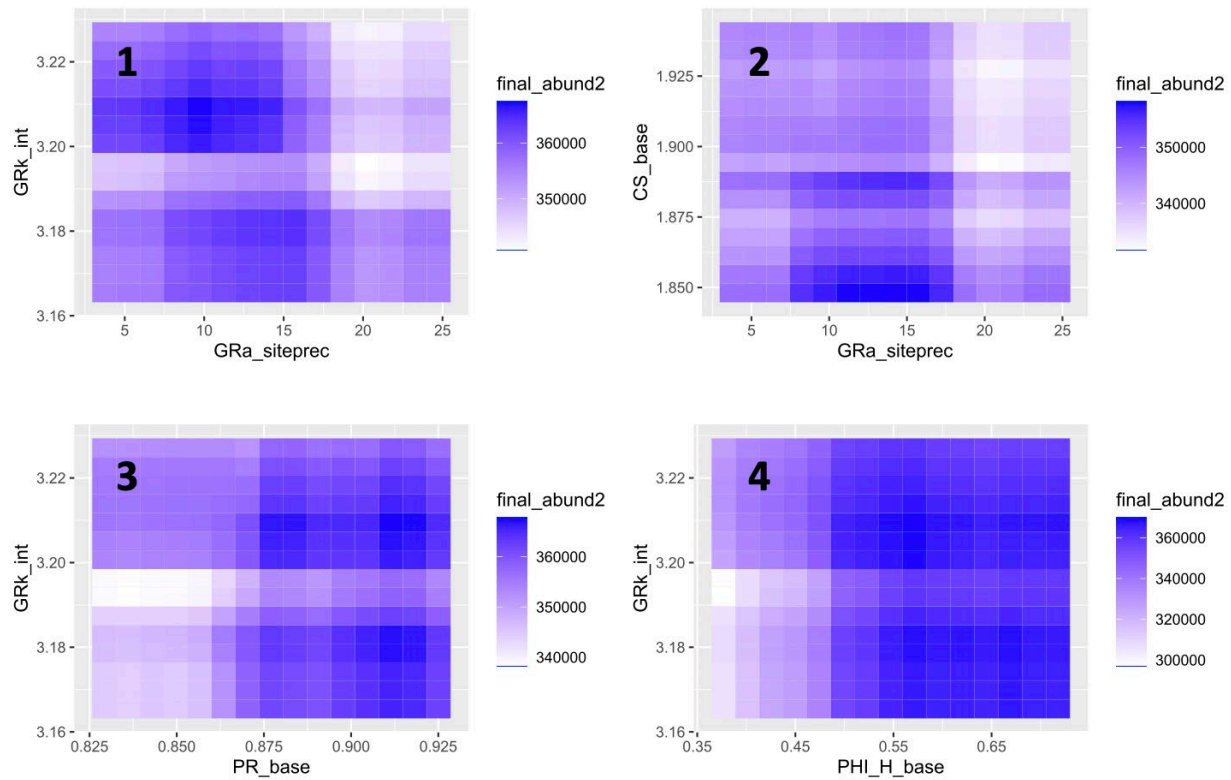


Figure 70. Bivariate partial dependence plots illustrating the four most important bivariate interactions determining simulated final abundance for Desert Tortoises. Plots produced using a random forest machine-learning algorithm fitted to the results from all simulation replicates. Panel numbers represent the relative importance of each interaction.

Gopher Tortoise results

Aside from baseline survival rates (higher survival rates strongly impacted the final results in the Gopher Tortoise model), the most important determinant of final simulated Gopher Tortoise abundance was the effect of prescribed burning on adult and juvenile survival rates, where local fire history (years since the most recent burn) negatively affected survival rates, resulting in less favorable population outcomes for scenarios with stronger burn effects on survival and/or lower burn frequencies; **Figure 71**; **Figure 72**). For this reason, the strongest simulated response of Gopher Tortoise populations to climate change was under scenarios where burn frequency was reduced as a result of increasing average temperatures. We observed high abundance only when parameter sampling resulted in extremely high adult and juvenile survival (**Figure 73**), suggesting either that estimates for these parameters from our data and from the literature are biased low, or that this species is likely to experience strong declines in the near future. Interestingly, we observed several interactions between scenarios and effects (**Figure 74**). In particular, the effects of baseline adult, juvenile, and hatchling survival rates on final abundance were more pronounced under scenarios in which the effect of burning on adult survival was turned off – likely because it was only under those scenarios in which many populations had a chance to grow or become sustainable.

Our results strongly suggest that the recovery of Gopher Tortoise populations across their range will require higher survival rates (most importantly, adult and juvenile survival rates), which is likely reduced relative to historical baseline conditions due to habitat degradation (e.g., fire suppression and other anthropogenic impacts across the southeastern USA) over the last century.

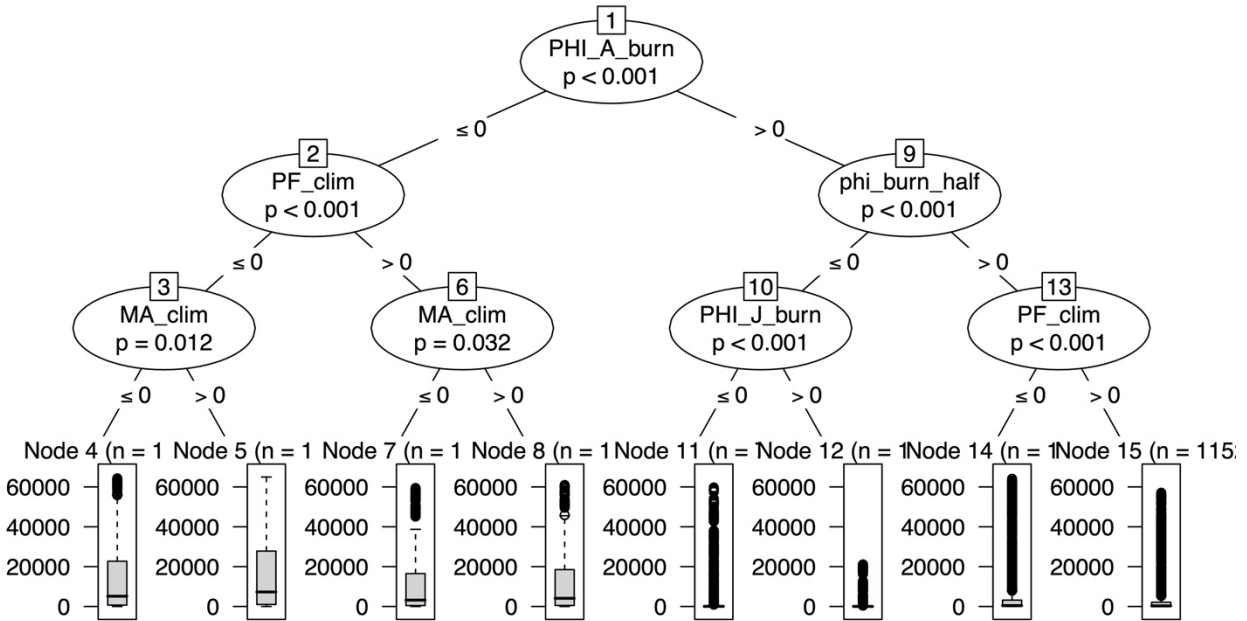


Figure 71. Conditional inference tree (similar to one of the many CART models comprising the random forest model) showing the impact of climate and burn effects on particular vital rates as determinants of simulated Gopher Tortoise final abundance. The highest final abundance is expected for scenarios with no effect of burn frequency on survival, and early age-at-maturity (earlier age at which survival jumps to adult levels).

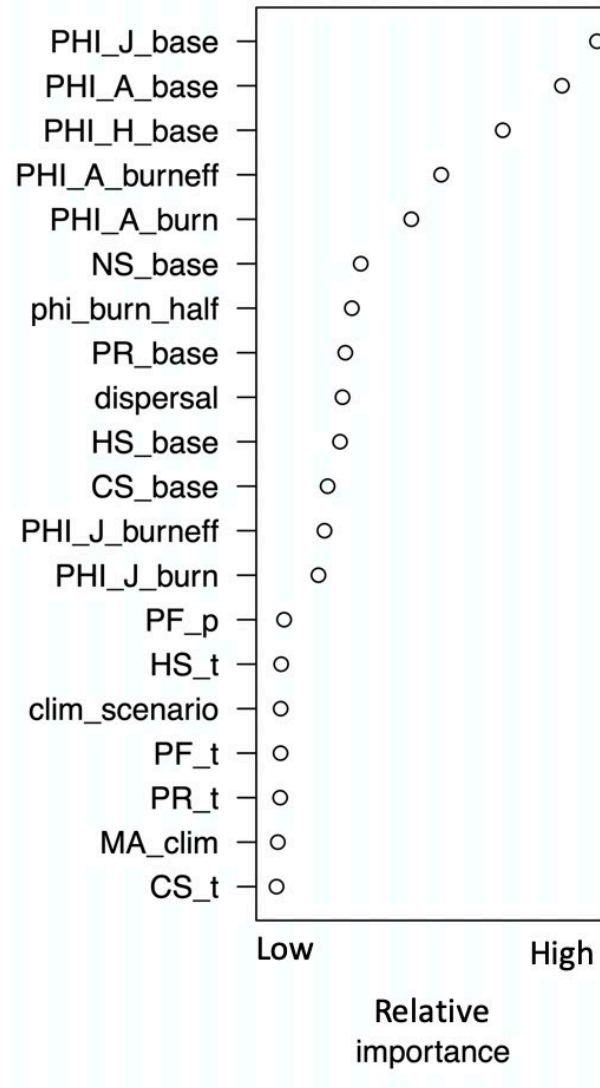


Figure 72. Relative importance of key simulation parameters in determining final simulated Gopher Tortoise abundance. The top four variables were: baseline juvenile survival, baseline adult survival, baseline hatchling survival, and the effect of fire history on adult survival.

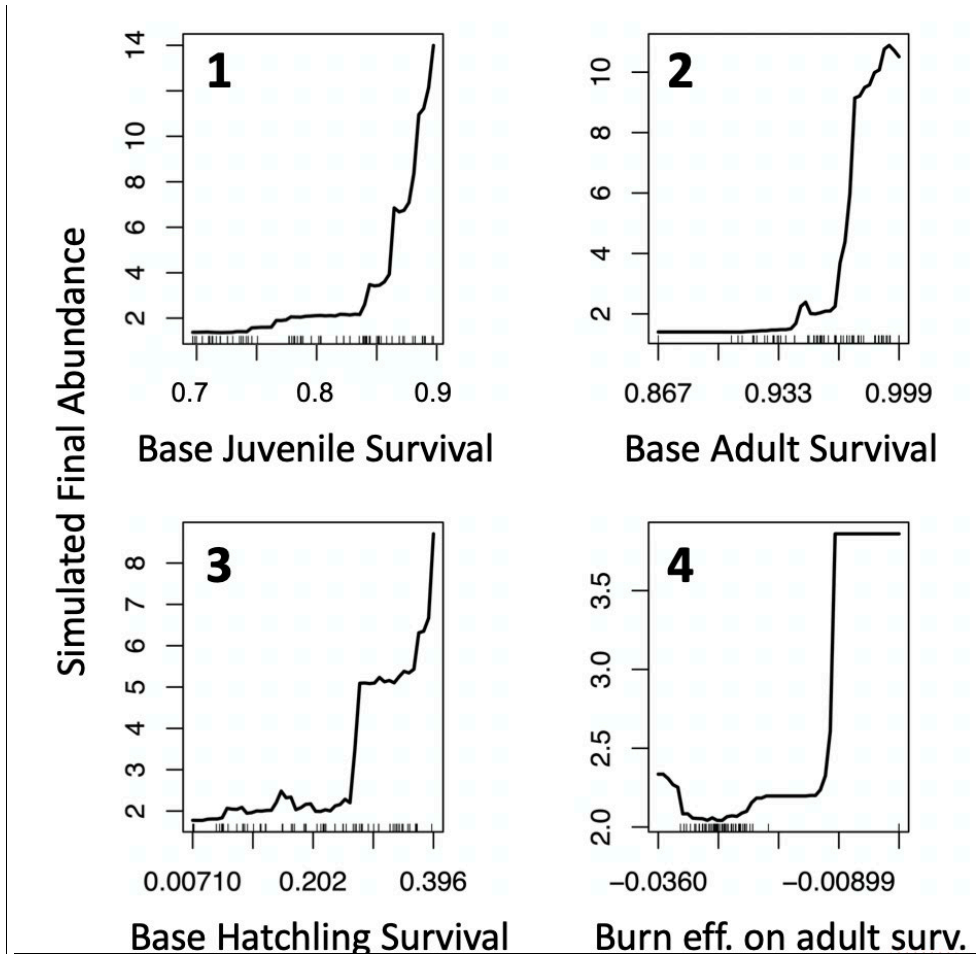


Figure 73. Univariate partial dependence plots illustrating the four most important determinants of simulated final abundance for Gopher Tortoises. Plots produced using a random forest machine-learning algorithm fitted to the results from all simulation replicates. Numbers represent the relative importance of the parameter as a determinant of final abundance.

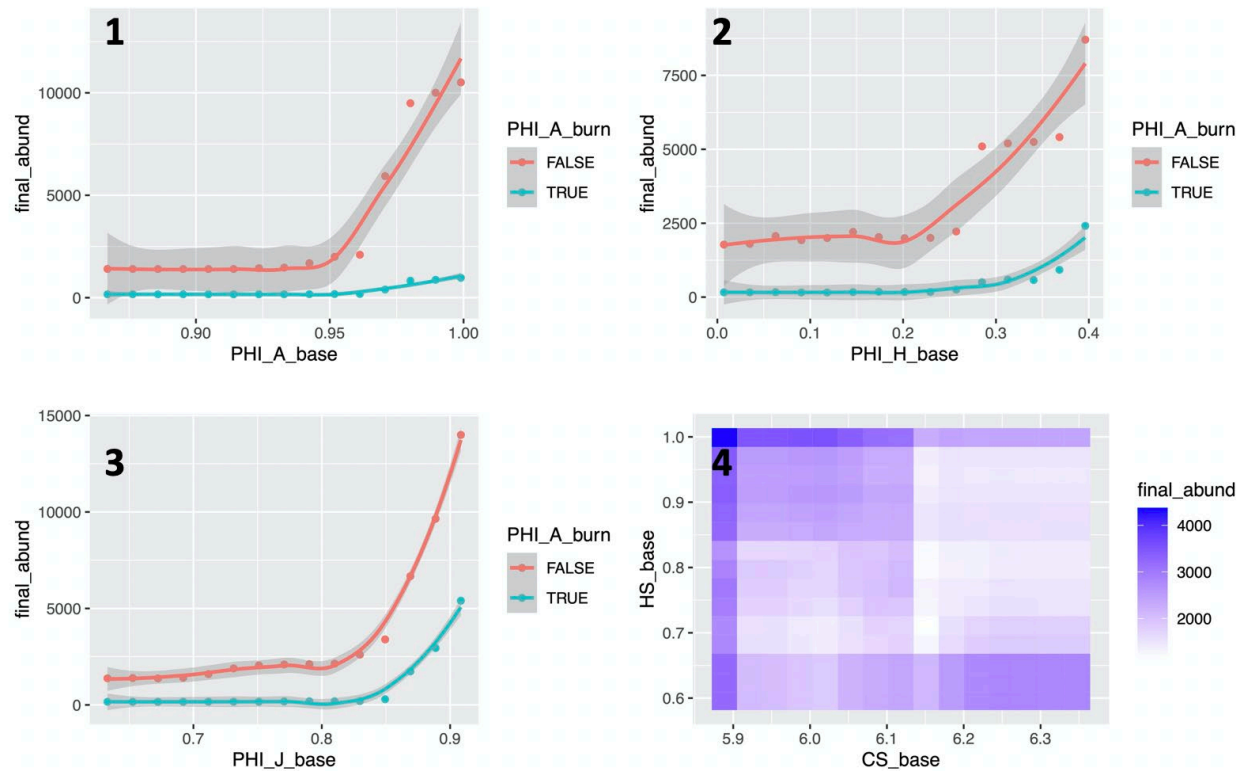


Figure 74. Partial dependence plots illustrating the four most important bivariate interactions determining simulated final abundance for Gopher Tortoises. Plots produced using a random forest machine-learning algorithm fitted to the results from all simulation replicates. Heat maps are used to visualize interactions between quantitative parameters (plot 4), whereas interaction plots are used when one of the variables is categorical. Panel numbers represent the relative importance of the interaction.

Conservation planning tool (Task 4)

We have developed and deployed a web-based application (*MITCH-Clim 0.1: Model-based Insights into Tortoise Critical Habitat Under Climate Change*) that enables users to visualize the expected impact of climate change on Desert Tortoise populations for a selected region of interest. The tool is able to access pre-run population simulation results across the range of the Desert Tortoise, including 20 replicates of all 256 scenarios. Users first select a scenario (i.e., which vital rates to model as functions of climate, which carbon emission scenario to use) and a region of interest (users can draw a polygon of interest on an interactive map, select from a list of pre-loaded polygons representing DoD installations, or upload a polygon from an ESRI shapefile). After making these selections, users are presented with several visualizations of tortoise population growth and critical habitat for the chosen region of interest, including (1) maps of abundance for any selected simulation year, (2) total abundance over time, and (3) age-structured abundance over time. Users can also compare abundance trajectories for multiple selected scenarios for their selected region of interest. The tool was created in the reactive programming environment, R-Shiny (Chang et al. 2022).

This application therefore enables a streamlined conservation planning workflow whereby users can identify regions of interest (e.g., DoD installations), and visualize/download maps for these regions of interest, including current and future (projected) population growth and viability under a number of plausible climate change scenarios. The tool enables users to identify land areas within their specified region of interest that are most likely to support viable populations across the widest possible range of present and future climate variation, while accounting for multiple sources of uncertainty. With this tool, it is relatively straightforward to rank proposed conservation areas and management/mitigation actions (e.g., altering fire regimes for Gopher Tortoises) according to their capacity to support viable tortoise populations under variable and uncertain climate conditions. Because the tool allows managers to assess the habitat present at their installation in context with habitat in other parts of the species' range, it allows for transparent inter-agency collaborative efforts to adaptively plan Desert Tortoise conservation actions. This tool could therefore prove valuable to managers across multiple public agencies as they work towards species recovery for *G. agassizii*.

Although models of land-use change are not yet incorporated into our simulation modeling framework, it would be a relatively straightforward task to incorporate land-used change scenarios (and other potential drivers of population dynamics) into our existing simulation models, which could be included in subsequent versions of the web application. Currently the tool is only capable of visualizing model results across for *G. agassizii*, but a similar application may be developed for *G. polyphemus* if the need arises. Finally, it will be straightforward to update the tool with new results as additional data (in the form of tortoise locations or updated climate layers) become available.

The web-based application can be found at https://kevintshoemaker.shinyapps.io/Mitch-clim_v01/. See Appendix C for a more detailed description of the application.

Conclusions and Implications for Future Research

We were able to accomplish most of the original objectives stated in our original project proposal. We identified numerous demographic parameters for both species that were sensitive to spatiotemporal climate variation, and quantified the magnitude of these effects (Task 1). In general, we found stronger climate effects on aspects of fecundity than survival, with probability of reproduction, egg production, hatching success, and hatchling sex ratios influenced by both temperature and precipitation (Hypothesis 1). While testing the role of local adaptation in driving apparent climate resilience (Task 2) proved difficult, the evidence we uncovered tended to indicate that local adaptations are unlikely to play a major role in preventing climate adaptation in either species (Hypothesis 2). A more detailed discussion of the potential correlations between genetic variability and climate variability across the range of the Desert Tortoise can be found in the final report for RC18-1207. In Task 3, we incorporated climate-driven, spatiotemporally variable demographic rates into comprehensive population simulation models for Desert and Gopher Tortoises. In Task 4, we created and deployed a web-based application enabling users to use our simulation results to gain insights into Desert Tortoise habitat and population viability for selected regions of interest.

Our models indicated that most Gopher Tortoise populations are likely to decline over time. Habitat effects had much greater influence on population viability than direct climate effects for Gopher Tortoise – in particular, the impact of prescribed fire (reduced survival under less frequent prescribed burns) was a primary driver of Gopher Tortoise population decline in our models. With prescribed burning frequencies likely to be further reduced under climate change, climate change could have highly adverse indirect consequences for Gopher Tortoise populations. The declines predicted by our models are generally more severe than the declines predicted by a recently published Gopher Tortoise population projection model (Folt et al. 2022), underscoring the importance of further research investigating how prescribed burning influences adult and juvenile survival in the southeastern USA.

Compared to the model predictions for Gopher Tortoise, Desert Tortoise populations were more likely to be self-sustaining throughout much of their range according to our models (Hypotheses 3 and 4; note that our models were focused on climate change, and did not directly consider other anthropogenic threats such as land conversion, increased raven predation, or disease).

Furthermore, the positive effects of climate change on certain demographic vital rates may counter some of the negative effects; for example, higher temperatures may reduce the age at maturity and increase the proportion of hatchlings born female (both positive population-level effects), countering the negative effect of increasing temperatures on hatching success (compensatory modulation; Hypothesis 1). However, there could be negative effects of highly skewed sex ratios (up to 80% female) that we do not model (such as mating failures). Task 4 (building a conservation planning tool for each species using the outputs from our simulation models) has recently been completed, and the latest version is available at https://kevintshoemaker.shinyapps.io/Mitch-clim_v01/.

Uncertainty and limitations

As with any large-scale simulation modeling study, our work is necessarily accompanied by many important limitations and sources of uncertainty. For the Desert Tortoise, the most

important limitation of this study is that our model (which focused on climate change specifically) did not account for major threats to Desert Tortoise populations, such as expansion of renewable energy development, increasing densities of ravens (*Corvus corax*), fragmentation due to roads, and other forms of habitat disturbance and degradation. Future versions of our simulation models could incorporate expected land-use change scenarios, resulting in estimates of the relative impacts of land-use vs climate change for both species, and enabling identification of key habitat areas that should be conserved to maintain viable metapopulations. Because our simulation models are built to handle spatially explicit information in the form of multi-band raster images (one band per simulation year), it will be straightforward to incorporate additional raster time-series information to these models. Nonetheless, a considerable amount of work would still be required to incorporate these additional threats to Desert Tortoise populations, with proper error checking and validation.

Another important limitation of our Desert Tortoise model is that we did not use several sources of data that are now available for fitting one of the most important parameters in the model; adult survival. In this study, we used capture recapture data from the “permanent study plots” as well as data from several long-term telemetry sites. While this is a large and robust dataset, there are two important limitations to these data: (1) these data are strongly biased geographically, with very little data available from the Western Mojave region in California, and (2) these data are old, with much of the data collected prior to 2000. Additional sources of data (including telemetry records and more recent capture-mark-recapture data) have since become available, largely due to recent work by Conservation Science Partners (CSP). I’m currently working with CSP to refine our model of adult survival model using additional long-term capture recapture and telemetry data, which mitigate the two gaps identified above. Preliminary findings with these additional data sources continue to indicate that precipitation in the several years prior to a given year is a strong predictor of annual survival. We will incorporate the updated survival model before publishing our Desert Tortoise modeling work.

Another notable limitation related to our Desert Tortoise model was the lack of reliable precipitation forecasts. Desert Tortoise populations are more sensitive to precipitation than temperature. However, the precipitation predictions available to us from downscaled General Circulation Models (GCMs) were highly variable from year to year, resembling white noise with little or no trend over time. The lack of reliable forecasts of precipitation trends may mask real threats to Desert Tortoise populations – for example, from extended droughts that are difficult to predict. To address this limitation, future scenario tests using this model should include hypothetical scenarios of increased drought frequency in addition to climate scenarios derived directly from GCMs.

One important limitation for both our Desert Tortoise and Gopher Tortoise models was our use of a relatively simplistic model of dispersal (the process by which individuals can move from one grid cell to another). Currently, our model only enables movement to adjacent grid cells (but is easily modified to enable dispersal across multiple grid cells). Even more importantly, our model does not account for barriers to dispersal such as roads, train tracks, and rivers. Neither does our dispersal model account for habitat factors that may impede or facilitate dispersal. With concerns about connectivity playing a key role in management decisions for both species, it will be valuable to incorporate a more detailed, evidence-based model of connectivity in our

simulation framework. We are currently working on developing ways to incorporate more realistic dispersal processes into our simulation models.

Important limitations relating to our Gopher Tortoise population model include (1) unexpected difficulties in identifying hatchling sex, and (2) lack of rangewide data relating fire frequency to survival. One ongoing component of this project is the development of a reliable sexing protocol for gopher tortoise hatchlings. Unfortunately, the hormone-based assays we developed proved to be only weakly indicative of hatchling sex, preventing us from accurately assessing sex from blood samples collected in the field. We're continuing to work on developing more effective sexing methods—for example, based on DNA methylation in red blood cells which will hopefully allow accurate sexing from a single blood sample.

For the Gopher Tortoise, the most important factor for determining population viability was whether or not adult and juvenile survival rates were dependent on the local fire history within each pixel. The data we used in this study to show that survival rates were dependent on fire history was based entirely on data from Fort Stewart in Georgia. With this burn effect included in our models, populations tended to decline steeply. Clearly, it is critically important to continue investigating the relationship between fire frequency and survival for Gopher Tortoises at other sites across the species' range.

Task 1 – Environmental sensitivities of population vital rates

We hypothesized (Hypothesis 1) that Mojave Desert and Gopher Tortoises would exhibit vital rates favorable to population growth across a wide range of environmental and climatic conditions. This was generally supported by results of Task 1, though the negative effects of warming temperatures on hatching success in Desert Tortoises, and the negative effects of warming on controlled burn frequency, and thus adult survival, in Gopher Tortoises, suggest there are limits to the range of climatic conditions that can support populations.

Desert Tortoise:

We were able to use a combination of existing and newly collected data to detect and quantify linkages between climate and demographic vital rates for Desert Tortoise. Overall, we fit models relating six Desert Tortoise vital rates to climate variation: clutch size, probability of reproduction, hatching success, sex ratio, age-at-maturity (via a model of somatic growth) and adult survival.

Adult Desert Tortoise survival was influenced by average precipitation (30-year normal), with higher precipitation resulting in higher mean survival rates (likely a consequence of increased hydration opportunities and forage production). Because our current model is fitted exclusively using data from the “permanent study plots”, which were mostly surveyed from 1980-2000, future work to incorporate more recent data sources (radiotelemetry records) into these models would be valuable, and may allow quantification of the impacts of severe droughts on desert tortoise survival. We are currently working with colleagues at Conservation Science Partners (CSP) to run our models of adult survival with additional sources of data, including multiple translocation sites across the species range.

We observed a positive relationship between prior-year precipitation and total reproductive output for Mojave Desert Tortoise. Both clutch size and probability of reproduction were

positively influenced by prior-year precipitation. This relationship was expected, as total precipitation is known to be correlated with the amount of vegetation resources available to Mojave Desert Tortoises. As expected, body size (mean carapace length) also had a strong impact on the number of eggs produced for Mojave Desert Tortoise. We also detected a positive relationship between egg production and spring temperatures, likely due to increased ability to produce more than one clutch with longer activity seasons.

Based on the results of our nesting surveys, we found that nest temperature (itself a function of air temperature) was a strong predictor of both hatching success and the fraction of hatchlings born female. Warmer nest temperatures tended to decrease hatching success, likely due to some eggs experiencing excessively hot temperatures. As expected, warmer temperatures resulted in increasingly female-biased sex ratios among successfully hatched eggs.

Growth rates and mean body size can have strong consequences for Desert Tortoise population growth by affecting the age at first reproduction, and by influencing key demographic rates like clutch size. We modeled mean somatic growth rates and body size in Desert Tortoise populations using a von Bertalanffy growth model in which the parameters (growth rate and asymptotic body size) were allowed to vary across spatiotemporal climate gradients. We found that growth rates increased (and age-at-maturity was reduced) for populations experiencing warmer temperatures and higher precipitation, providing a potential pathway for increased population viability under climate change. However, asymptotic body size was reduced under warmer temperatures, which is likely to result in lower clutch sizes, thereby negatively impacting population growth. By incorporating these relationships into our Desert Tortoise simulation model (Task 3), we were able to assess the holistic, population-level effects of these climate-vital rate relationships.

Gopher Tortoise:

Adult Gopher Tortoise survival and movement rates were primarily influenced by prescribed burning, and not by climate. How prescribed burning influences these vital rates is dependent on habitat condition. However, our models were not able to distinguish mortality from emigration, and identifying whether prescribed burning (or lack thereof) results in direct mortality should be a future research direction.

Both probability of reproduction and egg production (clutch size) were influenced by climate change in a population in the northern portion of the species' range, with warmer conditions in the prior spring and summer resulting in increased reproduction and egg production. Over a 25-year period of warming, these effects have resulted in an increase in average clutch size of approximately 1 egg (15% increase over a mean of 6.5 eggs). Whether this pattern only occurs in the northern portion of the species' range is unclear, and future research on climate effects on these vital rates in other parts of the species' range (particularly western and southern) would be valuable.

The effects of climate on hatching success varied across sites within the species' range. Some sites had strong nest temperature effects on hatching success, but others did not, indicating that drivers of hatching success may be site specific and dependent on other non-measured variables, such as soil type and moisture, and genetic conditions of a population. We did find that nest temperatures fell within a fairly narrow range, and therefore female Gopher Tortoises may be able to select nest sites that will result in high hatching success, no matter the conditions.

Hatchling sex ratios responded to both mean nest temperature and precipitation during the critical period; warmer temperatures and less precipitation produced more females. These findings indicate that wild nests are likely to experience sex ratio shifts in response to a changing climate. The statistical associations were strong, but there remains much variation in sex ratio that could be explained by complex interactions of temperature, precipitation, soil properties and genetic background.

Task 2 – Test for local adaptation

We hypothesized (Hypothesis 2) that Mojave Desert and Gopher Tortoise vital rates would exhibit minimal evidence for local adaptation. We were only able to test this in limited capacity for Desert Tortoises due to data limitations, finding no strong evidence that local adaptation may prevent or impede climate adaptation *in situ*. For Gopher Tortoise, we found subtle evidence for local adaptation (or at least, lack of plasticity) in Gopher Tortoise hatching success rates. However, these effects seem unlikely to reduce population resilience to climate change.

Desert tortoise:

Our analysis of the effect of translocation status on Desert Tortoise growth rates showed a change in climate from origin to translocation site affected how tortoises grew, although it may also indicate that tortoises from different climates than the translocation site simply have larger asymptotic sizes. In addition, we did not detect any difference in hatching success or sex ratio for nests deposited by translocated vs. resident Desert Tortoises. Nonetheless, our conclusions from this analysis are limited, and there remains a need for more research into how the effects of local adaptation could impede climate resilience. We did not incorporate any effects of local adaptation in our critical habitat breadth model for Desert Tortoise.

Gopher Tortoise:

We did not find evidence for local adaptation in Gopher Tortoise probability of reproduction, clutch sizes, or egg mass. Females had similar reproductive output no matter how far the environmental or geographic distance between their site of origin and the translocation site. We did find evidence for either local adaptation or lack of plasticity in nest site selection behaviors and hatching success. Tortoises that originated from sites with substantially different climates compared to the translocation site placed their nests in different locations and had different hatching success rates. However, the direction of these effects was complex, and remains challenging to interpret. For some measures of environmental distance between the origin and translocation sites, hatching success was lower for tortoises experiencing greater magnitude of climate change; whereas for other measures, hatching success was higher. These results indicate a changing climate may affect hatching success (and other vital rates dependent on nest conditions and temperatures, such as hatchling sex ratios) in difficult-to-predict ways. We accounted for this uncertainty in some of our scenarios in our critical habitat breadth model for Gopher Tortoise.

Task 3 – Model critical habitat breadth

We hypothesized that *Gopherus* populations (Hypothesis 3) and metapopulations (Hypothesis 4) are resilient to climate change. We found evidence of local resilience in many parts of the Desert Tortoise range, largely supporting our predictions. In contrast, Gopher Tortoise populations

across its range were predicted to decline sharply in total abundance. This effect was exacerbated by the effect of climate on habitat management affecting adult survival, showing lack of climate resilience for Gopher Tortoise and thereby running counter to our original hypothesis.

Desert tortoise:

For Desert Tortoise, simulated populations and metapopulations generally showed fairly strong resilience to climate changes across much of its range under several climate change scenarios (Hypotheses 3 and 4). Furthermore, the positive effects of climate change on certain demographic vital rates effectively countered some of the negative effects; for example, higher temperatures reduced the age-at-maturity and increased the proportion of hatchlings born female (both positive population-level effects), countering the negative effect of increasing temperatures on hatching success. Nonetheless, extreme warming scenarios (RCP 8.5) could result in slight overall population declines and greater loss of habitat throughout the Desert Tortoise range.

It is important to note that our models were focused on climate change, and did not directly consider other key threats to Desert Tortoise populations, including land-use change, increased raven predation, or disease. Nonetheless, the simulation modeling framework we developed has great potential to be expanded to include additional threat processes. Projections of land-use change across the Desert Tortoise range are currently being produced and published as part of a different SERDP-funded project (RC18-1207), and it will be relatively straightforward to integrate these projections in our simulation modeling framework, which is already fully capable of incorporating dynamic changes in space and time. Similarly, models predicting raven populations and the impacts of raven predation on tortoise populations are under development, and we look forward to incorporating this information into future versions of our model. Ultimately, our Desert Tortoise simulation modeling framework should continue to prove a useful and flexible tool for addressing the role of multiple competing threats to Desert Tortoise populations across their range and for testing how these effects may interact with anticipated future changes in climate.

Gopher Tortoise:

Our model of critical habitat breadth produced large declines across most simulation scenarios we ran. The only scenarios that resulted in persistent (albeit largely declining) populations were those in which there were no burning effects on adult or juvenile survival; even cutting the burn effect on survival in half was insufficient to substantially improve the probability of persistence. We were unable to distinguish whether the observed effect of burn history (years since burn) was due to increased mortality or increased long-term emigration. However, we believe it is likely that fire history exerts a negative effect on survival rates, particularly in isolated populations with little adjacent habitat to move into. Therefore, we contend that the population declines indicated by our simulation models (and driven largely by the effect of fire history on survival) are plausible. The predicted declines indicated by our simulation models were even more extreme than those indicated by a recently published Gopher Tortoise population viability assessment (Folt et al. 2022), in part due to improvements in the way we modeled age structure.

Baseline survival rates, especially for adults and juveniles, were highly influential in predicting Gopher Tortoise (and Desert Tortoise) population sustainability in our models. However, the available data for estimating these key demographic parameters is limited. For example, juvenile survival rates are rarely measured for natural populations (although many

estimates exist for head-started individuals), as juveniles are challenging to find in the field. In addition, both juvenile and adult survival rates are often confounded with movement rates (it is not possible to distinguish between mortality and permanent emigration). Future research should focus on gathering better estimates of survival rates, ideally using satellite/GPS telemetry to be able to monitor individuals no matter where they move.

The importance of prescribed burning (reduced habitat quality with time since fire) as a driver of population declines in our model is notable, and should be investigated further. We have no information on how juvenile Gopher Tortoises respond to prescribed fire (we only tested scenarios in which juvenile survival either did not change with fire history, or responded in an identical manner to adults). For adult Gopher Tortoises, testing the effects of prescribed burning across many different habitat types would be beneficial. In the meantime, we recommend that DoD sites should maintain frequent burning regimes in Gopher Tortoise habitats. In addition, we recommend further research into how Gopher Tortoises respond to prescribed fire activities on their sites, as there are likely site-specific population responses.

A warming climate had a slight compensatory effect on other Gopher Tortoise vital rates, but this is likely to be biased due to our use of information from the northern end of the Gopher Tortoise range (Fort Stewart Army Reserve) to fit our models of reproductive output. It is possible there will be different (and possibly detrimental) effects of climate change on aspects of fecundity for sites where climate is already very warm. Future research should focus on gathering fecundity data (monitoring egg production) at sites in the southern portion of the Gopher Tortoise range.

Task 4 – Build a conservation planning tool

This task has recently been completed. The web-based conservation planning application for Desert Tortoise is available at https://kevintshoemaker.shinyapps.io/Mitch-clim_v01/. More details on the application and how to use it are provided in Appendix C. We welcome feedback and suggestions about how to make this tool useful for conservation planning for DoD and other key stakeholders.

Literature Cited

- Agha, M., Augustine, B., Lovich, J. E., Delaney, D., Sinervo, B., Murphy, M. O., ... & Price, S. J. (2015). Using motion-sensor camera technology to infer seasonal activity and thermal niche of the Mojave Desert Tortoise (*Gopherus agassizii*). *Journal of thermal biology*, 49, 119-126.
- Alexander, J. M., Diez, J. M. & Levine, J. M. (2015). Novel competitors shape species' responses to climate change. *Nature* 525, 515–518.
- Allison, L. J., & McLuckie, A. M. (2018). Population trends in Mojave desert tortoises (*Gopherus agassizii*). *Herpetological Conservation and Biology*, 13(2), 433-452.
- Angilletta, Jr, M. J., & Dunham, A. E. (2003). The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *The American Naturalist*, 162(3), 332-342.
- Araújo, M. B., Cabeza, M., Thuiller, W., Hannah, L. & Williams, P. H. (2004). Would climate change drive species out of reserves? An assessment of existing reserve-selection methods. *Glob. Chang. Biol.* 10, 1618–1626.
- Archer, S. R., & Predick, K. I. (2008). Climate change and ecosystems of the southwestern United States. *Rangelands*, 30(3), 23-28.
- Armstrong, D. P., & Brooks, R. J. (2014). Estimating ages of turtles from growth data. *Chelonian Conservation and Biology*, 13(1), 9-15.
- Armstrong, D. P., & Brooks, R. J. (2013). Application of hierarchical biphasic growth models to long-term data for snapping turtles. *Ecological Modelling*, 250, 119-125.
- Ashton, K. G., Burke, R. L., & Layne, J. N. (2007). Geographic variation in body and clutch size of Gopher Tortoises. *Copeia*, 2007(2), 355-363.
- Ashton, K. G., & Feldman, C. R. (2003). Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution*, 57(5), 1151-1163.
- Atkinson D. (1994). Temperature and organism size—a biological law for ectotherms? *Adv Ecol Res* 25:1–58
- Averill-Murray, R. C., Darst, C. R., Strout, N., & Wong, M. (2013). Conserving population linkages for the Mojave Desert Tortoise (*Gopherus agassizii*). *Herpetological Conservation and Biology*, 8(1), 1-15.
- Averill-Murray, R. C., & Hagerty, B. E. (2014). Translocation relative to spatial genetic structure of the Mojave Desert Tortoise, *Gopherus agassizii*. *Chelonian Conservation and Biology*, 13(1), 35-41.
- Bachelet, D., Ferschweiler, K., Sheehan, T., & Strittholt, J. (2016). Climate change effects on southern California deserts. *Journal of Arid Environments*, 127, 17-29.
- Bailey, L. D., & Van De Pol, M. (2016). climwin: An R toolbox for climate window analysis. *PloS one*, 11(12), e0167980.

- Bailey, L. L., Simons, T. R., & Pollock, K. H. (2004). Estimating detection probability parameters for plethodon salamanders using the robust capture-recapture design. *The Journal of Wildlife Management*, 68(1), 1-13.
- Barrows, C. W., Henen, B. T. & Karl, A. E. (2016). Identifying climate refugia: A framework to inform conservation strategies for Agassiz's desert tortoise in a warmer future. *Chelonian Conserv. Biol.* 15, 2–11.
- Barrows, C. W. (2011). Sensitivity to climate change for two reptiles at the Mojave-Sonoran Desert interface. *J. Arid Environ.* 75, 629–635.
- Baskaran, L. M., Dale, V. H., Efroymson, R. A., & Birkhead, W. (2006). Habitat modeling within a regional context: an example using Gopher Tortoise. *The American Midland Naturalist*, 155(2), 335-351.
- Baxter, P. C., Wilson, D. S., & Morafka, D. J. (2008). The effects of nest date and placement of eggs in burrows on sex ratios and potential survival of hatchling desert tortoises, *Gopherus agassizii*. *Chelonian Conservation and Biology*, 7(1), 52-59.
- Beatley, J. C. (1974). Phenological events and their environmental triggers in Mojave Desert ecosystems. *Ecology*, 55(4), 856-863.
- Beever, E. A., O'leary, J., Mengelt, C., West, J. M., Julius, S., Green, N., ... & Hofmann, G. E. (2016). Improving conservation outcomes with a new paradigm for understanding species' fundamental and realized adaptive capacity. *Conservation Letters*, 9(2), 131-137.
- Berrigan D, Charnov, E.L. (1994). Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos* 70:474–478.
- Berry, K. H., & Aresco, M. J. (2014). Threats and conservation needs for North American tortoises.
- Bjurlin, C. D., & Bissonette, J. A. (2004). Survival during early life stages of the desert tortoise (*Gopherus agassizii*) in the south-central Mojave Desert. *Journal of Herpetology*, 527-535
- Blaustein, R. J. (2008). Biodiversity hotspot: the Florida panhandle. *BioScience*, 58(9), 784-790.
- Bock, S. L., Smaga, C. R., McCoy, J. A., & Parrott, B. B. (2022). Genome-wide DNA methylation patterns harbour signatures of hatchling sex and past incubation temperature in a species with environmental sex determination. *Molecular Ecology*, 31(21), 5487-5505.
- Bolker, B. M. (2008). Ecological models and data in R. In *Ecological Models and Data in R*. Princeton University Press.
- Breiman, L. (2001). Random forests. *Machine learning*, 45, 5-32.
- Butler, C. J. (2019). A Review of the Effects of Climate Change on Chelonians. *Diversity*, 11(8), 138.
- Cabanita, R., & Atkinson, D. (2006). Seasonal time constraints do not explain exceptions to the temperature size rule in ectotherms. *Oikos*, 114(3), 431-440.

- Calosi, P., Bilton, D. T. & Spicer, J. I. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biol. Lett.* 4, 99–102.
- Campbell, S. P., Zylstra, E. R., Darst, C. R., Averill-Murray, R. C., & Steidl, R. J. (2018). A spatially explicit hierarchical model to characterize population viability. *Ecological applications*, 28(8), 2055-2065.
- Carretero, M., Znari, M., Macé, J., & Harris, D. J. (2005). Morphological divergence among populations of *Testudo graeca* from west-central Morocco. *Animal Biology*, 55(3), 259-279.
- Carter, S., Nussear, K., Esque, T., Leinwand, IIF., Masters, E., Inman, R., Carr, N., Allison, L. (2020). Quantifying development to inform management of Mojave and Sonoran desert tortoise habitat in the American southwest. *Endangered Species Research*. 42. 10.3354/esr01045.
- Caswell, H. (2000). *Matrix population models* (Vol. 1). Sunderland, MA: Sinauer.
- Cayan, D. R. et al. (2010). Future dryness in the southwest US and the hydrology of the early 21st century drought. *Proc. Natl. Acad. Sci.* 107, 21271–21276.
- Ceballos, C. P., & Valenzuela, N. (2011). The role of sex-specific plasticity in shaping sexual dimorphism in a long-lived vertebrate, the snapping turtle *Chelydra serpentina*. *Evolutionary Biology*, 38, 163-181.
- Chang W, Cheng J, Allaire J, Sievert C, Schloerke B, Xie Y, Allen J, McPherson J, Dipert A, Borges B (2022). *_shiny: Web Application Framework for R_*. R package version 1.7.2, <<https://CRAN.R-project.org/package=shiny>>.
- Charmantier, A. et al. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*. 320, 800–803.
- Chevin, L. M., Lande, R. & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory. *PLoS Biol.* 8, e1000357.
- Cooch, E. (2008). Program MARK," A gentle introduction". <http://www.phidot.org/software/mark/docs/book/>.
- Crawford, B. A., Maerz, J. C., & Moore, C. T. (2020). Expert-informed habitat suitability analysis for at-risk species assessment and conservation planning. *Journal of Fish and Wildlife Management*, 11(1), 130-150.
- Daly, J.A., Buhlmann, K.A., Todd, B.D., Moore, C.T., Peaden, J.M. and Tuberville, T.D., (2019). Survival and movements of head-started Mojave desert tortoises. *The Journal of Wildlife Management*, 83(8), pp.1700-1710.
- Daly, C., Taylor, G. H., Gibson, W. P., Parzybok, T. W., Johnson, G. L., & Pasteris, P. A. (2000). High-quality spatial climate data sets for the United States and beyond. *Transactions of the ASAE*, 43(6), 1957.
- Dangremond, E. M., & Feller, I. C. (2016). Precocious reproduction increases at the leading edge of a mangrove range expansion. *Ecology and Evolution*, 6(14), 5087-5092.

- Davis, A. J., Jenkinson, L. S., Lawton, J. H., Shorrocks, B. & Wood, S. (1998). Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391, 783–786.
- Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C. & Mace, G. M. (2011). Beyond predictions: Biodiversity conservation in a changing climate. *Science*. 332, 53–58.
- Demuth, J. P. (2001). The effects of constant and fluctuating incubation temperatures on sex determination, growth, and performance in the tortoise *Gopherus polyphemus*. *Canadian Journal of Zoology*, 79(9), 1609-1620.
- Djurakic, M. R., & Milankov, V. R. (2020). The utility of plastron shape for uncovering cryptic diversity in Hermann's tortoise. *Journal of Zoology*, 310(2), 145-157.
- Doak, D., Kareiva, P. and Klepetka, B. (1994). Modeling population viability for the desert tortoise in the western Mojave Desert. *Ecological applications*, 4(3), pp.446-460.
- Drake, K. K., Esque, T. C., Nussear, K. E., Defalco, L. A., Scoles-Sciulla, S. J., Modlin, A. T., & Medica, P. A. (2015). Desert tortoise use of burned habitat in the eastern Mojave Desert. *The Journal of Wildlife Management*, 79(4), 618-629.
- Duda, J. J., Krzysik, A. J., & Freilich, J. E. (1999). Effects of drought on Desert Tortoise movement and activity. *The Journal of Wildlife Management*, 1181-1192.
- Dutcher, K. E., Vandergast, A. G., Esque, T. C., Mittelberg, A., Matocq, M. D., Heaton, J. S., & Nussear, K. E. (2020). Genes in space: what Mojave desert tortoise genetics can tell us about landscape connectivity. *Conservation Genetics*, 21, 289-303.
- Elith, J., & Leathwick, J. R. (2009). Species distribution models: ecological explanation and prediction across space and time. *Annual review of ecology, evolution, and systematics*, 40, 677-697.
- Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of animal ecology*, 77(4), 802-813.
- Elith, J. et al. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography*. 29, 129–151.
- Ennen, J. R., Lovich, J. E., Meyer, K. P., Bjurlin, C., & Arundel, T. R. (2012). Nesting ecology of a population of *Gopherus agassizii* at a utility-scale wind energy facility in southern California. *Copeia*, 2012(2), 222-228.
- Folt, B., Marshall, M., Emanuel, J. A., Dziadzio, M., Cooke, J., Mena, L., ... & McGowan, C. (2022). Using predictions from multiple anthropogenic threats to estimate future population persistence of an imperiled species. *Global Ecology and Conservation*, 36, e02143.
- Fordham, D. A. et al. (2012). Managed relocation as an adaptation strategy for mitigating climate change threats to the persistence of an endangered lizard. *Glob. Chang. Biol.* 18, 2743–2755.
- Fuller, A. et al. (2010). Physiological mechanisms in coping with climate change. *Physiol. Biochem. Zool.* 83, 713–720.

- Gaillard, D., Ennen, J. R., Kreiser, B. R., Qualls, C. P., Sweat, S. C., Birkhead, R., ... & Hentges, T. W. (2017). Range-wide and regional patterns of population structure and genetic diversity in the Gopher Tortoise. *Journal of Fish and Wildlife Management*, 8(2), 497-512.
- Gardali, T., Seavy, N. E., DiGaudio, R. T. & Comrack, L. A. (2012). A climate change vulnerability assessment of California's at-risk birds. *PLoS One* 7, e29507.
- Gaston, A. J., Gilchrist, H. G., & Hipfner, J. M. (2005). Climate change, ice conditions and reproduction in an Arctic nesting marine bird: Brunnich's guillemot (*Uria lomvia* L.). *Journal of Animal Ecology*, 832-841.
- Georges, A. (1989). Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters?. *Oecologia*, 81(3), 323-328.
- Gibbons, J. W. et al. (2000). The global decline of reptiles, déjà vu amphibians. *Bioscience* 50, 653–666.
- Gleick, P. H., (1986). Methods for Evaluating the Regional Hydrologic Effects of Global Climate Changes. *Journal of Hydrology* 88:97-116.
- Grace, J. B., Schoolmaster Jr, D. R., Guntenspergen, G. R., Little, A. M., Mitchell, B. R., Miller, K. M., & Schweiger, E. W. (2012). Guidelines for a graph-theoretic implementation of structural equation modeling. *Ecosphere*, 3(8), 1-44.
- Guida, R. J., Abella, S. R., Roberts, C. L., Norman, C. M., & Smith Jr, W. J. (2019). Assessing historical and future habitat models for four conservation-priority Mojave Desert species. *Journal of Biogeography*, 46(9), 2081-2097.
- Hagerty, B.E. and Tracy, C.R. (2010). Defining population structure for the Mojave Desert Tortoise. *Conservation genetics*, 11(5), pp.1795-1807.
- Hansen, M. C., Potapov, P. V., Moore, R., Hancher, M., Turubanova, S. A., Tyukavina, A., ... & Townshend, J. (2013). High-resolution global maps of 21st-century forest cover change. *science*, 342(6160), 850-853.
- Hart, E. M., Bell, K., & Butler, A. (2015). prism: Download data from the Oregon prism project. R package version 0.0, 6(10.5281).
- Hengl, T., Mendes de Jesus, J., Heuvelink, G. B., Ruiperez Gonzalez, M., Kilibarda, M., Blagotić, A., ... & Kempen, B. (2017). SoilGrids250m: Global gridded soil information based on machine learning. *PLoS one*, 12(2), e0169748.
- Hernandez-Divers, S. J., Stahl, S. J., & Farrell, R. (2009). An endoscopic method for identifying sex of hatchling Chinese box turtles and comparison of general versus local anesthesia for coelioscopy. *Journal of the American Veterinary Medical Association*, 234(6), 800-804.
- Hernandez-Divers, S. M., Hernandez-Divers, S. J., & Wyneken, J. (2002). Angiographic, anatomic and clinical technique descriptions of a subcarapacial venipuncture site for chelonians. *Journal of Herpetological Medicine and Surgery*, 12(2), 32-37.

- Hiers, J. K., Jackson, S. T., Hobbs, R. J., Bernhardt, E. S. & Valentine, L. E. (2016). The precision problem in conservation and restoration. *Trends Ecol. Evol.* 31, 820–830.
- Hijmans R (2023). *_terra: Spatial Data Analysis_*. R package version 1.7-23, <<https://CRAN.R-project.org/package=terra>>.
- Hijmans R (2022). *_raster: Geographic Data Analysis and Modeling_*. R package version 3.6-3, <<https://CRAN.R-project.org/package=raster>>.
- Hof, C., Levinsky, I., Araújo, M. B. & Rahbek, C. (2011). Rethinking species' ability to cope with rapid climate change. *Glob. Chang. Biol.* 17, 2987–2990.
- Hoffmann, A. A. & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature* 470, 479–485.
- Hoppers, A., Williams, L., Ponnaluri, V. C., Sexton, B., Saleh, L., Campbell, M., ... & Stewart, F. (2020). Enzymatic methyl-seq: next generation methylomes. *Journal of Biomolecular Techniques*, 31(Suppl), S15.
- Hothorn, T., Hornik, K., & Zeileis, A. (2006). Unbiased Recursive Partitioning: A Conditional Inference Framework. *Journal of Computational and Graphical Statistics*, 15(3), 651–674.
- Howell, H. J., Rothermel, B. B., White, K. N., & Searcy, C. A. (2020). Gopher Tortoise demographic responses to a novel disturbance regime. *The Journal of Wildlife Management*, 84(1), 56-65.
- Hunter, E. A., Loope, K. J., Drake, K. K., Hanley, K., Jones Jr, D. N., Shoemaker, K. T., & Rostal, D. C. (2021). Warming conditions boost reproductive output for a northern Gopher Tortoise population. *Endangered Species Research*, 46, 215-226.
- Hunter, E. A., & Rostal, D. C. (2021). Fire management effects on long-term Gopher Tortoise population dynamics. *The Journal of Wildlife Management*, 85(4), 654-664.
- Hunter, E. A. et al. (2015). Coastal vertebrate exposure to predicted habitat changes due to sea level rise. *Environ. Manage.* 56, 1528–1537.
- Inman, R., Fotheringham, A.S., Franklin, J., Esque, T., Edwards, T. and Nussear, K. (2019). Local niche differences predict genotype associations in sister taxa of Desert Tortoise. *Diversity and Distributions*.
- Iverson, J. B. (1980). The reproductive biology of *Gopherus polyphemus* (Chelonia: Testudinidae). *American Midland Naturalist*, 353-359.
- James, F. C., Hess, C. A., Kicklighter, B. C. & Thum, R. A. (2001). Ecosystem management and the niche gestalt of the Red-cockaded Woodpecker in longleaf pine forests. *Ecol. Appl.* 11, 854–870.
- Janzen, F. J. (1994). Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. *Ecology*, 75(6), 1593-1599.
- Jones, O. and Wang, J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10: 551–555.

- Karl, A.E. (1998). Reproduction strategies, growth patterns, and survivorship of a long-lived herbivore inhabiting a temporally variable environment. Ph.D. Thesis., Univ. California, Los Angeles.
- Kawecki, T. J. & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecol. Lett.* 7, 1225–1241.
- Kendall, B. E., Fujiwara, M., Diaz-Lopez, J., Schneider, S., Voigt, J., & Wiesner, S. (2019). Persistent problems in the construction of matrix population models. *Ecological modelling*, 406, 33-43.
- Kingsolver JG, Diamond SE. (2011). Phenotypic selection in natural populations: what limits directional selection? *Am. Nat.* 177, 346–357.
- Kreiser, B. R., Ennen, J. R., Gaillard, D. L., & Qualls, C. P. (2013). Isolation and characterization of polymorphic microsatellite loci for the Gopher Tortoise (*Gopherus polyphemus*) and cross-amplification in other species of *Gopherus*. *Conservation Genetics Resources*, 5, 255-259.
- Kupfer, J. A., Terando, A. J., Gao, P., Teske, C., & Hiers, J. K. (2020). Climate change projected to reduce prescribed burning opportunities in the south-eastern United States. *International Journal of Wildland Fire*, 29(9), 764-778.
- Laloë, J. O., Cozens, J., Renom, B., Taxonera, A., & Hays, G. C. (2017). Climate change and temperature-linked hatchling mortality at a globally important sea turtle nesting site. *Global change biology*, 23(11), 4922-4931.
- Lamb, J. Y., Ennen, J. R., & Qualls, C. P. (2013). Environmental characteristics of nest sites selected by Gopher Tortoises (*Gopherus polyphemus*) in southern Mississippi. *Chelonian Conservation and Biology*, 12(2), 227-234.
- Lance, V. A., Valenzuela, N., & Von Hildebrand, P. (1992). A hormonal method to determine sex of hatchling giant river turtles, *Podocnemis expansa*: application to endangered species. *Journal of Experimental Zoology*, 270, 16A.
- Lawler, J. J. (2009). Climate change adaptation strategies for resource management and conservation planning. *Ann. N. Y. Acad. Sci.* 1162, 79–98.
- Lawrence, D.J., Runyon, A.N., Gross, J.E., Schuurman, G.W. and Miller, B.W. (2021). Divergent, plausible, and relevant climate futures for near-and long-term resource planning. *Climatic Change*, 167(3-4), p.38.
- Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R news*, 2(3), 18-22.
- Lolavar, A., & Wyneken, J. (2020). The impact of sand moisture on the temperature-sex ratio responses of developing loggerhead (*Caretta caretta*) sea turtles. *Zoology*, 138, 125739.
- Lolavar, A., & Wyneken, J. (2017). Experimental assessment of the effects of moisture on loggerhead sea turtle hatchling sex ratios. *Zoology*, 123, 64-70.

- Longshore, K. M., Jaeger, J. R., & Sappington, J. M. (2003). Desert tortoise (*Gopherus agassizii*) survival at two eastern Mojave desert sites: death by short-term drought?. *Journal of Herpetology*, 37(1), 169-178.
- Loope, K. J., Rostal, D. C., Walden, M. A., Shoemaker, K. T., & Hunter, E. A. (2022). A comparison of non-surgical methods for sexing young Gopher Tortoises (*Gopherus polyphemus*). *PeerJ*, 10, e13599.
- Lovich, J. E., Puffer, S. R., Agha, M., Ennen, J. R., Meyer-Wilkins, K., Tennant, L. A., ... & Vamstad, M. S. (2018). Reproductive Output and Clutch Phenology of Female Agassiz's Desert Tortoises (*Gopherus agassizii*) in the Sonoran Desert Region of Joshua Tree National Park. *Current herpetology*, 37(1), 40-57.
- Lovich, J., Agha, M., Meulblok, M., Meyer, K., Ennen, J., Loughran, C., ... & Bjurlin, C. (2012). Climatic variation affects clutch phenology in Agassiz's Desert Tortoise *Gopherus agassizii*. *Endangered Species Research*, 19(1), 63-74.
- Lovich, J. E., Yackulic, C. B., Freilich, J., Agha, M., Austin, M., Meyer, K. P., ... & Root, S. A. (2014). Climatic variation and tortoise survival: has a desert species met its match?. *Biological Conservation*, 169, 214-224.
- McKee, R. K., K. A. Buhlmann, C. T. Moore, J. Hepinstall-Cymerman, and T. D. Tuberville. (2021). Waif Gopher Tortoise survival and site fidelity following translocation. *The Journal of wildlife management*.
- McLuckie, A. M., & Fridell, R. A. (2002). Reproduction in a desert tortoise (*Gopherus agassizii*) population on the Beaver Dam Slope, Washington County, Utah. *Chelonian Conservation and Biology*, 4(2), 288-294.
- McMahon, S. M. et al. (2011). Improving assessment and modelling of climate change impacts on global terrestrial biodiversity. *Trends Ecol. Evol.* 26, 249–259.
- Medica, P. A., Nussear, K. E., Esque, T. C., & Saethre, M. B. (2012). Long-term growth of desert tortoises (*Gopherus agassizii*) in a southern Nevada population. *Journal of Herpetology*, 213-220.
- Millar, C. I., Stephenson, N. L. & Stephens, S. L. (2007). Climate change and forests of the future: Managing in the face of uncertainty. *Ecol. Appl.* 17, 2145–2151.
- Milsum, T. P. et al. (2000). Habitat models of bird species' distribution: An aid to the management of coastal grazing marshes. *J. Appl. Ecol.* 37, 706–727.
- Mitchell, C. I., Friend, D. A., Philips, L. T., Hunter, E. A., Lovich, J. E., Agha, M., Puffer, S. R., Cummings, K. L., Medica, P. A., Esque, T. C., Nussear, K. E., Shoemaker, K. T. (2021). Unscrambling the drivers of egg production in Agassiz's desert tortoise: climate and individual attributes predict reproductive output. *Endangered Species Research*, (44), 217-230.
- Mitchell, N. J., & Janzen, F. J. (2010). Temperature-dependent sex determination and contemporary climate change. *Sexual Development*, 4(1-2), 129-140.

- Mitchell, M. J. (2005). Home range, reproduction, and habitat characteristics of the female Gopher Tortoise (*Gopherus polyphemus*) in southeast Georgia.
- Monsinjon, J. R., Guillon, J. M., Wyneken, J., & Girondot, M. (2022). Thermal reaction norm for sexualization: The missing link between temperature and sex ratio for temperature-dependent sex determination. *Ecological Modelling*, 473, 110119.
- Morafka, D. J. & Berry, K. H. (2002). Is *Gopherus agassizii* a desert-adapted tortoise, or an exaptive opportunist? Implications for tortoise conservation. *Chelonian Conserv. Biol.* 4, 263–287.
- Moritz, C., & Agudo, R. (2013). The future of species under climate change: resilience or decline?. *Science*, 341(6145), 504-508.
- Mueller, J. M., Sharp, K. R., Zander, K. K., Rakestraw, D. L., Rautenstrauch, K. R., & Lederle, P. E. (1998). Size-specific fecundity of the Desert Tortoise (*Gopherus agassizii*). *Journal of Herpetology*, 313-319.
- Murray, D. L. (2006). On improving telemetry-based survival estimation. *The Journal of Wildlife Management*, 70(6), 1530-1543.
- Nafus, M. G., Todd, B. D., Buhlmann, K. A., & Tuberville, T. D. (2015). Consequences of maternal effects on offspring size, growth and survival in the desert tortoise. *Journal of Zoology*, 297(2), 108-114.
- Nicotra, A. B., Beever, E. A., Robertson, A. L., Hofmann, G. E., & O'Leary, J. (2015). Assessing the components of adaptive capacity to improve conservation and management efforts under global change. *Conservation Biology*, 29(5), 1268-1278.
- Nussear, K.E., Esque, T.C., Inman, R.D., Gass, L., Thomas, K.A., Wallace, C.S., Blainey, J.B., Miller, D.M. and Webb, R.H. (2009). *Modeling habitat of the Desert Tortoise (Gopherus agassizii) in the Mojave and parts of the Sonoran Deserts of California, Nevada, Utah, and Arizona* US Geological Survey Open File Report No. 2009-1102.
- Nussear, K.E., Tracy, C.R., Medica, P.A., Wilson, D.S., Marlow, R.W., Corn, P.S., (2012). Translocation as a conservation tool for Agassiz's Desert Tortoises: Survivorship, reproduction, and movements. *J. Wildl. Manage.* 76, 1341–1353.
- Ohlberger J. (2013). Climate warming and ectotherm body size – from individual physiology to community ecology. *Functional Ecology* 27:991-1001.
- Ott, J. A., Mendonça, M. T., Guyer, C., & Michener, W. K. (2000). Seasonal changes in sex and adrenal steroid hormones of Gopher Tortoises (*Gopherus polyphemus*). *General and Comparative Endocrinology*, 117(2), 299-312.
- Pearson, R. G. et al. (2014). Life history and spatial traits predict extinction risk due to climate change. *Nat. Clim. Chang.* 4, 217–221.
- Pearson, R. G. & Dawson, T. P. (2003). Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Glob. Ecol. Biogeogr.* 12, 361–371.

- Perez-Heydrich, C., Jackson, K., Wendland, L.D. and Brown, M.B. (2012). Gopher Tortoise hatchling survival: field study and meta-analysis. *Herpetologica*, 68(3), pp.334-344.
- Pilowsky, J. A., Haythorne, S., Brown, S. C., Krapp, M., Armstrong, E., Brook, B. W., ... & Fordham, D. A. (2022). Range and extinction dynamics of the steppe bison in Siberia: A pattern-oriented modeling approach. *Global Ecology and Biogeography*, 31(12), 2483-2497.
- Plummer, M. (2015). JAGS Version 4.0. 0 user manual. See <https://sourceforge.net/projects/mcmc-jags/files/Manuals/4x>.
- Porter WP, Mitchell JW, Beckman WA, DeWitt CB. (1973). Behavioral implications of mechanistic ecology. *Oecologia* 13:1-54.
- PRISM Climate Group. (2021). [Http://prism.oregonstate.edu](http://prism.oregonstate.edu). Oregon State University.
- R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rebaudo, F. (2014). SimAdapt (Version 1.8.0) CoMSES Computational Model Library <https://www.comses.net/codebases/3137/releases/1.8.0/>
- Rebaudo, F., Le Rouzic, A., Dupas, S., Silvain, J.F., Harry, M., Dangles, O. (2013). SimAdapt: An individual-based genetic model for simulating landscape management impacts on populations. *Methods in Ecology and Evolution* 4:595–600.
- Reed, T. E., Schindler, D. E. & Waples, R. S. (2010). Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv. Biol.* 25, 56–63.
- Reed, J.M., Fefferman, N. and Averill-Murray, R.C., (2009). Vital rate sensitivity analysis as a tool for assessing management actions for the desert tortoise. *Biological Conservation*, 142(11), pp.2710-2717.
- Richter, S. et al. (2012). Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia* 169, 269–279.
- Roberson, J. B., B. L. Burge, and P. Hayden. (1985). Nesting observations of free-living Desert Tortoises (*Gopherus agassizii*) and hatching success of eggs protected from predators, p. 91-99. In: *Proceedings of the Desert Tortoise Council Symposium 1985*.
- Rostal, D. C., & Wibbels, T. (2014). Embryonic development, hatching success, and temperature-dependent sex determination in North American tortoises. *Biology and conservation of North American tortoises*. Johns Hopkins University Press, Baltimore, MD, 46-52.
- Rostal, D. C., & Jones, D. N. (2002). Population biology of the Gopher Tortoise (*Gopherus polyphemus*) in southeast Georgia. *Chelonian Conservation and Biology*, 4(2), 479-487.
- Rostal, D. C., Grumbles, J. S., Lance, V. A., & Spotila, J. R. (1994). Non-lethal sexing techniques for hatchling and immature desert tortoises (*Gopherus agassizii*). *Herpetological Monographs*, 83-87.
- Royle, J. A., Chandler, R. B., Sollmann, R., & Gardner, B. (2013). *Spatial capture-recapture*. Academic Press.

- Royle, J. A., & Dorazio, R. M. (2008). Hierarchical modeling and inference in ecology: the analysis of data from populations, metapopulations and communities. Elsevier.
- Sadoti, G., Gray, M. E., Farnsworth, M. L., & Dickson, B. G. (2017). Discriminating patterns and drivers of multiscale movement in herpetofauna: The dynamic and changing environment of the Mojave Desert Tortoise. *Ecology and evolution*, 7(17), 7010-7022.
- Sadeghi, M., Nguyen, P., Naeini, M. R., Hsu, K., Braithwaite, D., & Sorooshian, S. (2021). PERSIANN-CCS-CDR, a 3-hourly 0.04 global precipitation climate data record for heavy precipitation studies. *Scientific data*, 8(1), 157.
- Sánchez-Ramírez, S., Y. Rico, K.H. Berry, T. Edwards, A.E. Karl, B. T. Henen, and R.W. Murphy. (2018). Landscape limits gene flow and drives population structure in Agassiz's Desert Tortoise (*Gopherus agassizii*). *Scientific reports* 8(1), pp11231.
- Schaub, M., & Abadi, F. (2011). Integrated population models: a novel analysis framework for deeper insights into population dynamics. *Journal of Ornithology*, 152(1), 227-237.
- Schloss, C. A., Nuñez, T. A. & Lawler, J. J. (2012). Dispersal will limit ability of mammals to track climate change in the Western Hemisphere. *Proc. Natl. Acad. Sci. U. S. A.* 109, 8606–11.
- Schwartz, T. S., Osentoski, M., Lamb, T., & Karl, S. A. (2003). Microsatellite loci for the North American tortoises (genus *Gopherus*) and their applicability to other turtle species. *Molecular Ecology Notes*, 3(2), 283-286.
- Schwarz, C. J., & Arnason, A. N. (1996). A general methodology for the analysis of capture-recapture experiments in open populations. *Biometrics*, 860-873.
- Schwarzkopf, L., & Brooks, R. J. (1985). Sex determination in northern painted turtles: effect of incubation at constant and fluctuating temperatures. *Canadian Journal of Zoology*, 63(11), 2543-2547.
- Scott, P. A., L. J. Allison, K. J. Field, R. C. Averill-Murray, and H. B. Shaffer. (2020). Individual heterozygosity predicts translocation success in threatened desert tortoises. *Science* 370:1086–1089.
- Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., ... & Naik, N. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, 316(5828), 1181-1184.
- Selmoni, O., Vajana, E., Guillaume, A., Rochat, E., Joost, S. (2019). Sampling strategy optimization to increase statistical power in landscape genomics: a simulation-based approach. *Molecular Ecology Resources* 2019;00:1-16.
- Sheridan JA, Bickford D. 2011. Shrinking body size as an ecological response to climate change. *Nature Climate Change* 1:401-406.
- Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*. 301, 65.

- Spotila, J. R., Zimmerman, L. C., Binckley, C. A., Grumbles, J. S., Rostal, D. C., List Jr, A., ... & Kemp, S. J. (1994). Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. *Herpetological Monographs*, 103-116.
- Taylor, K. E., Stouffer, R. J., & Meehl, G. A. (2012). An overview of CMIP5 and the experiment design. *Bulletin of the American meteorological Society*, 93(4), 485-498.
- Thomas, K. A., Guertin, P. P. and Gass, L. (2012). Plant distributions in the southwestern United States; a scenario assessment of the modern-day and future distribution ranges of 166 species. US Geological Survey. Open File Report 2012-1020, 83pp.
- Thomson, D. L., Cooch, E. G., & Conroy, M. J. (Eds.). (2008). Modeling demographic processes in marked populations (Vol. 3). Springer Science & Business Media.
- Thornton, P.E., Shrestha, R., Thornton, M. et al. Gridded daily weather data for North America with comprehensive uncertainty quantification. *Sci Data* 8, 190 (2021). <https://doi.org/10.1038/s41597-021-00973-0>
- Tracy, C. R., R. C. Averill-Murray, W. I. Boarman, D. J. Delehanty, J. S. Heaton, E. D. McCoy, D. J. Morafka, K. E. Nussear, B. E. Hagerty, and P. A. Medica. (2004). Desert Tortoise Recovery Plan Assessment. Technical Report. US Fish and Wildlife Service, Reno, NV.
- Tuberville, T.D., Buhlmann, K.A., Sollmann, R., Nafus, M.G., Peaden, J.M., Daly, J.A. and Todd, B.D., (2019). Effects of short-term, outdoor head-starting on growth and survival in the Mojave desert tortoise (*Gopherus agassizii*). *Herpetological Conservation and Biology*, 14(1), pp.171-184.
- Tuberville, T. D., Norton, T. M., Waffa, B. J., Hagen, C., & Glenn, T. C. (2011). Mating system in a Gopher Tortoise population established through multiple translocations: apparent advantage of prior residence. *Biological Conservation*, 144(1), 175-183.
- Turner, F. B., Hayden, P., Burge, B. L., & Roberson, J. B. (1986). Egg production by the Desert Tortoise (*Gopherus agassizii*) in California. *Herpetologica*, 93-104.
- Turner, F. B., Medica, P. A., and Lyons, C. L. (1984). Reproduction and survival of the Desert Tortoise (*Scaptochelys agassizii*) in Ivanpah Valley, California. *Copeia* 1984: 811-820.
- Turner, F. B., & Randall, D. C. (1989). Net production by shrubs and winter annuals in southern Nevada. *Journal of Arid Environments*, 17(1), 23-36.
- Turner, F.B., Berry, K.H., Randall, D.C. and White, G.C., (1987). Population ecology of the desert tortoise at Goffs, California, 1983-1986. eScholarship, University of California.
- U.S. Fish and Wildlife Service, U.S. Department of the Interior. (2011). 2011 Revised Recovery Plan for the Mojave Population of the Desert Tortoise (*Gopherus agassizii*). U.S. Fish and Wildlife Service, Pacific Southwest Region, Sacramento, California. 222 pp. <https://www.fws.gov/media/2011-revised-recovery-plan-mojave-desert-tortoise>
- Valenzuela, N., Litterman, R., Neuwald, J. L., Mizoguchi, B., Iverson, J. B., Riley, J. L., & Litzgus, J. D. (2019). Extreme thermal fluctuations from climate change unexpectedly

- accelerate demographic collapse of vertebrates with temperature-dependent sex determination. *Scientific reports*, 9(1), 4254.
- Valladares, F. et al. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol. Lett.* 17, 1351–1364.
- Valverde, R. A., Wingard, S., Gómez, F., Tordoir, M. T., & Orrego, C. M. (2010). Field lethal incubation temperature of olive ridley sea turtle *Lepidochelys olivacea* embryos at a mass nesting rookery. *Endangered Species Research*, 12(1), 77-86.
- van de Pol, M., Bailey, L. D., McLean, N., Rijdsdijk, L., Lawson, C. R., & Brouwer, L. (2016). Identifying the best climatic predictors in ecology and evolution. *Methods in Ecology and Evolution*, 7(10), 1246-1257.
- Van der Putten, W. H. et al. (2010). Predicting species distribution and abundance responses to climate change: Why it is essential to include biotic interactions across trophic levels. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365, 2025–34.
- van Vuuren D.P., Riahi K., Moss R., Edmonds J., Thomson A., Nakicenovic N., Kram T., Berkhout F., Swart R., Janetos A., Rose S.K., Arnell N. (2012). A proposal for a new scenario framework to support research and assessment in different climate research communities. *Global Environmental Change* 22:21–35
- Vandergast, A. G., W. M. Perry, R.V. Lugo, and S.A. Hathaway. (2011). Genetic landscapes GIS Toolbox: tools to map patterns of genetic divergence and diversity. *Molecular Ecology Resources* 11(1), pp158-161.
- Walden, M., (2022). Body Condition, Hatchling Sex Identification, and Somatic Growth of the Mojave Desert Tortoise, *Gopherus agassizii* (Doctoral dissertation, University of Nevada - Reno).
- Wallis, I. R., Henen, B. T., & Nagy, K. A. (1999). Egg size and annual egg production by female Desert Tortoises (*Gopherus agassizii*): the importance of food abundance, body size, and date of egg shelling. *Journal of Herpetology*, 394-408.
- Weisrock, D. W., & Janzen, F. J. (1999). Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Functional Ecology*, 13(1), 94-101.
- Wilensky, U. (1999). NetLogo. Center for Connected Learning and Computer-Based Modeling. Northwestern University, Evanston IL. <http://ccl.northwestern.edu/netlogo/>
- Williams, S. E., Shoo, L. P., Isaac, J. L., Hoffmann, A. A., & Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS biology*, 6(12), e325.
- Wisz, M. S. et al. (2013). The role of biotic interactions in shaping distributions and realised assemblages of species: Implications for species distribution modelling. *Biol. Rev.* 88, 15–30.
- Wright, M. N., & Ziegler, A. (2017). Ranger: A fast implementation of random forests for high dimensional data in C++ and R. *J. Stat. Softw.* 77, 1–17. doi:10.18637/jss.v077.i01

- Yuan, M. L., White, K. N., Rothermel, B. B., Zamudio, K. R., & Tuberville, T. D. (2019). Close-kin mating, but not inbred parents, reduces hatching rates and offspring quality in a threatened tortoise. *Journal of evolutionary biology*, 32(10), 1152-1162.
- Zimmerman, L. C., O'Connor, M. P., Bulova, S. J., Spotila, J. R., Kemp, S. J., & Salice, C. J. (1994). Thermal ecology of Desert Tortoises in the eastern Mojave Desert: seasonal patterns of operative and body temperatures, and microhabitat utilization. *Herpetological Monographs*, 45-59.
- Zuur, A., Ieno, E., Walker, N., Saveliev, A., & Smith, G. (2009). Zero-Truncated and Zero-Inflated Models for Count Data. 10.1007/978-0-387-87458-6_11.
- Zylstra, E. R., Steidl, R. J., Jones, C. A., & Averill-Murray, R. C. (2013). Spatial and temporal variation in survival of a rare reptile: a 22-year study of Sonoran Desert Tortoises. *Oecologia*, 173(1), 107-116.

APPENDICES

Appendix A: Supporting Data

Note: we will continue to include links to all data collected as part of this project in all publications emerging from this project. We will also include links to code for running all analyses and simulation models described in this report. We will also provide links to unpublished data collected as part of this project, and will work with our program manager to discuss a temporary embargo to allow us to publish results from these datasets prior to releasing the data for broader public consumption.

R Code for running the Desert Tortoise and Gopher Tortoise simulation models presented herein: <https://github.com/kevintshoemaker/GopherusPVA>.

Effects of climate on Desert Tortoise reproductive output (Mitchell et al. 2021): <https://github.com/kevintshoemaker/TortoiseEggModel>. Note: Desert Tortoise egg production data sets collected as part of several studies associated with the manuscript can also be accessed at Puffer et al. (2020).

Effects of fire on Gopher Tortoise survival (Hunter and Rostal 2021): https://github.com/elizabethahunter/Tortoise_Prescribed_Fire

Appendix B: List of Scientific/Technical Publications: RC18-1103

Published papers:

- Loope, K.J., Rostal, D.C., Walden, M.A., Shoemaker, K.T., Hunter, E.A. 2022. A comparison of non-surgical methods for sexing young Gopher Tortoises (*Gopherus polyphemus*). *PeerJ* 10:e13599. <https://doi.org/10.7717/peerj.13599>
- Walden, M.A., Jania, R., Kinney, M.E., Devan-Song, A., Drake, K.K., Esque, T.C., Shoemaker, K.T. 2022. Computed tomography for measuring body fat reserved in threatened Agassiz's

desert tortoise (*Gopherus agassizii*). Journal of Zoo and Wildlife Medicine 53(2), 412-423.
<https://doi.org/10.1638/2020-0168>

Hunter, E.A., and Rostal, D.C. 2021. Fire management effects on long-term Gopher Tortoise population dynamics. The Journal of Wildlife Management 85(4):654-664.
<https://doi.org/10.1002/jwmg.22033>

Mitchell, C.I., Friend, D.A., Phillips, L.T., Hunter, E.A., Lovich, J.E., Agha, M., Puffer, S.R., Cummings, K.L., Medica, P.A., Esque, T.C., Nussear, K.E., Shoemaker, K.T. 2021. 'Unscrambling' the drivers of egg production in Agassiz's desert tortoise: climate and individual attributes predict reproductive output. Endangered Species Research 44:217-230.
<https://doi.org/10.3354/esr01103>

Hunter, E.A., Loope, K.J., Drake, K.K., Hanley, K., Jones, Jr., D.A., Shoemaker, K.T., Rostal, D.C. 2021. Warming conditions boost reproductive output for a northern Gopher Tortoise population. Endangered Species Research 46:215-226. <https://doi.org/10.3354/esr01155>

Publications in preparation:

Walden, M.A., Loope, K.J., Hunter, E.A., Divers, S.J., Comolli, J.R., Esque, T.C., and Shoemaker, K.T. *Submitted*. Testosterone identifies hatchling sex for Mojave desert tortoises (*Gopherus agassizii*). *Scientific Reports*.

Walden, M.A., Devan-Song, A., Drake, K.K., Esque, T.C., Nussear, K.E., Hunter, E.H, Shoemaker, K.T. *In prep*. Faster somatic growth may contribute to climate change resilience for a terrestrial vertebrate ectotherm. *Endangered Species Research (ready to submit)*

DeSha, N.D., K.J. Loope, K.T. Shoemaker, M. Aresco, and E.A. Hunter. *In prep*. Imperfect plasticity in Gopher Tortoise nesting behavior and fecundity in a translocated population. Target: *Animal Conservation*

Planned publications:

Loope, K.J., DeSha, N.D, K.T. Shoemaker, M. Aresco, and E.A. Hunter. Does source population influence mate choice in a translocated tortoise population of mixed origins? Target: *Conservation Genetics*

Arneson, A., K.J. Loope, K.T. Shoemaker, E.A. Hunter. Hindcasting nest thermal environments from air temperature and precipitation datasets. Target: *Ecological Modelling*.

Loope, K.J., A. Arneson, N.D. DeSha, K.T. Shoemaker, M. Aresco, B. Rothermel, L. Smith, D. Rostal, M. Legare, and E.A. Hunter. Nest site selection, nest thermal environment and hatching success in natural nests of the Gopher Tortoise. Target: *Frontiers in Ecology and Evolution*

Loope, K.J. & M.A. Walden (co-1st authors), E.A. Hunter, and K.T. Shoemaker. Warming temperatures influence hatching success and hatchling sex ratio in an imperiled reptile. Target: *Proceedings of the Royal Society: B*

Loope, K.J., D.R. Rostal, K.T. Shoemaker, and E.A. Hunter. Nest temperature and local precipitation influence wild Gopher Tortoise sex ratios. Target: *Endangered Species Research*

Loope, K.J., M.E. Berres, D.R. Rostal, K.T. Shoemaker, and E.A. Hunter. Blood methylome profiling reveals hatchling sex in monomorphic tortoises. Target: *Molecular Ecology*

Hromada et al. Rangewide environmental sensitivities of Mojave desert tortoise demographic rates. Target: *Ecosphere*.

Shoemaker K.T., K.J. Loope, M.A. Walden, E.A. Hunter. Simulation modeling reveals local climate change effects but range-wide resilience in the imperiled desert tortoise. Target: *Ecological Applications*

Shoemaker K.T., K.J. Loope, B. Folt, E.A. Hunter. Range-wide simulation modeling reveals critical role of climate change impacts on habitat management in a declining tortoise. Target: *Journal of Applied Ecology*

Presentations

Shoemaker, K.T., Walden, M., Hunter, E.A., Nussear, K.E., Esque, T.C., Drake, K.K., Averill-Murray, R. Contributed talk. July, 2022. Modeling the range-wide environmental drivers of population dynamics for desert tortoises using long-term capture-recapture data. North American Congress for Conservation Biology annual meeting, Reno, NV.

Hunter, E.A., Loope, K.J., DeSha, J.N., Aresco, M.J., Rostal, D.C., Rothermel, B.B., Smith, L.L., Shoemaker, K.T. Contributed talk. July, 2022. Natural variation and plasticity in response to climate change in Gopher Tortoise nest-site selection, nest thermal environment, and hatching success. North American Congress for Conservation Biology annual meeting, Reno, NV.

Loope, K.J., DeSha, J.N., Aresco, M., Shoemaker, K.T., Hunter, E.A. Poster presentation. July, 2022. Reproductive integration of Gopher Tortoises in a translocated population with multiple source populations. North American Congress for Conservation Biology annual meeting, Reno, NV.

Walden, M.A., Loope, K.J., Hunter, E.A., Divers, S.J., Comolli, J.R., Esque, T.C., Shoemaker, K.T. Feb, 15-25, 2022. Innate testosterone identifies sex of hatchling Mojave desert tortoises (*Gopherus agassizii*). Desert Tortoise Council 47th Annual Meeting and Symposium, Virtual meeting.

Hunter, E.A., Loope, K.J., Rostal, D.C., Shoemaker, K.T. 2021. "Climate variability influences aspects of Gopher Tortoise fecundity." Virginia Chapter of the Wildlife Society, Virtual meeting.

Mitchell, C.I., Friend, D.A., Philips, L.T., Hunter, E.A., Lovich, J.E., Agha, M. Puffer, S.R., Cummings, K.L., Medica, P.A., Esque, T.C., Nussear, K.E., and Shoemaker, K.T. Feb 9-25, 2021. "'Unscrambling' the drivers of egg production in Agassiz's desert tortoise: Climate and individual attributes predict reproductive output." Desert Tortoise Council 46th Annual Meeting and Symposium, Virtual meeting.

Loope, K.J., J.N. DeSha, M. Aresco, K.T. Shoemaker, E.A. Hunter. 2021. "Reproductive integration of Gopher Tortoises in a translocated population with multiple source populations." The Wildlife Society Annual Conference, Nov 2-5 2021, Virtual meeting.

Loope, K.J., JN DeSha, M Aresco, KT Shoemaker, EA Hunter. 2021. Reproductive integration of Gopher Tortoises in a translocated population with multiple source populations. 43rd Annual Gopher Tortoise Council Meeting, Oct 29 2021, Virtual meeting.

Loope, K.J., DeSha, J.N., Rothermel, B.B., Smith, L.L., Rostal, D.C., Shoemaker, K.T., Hunter, E.A. 2020. "Nest site, nest temperature, and hatching outcomes across an environmental gradient in the Gopher Tortoise." 42rd Annual Gopher Tortoise Council Meeting, Oct 2020, Virtual meeting.

DeSha, J.N., Loope, K.J., Aresco, M.J., Shoemaker, K.T., Hunter, E.A. 2020. "Plasticity in Gopher Tortoise Nesting Behaviors and Fecundity in a Translocated Population." 42rd Annual Gopher Tortoise Council Meeting, Oct 2020, Virtual meeting.

Hunter, E.A., Rostal, D.C., and Shoemaker, K.T. 2020. "Climate variability influences aspects of fecundity but not survival in a long-lived species, the Gopher Tortoise." 42rd Annual Gopher Tortoise Council Meeting, Oct 2020, Virtual meeting.

Hunter, E.A., Rostal, D.C., and Shoemaker, K.T. 2020. "Climate variability influences aspects of fecundity but not survival in a long-lived species, the Gopher Tortoise." The Wildlife Society National Conference, Virtual meeting.

DeSha, J.N., Loope, K.J., Aresco, M.J., Shoemaker, K.T., Hunter, E.A. 2020. "Can Gopher Tortoise nesting behavior compensate for the effects of climate change?" The Wildlife Society National Conference, Virtual meeting.

Loope, K.J., DeSha, J.N., Rothermel, B.B., Smith, L.L., Rostal, D.C., Shoemaker, K.T., Hunter, E.A. 2020. "Nest site, nest temperature, and hatching outcomes across an environmental gradient in the Gopher Tortoise." The Wildlife Society National Conference, Virtual meeting.

Shoemaker, K.T., Walden, M., Esque, T., Nussear, K., Hunter, E.A., Presentation. Aug 2020. "Climatic and environmental sensitivities of Mojave Desert Tortoise demographic rates: New insights from old data". Ecological Society of America, Virtual meeting.

Shoemaker, K.T., Walden, M.A., Hunter, E.A., Esque, T.C., Nussear, K. Invited Symposium Paper. Feb 23, 2020. Critical habitat breadth for *Gopherus* Tortoises. Annual Meeting of the Desert Tortoise Council, Las Vegas, NV.

Loope, K.J., Rostal, D.C., Baker, H., Walden, M.A., Shoemaker, K.T., Hunter, E.A. Poster presentation. Nov 16, 2019. Non-invasive methods for sexing hatchling Gopher Tortoises (*Gopherus polyphemus*). Annual meeting of the Gopher Tortoise Council, Gulf Shores, AL.

Hunter, E.A., and Rostal, D.C. Oral presentation. Nov 16, 2019. Effects of fire management on Gopher Tortoise population dynamics depend on habitat context. Annual meeting of the Gopher Tortoise Council, Gulf Shores, AL.

DeSha J.N., Loope, K.J., Aresco, M.J., Shoemaker, K.T., Hunter, E.A. Poster presentation. Nov 16, 2019. Can Gopher Tortoise nesting behavior compensate for the effects of climate change? Annual meeting of the Gopher Tortoise Council, Gulf Shores, AL.

Hunter E.A., Shoemaker, K.T., and Rostal, D.C. Poster presentation. Nov 7, 2019. Effects of climatic variation of Gopher Tortoise population vital rates. Georgia Climate Conference, Atlanta, GA.

Shoemaker, K.T., Walden, M.A., Hunter, E.A., Esque, T.C., Nussear, K. Contributed Paper. Oct 2, 2019. Rangewide environmental sensitivities of Mojave desert tortoise demographic rates: New insights from old data. Annual meeting of the Wildlife Society, Reno, NV.

Loope K.J., Rostal, D.C., Baker, H., Walden, M.A., Shoemaker, K.T., Hunter, E.A. Poster presentation. Sept 30, 2019. Non-invasive methods for sexing hatchling Gopher Tortoises (*Gopherus polyphemus*). Annual meeting of The Wildlife Society, Reno, NV.

Hunter E.A., and Rostal, D.C. Poster presentation. Sept 30, 2019. Effects of fire management on Gopher Tortoise population vital rates: results from a 21-year mark-recapture study. Annual meeting of The Wildlife Society, Reno, NV.

Hunter, E.A., K.J. Loope, J.N. DeSha, D.C. Rostal, L.L. Smith, M.J. Aresco, and K.T. Shoemaker. 2018. Accounting for demographic plasticity in climate change planning for Gopher Tortoises. Gopher Tortoise Council annual meeting. Venus, FL

MITCH-Clim v0.1 User Guide

Model-based Insights into Tortoise Critical Habitat under Climate Change

Authors: Kevin Shoemaker, Elizabeth Hunter, Kevin Loope, Michael Lam

Last Modified 7/3/2023

Table of Contents

Preface.....	159
Introduction.....	159
Purpose.....	159
Accessibility.....	160
Application.....	160
Quick Start Guide	161
Loading Scenarios (Step 1).....	162
Selecting Inputs.....	162
Activate Climate Effects on Tortoise Vital Rates:.....	163
Climate Scenarios:	163
Choosing a Region of Interest (Step 2).....	164
Method 1 - Drawing a polygon.....	164
Method 2 - Selecting a polygon from dropdown menu	166
Method 3 - Uploading a polygon from file.....	166
Analysis (Step 3).....	167
Understanding the Outputs	168
Abundance over Time (Current Run)	168
Abundance over Time by Age Structure	169
Abundance over Time (Scenario Comparison).....	169
Abundance Map	170
Downloading Results.....	171
Starting New Analyses.....	171

Preface

Introduction

Conserving threatened and endangered species (TES) on Department of Defense (DoD) installations, without sacrificing vital military objectives, is necessarily a complex balancing act – especially given the uncertainties and risks associated with climate change.

This project advances the concept of critical habitat breadth as the foundation for rigorous TES conservation planning and vulnerability assessment in the face of climate change. In this application we apply the critical habitat breadth concept to the Desert Tortoise (*Gopherus agassizii*) to understand and predict the range of climate conditions capable of harboring viable populations of this species now and in the future.

We compiled and re-analyzed previously collected data from across the range of the Desert Tortoise to assess how Desert Tortoise demographic vital rates (e.g., survival, fecundity, age-at-maturity) respond to spatiotemporal environmental and climatic gradients. In addition, we conducted intensive nest monitoring surveys to investigate how hatching success and hatchling sex ratios respond to climate gradients. We integrated these statistical models into comprehensive, spatially explicit predictive models capable of forecasting annual range-wide population dynamics for both species. We used this simulation model to forecast and visualize population dynamics through the year 2099 under multiple plausible scenarios, and to predict when and where populations are most likely to be self-sustaining.

Purpose

The main purpose of this application is to assist resource managers in identifying mitigation and conservation strategies capable of sustaining viable Desert Tortoise metapopulations under changing and uncertain future climate conditions. This application allows the user to select a scenario and define a region of interest within the Desert Tortoise range and the application provides detailed information on projected spatiotemporal population dynamics. The application also allows users to compare expected conservation outlooks under multiple scenarios.

The sections below provide detailed instructions on how to utilize this application.

Accessibility

The MITCH-Clim v0.1 application is hosted on shinyapps.io: https://kevintshoemaker.shinyapps.io/Mitch-clim_v01/, and also available for download through [GitHub](#).

If using the PC version downloaded from GitHub, user must also have R and RStudio downloaded to run the application:

- Open the App.R file in the RStudio integrated development environment (IDE). App.R is located in the DTApp folder within the MitchClim_v01 folder in GitHub.
- RStudio should automatically search for and ask the user to download any package dependencies upon opening of App.R. Make sure all required packages are installed prior to running the application.
- Click on ‘Run App’ on the top right of the RStudio environment.

Application

Instructions on how to use the application can also be found in the default ‘Overview’ tab of the application itself.

MITCH-CLIM v0.1

Model-based Insights into Tortoise Critical Habitat under CLIMate change

Overview Application Figures About More Info

Intro & Purpose

The main purpose of this application is to assist resource managers in identifying mitigation and conservation strategies capable of sustaining viable metapopulations of Desert Tortises under changing and uncertain future conditions. Defining the critical habitat breadth allows us to understand and predict the full range of habitat and environmental conditions capable of harboring viable populations now and in the future. This application allows the user to define a region of interest from which to display critical habitat breadth over time.

We used these range-wide, spatially explicit population simulation models to produce projections of population growth rates and critical habitat over the next century, and to assess metapopulation viability under multiple plausible future climate scenarios. In this study, each cell represents a 5 X 5 km grid area.

The sections below provide detailed instructions on how to utilize this application.

Selecting Inputs

The user must <check> which climate effect variables to include in the display of results. All CE variables are checked ‘on’ as the default setting. Once inputs are selected, click on the ‘Load Scenario’ button to begin loading. The loading process takes 60-90 seconds.

Tortoise Climate Effect Variables:

Key vital rates modeled for fecundity and survival.

Adult survival : This parameter represents the annual survival rate for all reproductive adults (individuals above the age-at-maturity). Modeled using data from existing capture-mark-recapture and telemetry data, and published literature.

Egg production : This parameter was measured as the total annual per-capita egg production, also known as clutch size. Modeled using data from diagnostic imaging technology, and telemetry.

Probability of reproduction : This parameter was drawn directly from our range-wide analysis of Desert Tortoise reproductive output. Modeled using data from diagnostic imaging

Quick Start Guide

1. At top of navigation panel, click on the 'Application' tab

MITCH-CLIM v0.1

Model-based Insights into Tortoise Critical Habitat under CLimate change

[Overview](#) [Application](#) [Figures](#) [About](#) [More Info](#)

STEP 1: Select Scenario

Select climate effects to be activated:

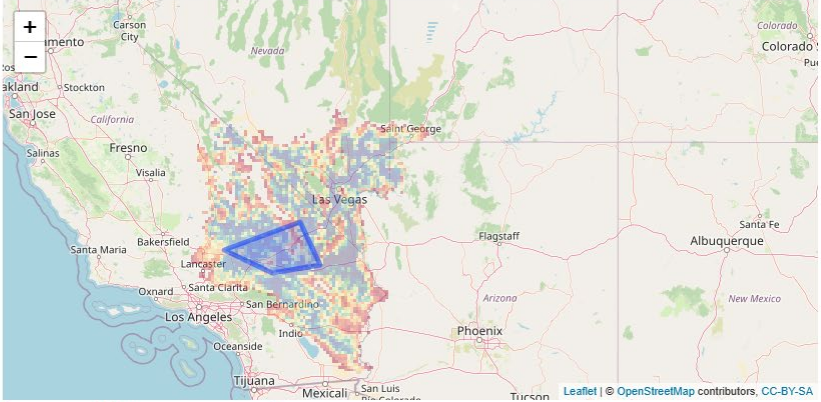
- ☒ Adult survival
- ☒ Egg production
- ☒ Probability of reproduction
- ☒ Hatching success
- ☒ Sex ratio
- ☒ Somatic growth

Climate scenario

rcp2.6

Load Scenario

typical download time: 90 seconds



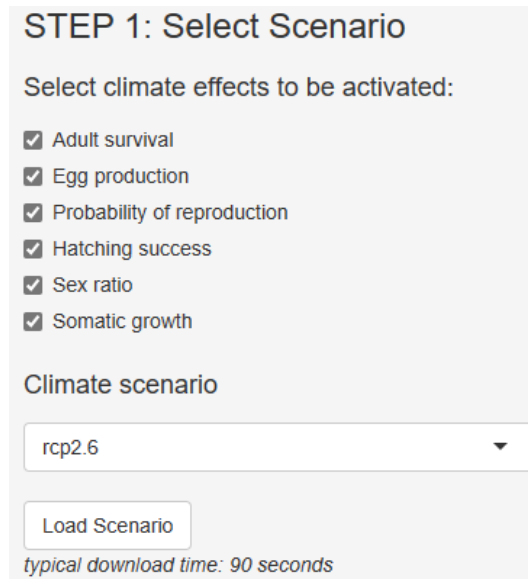
Clear ROI Download Polygon

2. Select vital rates to activate (all boxes checked means that each of the six vital rates are allowed to change across space and time as a function of climate, in turn driving Desert Tortoise abundance)
3. Choose one of the four climate scenarios (RCP2.6, RCP4.5, RCP6.0, or RCP8.5)
4. Load scenario (unique vital rate x RCP combination) by clicking the 'Load Scenario' button
5. Choose an ROI (region of interest) using one of three methods
 - a. Draw polygon directly on interactive map window
 - b. Select polygon from pre-set list
 - c. Upload polygon from file (ESRI Shapefile format)
6. Click on the 'Run Analysis' button (takes two minutes or longer to process)
7. Click on the 'Output Viewer' tab to display results (tab displays after analysis is complete)
8. Load additional scenarios (Application tab) to compare by re-running the above steps

Loading Scenarios (Step 1)

Selecting Inputs

First, the user can use the checkbox interface (see below) to indicate which Desert Tortoise population vital rates, if any, they wish to model as a function of climate. The default scenario is for all vital rates to respond to climate: since all climate-vital rate relationships were fitted empirically, we consider this condition to be the most realistic. Next, use the dropdown menu to select which RCP scenario to include in the analysis. See next section below for what each variable represents.



STEP 1: Select Scenario

Select climate effects to be activated:

- ☒ Adult survival
- ☒ Egg production
- ☒ Probability of reproduction
- ☒ Hatching success
- ☒ Sex ratio
- ☒ Somatic growth

Climate scenario

rcp2.6 ▼

Load Scenario

typical download time: 90 seconds

Once all scenario inputs are selected, click on the 'Load Scenario' button to begin downloading simulation results from the cloud. The loading process takes about 60-90 seconds.

A progress bar will appear in the bottom right of the screen, saying 'Download in Progress'. Once completed, the message 'Your selected scenario has been successfully downloaded!' will appear on the screen.

Activate Climate Effects on Tortoise Vital Rates:

Six key Desert Tortoise vital rates can be modeled as a function of climate:

Adult survival: This parameter represents the annual survival rate for all reproductive adults (individuals above the age-at-maturity). Modeled using data from existing capture-mark-recapture and telemetry data, and published literature.

Egg production: This parameter was measured as the total annual per-capita egg production, also known as clutch size. Modeled using data from diagnostic imaging technology, and telemetry.

Probabability of reproduction: This parameter was drawn directly from our range-wide analysis of Desert Tortoise reproductive output. Modeled using data from diagnostic imaging technology, and telemetry.

Hatching success: This parameter represents the fraction of eggs within each nest that hatch successfully, assuming the nest does not fall victim to predation. Modeled using data from nest site monitoring.

Sex ratio: The sex ratio parameter (proportion of hatchlings born female; PF) reflects the fraction of successful hatchlings in each nest that are born female. Since this species has temperature-dependent sex determination, this parameter was modeled as a function of temperature, using data from nest site monitoring and hormone assays.

Somatic growth: This parameter represents growth by age as modeled using the von Bertalanffy growth function, involving a growth coefficient k and an asymptotic body size coefficient a . Both coefficients are modeled separately as a function of climate. Modeled using data from diagnostic imaging technology, and morphometrics.

Climate Scenarios:

The Representative Concentration Pathways (RCPs) describe four different 21st century pathways of greenhouse gas (GHG) emissions and atmospheric concentrations, air pollutant emissions and land use.

RCP 2.6: This scenario aims to keep global warming likely below 2°C above pre-industrial temperatures. This is the most stringent of the scenarios.

RCP 4.5: This intermediate scenario assumes a consistent decrease in emissions as a consequence of assumed air pollution control and GHG mitigation policy. More likely than not to exceed 2°C temperature increase by end of the 21st century.

RCP 6.0: This intermediate scenario assumes a consistent decrease in emissions as a consequence of assumed air pollution control and GHG mitigation policy. Likely to exceed 2°C temperature increase by end of the 21st century.

RCP 8.5: This 'baseline' scenario follows higher GHG emissions compared to the other three scenarios.

Choosing a Region of Interest (Step 2)

In this study, each cell/pixel represents a 5 X 5 km grid area.

There are three ways in which the user can select an ROI. Note that only one region of interest is stored at any given time.

STEP 2: Select ROI

To select a region of interest (ROI), you can (1) Draw a polygon to define the ROI on the map window to the right (one vertex generated per click), (2) Select a DoD installation from a preset list, or (3) upload a polygon from a shapefile (.shp)

Choose DoD Installation

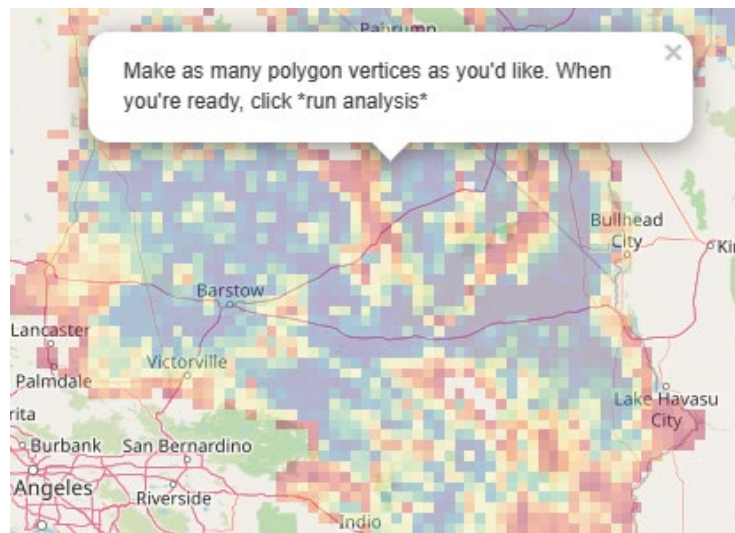
(Optional) select installation ▼

Upload a polygon ?

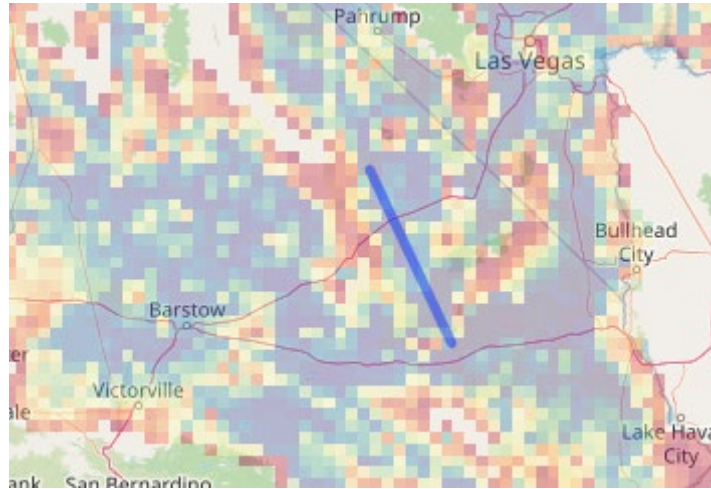
Browse... No file selected

Method 1 - Drawing a polygon

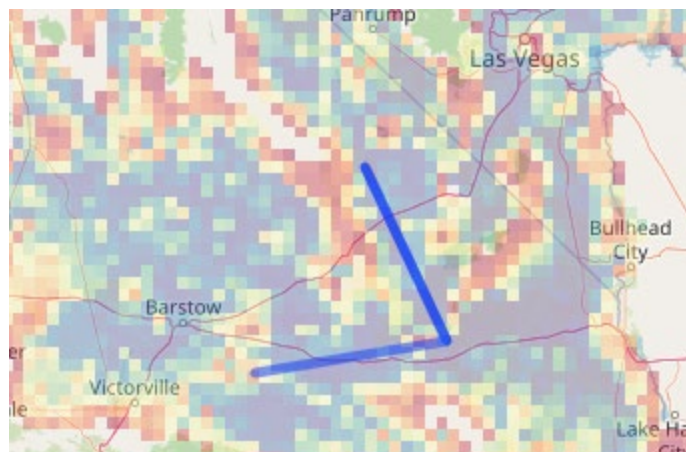
1. Click inside the Map box to initiate first vertex of polygon. If you make a wrong vertex at any point, click on 'Clear ROI' to start over.
2. A vertex will be created where 'clicked' and a message will appear. You can click on the “X” button in the top righthand corner of the pop-up window to clear it from the screen.



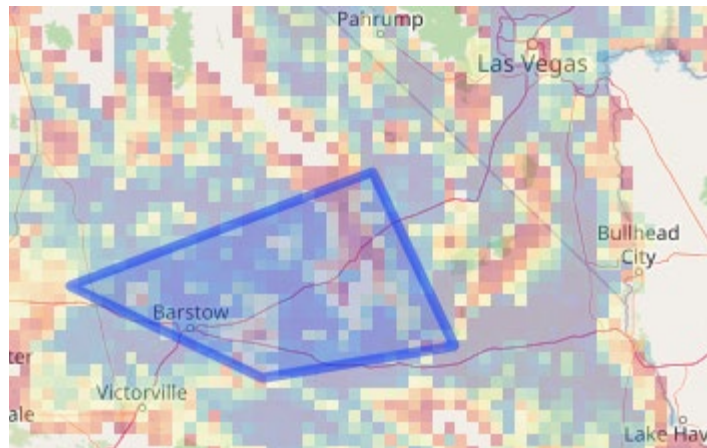
- Click on a second location in the Map box to draw a second vertex. A blue line connecting these two points will now display.



- Continue to add vertices to your polygon until you have a least 4 vertices.

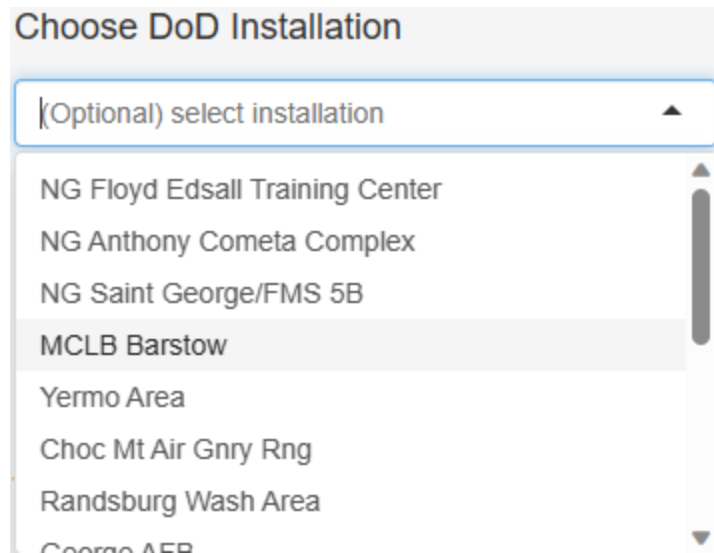


- Your polygon should now be a closed figure with the last line/vertex connected back to your first vertex. You can continue to add as many vertices as you would like to define your region of interest.



Method 2 - Selecting a polygon from dropdown menu

1. Click on dropdown menu



2. Select the desired preset DoD installation site (Note that this menu lists only the DoD sites that overlap with the desert tortoise range).
3. The chosen DoD site polygon will automatically generate on the map.

Method 3 - Uploading a polygon from file

1. This must be an associated set of polygon files in the form of a shape files, with file extensions .dbf, .prj, .shp, and .shx.
2. Select all four file components together and then click ok.
3. A popup should then appear in a second to confirm that the file selected has been successfully downloaded.
4. The polygon should automatically render onto the map.

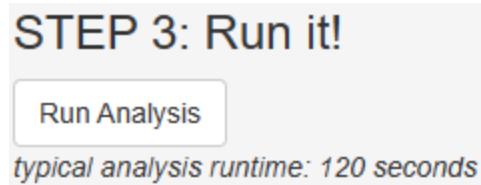
NOTE you can download the current polygon (regardless of how it was generated) to your computer by clicking the green 'Download Polygon' button under the Map box.

 Download Polygon

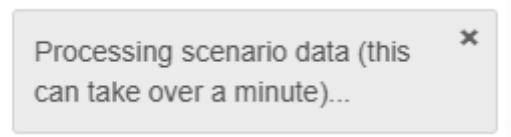
Once an ROI is made, you may proceed to 'Run Analysis' (if scenario already downloaded). Clicking on the "Clear ROI" button will start a new polygon of interest.

Analysis (Step 3)

Upon clicking the 'Run Analysis' button, a popup message on the bottom right should appear saying 'processing started'.



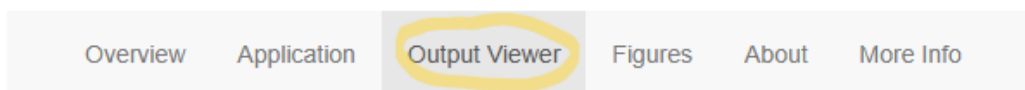
Please wait for the duration for the analysis to run (this typically takes 2-4 minutes).



A second message will appear once the analysis is complete. A new tab called 'Output Viewer' will appear next to the 'Application' tab. Click on this tab.

MITCH-CLIM v0.1

Model-based Insights into Tortoise Critical Habitat under CLIMate change



Understanding the Outputs

The analysis process automatically clips the portion of the ROI that is within the boundaries of the desert tortoise range and runs the analysis for both the ROI as well as the full desert tortoise range extent. Each output tab will display results for both ROI and full range. If no ROI has been specified, the full range is used as the ROI and there should be no difference between the ROI and full range outputs.

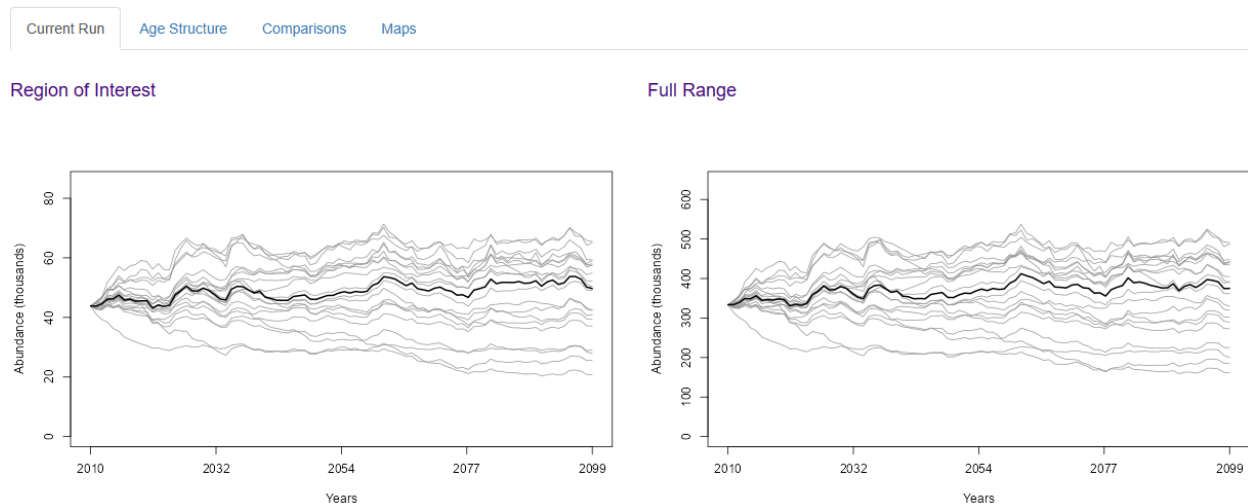
The ‘Output Viewer’ tab contains several results panels which display the following:

- Current Run -- Plot of final abundance for ROI vs Full Range for the most recently run scenario
- Age Structure -- Plot of final abundance broken down by age structure (hatchling, juvenile, and adult stages) of ROI vs Full Range of user-selected scenarios for comparison
- Comparison -- Plot output of ROI vs all user-selected scenarios run during this session (enabling comparison of multiple scenarios)
- Map -- Map output of ROI vs Full Range of user-selected scenarios for comparison

Abundance over Time (Current Run)

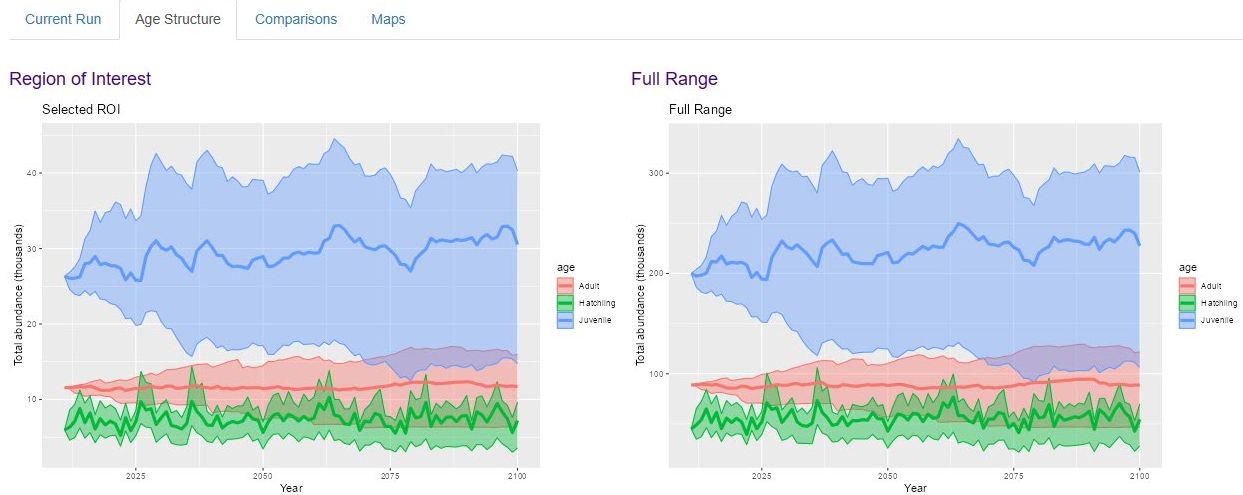
The default tab in the Output Viewer page tab is the ‘Current Run’ tab, which plots the 20 replicates of abundance (by the thousands) by year from 2010 to 2099. The dark line represents the median replicate abundance. Note the scales can differ in the left Y axis between the ROI and full range.

Main Results:



Abundance over Time by Age Structure

The second tab in Output Viewer displays the same results from the 'Current Run' tab, but broken down by the three primary tortoise age groups (hatchling, juvenile, and adult). As you can see in the example below, the majority of the desert tortoise population tends to be in the juvenile phase (it takes many years to reach adulthood). Again, note the scales can differ in the left Y axis between the ROI and full range.



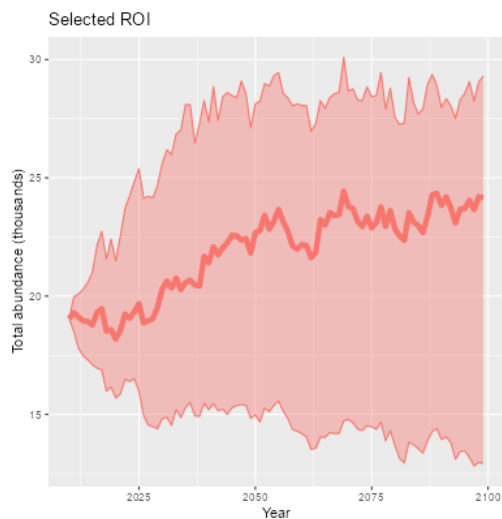
Abundance over Time (Scenario Comparison)

The third tab in Output Viewer allows you to check from a list of previously loaded scenarios in this open session of the application to display. It will show the current/recent ROI data on the left, and the full range on the right, as usual.

Region of Interest

Select scenario(s) to display:

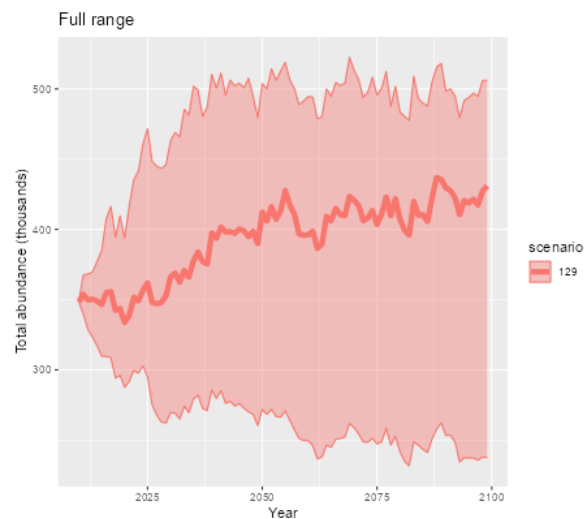
☒ 129



Full Range

Select scenario(s) to display:

☒ 129



You will be able to check on/off which scenarios to display. Please use the accompanied chart on the top portion of the tab to remember what parameter settings each scenario index (right-most column in the table that appears at the top of the scenario comparison tab) corresponds to:

phi.a_clim	cs_clim	pr_clim	hs_clim	pf_clim	growth_clim	clim_scenario	index
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	rcp26	1.00

phi.a_clim = Adult survival

cs_clim = Egg production

pr_clim = Probability of reproduction

hs_clim = Hatching success

pf_clim = Sex ratio

growth_clim = Somatic growth

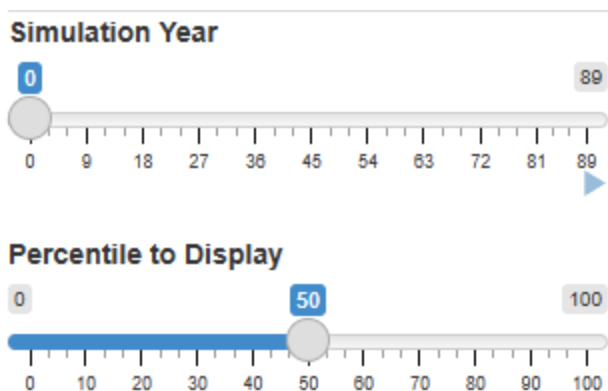
clim_scenario = Climate scenario

‘TRUE’ signifies that the parameter was toggled ‘on’, meaning that it is impacted by climate factors.

‘FALSE’ signifies that the parameter was toggles ‘off’, meaning that it is NOT impacted by climate factors.

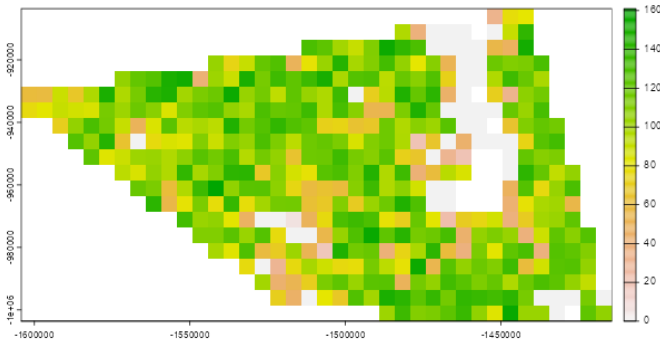
Abundance Map

The fourth and final tab in the output viewer allows the user to select the year and percentile of abundance to display spatially, both for the selected ROI (left map) and the full range (right map). Drag the slider bar to select a specific simulation year and a specific abundance percentile to display. You may also use the play button located on the right-hand side of the simulation year to automatically load the next year’s results every 5 seconds. Note that the results take some time to process and refresh for each new year displayed.

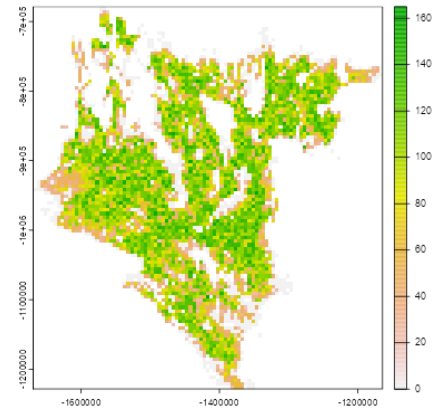


X (bottom) and Y (left) axes show the geographic coordinates, while legend (color ramp) indicates the total population abundance in each pixel:

Region of Interest



Full Range



Downloading Results

Users will be able to download the maps and/or plots individually to their own device as a Tif file. Users will also be able to download any current selected polygon ROI as a shapefile, which can be reuploaded in future app sessions to run more analyses. [NOTE: this feature has not yet been implemented as of 7/3/2023]

Starting New Analyses

- *To begin a new analysis, click back to the 'Application' tab*
- *Click on the red 'Clear ROI' button located under the Map panel*

✕ Clear ROI

- *This will get rid of the polygon(s) as well as clear any tables and maps displayed in the results area (Output Viewer tab)*