OXFORD UNIVERSITY PRESS | Integrative and Comparative Biology

Physiological trade-offs in lizards: Costs for individuals and populations

Journal:	*Integrative and Comparative Biology*
Manuscript ID	ICB-2017-0068
Manuscript Type:	Symposium article
Date Submitted by the Author:	31-Mar-2017
Complete List of Authors:	Smith, Geoffrey; Utah State University, Biology French, Susannah; Utah State University, Biology
Keywords:	ecoimmunology, reptiles, reproductive, energy

Physiological trade-offs in lizards: Costs for individuals and populations

G.D. Smith[1,2] and S.S. French[1,2]

[1]Department of Biology, Utah State University, Logan, Utah 84322-5305, USA; [2]Ecology Center, Utah State University, Logan, Utah 84322-5205

Synopsis

The immune system is a critical component of health and fitness, whereby organisms must maintain sufficient health to survive to reproduce. Because of the key role of immunity in an organism's fitness, the use of immunological indices is widespread. However, there is a paucity of empirical support for the best way to interpret immunological data, and the internal energetic state of the organism, as well as the external environmental pressures it faces, are often not considered concurrently. Presently, a more robust immune response is thought to be advantageous, regardless of context. In reality, a "weaker" response may ultimately lead to improved fitness if the animal incurs fewer performance costs on competing systems, especially reproduction. To determine the fitness consequences of immunity, individual immunity and reproduction must be linked to population performance. A synthesis of results are presented using a well-studied model organism, the side-blotched lizard, from a combination of field and laboratory studies to test the hypothesis that resource availability alters energy allocation among the immune and reproductive systems. Specifically, experiments involving specific immune and reproductive metabolic and performance costs in a laboratory setting are discussed, as well as associated demographic trade-offs between survival and reproductive success, demonstrating essential links between immunity and the population.

Introduction

To reproduce, organisms must survive. Central to an organism's survival is its immune system (Janeway et al. 2001), but the myriad trade-offs associated with immunity and reproduction muddle the picture (Lochmiller and Deerenberg 2000; Sheldon and Verhulst 1996), and external stressors and internal energy budgets must be taken into consideration (Norris and Evans 2000; Wingfield and Sapolsky 2003). Indeed, just as Dobzhansky famously stated that "Nothing in biology makes sense except in the light of evolution," we can also say that nothing makes sense in without understanding an animal's energy status. Our objective is to view the organism in context, by combining controlled laboratory experiments and long-term field studies, and measuring a multitude of physiological and ecological parameters. By using this integrative approach, we can extricate the costs of reproduction and immunity, and, ultimately, link these two systems to understand how they dictate an organism's reproductive success and lifetime fitness.

Is a Bigger Immune Response Better?

In some situations, mounting a large immune response is beneficial because clears the organism of the infection quickly, improving survival probability and the likelihood of reproduction (Kluger et al. 1975). However, given the costly nature of immunity and its direct competition with other systems, reproductive output, and ultimately fitness, might benefit from an attenuated response (Ardia and Schat 2008; Demas et al. 2012). In fact, many wild animals live with chronic, life-long infections (Moret and Schmid-Hempel 2000; Ressel and Schall 1989). The countless examples of parasites coevolving with their hosts indicate that this is not the exception, but the norm (Lively 1996). Unfortunately, the complicated and numerous relationships among the immune, neurological, and endocrine systems makes it difficult to separate the different energetic costs and response to stimuli, and highly-controlled laboratory studies rarely mirror field conditions. Because natural selection occurs on the landscape of ecological variation, it is important to consider those pressures and restrictions.

Reptiles as a Model

Ectothermic- Reptiles are excellent models to study these trade-offs. They are ectothermic, so unlike mammalian models, the body temperature of the organism can be altered experimentally (Maung 1963) or held constant (Angilletta and Sears 2000), making the animals functionally homeothermic. This allows us to test for optimal temperatures for certain immunological pathways that evolved before metabolic thermoregulation held everything constant (Zimmerman et al. 2010). Using readily-available environmental chambers, temperature treatments can be given to the animals, and by using temperature-loggers, the behavioral thermoregulation of free-living individuals can be correlated with other measures of performance (Taylor et al. 2004).

Discrete Reproductive Investment- The costs of reproduction can come in many forms. The energy required for gamete production (Olsson et al. 1997) and follicular development (Krawchuk and Brooks 1998) are straightforward, but courtship (Sullivan and Kwiatkowski 2007), carotenoid deposition (Vinkler and Albrecht 2010), and parental care (Case 1978) can also demand large energetic costs. Although viviparity has evolved multiple times in reptiles (Shine 1995), ~85% of species lay eggs (Tinkle and Gibbons 1977), which are discrete reproductive investment units that can be further broken down by nutrient composition to the yolk and other components (Congdon and Gibbons 1985). The rare, or infrequent, parental care in reptiles simplifies the complicated behavioral investment made by most birds, and the relatively huge energetic investment of lactation in mammals. Furthermore, noninvasive techniques like ultrasonagraphy are useful in determine accurate clutch numbers and follicle sizes (Gilman and Wolf 2007).

Seasonality- Most reptiles (unlike humans and many other animals) have predictable seasonal patterns. Even in tropical regions where temperature in largely constant and hibernation does not occur, water availability causes animals to utilize favorable periods of time to reproduce and other periods to restore energy reserves or preserve them via torpor or estivation (Logan et al. 2014; Moritz et al. 2012). Comparing animals from the breeding season and nonbreeding season is a useful way to measure the energetic and nutritional cost of reproductive effort, and the effects of reproductive investment on immunity.

Territoriality and Housing- Many reptiles are territorial, meaning they are conspicuous and easy to catch. We can also take advantage of their site fidelity to recapture them days later and actually conduct immune trials in the field, which can be exceedingly difficult for other animals with low chances of recapture (Smith et al. 2013). Although some reptiles can get very large, most are small, and many are easily housed in commercially-available terraria. The large and active herpetocultural community is also a good resource for husbandry information. The ease of captive care makes these animals prime for laboratory studies.

Methodological Approaches

In our lab, we use several physiological tests to determine an animal's survival and reproductive viability. Wound healing is an immune challenge that involves multiple aspects of the immune system. Furthermore, it is ecologically relevant as lizards are often wounded in the wild by predators or conspecifics. Sterile biopsy punches are used on anesthetized lizards, and digital images immediately after the wound and throughout the healing profile allow us to determine healing rate (French et al. 2006). Bactericidal assays are similarly relevant to wild animals, which must respond to infections. Briefly, a known concentration of bacteria is added to the animal's plasma, which contains important immunological components. Natural antibodies, phagocytes, and other components kill the bacteria. Measuring the growth of resulting bacteria allows us to determine the bactericidal capacity of the plasma. The assay is customizable, and can be used with many types of bacteria and different temperatures depending on the question (French and Neuman-Lee 2012). In addition to these tests and others (lysis and agglutination, mitogen challenges, and more) we use a portable ultrasound to measure reproductive investment, often in conjunction with sex steroid analysis. Measuring the immunocompetence and reproductive investment of an animal can help us determine what energetic decision are occurring, but other simultaneous processes can complicate the results. For this reason, we also utilize stable isotope analysis to track resource movement through the animal, along with energy metabolites in the blood and metabolic rate to assess energy use and status. Circulating and challenge-induced hormone concentrations are measured, and so is oxidative stress.

Results and Discussion

The costs of reproduction and immunity are both material (proteins and essential minerals) and energetic, and if resources are limited the reproductive and immune systems are in competition for them. By using ecologically relevant techniques, we have demonstrated that trade-offs between the immune and reproductive systems are highly context-dependent (see Table 1). In wild tree lizards (*Urosaurus ornatus*), investment into reproduction came at the expense of wound healing but only during the energetically-taxing period of vitellogenesis (French and Moore 2008). Administration of follicle-stimulating hormone (FSH) caused a further increase in reproductive investment that resulted in suppressed healing when resources were held constant (French et al. 2007). In another study, N^{15} (a stable isotope) was injected into side-blotched lizards (*Uta stansburiana*) during vitellogenesis and a cutaneous biopsy was administered as an immune challenge. The animals were euthanized and dissected at the end of the experiment, and lizards that invested more protein into their scabs had reduced enrichment into the follicles, indicating that the immune challenge reduced reproductive investment in a trade-off. However, when animals were also injected with FSH, the trade-off reversed and animals that invested more into their scabs also invested more into their follicles (Smith et al., *in prep.*). These animals also had higher metabolic rates, and the simultaneous investment into two competing systems could indicate that the animals were manipulated toward a terminal investment. To further complicate these relationships, the immune system itself prioritizes energy and responds differently depending on the challenge. For instance, side-blotched lizards receiving lipopolysaccharide, did not show an increase in metabolic rate as expected, while lizards receiving a cutaneous biopsy dropped their metabolic rates in response, probably due to sickness behavior (Smith et al., *in review*).

When animals make resource allocations to immune and reproductive processes, other important aspects of their performance can suffer as well. We observed sprint speed, a factor important for escaping predators (Husak et al. 2006), in control lizards and lizards that received a cutaneous biopsy. Lizards that healed their wounds more quickly had slower sprint speeds, perhaps due to energetic or protein prioritization, or behavioral changes (Hudson, *unpublished data*). It may also be due to an overall downregulation of metabolism following a wound that we have observed repeatedly in side-blotched lizards. These behavioral parameters are important to consider because they can affect survival, and ultimately fitness, in wild populations.

What is Natural?

The strength of laboratory studies comes from the high degree of control researchers can achieve on their experimental treatments. However, the very mechanisms we are trying to understand evolved in a variable landscape, so field studies are crucial (see Table 2). Further complicating matters is the anthropogenic impact on virtually every ecosystem on the planet. To address this effect, we have sampled urban and rural populations of side-blotched lizards (*Uta stansburiana*) inside and near the city of Saint George, Utah. These short-lived species were expected to display differences in resource allocation across urban and rural sites, and in 2010 urban animals had higher levels of corticosterone, increased reproductive investment, and decreased immunocompetence (Lucas and French 2012). However, subsequent years of study have shown that precipitation can influence these factors. Lizards had higher reproductive investment and lower immune capacity during wetter years, and urban animals had more variation among these traits, perhaps indicating greater phenotypic plasticity (Smith et al., *in prep.*). A disparity in diet, based on isotope signature, also emerged between urban and rural sites (Durso et al., *in review*)

The ultimate goal of the long-term sampling effort in Saint George is to determine how physiological attributes of individuals contribute to populations over time. Lucas and French (2012) found reduced survival at the urban sites within a season, and a multi-year population model corroborates that finding (Smith et al., *in prep.*).

Short-lived and Long-lived Species

Certain attributes must be known to measure an animal's success, including lifespan. Side-blotched lizards are known to be longer-lived at northern latitudes than in the south (Tinkle 1967), so it stands to reason that physiological criteria vary along a latitudinal gradient as well. In 2012, lizards in Oregon had higher corticosterone concentrations and innate immune ability, but decreased reproductive investment compared to lizards in southern Utah. We hypothesized that the longer lifespans in northern animals would allow them future reproductive opportunities if they survived to the next breeding season, so increasing immune function at the expense of immediate reproduction was favorable. More southerly lizards were limited in terms of lifespan and were investing in immediate reproduction (Smith et al., *in prep.*).

Even the longest-lived side-blotched lizards rarely exceed 7-years old, and these individuals are exceptional (P.A. Zani, pers. comm.), but reptiles also include some of the longest-living vertebrates known to science, with some species living more than 100 years (Garrick et al. 2012). Marine iguanas (*Amblyrhynchus cristatus*) have an extended reproductive life (Wikelski and Wrege 2000) and appear to utilize a different strategy in response to anthropogenic disturbance, making them a good model to study how longer-lived species cope with change. Marine iguanas subjected to higher levels of tourism intensity have decreased bactericidal ability and smaller clutches sizes. This could be because these animals are entering a resource-saving state and reducing unnecessary expenditures, or because the stress of living with tourists is detrimental to these animals. Higher oxidative stress in the form of reactive oxygen metabolites at the tourist sites are further evidence in support of this hypothesis (French et al., *in review*).

The northern Bahamian rock iguana (*Cyclura cychlura*) is also long-lived, with an estimated lifespan of at least 30 years of age (Iverson et al. 2004). Endemic to the Bahamas, these lizards are listed as vulnerable on the International Union for Conservation of Nature Red List (Knapp et al. 2004). We are working in collaboration with the Shedd Aquarium to follow up on studies by Knapp et al. (2013), who found altered blood chemistry, including elevated blood glucose at sites frequented by tourists. Researchers also found that along with altered diet at tourist sites there is increased population abundance and decreased survival, (Hines 2011; Iverson et al. 2006; Smith and Iverson 2015), hinting at similar population responses to those observed in side-blotched lizards in response to urbanization. More recently we have also been measuring reproductive investment, immunity and oxidative stress across these populations.

Conclusions

So, is a bigger immune response a better immune response? Not necessarily. Being efficient with energetic and material resources among physiological systems, while responding appropriately to different stressors, appears to be most important. Our research up to this point indicates that there is a direct competition for resources between the immune and reproductive system, and these resources can include both energy and proteins. However, these trade-offs are not always predictable, and vary with reproductive stage, energy availability, and external stressors. Furthermore, behavioral changes like reduced sprint speed or sickness behavior must be considered when determining the animal's overall performance. Likely, subtle differences in energy status and timing lead to variations in trade-offs that increase lifetime fitness. Along the same lines increased immune efficiency rather than maximize response robustness is likely the most effective strategy. When individuals utilize different strategies that alter their own survival or reproductive output, the population as a whole can be affected by the life-history choices. In a world dominated by anthropogenic change, it is crucial to understand how individuals inform population dynamics that in turn determine the persistence of a species.

Acknowledgements

We would like to thank Simon Lailvaux and Jerry Husak for organizing this symposium. Many collaborators and lab members have made this work possible, including Lori Neuman-Lee, Andrew Durso, Gareth Hopkins, Alison Webb, Spencer Hudson, Emily Virgin, Austin Spence, Marilize Van der Walt, Heather Skinner, Tyler Hansen, Sydney Greenfield, Nick Kiriazis, John Petit, Taylor Petit, Julianne Wood, Edmund Brodie, Jr., Charles Knapp, John Iverson, Alan Savitzky, David Koons, Lise Aubry, Dale DeNardo, Michael Angilletta, Patricia Terletzky, Eric Gese, Bobby Fokidis, Peter Zani, Erick Yucailla, Trevor Brown, and Leilani Lucas. Funding for this work was provided by NSF IOS CAREER (#1350070) awarded to SSF, Utah State University Research Catalyst grant awarded to SSF, and Utah Agricultural Experiment Station (project #UA01104) to SSF. The Utah State University Ecology Center has also been very generous with funding for graduate students and we thank them.

References

Angilletta, M., Sears, M. (2000) The metabolic cost of reproduction in an oviparous lizard. Functional Ecology 14, 39-45.
Ardia, D., Schat, K. (2008) Ecoimmunology, Avian Immunology. Academic Press, London, 421-441.
Case, T.J. (1978) Endothermy and parental care in the terrestrial vertebrates. The American Naturalist 112, 861-874.
Congdon, J.D., Gibbons, J.W. (1985) Egg components and reproductive characteristics of turtles: relationships to body size. Herpetologica, 194-205.
Demas, G., Greives, T., Chester, E., French, S. (2012) The Energetics of Immunity, in: G.E. Demas, R.J. Nelson (Eds.), Ecoimmunology. Oxford University Press, 259-296.
French, S.S., DeNardo, D.F., Moore, M.C. (2007) Trade-Offs between the Reproductive and Immune Systems: Facultative Responses to Resources or Obligate Responses to Reproduction? The American Naturalist 170, 79-89.
French, S.S., Matt, K.S., Moore, M.C. (2006) The effects of stress on wound healing in male tree lizards (*Urosaurus ornatus*). General and comparative endocrinology 145, 128-132.
French, S.S., Moore, M.C. (2008) Immune function varies with reproductive stage and context in female and male tree lizards, *Urosaurus ornatus*. Gen. Comp. Endocrinol. 155, 148-156.
French, S.S., Neuman-Lee, L.A. (2012) Improved *ex vivo* method for microbiocidal activity across vertebrate species. Biology Open 1, 482-487.
Garrick, R.C., Benavides, E., Russello, M.A., Gibbs, J.P., Poulakakis, N., Dion, K.B., Hyseni, C., Kajdacsi, B., Márquez, L., Bahan, S. (2012) Genetic rediscovery of an 'extinct' Galápagos giant tortoise species. Current Biology 22, R10-R11.
Gilman, C.A., Wolf, B.O. (2007) Use of portable ultrasonography as a nondestructive method for estimating reproductive effort in lizards. Journal of Experimental Biology 210, 1859-1867.
Hines, K.N. (2011) Effects of ecotourism on endangered northern Bahamian Rock Iguanas (*Cyclura cychlura*). Herpetological Conservation and Biology 6, 250-259.
Husak, J.F., Fox, S.F., Lovern, M.B., Van Den Bussche, R.A. (2006) Faster lizards sire more offspring: sexual selection on whole-animal performance. Evolution 60, 2122-2130.
Iverson, J.B., Converse, S.J., Smith, G.R., Valiulis, J.M. (2006) Long-term trends in the demography of the Allen Cays Rock Iguana (*Cyclura cychlura inornata*): Human disturbance and density-dependent effects. Biological Conservation 132, 300-310.
Iverson, J.B., Smith, G.R., Pieper, L. (2004) Factors affecting long-term growth of the Allen Cays rock iguana in the Bahamas. Iguanas: biology and conservation, 176-192.
Janeway, C.A., Travers, P., Walport, M., Shlomchik, M.J. (2001) Immunobiology: the immune system in health and disease. 5 ed. Churchill Livingstone London.
Kluger, M.J., Ringler, D.H., Anver, M.R. (1975) Fever and survival. Science 188, 166-168.
Knapp, C.R., Hines, K.N., Zachariah, T.T., Perez-Heydrich, C., Iverson, J.B., Buckner, S.D., Halach, S.C., Lattin, C.R., Romero, L.M. (2013) Physiological effects of tourism and associated food provisioning in an endangered iguana. Conservation Physiology 1, cot032.
Knapp, C.R., Iverson, J.B., Buckner, S. (2004) *Cyclura cychlura*. The IUCN Red List of Threatened Species 2004: e.T6035A12356382, 1-10.
Krawchuk, M.A., Brooks, R.J. (1998) Basking behavior as a measure of reproductive cost and energy allocation in the painted turtle, *Chrysemys picta*. Herpetologica, 112-121.
Lively, C.M. (1996) Host-parasite coevolution and sex. BioScience 46, 107-114.

Lochmiller, R.L., Deerenberg, C. (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88, 87-98.
Logan, M.L., Cox, R.M., Calsbeek, R. (2014) Natural selection on thermal performance in a novel thermal environment. Proceedings of the National Academy of Sciences 111, 14165-14169.
Lucas, L.D., French, S.S. (2012) Stress-Induced Tradeoffs in a Free-Living Lizard across a Variable Landscape: Consequences for Individuals and Populations. PloS one 7, e49895.
Maung, R.T. (1963) Immunity in the tortoise *Testudo ibera*. The Journal of Pathology 85, 51-66.
Moret, Y., Schmid-Hempel, P. (2000) Survival for immunity: the price of immune system activation for bumblebee workers. Science 290, 1166-1168.
Moritz, C., Langham, G., Kearney, M., Krockenberger, A., VanDerWal, J., Williams, S. (2012) Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 1680-1687.
Norris, K., Evans, M.R. (2000) Ecological immunology: life history trade-offs and immune defense in birds. Behavioral Ecology 11, 19-26.
Olsson, M., Madsen, T., Shine, R. (1997) Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. Proceedings of the Royal Society of London B: Biological Sciences 264, 455-459.
Ressel, S., Schall, J. (1989) Parasites and showy males: malarial infection and color variation in fence lizards. Oecologia 78, 158-164.
Sheldon, B.C., Verhulst, S. (1996) Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution 11, 317-321.
Shine, R. (1995) A new hypothesis for the evolution of viviparity in reptiles. The American Naturalist 145, 809-823.
Smith, G.D., Lucas, L.D., French, S.S. (2013) Town and country lizards: Physiological ecology of side-blotched lizards across a variable landscape, in: W.I. Lutterschmidt (Ed.), Reptiles in Research: Investigatiions of Ecology, Physiology, and Behavior from Desert to Sea. Nova Science Publishers, Incorporated, New York City, New York, USA, 29-50.
Smith, G.R., Iverson, J.B. (2015) Effects of tourism on body size, growth, condition, and demography in the Allen Cay Iguana, *Cyclura cychlura inornata*, on Leaf Cay, The Bahamas. Herpetological Conservation and Biology.
Sullivan, B.K., Kwiatkowski, M.A. (2007) Courtship displays in anurans and lizards: theoretical and empirical contributions to our understanding of costs and selection on males due to female choice. Functional Ecology 21, 666-675.
Taylor, E.N., DeNardo, D.F., Malawy, M.A. (2004) A comparison between point-and semi-continuous sampling for assessing body temperature in a free-ranging ectotherm. Journal of Thermal Biology 29, 91-96.
Tinkle, D.W. (1967) The life and demography of the side-blotched lizard, *Uta stansburiana*. Miscellaneous Publications of the Museum of Zoology, University of Michigan 132, 1-182.
Tinkle, D.W., Gibbons, J.W. (1977) The distribution and evolution of viviparity in reptiles. Museum of Zoology, University of Michigan Ann Arbor, MI.
Vinkler, M., Albrecht, T. (2010) Carotenoid maintenance handicap and the physiology of carotenoid-based signalisation of health. Naturwissenschaften 97, 19-28.
Wikelski, M., Wrege, P.H. (2000) Niche expansion, body size, and survival in Galápagos marine iguanas. Oecologia 124, 107-115.

Wingfield, J., Sapolsky, R. (2003) Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15, 711-724.
Zimmerman, L., Vogel, L., Bowden, R. (2010) Understanding the vertebrate immune system: insights from the reptilian perspective. The Journal of Experimental Biology 213, 661-671.

Table 1. Immune and reproductive trade-offs via laboratory experiments.

Species	Challenge	Outcome	Citation
Urosuarus ornatus	Cutaenous biopsy & food restriction	Trade-offs between immune and reproductive systems only occurred with limited resources	(French et al. 2007a)
Urosuarus ornatus	Cutaneous biopsy, follicle-stimulating, hormone (FSH), & food restriction	Suppressed wound healing during vitellogenesis when food was limiting; FSH increased reproductive investment and suppressed wound healing	(French et al. 2007b)
Urosuarus ornatus	Cutaneous biopsy	Suppressed wound healing during vitellogenesis in females in the wild; suppressed wound healing in mid-reproductive season in laboratory-housed males	(French and Moore 2007)
Uta stansburiana	Cutaneous biopsy & restraint stress	Suppressed bactericidal ability after restraint stress in wounded lizards, but faster early wound healing correlated positively with bactericidal ability	(Neuman-Lee and French 2014)
Uta stansburiana	Cutaneous biopsy	Advanced reproductive stage correlated with more N^{15} being deposited into follicles at the expense of healing wounds	(Durso & French, *in review*)
Uta stansburiana	Cutaneous biopsy & lipopolysaccharide (LPS)	Biopsies deceased metabolic rate while LPS did not, although it did increase bactericidal ability. The greater the decrease in metabolic rate, the greater the wound healing rate	(Smith et al., *in review*)
Uta stansburiana	Cutaneous biopsy	Greater wound healing rate correlated negatively with spring speed	(Hudson et al., *in prep*)
Uta stansburiana	Cutaneous biopsy & FSH	Biopsies decreased metabolic rate; FSH increased it. N^{15} trade-off observed In absence of FSH, but FSH injected reversed the trade-off	(Smith et al., *in prep*)

Table 2. Immune and reproductive trade-offs observed in wild animals (field studies).

Species	Challenge	Outcome	Citation
Urosuarus ornatus	Cutaneous biopsy	Suppressed wound healing during vitellogenesis in females in the wild; suppressed wound healing in mid-reproductive season in laboratory-housed males	(French and Moore 2007)
Urosuarus ornatus	Urban-rural gradient	Urban lizards had lower heterophil:lymphocyte ratios but higher overall leukocyte counts	(French et al. 2008)
Amblyrhynchus cristatus	Ecotourism, cutaneous biopsy, & restraint stress	Suppressed wound healing & hemolytic complement activity at tourist sites. Baseline bactericidal ability similar, but post-stress ability suppressed at tourist sites	(French et al. 2010)
Uta stansburiana	Urban-rural gradient	Reduced survival and immunocompetence at urban sites, but increased reproductive investment	(Lucas and French 2012)
Uta stansburiana	Urban-rural gradient	Isotope signatures between urban and rural lizards indicate different diets. These nutritional differences can be predictive of bactericidal ability & stress reactivity	(Durso et al., *in prep*)
Uta stansburiana	Latitudinal gradient	Longer-lived northern animals had increased bactericidal ability & reduced reproductive investment compared to southern lizards in 2012, but drought conditions reversed this trend in 2013	(Smith et al., *in prep*)
Uta stansburiana	Urban-rural gradient	Reproductive investment increased at the expense of bactericidal ability in wetter years in urban & rural sites. Trend reversed in drier years, & urban lizards changed more in response to precipitation. Survival greater in rural populations	(Smith et al., *in prep*)