Ecological and Phenotypic Divergence among Ornate Tree Lizard (*Urosaurus ornatus*)

Color Morphs in Response to Environmental Variation

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This dissertation titled
Ecological and Phenotypic Divergence among Ornate Tree Lizard (*Urosaurus ornatus*)
Color Morphs in Response to Environmental Variation

by

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has been approved for
the Department of Biological Sciences
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ABSTRACT

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Ecological and Phenotypic Divergence among Ornate Tree Lizard (*Urosaurus ornatus*)
Color Morphs in Response to Environmental Variation

Director of Dissertation: Donald B. Miles

Anthropogenic disturbance has been described as an agent of ecological divergence, yet our understanding of the processes linking these phenomena are limited. The changes in resource availability (i.e., resource limitation) following a disturbance may favor variation in physiology, behavior, or ecology (e.g., habitat use and diet) in a species in order to minimize competition and satisfy life-history demands. Consequently, populations in disturbed environments may differ in these characteristics from populations in environments where resources are abundant. In this dissertation I address these considerations for tree lizards (*Urosaurus ornatus*) from grassland regions varying in prescribed burn history. In the southwestern US, burning induces environmental shifts towards structural homogenization and grass-dominance, resulting in resource-limited environments. Moreover, tree lizards are polymorphic in reproductive behavior and throat color that is maintained by socially-mediated sexual selection. In the following chapters I first introduce my study system and the role of anthropogenic disturbance in generating environmental variation (Chapter 1). I then validate the use of stable isotope analysis to describe one of the major consequences of resource limitation, trophic niche divergence, among color morphs of my focal taxon (Chapter 2). I then use field-collected isotopic data to demonstrate that color morphs differing in reproductive behavior may also
diverge in ecology and morphology (Chapter 3). Thus, color polymorphic species are likely maintained in part by both divergent natural and sexual selection, and consequently, not all morphs may respond equally to environmental perturbations. Using a novel approach, I link morphological and ecological traits with environmental variation, illustrating that *U. ornatus* color morphs differing in morphological trait combinations also differ in the degree they ‘fit’ their microhabitats (Chapter 4). Specifically, dominant morphs, and those lizards that exhibit divergent (non-average) morphologies, tended to ‘fit’ best. I use capture-mark-recapture data to explicitly demonstrate that those lizards also exhibited a survival advantage in more-disturbed sites. Finally, I demonstrate that environmental variation alters microhabitat use and spatial segregation of *U. ornatus* morphs, resulting in increased spatial overlap and more-intense social interactions among male lizards, favoring both ecological and phenotypic divergence in burned habitats (Chapter 5). Altogether, my findings suggest that divergence in phenotypic and ecological traits in *U. ornatus* may be an adaptive response to resource limitation resulting from environmental variation.
DEDICATION

This work is dedicated to my parents Stephen and Leslie Lattanzio, my sister Janelle and her family, and my love, Kortney Jaworski.

I thank each of you for believing in me and providing me with encouragement and support along my journey to becoming a Doctor of Philosophy in Biology.
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CHAPTER 1: INTRODUCTION

I. Anthropogenic Disturbance and Environmental Variation

A major challenge in ecology is identifying the consequences of large-scale anthropogenic disturbances for wildlife. Indeed, human activities are advancing environmental change around the world at an increasingly rapid pace, with potentially major consequences for animal populations. In general, anthropogenic disturbances result in the redistribution of the types and availability of vegetation and structural habitat characteristics in a region. Depending on the region affected, these alterations may enhance (Palmer et al. 2010) or reduce (Peterson and Reich 2008) habitat structural heterogeneity and cover. These disturbances may also be direct or indirect. Direct anthropogenic disturbances, including primarily land development and land management practices, have immediate effects and typically reduce habitat heterogeneity at higher frequencies (e.g. prescribed burning, Peterson and Reich 2008). The effects of indirect environmental disturbances, such as how carbon emissions influence ambient temperatures, may be less immediate and not necessarily decrease habitat heterogeneity. In most cases, these types of disturbance interact to contribute to broader-scale phenomena like global climate change (D'Antonio and Vitousek 1992).

Prescribed burning is a common land management practice in the southwestern United States that is used to mitigate wildfire risk after decades of active fire prevention (e.g. Boer et al. 2009, Russell et al. 2009). Historically, many arid ecosystems were maintained by periodic wildfires sparked by lightning during storms. Implementation of a
fire suppression policy in the early 1900's reduced the frequency of wildfires and changed forest composition and structure throughout the US. In grassland regions, fire suppression favored shrub encroachment, which in turn causes fires to burn hotter and more intensely, resulting in often catastrophic, uncontrolled wildfires.

In the southwestern US, like many other regions, variation in prescribed burn frequency prompts the reorganization of dominant vegetation communities. Unique to arid environments however are the stark contrast in the properties of vegetation exhibiting C₃ and C₄ photosynthetic pathways, including cover, quality (e.g. nitrogen content), and structure (e.g. Wolf et al. 2002, Warne et al. 2010). Frequent burning promotes grass (C₄) recruitment, given that most species are fire-adapted to seed and re-establish populations quickly following a burn (both native and invasive species, see D'Antonio and Vitousek 1992). Areas burned more frequently are subsequently open habitats characterized by near-100% grass cover. In contrast, unmanaged areas protected from wildfires are more heterogeneous and largely dominated by C₃ shrubs, forbs, and trees. These habitats also have greater canopy cover and structure, and support more thermally-variable microhabitats. Significant environmental variation is thus generated across burned and non-burned regions.

II. Limitations to Our Understanding of the Effects of Anthropogenic Disturbance on Animal Populations

Although the economic and environmental implications of prescribed burning are clear (e.g. reduced risk of wildfires), its effects on wildlife remain poorly understood.
Whereas grassier habitats will promote more controllable, low-intensity burns that pose little risk to human populations (Boer et al. 2009), more-heterogeneous habitats that are burned less-frequently likely provide a greater diversity of resources for wildlife to exploit (Engle et al. 2008).

The majority of studies focusing on the effects of anthropogenic disturbance in the form of land management practices take a numerical approach. In particular, these studies utilize changes in abundance, occupancy, and/or diversity of species or communities in disturbed regions to infer effects (e.g. Russell et al. 2009, Grant et al. 2010). Often, these studies report negative or contrasting effects on wildlife communities. For example, Russell et al. (2009) demonstrate that some types of birds (e.g. Picoides woodpeckers) benefit from burning whereas others (e.g. Pine Siskin, Carduelis pinus) decline. These bird species contrast in their microhabitat preferences, namely, trees versus seeds. The increased openness of habitats following burns may better support species that utilize unburned aspects of the landscape (e.g. tall trees) over others that rely on resources that may be consumed by fire or available following recruitment some time post-fire. Other studies indicate that any negative numerical effects of burning are limited to the year immediately following a burn (Grant et al. 2010).

Anthropogenic disturbances like prescribed burning impact species within the same habitat in different, often contrasting ways (Driscoll and Henderson 2008, Hodson et al. 2010). These studies illustrate that the numerical responses (i.e. distributional shifts) of wildlife in response to anthropogenic disturbance depends on disturbance intensity as well as population density prior to the event. These snapshots of the impact on species
diversity (see also Langford et al. 2007) unfortunately do little to indicate long-term fitness effects, including capturing variation in evolutionary and ecological dynamics that may underlie population responses (Weese et al. 2011). In particular, a species’ ability to cope with these disturbances depends on several integrated factors, including its specific life-history attributes, resource requirements, and microhabitat preferences. Moreover, disturbance predictability and intensity, as well as the resulting changes in community (species) composition that follow a disturbance, affect how species can adapt to altered environments. These compositional changes can also alter prey availability and habitat quality as well (Webber et al. 2013). This multi-scale interaction necessitates a transition from single-season estimates of species abundance and range patterns (e.g. Langford et al. 2007) towards more integrative, long-term approaches (Parr and Chown 2003).

III. The Role of Trait Variation in Modulating Disturbance Effects, and a New Perspective for Studying these Effects

The nature of the interaction between animals and environmental changes will influence the selective regimes organisms experience across their range, their fitness, and ultimately, their evolutionary fate (e.g. Vander Wal et al. 2013). Evolutionary theory suggests that both genetic and phenotypic variation can act as fundamental buffers to the negative effects of environmental stressors like anthropogenic disturbance. Thus in order to understand the effects of disturbance or, more broadly, the implications of environmental variation on a species, a departure from numerically-driven focus is
Critical. Indeed, shifts from numerical to trait-based approaches are growing in popularity (e.g. Langlands et al. 2011).

These approaches capitalize on the knowledge that because species interact with their environment through their traits, that a better understanding of the responses of those species to disturbance may be obtained by studying variation in those traits. Indeed, species traits have been shown to be important in describing trophic community disassembly following environmental change (Lindo et al. 2012), and these traits may also play a role in determining species-area relationships across regions (Franzén et al. 2012). Trait variation undoubtedly matters for populations from an ecological standpoint (Bolnick et al. 2011), and may have significant effects on community dynamics.

Both phenotypic and trophic variation may contribute to the persistence of species in disturbed regions (Chevin et al. 2013, Kovach-Orr and Fussmann 2013), but largely these phenomena have been studied using single-trait approaches (e.g. fourth-corner methods, see Dray and Legendre 2008, ter Braak et al. 2012). The major problem with these approaches is that although they successfully link environmental variation with trait variation, linkages are determined for independent traits. This can cause problems when attempting to infer broad generalizations from several traits that do not necessarily all correlate with the same environmental variables, let alone in the same direction or magnitude (e.g. see Ernst et al. 2012). The fact that these traits are not independent and are correlated components of each organism’s phenotype prompts a transition to multivariate approaches in order to understand the consequences of disturbance for species. This transition will also allow for a more comprehensive understanding of how
trait variation contributes to overall phenotypic integration among individuals in a species, especially given that greater variation may help buffer species against perturbations.

IV. The Case for Color Polymorphic Species

Color polymorphic species may be less vulnerable to perturbations than monomorphic species, given the complex heritable phenotypic syndromes characterizing each morph (Gray and McKinnon 2007, Forsman et al. 2008). Indeed, physiological, morphological, and behavioral variation is common among discrete morphs (e.g. Sinervo et al. 2000, Forsman 2001, Huyghe et al. 2007). In contrast to monomorphic species, polymorphic species also tend to exploit a wider range of habitat types and are more likely to display higher rates of range expansion (Forsman and Aberg 2008). Altogether, these traits likely serve to enhance the resilience of polymorphic species to habitat change.

Several papers have treated this prediction and shown that, in general, polymorphic species do appear to fare better than monomorphic taxa. In a recent study on snake evolution, Pizzatto and Dubey (2012) demonstrated that color polymorphic snake taxa are significantly older than monomorphic taxa. These greater ages suggest that polymorphic taxa experienced, and persisted through, more climatic variation in their lifetime and as a consequence, may suffer less from future climatic variations (Pizzatto and Dubey 2012). Moreover, experimental data suggest that individual arthropods in more-phenotypically variable groups may accrue greater survival benefits, primarily from
a reduction in competition intensity, than individuals in phenotypically more homogeneous groups (Caesar et al. 2010). Forsman and Hagman (2009) even go so far as to propose that, for Australian frogs at least, color pattern might be predictive of endangerment status.

Despite discrete variation among morphs in their behavior and use of ecological resources, almost no attention has been paid to whether morphs differ in other components of their ecology or life history, as well as their ability to respond to environmental stressors. The diversity and availability of resources often decline significantly in disturbed areas (e.g. Rowe et al. 2011). These resources typically include prey availability, habitat heterogeneity, and thermal suitability, among others, and are essential for individual organisms to meet life-history demands. Changes in any of these properties by environmental perturbations should thus alter the selective environment each individual organism experiences. Because there is sufficient evidence that both divergent natural selection and selection on social traits (behaviors) act to maintain equal fitness among discrete morphs (Sinervo et al. 2001, Gray and McKinnon 2007, Calsbeek et al. 2010), these changes will likely act to counter the fitness balance among the morphs. In order to better understand why polymorphic species are generally less vulnerable to environmental change than monomorphic species it is essential to characterize the mechanisms contributing to their success, including (any) inter-morph response differences.
V. Study Species: *Urosaurus ornatus*

*A. Life History and Ecology of U. ornatus*

The ornate tree lizard (*Urosaurus ornatus*) is one of the most common color polymorphic species in the world. In the southwestern United States, *U. ornatus* ranges from near sea-level to habitats > 2500 m in altitude. Throughout its range *U. ornatus* exploit diverse microhabitats, but most populations are arboreal or saxicolous (Zucker 1989, Smith 1996, Herrel *et al.* 2001).

Variation in habitat use is also tied with morphological variation in this species. For instance, Herrel *et al.* (2001) found that arboreal populations of *U. ornatus* prefer microhabitats with greater amounts of leaf litter and shrub cover. These populations tend to exhibit longer heads, narrower bodies, and longer limbs than rock-dwelling populations (Herrel *et al.* 2001). In the same study, Herrel *et al.* (2001) also documented that arboreal populations seemed to be in better condition than other populations, suggesting that arboreal habitats might exhibit greater resource availability (Smith 1996).

In a study investigating male and female territoriality, M'Closkey *et al.* (1987) demonstrated that in less-productive (dry), resource-poor seasons, lizards tended to prefer the largest available trees. In wet seasons, where habitat productivity and resource availability is higher, habitat choice was random with respect to tree availability or quality. Furthermore, the results of Thompson and Moore (1991a) suggest that mesquite (*Prosopis* sp.) forests support a greater diversity of morphs than boulder sites. These sites also support larger mean clutch sizes and larger adult body sizes (Thompson and Moore...
Altogether, the findings of these studies suggest that arboreal microhabitats are better quality and may be preferred by *U. ornatus*.

Tree lizards may also express one of the most complex examples of color polymorphism, with both males (six, Figure 1) and females (three, Figure 2) exhibiting multiple phenotypes (Zucker and Boecklen 1990, Thompson and Moore 1991a). For males, data suggest that these morphs are genetically fixed and are associated with varying behavioral strategies linked to reproduction (*e.g.* Thompson and Moore 1991b, 1992). In particular, blue males are aggressive and actively patrol and defend territories. Yellow males on the other hand are satellite males which may sometimes defend smaller territories adjacent to blue male sites. Orange males are typically nomadic but, during productive, non-drought seasons, have been demonstrated to switch tactics and defend territories (Knapp *et al.* 2003). Throat color manipulation (*i.e.* painting) does not induce behavioral shifts in the signaler, but often did in the receiver of those signals, hinting at a role for sexual selection in maintaining differences in dewlap coloration (Thompson and Moore 1991b). The frequencies of these morphs tend to vary microgeographically, hinting that shifts in selective regimes resulting from environmental variation may not favor all morphs equally.
Physiological differences appear to underlie expression of the discrete male morphs in this species, particularly variation in testosterone (Weiss and Moore 2004), corticosterone (Knapp and Moore 1995), and progesterone (Weiss and Moore 2004) levels. For instance, although more-aggressive orange-blue morph males overall possess
similar levels of testosterone as nomadic orange males, and exhibit similar responses in those hormones to a behavioral challenge (Thompson and Moore 1992), some physiological differences in testosterone regulation occur among these males. In particular, castrated males (no testosterone) were unable to maintain territories in this experiment (Thompson and Moore 1992).

Much less is known about the three female *U. ornatus* color morphs. In particular, studies have suggested both female phenotypes may be plastic or fixed, and that like the frequency of male morphs, this phenomenon may vary microgeographically (Zucker and Boecklen 1990). When color change is apparent, it is associated with reproductive condition: gravid females tend to develop deeper orange coloration than non-gravid females (Zucker and Boecklen 1990). The authors acknowledge that this phenomenon was not ubiquitous, and, given the additional fact that female *U. ornatus* are capable of storing sperm (Villaverde and Zucker 1998), multiple mating is likely a common occurrence. Some evidence also exists that females may exhibit territoriality or at least, site fidelity (Zucker 1989, Mahrt 1998). Females though appear to select smaller ranges than males which often overlap with a males’ territory but rarely if ever overlap with the territory of another female (Zucker 1989). The small sizes of these territories may be linked to preference for and defense of potential nest sites. In particular, Mahrt (1998) documented that once females became gravid, the size of their home range decreased significantly. This phenomenon may be the outcome of female selection of a particular nest site, or a reduction in the ability of a female to defend larger areas due to a trade-off between performance ability and clutch size (Mahrt 1998). Female *U. ornatus* also tend
to become more aggressive when gravid (Mahrt 1998), suggesting that the physiological changes associated with egg development may underlie shifts in female behavior.

In addition to shifts in behavior, some workers have suggested that females may also shift coloration to deeper orange tones when gravid (Zucker and Boecklen 1990). If this is the case, then at least yellow and orange morph females may be transient phases of the same morph, with differentiation linked to within-season reproductive state, and not indicative of discrete differences in phenotype. In that study, Zucker and Boecklen (1990) document that although yellow females tended to lay more eggs in their first clutch, most of the females became more orange in throat coloration over time. The authors interpret this finding broadly, suggesting that microgeographic variation in selection may be at play for female *U. ornatus* at different sites (Zucker and Boecklen 1990). If color change is a common occurrence, then this may be a cue for males of female receptivity. When multiple female morphs exist, this may also be a cue for females to recognize other females (Zucker and Boecklen 1990). Given that female *U. ornatus* are capable of sperm storage (Villaverde and Zucker 1998), multiple mating may be a common occurrence and thus orange coloration probably does not indicate a lack of receptivity *per se*. At any rate, the potential for females in some populations to change coloration, and other to exhibit fixed coloration, suggests that the traits associated with female throat color may be the result of both genetic and plastic mechanisms.

Given their broad behavioral variation, limited dispersal capabilities but use of a wide variety of microhabitats across their range, tree lizards are excellent candidates for investigating the links between environmental variation and ecological differentiation.
B. Study Sites

Sites were chosen within the Appleton-Whittell Research Ranch near Elgin, in Santa Cruz County, Arizona (31° 35.428 N, 110° 30.388 W; http://researchranch.audubon.org/). Since around 1970, this semi-desert grassland has been managed primarily through prescribed burning. Vegetation and structural habitat vary microgeographically at the ranch however as a function of variation in burn history: areas burned more frequently are more open, grass-dominated systems. In contrast, areas burned less frequently are more heterogeneous in vegetation and structural cover. For my dissertation I surveyed three study sites representing three different burn histories (Figures 3-5): two burned sites and one protected from burning. The non-burned (hereafter NB) site has not been burned since about 1980, when the ranch was first established as a research station. The once-burned (hereafter low-frequency burn, LB) site was affected by a fire in 2002 that burned most of the ranch. The twice-burned (hereafter high-frequency burn, HB) site was also impacted by the same burn in 2002. The HB site was previously burnt in 1980. Altogether, these sites exhibit a continuum of microhabitat variation that should have dramatic effects on wildlife (Figure 6).

C. General Methodology

Two 1-ha survey plots were established within each of these three sites. Since 2009, these plots have served as sites to monitor nutrient fluctuations across each of three trophic levels: base vegetation resources, arthropods, and focal tree lizards. I also used these plots as sites to monitor *U. ornatus* populations from 2010-2012.
Figure 3. Map of non-burned (NB) site located in the Appleton-Whittell Research Ranch near Elgin, in Santa Cruz County, Arizona. This region has not been managed by prescribed burning since 1980. The bold dotted line estimates the total area of this study site (highlighted in blue). Red and yellow dashed boxes represent paired 1-ha survey areas (see Study Sites, above). Edges of survey areas are 100 m in length for scale. Inset: map of ranch with star indicating position of study site.

Figure 4. Map of low-frequency burned (LB) site located in the Appleton-Whittell Research Ranch near Elgin, in Santa Cruz County, Arizona. This site was burned once since 1980. The bold dotted line encompasses the approximate search area at this site (highlighted in blue). Red and yellow dashed boxes represent paired 1-ha survey areas (see Study Sites, above). Edges of survey areas are 100 m in length for scale. Inset: map of ranch with star indicating position of study site.
Figure 5. Map of high-frequency burned (HB) site located in the Appleton-Whittell Research Ranch near Elgin, in Santa Cruz County, Arizona. This site was burned twice since 1980. The bold dotted line encompasses the approximate search area at this site (highlighted in blue). Red and yellow dashed boxes represent paired 1-ha survey areas (see Study Sites, above). Edges of survey areas are 100 m in length for scale. Inset: map of ranch with star indicating position of study site.

Resource availability was monitored within 16 100 m² (~5.6 m radius) points aligned along two stratified grids within the survey area each field season (following a modified BBIRD protocol, http://www.umt.edu/bbird/protocol/veg.htm) (Figures 3-5). These points were geo-referenced in 2009 (LB and HB sites) and 2010 (NB site) with the aid of a handheld GPS unit. The size of the radius plots approximate the typical home-range for tree lizards (M’Closkey et al. 1987, Zucker 1989), and are strongly amenable to isotopic sampling, estimations of vegetation cover (spatial and temporal), and arthropod sampling. To quantify variation in habitat use and resource use, I also centered these plots at capture points of every focal lizard. Within all vegetation plots, I placed four 1 m² frames to sample vegetation tissues, estimate ground cover, and measure spatial microhabitat characteristics (Figure 7).
Figure 6. Photos of the three study sites monitored during this study at the Appleton-Whittell Research Ranch near Elgin, in Santa Cruz County, Arizona. These sites include a non-burned control (A), low-frequency burn (B), and high-frequency burn (C) site.

Figure 7. Methodology used to measure habitat structure and vegetation cover at capture (use) and random (available) points in this study. In the first panel (A), the outer circle designates the 100 m² plot used to collect vegetation and structural data. Each smaller circle (a-d) represents one of four 1 m² frames used to collect these data within each plot. One of these plots is expanded in the second panel (B), to illustrate hoop-frame (with 10 cm blocks) on typical desert vegetation. All data were recorded from within these frames, with the exception of tree distances (see General Methodology above). The first frame was centered on the point of capture, and the remaining three at 120° from each other, beginning at a random distance (between 1 and 5 m) and angle from the center frame. A similar frame was used to sample the available habitat at a random point near each capture point.
For ground cover, I recorded percent cover (%) of grass, shrub, tree, and forb vegetation types, as well as rock, bare ground, leaf litter, and woody debris. I also recorded the distance from the capture point to the two nearest trees to the nearest 0.1 meter. I also recorded the percent canopy cover of those trees as an additional estimate of microhabitat quality. I collected leaf, flower, and stem tissues from every individual plant identified within the four 1 m² frames for isotopic sampling (see below). I monitored arthropod prey availability by systematically sweep netting along each stratified grid within each study area twice each season. On arthropod sampling days, I made certain to sweep-net each area once in the mid-morning and then again mid-afternoon, to account for activity period variation among prey. I identified all vegetation and arthropods to family, and further to genus or species whenever possible, using field guides. All arthropod and vegetation samples were frozen immediately after collection until stable isotope processing (see below). I also obtained annual temperature and humidity data from nearby meteorological stations maintained by the research ranch.

All lizards were transported in a climate-controlled vehicle to a field laboratory for morphological, physiological, and behavioral measures, in addition to tissue collections for isotopic analysis. Lizards were housed individually in separate 5.7 liter aquaria (27.9 cm L × 17.8 cm W × 12.7 cm H, Frey Scientific, Nashua, NH, USA). During this time water was provided ad libitum. Aside from collections of claw tissues from toe-clips each year for isotopic processing (see below), specific laboratory protocols and housing durations varied by year and thus also vary by chapter in this document. Specific laboratory methods will be discussed in their respective chapter(s).
D. Isotopic Analysis

An integral component of this research design involves the use of stable isotopes as a means to detect trophic variation among members of my study species (*U. ornatus*) across my three study sites. For most elements, there typically exists one light isotope in overwhelming majority, and one to two heavier isotopes of minor abundance (Dawson and Brooks 2001). The ratios of heavy to light isotopes of a given element is expressed most often in delta (δ) notation, using the following standard equation,

\[
\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where \(R_{\text{sample}}\) and \(R_{\text{standard}}\) are ratios of heavy to light isotopes in the sample and standard, respectively. Delta (δ) values thus represent the isotopic ratio of the sample relative to a standard, expressed in per-mil (‰) notation. Previous work using stable isotopes as biogeochemical markers has demonstrated their broad utility in identifying prey sources, habitat use, water source (*i.e.* drinking rainwater or obtaining water from a food source), carbon source (*e.g.* C\(_3\) or C\(_4\) vegetation), and reproductive allocation of wildlife (Magnusson *et al.* 1999, O’Brien *et al.* 2000, Bolnick *et al.* 2002, Struck *et al.* 2002, Urton and Hobson 2005, Newsome *et al.* 2007). While identification of base-resource use variation is informative, the true potential of isotopic analyses requires measurement of prey (or resources) occupying trophic levels as well as monitoring of the availability of those resources. To my knowledge, this task has not yet been attempted in full. A comprehensive approach however will allow for a significantly better understanding of
the variation and consequences of resource-use by focal populations, as well as the influence of environmental variation on trophic linkages.

In this study, I adopted an integrative approach to monitor carbon and nitrogen nutrient pools and trace their movements across all known trophic linkages, from resources to arthropod consumers to my focal species (lizards), within each site. Carbon isotopes provide information on the base-resources used by consumers, including whether food webs originate from C3 or C4 sources. Nitrogen, in contrast, provides information on the trophic level and physiological state of each consumer. By combining results from both elements, it has been possible to map out resources on a spatial (within season) and temporal (between seasons) isotopic landscape and determine how those resource pathways shift in accordance with environmental variation (e.g. Herrera et al. 2003). Any shift in resource pool or linkages could lead to loss of functioning of an ecosystem (i.e. loss of functional groups, Elmqvist et al. 2003), as well as the loss of a consumer (e.g. tree lizards).

At the end of each field season, all frozen isotope samples (lizard, arthropod, plant) were thawed and heated in a drying oven at 45 °C for 48 hours. Samples were then ground with a mortar and pestle, and placed into individual 5 x 8 mm tin capsules (Costech Inc., Valencia, CA, USA). All samples were then shipped to the University of California at Davis Isotope Facility (http://stableisotopefacility.ucdavis.edu/) for processing.
VI. Research Objectives

In this study I investigate several hypotheses related to the linkages between environmental variation and ecological differentiation in tree lizard populations. I addressed several questions to this end. In Chapter 2, I developed and tested a protocol for validating the use of stable isotope analysis for quantifying variation in species’ diet selection, laying the foundation for my next chapter. In Chapter 3, I addressed the hypothesis that environmental variation interacts with population structuring and prey availability to generate trophic niche variation among *U. ornatus* color morphs. I extend this investigation into Chapter 4, where I explore the selective consequences of ecological, morphological, and physiological variation for male and female *U. ornatus* in altered landscapes. Finally, in Chapter 5, I explore the interaction between two key processes underlying color polymorphisms in many species, divergent natural selection and socially-mediated selection, in modulating male behavior and social network structure across environments.
CHAPTER 2: OUT OF THE LAB AND INTO THE FIELD: A PROTOCOL FOR VALIDATING ISOTOPIC DIET-SWITCH STUDIES

I. Introduction

Studies relying on stable isotope data to model the trophic ecology of animals are increasingly being implemented, in large part because of variations in isotopic composition among producers and different consumer levels (Willson et al. 2010, Oelze et al. 2011). Among terrestrial systems, the majority of studies focus on mammals or birds (for a review, see Kelly 2000). Fewer studies are devoted to the trophic ecology of squamate reptiles (but see Struck et al. 2002, Castillo and Hatch 2007, Warne et al. 2010), despite their generally high susceptibility to factors that influence diet selection and thus isotopic tissue compositions (e.g. seasonality, Warne et al. 2010).

The natural variation in abundances of carbon and nitrogen isotopes in particular facilitates the assessment of trophic relationships among populations or individuals (e.g. the isotopic niche concept, Newsome et al. 2007, individual specialization, Araújo et al. 2007). Individually, these isotopes provide information on resource-variation at the base of a food web (carbon) and the approximate trophic level (nitrogen) of the study organism. Mixing models (e.g. Parnell et al. 2010) then allow for information from both elements to be combined to develop plausible contribution scenarios (i.e. trophic linkages) between each potential consumer-resource combination. To this end, mixing models are exceptionally useful tools for researchers interested in modeling the trophic ecology of animals in the wild.
A major limitation with this approach concerns the estimation of the expected isotopic change between resources and a consumer (i.e. fractionation, Tieszen et al. 1983). Fractionation coefficients are typically determined using diet-switch experiments, which involves switching the diet of a focal species in a laboratory and then monitoring the isotopic change in its tissues to determine when the new diet is incorporated. The isotopic difference between a consumer and its diet after this point is then retained as a fractionation coefficient. Studies attempting to reconstruct trophic relationships however generally fall into one of three categories: those that estimate fractionation coefficients directly, those that rely on estimates derived from the literature, and those that estimate a consumers’ diet without fractionation data (e.g. Struck et al. 2002).

Aside from a recognized need for fractionation information to reconstruct consumer diets, no studies to-date have attempted to ensure that any fractionation coefficients derived (experimentally or from the literature) have ecological value for the study species. Inclusion of relevant (i.e. species or functional group-specific) fractionation information is essential because it increases the precision of the isotopic mixing models used to reconstruct diet. For example, general estimates available for these fractionations are +1‰ carbon and +3‰ for nitrogen isotopes per trophic step (DeNiro and Epstein 1981, Kelly 2000), yet the majority of reported fractionation coefficients vary considerably around these estimates (reviewed by Caut, Angulo and Courchamp 2009). In some cases, fractionation coefficients are approximated using literature values from related species that differ significantly in diet (Painter et al. 2009, Sorensen et al. 2009), despite the fact that variation in energetic demands is common
among functionally-distinct taxa (Ethier et al. 2010). Indeed, several studies have shown that fractionation estimates display considerable variation given different diet and consumer characteristics (see Table 1). Thus, estimating the fractionation coefficient(s) for a focal taxon or functional group is critical. This need for more laboratory studies has been advocated for well over a decade now (see Gannes, O’Brien, and Martinez del Río 1997, Gannes et al. 2007). However, the guidelines provided in these studies do not recognize the importance of validating the lab diet by experimentally determining the similarities between it and the consumers’ natural diet. If substantial dissimilarities exist between the lab diet and natural diet, the fractionation coefficients generated will be inappropriate for drawing field-based conclusions.

Table 1
Factors determined to influence diet-tissue fractionation coefficients ($\Delta$) of carbon and nitrogen isotopes during diet-switch experiments.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Isotope(s) affected</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>diet isotopic ratios</td>
<td>carbon, nitrogen</td>
<td>Hilderbrand et al. 1996, Caut, Angulo and Courchamp 2009</td>
</tr>
<tr>
<td>consumer physiological state</td>
<td>nitrogen</td>
<td>Hobson et al. 1993, Castillo and Hatch 2007</td>
</tr>
<tr>
<td>consumer age/size</td>
<td>nitrogen</td>
<td>Roth and Hobson 2000, Sweeting et al. 2007</td>
</tr>
<tr>
<td>air temperature</td>
<td>carbon, nitrogen</td>
<td>Barnes et al. 2007</td>
</tr>
</tbody>
</table>
In this paper I provide a validation protocol for conducting isotopic diet-switch studies (Figure 8). Adherence to this protocol will ensure that the prey type used in a laboratory feeding experiment is representative of the organisms’ natural diet. This is an essential condition which, when met, will validate the use of any derived fractionation coefficients for modeling the trophic ecology of their focal species in the wild. I take advantage of experimental data I collected on an insectivorous lizard (*Urosaurus ornatus*) to demonstrate this protocol. By following the steps outlined in this protocol (see Figure 8) I am able to demonstrate that my lab diet is representative of the natural prey available to *U. ornatus* during the timeframe of my study. Thus, the fractionation (Δ) values I report are ecologically relevant for modeling the diet selection of this (and possibly other) insectivorous lizard species. More importantly, this demonstration highlights the practicality of my protocol and the ease in which it may be integrated into other research programs.

*Figure 8.* Protocol for validating isotopic diet-switch studies.
II. Materials and Methods

A. Study Site

I conducted this study June through August 2009 at the Appleton-Whittell Research Ranch in Santa Cruz County, Arizona (31° 35' N, 110° 30' W). Vegetation at the site is dominated primarily by bunch grasses (*Bouteloua* sp.) and invasive lovegrass (*Eragrostris* sp.). Non-grassy species comprise less than 25% of the overall vegetation at the study site, with the most common species including mesquite (*Prosopis* sp.), acacia (*Acacia* sp.), and sagebrush (*Artemisia* sp.). The distributions and relative abundance of these species has been affected by a history of prescribed burns. In particular, this variation in the relative dominance of C3 and C4 plant types facilitates identifying trophic variation among individuals using isotopic methods.

B. Focal Species

The tree lizard (*Urosaurus ornatus*) is a common lizard species that occurs throughout the southwestern US. It is a small-sized species with a mean adult snout-vent length (SVL) of 46.9 ± 1.8 mm and mass of 4.7 ± 0.5 g. At my study site, *U. ornatus* lizards are arboreal and occupy scrub oak (*Quercus emoryi* and *Q. arizonica*) and mesquite trees, which are surrounded by a mixture of grass and shrub vegetation.

C. Validation Protocol

My validation protocol (Figure 8) emphasizes 1) use of prior knowledge to inform the choice of an appropriate lab diet, followed by 2) the use of field-based collections or published data to identify the species natural diet, and then 3) comparisons of the characteristics of the chosen lab diet to those of the natural diet. These comparisons are
essential for evaluating the ecological relevance of data obtained from a diet-switch experiment. Because tree lizards are insectivorous (Dunham 1980), I elected to use domestic crickets (Acheta domestica) as a lab diet for the diet-switch experiment. Prior to that experiment, I first estimated wet mass to the nearest 0.001g for a random sample (n = 20) of these crickets. I then placed individual crickets into separate plastic vials and stored them in a freezer (-20 °C) until further analysis.

I collected arthropods in the same site where I captured lizards for this study in order to obtain data on the potential prey of U. ornatus in this region. I sampled arthropods using a sweep net for a period of six hours on 3- and 7-June-2009. I identified all arthropods that I collected to the family level (Table S1). I estimated the wet mass of all arthropod samples to the nearest 0.001 g. I then placed individual arthropods into separate plastic vials and stored them in a freezer (-20 °C) within 6 h after collection. For stable isotope analysis, I divided arthropod families into one of four functional groups: C3 herbivores, C4 herbivores, non-spider predators, and spiders (following Gratton and Denno 2006).

D. Diet-Switch Experiment

I captured adult U. ornatus lizards (n = 11) by noose or hand in June. The study site is dominated by a high abundance of C4 grasses (mean δ13C = -13.5 ± 0.6‰) and scattered oak and mesquite trees. I determined that the diets of tree lizards at this site are in fact dominated by prey embedded within C4-based food webs, based on the average isotopic delta-values of other tree lizards collected from this area (δ13C: -16.2 ± 0.8‰; δ15N: 8.3 ± 1.2‰; n = 26 lizards). The high abundance of C4 vegetation ensured that
captured lizards exhibited a dietary isotope delta-value that differed from the C3-based diet (crickets) I provided during the experiment. This is a necessary prerequisite for isotopic diet-switch studies (Gannes, O’Brien and Martinez del Rio 1997). I measured the body temperature of each lizard immediately upon capture by placing a Raytek infrared thermometer (Fluke Corporation, Everett, WA, USA) directly against the abdomen. Lizards were individually housed for the duration of the experiment (approximately 21 days) in 5.7 liter aquaria (27.9 cm L x 17.8 cm W x 12.7 cm H, Frey Scientific, Nashua, NH, USA) in a laboratory at the study site. I held lizards in a climate-controlled room (air temperature \([T_a]\): 36.2 ± 2.4 °C) and used timers on lights suspended above lizard cages to mimic a natural photoperiod (15:9 h light:dark). I measured snout-vent length (SVL) to the nearest 0.1 mm and mass to the nearest 0.1 g upon capture. I also recorded mass every two days throughout the experiment. I provided lizards water \textit{ad libitum} during the experiment. I fed each lizard two crickets (\textit{A. domestica}) daily. Feeding took place in the morning and I randomly varied the order that individual lizards were fed each day. I removed any uneaten crickets from each cage at the end of each feeding day (ca. 20:00 h). I fed crickets a diet of Fluker’s™ High-Calcium Cricket Diet as a food source and Fluker’s™ Cricket Quencher as a water source, both \textit{ad libitum}. All crickets used in this study have been maintained on this diet since hatching. A constant diet for the prey is essential to minimize their isotopic variation, which could influence the delta-values obtained for the lizards and affect the carbon and nitrogen isotope fractionation (\(\Delta\)) values (Castillo and Hatch 2007).
I used claw tissue in my validation study because claws are continuously generated and inert once formed and thus should represent a relatively stable time series of isotopic data (Bearhop et al. 2003). Claw clips are also a preferred source for isotopic data for species like *U. ornatus* that have limited dispersal capacities and do not migrate (Ethier et al. 2010). A further advantage of using claw clips is the non-invasive nature of the protocol. I clipped the tips of all claws at day zero (day of capture) to determine initial isotopic delta-values. I clipped two claws every two days after initiating the experiment to quantify the temporal changes in the isotopic signal. I randomized which toes were clipped at each time step.

Finally, temporal changes in body condition, or body temperature (through its effects on prey assimilation), may enrich isotopic delta-values and thus these traits need to be monitored during feeding experiments to ensure minimal variation occurs within and among individual lizards (Congdon 1989, Castillo and Hatch 2007). I estimated body condition for each lizard as the residuals of the regression of mass every two days with the initial SVL. I also recorded lizard body temperature every two days.

**E. Isotopic Analysis**

At the end of the experiment, I dried all tissue samples in a drying oven at a constant temperature (60° C) for a minimum of 48 h. After drying I reweighed each sample to the nearest 0.1 g. I then crushed and homogenized each sample with a mortar and pestle. I enclosed 1-2 mg of each homogenate into a 5x8 mm tin capsule (Costech Inc., Valencia, CA, USA) for isotopic processing. I pooled some smaller individuals (*e.g.* some ants, leafhoppers) by family in order to generate enough tissue for processing. I had
my samples analyzed for $\delta^{13}C$ and $\delta^{15}N$ simultaneously using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Crewe, Cheshire, UK) at the University of California Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu). The error deviation for $\delta^{13}C$ was 0.2‰ and 0.3‰ for $\delta^{15}N$. All isotopic abundances are expressed in standard per-mil delta ($\delta$) notation:

$$X^{\%} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000^{\%}$$

where $X = \delta^{13}C$ or $\delta^{15}N$, and $R = ^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

\textit{F. Statistical Analysis}

I determined the mean $\delta^{13}C$ and $\delta^{15}N$ delta-values of cricket tissues using the sample (n = 20) preserved during the validation protocol. I determined the similarity in $\delta^{13}C$ and $\delta^{15}N$ delta-values of the field-collected arthropod samples with an Analysis of Similarities (ANOSIM) as implemented in PRIMER 5.1 (PRIMER-E Ltd., Ivybridge, UK). For this analysis, I first calculated a matrix of Euclidean distances using the isotopic carbon and nitrogen data. I then applied the ANOSIM using functional group as a factor and the Euclidean distances as the response variables in the model. This analysis also generates post-hoc pairwise comparisons of the main results with $P$-values adjusted for multiple comparisons. I compared isotopic variation in the crickets to the arthropod samples using a multivariate analysis of variance (MANOVA). I used the carbon and nitrogen isotopic delta-values as response variables and prey type ($C_3$-herbivores, $C_4$-herbivores, non-spider predators, spiders, and crickets) as the predictor variable. Post-hoc
comparisons of the lab diet to the four arthropod groups were conducted using a Tukey’s Honest Significant Difference (HSD) test.

Because water conservation is a significant concern for species inhabiting arid environments (Hadley 1970), prey water content may be indicative of prey quality. I used the formula from Seccombe-Hett and Turkington (2008) to obtain water content (%) for all arthropods:

\[ \text{Water content} = 1 - \left(\frac{\text{sample dry mass}}{\text{sample wet mass}}\right) \times 100\% \]

I compared water content and wet masses using separate ANOVAs to determine if these values (i.e. water content [%] or wet mass [g]) differed between the lab diet (crickets) and field-collected arthropods. I applied an arc-sine transformation of water content and a log(10)-transformation of wet mass to assure the variables met the assumptions of each ANOVA. I used a pairwise comparison among groups (Tukey’s HSD test) to evaluate which groups differed in these values.

I applied an exponential model to describe the change in isotopic composition of lizard claw tissues as a function of time (Tieszen et al. 1983):

\[ \delta_t = \delta_f + (\delta_i - \delta_f)\exp(-\nu T) \]

where \( \delta_t \) is the mean isotopic value for lizard claws at time \( t \), \( \delta_i \) is the initial isotopic value of lizard claws at the start of the experiment, \( \delta_f \) is the equilibrium isotopic value, \( T \) is the total time of the experiment (d), and \( \nu \) is the turnover rate (time\(^{-1}\)). From this model I can estimate the time lag for each element by taking \( 1/\nu \). I fit this model using the least-squares method in SigmaPlot 12 (Systat Software Inc., San Jose, CA, USA). I applied repeated-measures ANOVAs to the claw tissue isotopic delta-values after that period to
verify that they remained unchanged (i.e. attained isotopic equilibrium). Finally, I also used repeated-measures ANOVAs to quantify temporal changes in 1) body temperature and 2) body condition throughout the experiment.

All means are present ± 1.0 standard deviation (SD) except where noted. I conducted all statistical analyses, except those noted above, using the R software environment (R Development Core Team 2009) version 2.11.

III. Results

A. Isotopic Patterns in Domestic Crickets

The carbon and nitrogen isotopic delta-values of domestic cricket tissues showed no evidence of temporal variation during the experiment ($\delta^{13}C = -20.2 \pm 0.8\%$, $\delta^{15}N = 4.5 \pm 0.2\%$).

B. Isotopic Patterns in Field-Collected Arthropods

Overall I identified 38 families of arthropods present at the field site that are known prey of tree lizards (e.g. Asplund 1964, Dunham 1980). The three most abundant families (based on total number of individuals collected per family) within each functional group are as follows: $C_4$ herbivores (Acrididae, Cercopidae, Tettigoniidae), $C_3$ herbivores (Chrysomelidae, Flatidae, Rhopalidae), non-spider predators (Cleridae, Formicidae, Lycaenidae), and spiders (Araneidae, Salticidae, Thomisidae) (Table S1). Overall these functional groups differed significantly in their patterns of carbon and nitrogen isotopic delta-values (ANOSIM: Global $R = 0.39$, $P = 0.001$). Only spiders and non-spider predators were isotopically similar in carbon and nitrogen delta-values (both
pairwise $P > 0.1$). Both predator groups also exhibited significantly enriched nitrogen isotopic delta-values compared to the herbivorous groups (all pairwise $P < 0.01$). C$_4$ herbivores exhibited more-enriched carbon isotopic delta-values compared to C$_3$ herbivores (C$_4$ herbivores, $\delta^{13}$C = -23.4 ± 2‰; C$_3$ herbivores, $\delta^{13}$C = -16.6 ± 2.9‰).

C. Validation Protocol

Water content of field-collected arthropods varied from 54 to 74% among the four groups. Crickets and the four field-collected arthropod functional groups differed significantly in water content (ANOVA: $F_{1,73} = 9.276$, $P = 0.001$). Post-hoc analyses indicate that this difference was largely driven by the lower water content of non-spider predators and high water content of domestic crickets (Table 2).

Table 2
Comparison of Characteristics of the Lab Diet (Crickets) to those of Four Arthropod Groups that are Known Prey of *Urosaurus ornatus* in the Wild.

<table>
<thead>
<tr>
<th>Arthropod Type</th>
<th>Wet mass (g)</th>
<th>Dry mass (g)</th>
<th>Water Content (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crickets (lab diet)</td>
<td>0.078 ± 0.005</td>
<td>0.024 ± 0.003</td>
<td>71.0 ± 1.9</td>
<td>--</td>
</tr>
<tr>
<td>Pooled arthropods</td>
<td>0.061 ± 0.015</td>
<td>0.023 ± 0.005</td>
<td>62.5 ± 2.2</td>
<td>0.001</td>
</tr>
<tr>
<td>C$_3$ herbivores</td>
<td>0.057 ± 0.012</td>
<td>0.016 ± 0.002</td>
<td>71.9 ± 3.1</td>
<td>0.998</td>
</tr>
<tr>
<td>C$_4$ herbivores</td>
<td>0.16 ± 0.044</td>
<td>0.059 ± 0.016</td>
<td>62.9 ± 2.2</td>
<td>0.113</td>
</tr>
<tr>
<td>Non-spider predators</td>
<td>0.023 ± 0.006</td>
<td>0.011 ± 0.003</td>
<td>53.6 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spiders</td>
<td>0.018 ± 0.002</td>
<td>0.005 ± 0.001</td>
<td>74.0 ± 4.4</td>
<td>0.916</td>
</tr>
</tbody>
</table>

Because prey body size can be an important concern for gape-limited predators like *U. ornatus*, I also compared masses of crickets to the field-collected arthropods that are known prey items to *U. ornatus*. Cricket mass did not significantly differ from that of
the pooled field-collected arthropod set ($F_{2,73} = 0.299, P = 0.586$). Domestic cricket tissues fed Fluker’s cricket food are also relatively central in isotopic space compared to the four arthropod functional groups (Figure 9). Specifically, crickets $\delta^{13}C$ values are central to those of the herbivorous arthropods and are depleted in $\delta^{15}N$ values relative to non-spider predators and spiders (MANOVA: $F_{4,99} = 35.422, P < 0.001$; Table 3). Crickets are therefore an appropriate lab diet for a diet-switch experiment on *U. ornatus*.

**D. Diet-Switch Experiment**

All lizards readily ate the feeder crickets. During this experiment the lizard claw tissues shifted towards a new isotopic equilibrium as a result of the cricket diet (summarized in Figures 10 and 11 for carbon and nitrogen isotopes, respectively). Both $\delta^{13}C$ and $\delta^{15}N$ incorporation models were significant ($\delta^{13}C$: $\delta_f = -18.99 \pm 0.14$, $\nu = 0.0725 \pm 0.02$, $R^2 = 0.92$; $\delta^{15}N$: $\delta_f = 5.14 \pm 0.17$, $\nu = 0.0746 \pm 0.011$, $R^2 = 0.95$; both $P < 0.05$). I estimated a similar lag time ($1/\nu$) for the cricket carbon and nitrogen isotopes to be fully incorporated into the lizard claw tissues (13.8 versus 13.4 days, respectively). From this point (ca. day 13) forward, repeated-measures ANOVAs verified that lizard tissues had indeed attained equilibrium ($\delta^{13}C$: $F_{3,30} = 0.019, P = 0.980$, Figure 10; $\delta^{15}N$: $F_{3,30} = 0.029, P = 0.993$; Figure 11). My estimates of the fractionation coefficients during this equilibrium period for *U. ornatus* are therefore $1.2 \pm 0.4‰$ for carbon and $0.7 \pm 0.3‰$ for nitrogen isotopes.
Figure 9. Relationship of lab diet (crickets) to four arthropod consumer groups (C₄ herbivores, C₃ herbivores, non-spider predators, and spiders) collected at the study site in carbon and nitrogen isotopic delta-space. Points are mean ± standard deviation (SD).

E. Temporal Variation in Lizard Body Temperature and Body Condition

Lizard body temperatures averaged 35.1 ± 3.2°C immediately following capture. During the experiment, temperature fluctuations were minimal and lizard body temperatures overlapped with field values (repeated-measures ANOVA, $F_{9,90} = 1.696$, $P = 0.101$). The body mass and condition of individual lizards did not differ over time (repeated-measures ANOVA: $F_{9,90} = 0.882$, $P = 0.545$).
Table 3
Results of Tukey’s Honestly-Significant Difference Test on a Multiple Analysis of Variance (MANOVA) Comparing the Carbon and Nitrogen Isotopic Variation among the Lab Diet (Crickets) to four Arthropod Functional Groups that are Known Prey of *Urosaurus ornatus* in the Wild.

<table>
<thead>
<tr>
<th>Arthropod group</th>
<th>Mean δ(^{13})C value difference (‰)</th>
<th>P-value</th>
<th>Mean δ(^{15})N value difference (‰)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_3) herbivores</td>
<td>-3.05 ± 0.85</td>
<td>&lt;0.001</td>
<td>0.45 ± 0.62</td>
<td>0.95</td>
</tr>
<tr>
<td>C(_4) herbivores</td>
<td>3.64 ± 0.94</td>
<td>&lt;0.001</td>
<td>1.1 ± 0.55</td>
<td>0.28</td>
</tr>
<tr>
<td>Non-spider predators</td>
<td>-1.5 ± 0.86</td>
<td>0.41</td>
<td>-2.65 ± 0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spiders</td>
<td>-1.27 ± 0.97</td>
<td>0.69</td>
<td>-3.56 ± 0.68</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Figure 10.* Temporal pattern of carbon isotope integration of the lab diet (crickets) into tree lizard (*U. ornatus*) claw tissues over a 21 day period. Points are mean ± standard deviation (SD).
IV. Discussion

Numerous studies have articulated the need for researchers to estimate fractionation values for their focal species. However, available guidelines for diet-switch experiments do not consider the implications of any differences between a lab diet and a consumers’ natural prey. In this paper I outlined a validation protocol for diet-switch studies that capitalizes on the integration of lab and field data (see Figure 8). This integration is essential in order to evaluate the ecological relevance of fractionation values derived from a diet-switch experiment. I illustrated the applicability of this protocol using experimental data I collected on my focal species, the tree lizard \textit{(Urosaurus ornatus)}. Domestic crickets fell within the range of available prey types with respect to their size, quality, and isotopic characteristics. I therefore argue that my
estimates are appropriate for use in field studies of the trophic ecology of this and potentially other insectivorous lizard species. To my knowledge, no other studies have attempted to validate their diet-switch experiments in this manner despite mounting evidence that fractionation is influenced by numerous consumer and diet properties (e.g. Table 1). I thus suggest that my protocol ensures that lab-derived fractionation coefficients are also of sufficient ecological value for a focal taxon.

A. Application of My Protocol: An Experiment on U. ornatus

For the diet-switch experiment, I used a sample of wild-caught U. ornatus that displayed the expected C₄-dominated delta values for a grassland field site and switched their prey to domestic crickets fed a C₃-dominated diet. However, my fractionation data differ substantially from the 1‰ for carbon and 3‰ for nitrogen isotopes typically provided in the literature (DeNiro and Epstein 1981, Kelly 2000). Similar mismatches actually occur quite often in the literature (for a review see Caut, Angulo and Courchamp 2009), stressing a need for fractionation coefficients that are specific to each species or functional group.

Initially, I clipped each claw to generate a baseline sample, and then clipped claws again throughout the study. Based on my results, there is a time lag of about 13.6 days (average lag for carbon and nitrogen isotopes) for a new diet to be incorporated into the claw tissue. This is a relatively short time frame; however, U. ornatus claws are extremely small (0.8 – 1.2 mm in total length). Other studies indirectly support my estimates. Previous work using avian claw tip samples suggested average rates of incorporation into claws of approximately 0.04 mm/day (Bearhop et al. 2003). If tree
lizards incorporate cricket tissues into claws at a rate similar to birds in that study, it would take ~ 20 – 30 days total for whole-claw equilibrium to be reached. I ran my experiment for a total of 21 days, which is within that time frame. Because claw tissue integrates from base to tip, and because I was collecting tissues between mid-claw and the base of the claw, I propose that my estimates are robust.

The duration of my study is relevant for field work at this and other sites in the Sonoran desert. Vegetation in this region typically undergoes two pulses of growth that follow the winter rain and summer monsoon periods, respectively (Warne et al. 2010). I sampled all vegetation, arthropods, and lizard tissues prior to the onset of the summer rains (ca. early July). Thus my sampling characterizes resources (vegetation and arthropod) incorporated during the post-winter wet season and spring drought. The lizard tissue isotopic delta-values, after corrected by estimated fractionation values, represent the average carbon and nitrogen isotopic signature of the lizards’ diet during these months (depending on initial capture date).

During a diet-switch experiment, some additional factors need to be considered that might influence fractionation (Codron et al. 2011). The factors that may stress lizards and thereby influence the capacity for these animals to assimilate prey include body temperature, body condition, and prey availability (Congdon 1989, Castillo and Hatch 2007, Kempster et al. 2007, Sweeting et al. 2007). Lizards were maintained in a room at a constant temperature, which should minimize any effects of temperature on discrimination (e.g. Barnes et al. 2007). And, when environmental thermal variability is low (i.e. in a controlled lab), the ability to behaviorally thermoregulate is restricted and
lizard body temperatures should track ambient temperature (Wang and Xu 1987). Consequently, lizard body temperatures did not significantly fluctuate during the experiment. In addition, individual lizard body condition was similar throughout the duration of the experiment. Body condition is a measure of the mass per unit length (in this case, mm) of each lizard. If body condition fluctuated, it would likely influence the ability of these lizards to assimilate nitrogen isotopes from the cricket prey I provided to them. In particular, low-body condition individuals may metabolize their own energy stores, a process which inflates measurements of δ¹⁵N delta values and therefore introduces bias into fractionation estimates (Hobson, et al. 1993, Castillo and Hatch 2007). I also controlled for prey availability by providing all lizards with food at the same frequency. For these reasons, I believe my model of δ¹⁵N incorporation accurately represents the incorporation of cricket nitrogen isotopic values into the lizard consumer tissues.

B. Validation Protocol: Going beyond a Diet-Switch Experiment

Historically, diet-switch experiments were only focused on estimating the fractionation coefficient(s) for the isotope(s) of interest. However, I argue that the primary goal of these experiments for isotopic ecologists should be to estimate ecologically-relevant fractionation values, which may be accomplished by following my guidelines (Figure 8). In particular, Hobson and Clark (1992) demonstrated that crow tissues fractionate nitrogen isotopes differently when the birds are fed either a plant- or fish-based diet. Other studies have implicated diet quality in general (Mirón et al. 2006), as well as the degree of fractionation by the prey base (Vander Zanden and Rasmussen...
2001), as additional factors that directly influence fractionation in consumer tissues. These studies (and others, see Table 1) clearly emphasize the need for guidelines such as mine to validate diet-switch experiments for each focal species or functional group. Departures from this protocol would therefore induce biases in fractionation estimates and lead to erroneous conclusions concerning the linkages between a consumer and its available resources.

In this study I compared characteristics of the cricket lab diet to those of arthropod groups recognized as being prey taken by *U. ornatus* lizards in nature (Asplund 1964, Dunham 1980) as part of the validation procedure. Any significant departures in these characteristics would therefore limit the potential for using crickets as a standard by which to evaluate field data on this species. Prey water content may be of significant concern for *U. ornatus* given the relatively pulsed annual resource availability characteristic of arid systems in the southwestern US (Warne *et al.* 2010). Lizards may favor potentially water-rich prey types (*e.g.* arthropod herbivores) when they are available. The overall water content of field-collected arthropods was lower than that of the crickets used in this study (see Table 3), owing primarily to the effects of the relatively low water content of non-spider predators (less than 54%). However, the mean water content of crickets was in fact similar to both C$_3$ herbivores and spiders available to *U. ornatus* in the wild.

In addition, because gape limitation may constrain diet selection by lizards like *U. ornatus* and because prey mass may influence fractionation rates (*e.g.* fat content of prey) I also compared the size (mass) of domestic crickets to field-collected arthropods. I found
that crickets were of similar size to the average size of all arthropods collected in the field, suggesting that on average, crickets are a reasonable proxy for the size of prey *U. ornatus* would likely encounter in the wild. And finally, because carbon and nitrogen isotopic delta-values are used to specifically reflect trophic relationships (Newsome *et al.* 2007), and prey isotopic ratios may affect fractionation values (see Table 1), I believe it is also important to use fractionation coefficients derived from diet sources that fall within the range of available prey delta-values. Indeed, the isotopic $\delta^{13}$C and $\delta^{15}$N of crickets were within the range of delta-values for the arthropod prey available to lizards in the wild. This means that from an isotopic perspective, crickets represent a potential prey type that lizards would likely encounter in the wild.

**C. Conclusions and the Future of Isotopic Validation Studies**

In this paper I described a validation protocol for isotopic diet-switch studies and illustrated through an example the ease in which that protocol may be integrated into those experiments. This protocol is especially appropriate for studies in which a diet-switch experiment is feasible (*e.g.* small-bodied animals), and the characteristics I monitored are particularly applicable to consumers in arid environments. However, using this protocol in other regions requires consideration of characteristics that are likely more relevant to that focal species. These characteristics may be easily obtained from life history data either directly (*e.g.* observation or experimentation) or indirectly (*e.g.* literature surveys). Although directly conducting validation experiments on a focal taxon is the preferred method in stable isotopic research, it should be recognized that for some animals (*e.g.* some large-bodied mammals), diet-switch experiments may be difficult if
not impossible to conduct or validate. In these cases, I recommend that researchers nevertheless take steps to ensure the relevancy of any fractionation (e.g. estimates provided by Caut, Angulo and Courchamp 2009) or diet data they extract from the literature. Before applying any published fractionation coefficient(s), it is critical to 1) establish (quantitatively or qualitatively) that the lab diet used in the reference study is representative of the diet available to their focal species, as well as 2) sample the same tissue type. These steps will allow other researchers to ensure the relevancy of published values for their own focal species.

A growing interest in the use of stable isotopes to accurately reconstruct consumer diets in a natural setting clearly emphasizes the need for a protocol to be incorporated into the typical diet-switch experiment procedure. The guidelines I propose are reasonable, involving data either readily available (e.g. published estimates) or easily collected, and therefore should be applicable to several consumer-resource combinations. I encourage isotopic ecologists to adapt this protocol for use during their diet-switch experiments. In doing this, the researchers conducting the study, as well as others relying on their results, will be able to assure the ecological relevance of the fractionation coefficient(s) for their study species.
CHAPTER 3: TROPHIC NICHE DIVERGENCE AMONG COLOR MORPHS OF THE ORNATE TREE LIZARD, *UROSAURUS ORNATUS*

I. Introduction

Mechanisms involved in the maintenance of discrete behavioral and morphological polymorphisms within a species include frequency dependent selection, spatial variation in selection, or disruptive selection (Gray and McKinnon 2007, Forsman et al. 2008). Color polymorphisms in particular are inferred to represent alternative reproductive strategies in a species and their maintenance requires that each morph has equal fitness in the long term (Gray and McKinnon 2007). Indeed, the fixation of divergent phenotypic traits to each color morph by correlational selection may enhance reproductive success. These phenotypic syndromes typically include physiological and whole-organism performance traits which shape asymmetries in resource-holding potential and relative dominance of the discrete male morphs (e.g. Sinervo et al. 2000, Knapp et al. 2003, Calsbeek et al. 2010). Variation in these factors should affect the relative mating success of each morph (Huyghe et al. 2007).

An underlying assumption in these studies is that apart from behavioral differences, morphs exhibit similar ecological roles. This assumption is counterintuitive given that behavioral dissimilarities among morphs are linked to differences in the spatial dispersion of morphs, the size of their territories or home ranges, and the quality of those habitats (Calsbeek and Sinervo 2002, Calsbeek et al. 2002, Forsman et al. 2008). For instance, in a study on Australian reptiles, Forsman and Åberg (2008) demonstrate that
polymorphic species utilize larger areas and occupy a greater diversity of distinct microhabitats than their monomorphic congeners, even when controlling for range size. It therefore follows that if discrete morphs are capable of utilizing different components of a shared resource spectrum, then their trophic niches may also differ (e.g. McLaughlin 2001). This is an underappreciated yet key ecological consequence of a color polymorphism.

Numerous studies have documented resource polymorphisms in a diverse array of taxa (Smith and Skúlason 1996). Yet evidence for discrete dietary variation among color morphs is scant (but see Roulin 2004, Anthony et al. 2008). I am unaware of examples of dietary variation among morphs associated with alternative reproductive tactics. Dietary divergence among morphs may arise through two mechanisms (Karpestam and Forsman 2011): morph-specific differences in microhabitat exploitation or morphological constraints (e.g. gape limitation) that influence prey selectivity among morphs (Roulin 2004, Komiya et al. 2011). These two hypotheses differ in that the first proposes that a resource polymorphism among morphs is unrelated to individually-based diet selection and instead is a function of differences in prey availability arising from segregation of morphs into different microhabitats. An implicit assumption with the first hypothesis is that morphs are opportunistic feeders and a resource polymorphism is an indirect outcome of habitat selection. In contrast, the second hypothesis assumes the absence of habitat segregation among morphs and instead implies prey selectivity in a shared resource environment.
A third hypothesis integrates habitat variation (e.g. frequency and magnitude of disturbance) with prey availability (types and trophic levels) and the spatial segregation of morphs. In this scenario a resource polymorphism is generated as a consequence of habitat variation, which favors spatial partitioning among morph home ranges and patch-level variation in prey types. Jointly, these factors enhance dietary variation among morphs. Here I envisage a scenario for a polymorphic lizard in which color morphs occupy habitats that vary in structural elements such as the relative dominance of grasses (C₄) versus woody (C₃) vegetation. Whereas the diet of each morph may consist of arthropods, the underlying trophic linkages may involve prey selection of arthropods utilizing woody vegetation (i.e. C₃-based) versus grass vegetation (C₄-based). In addition, because C₄-dominated habitats have limited amounts of coverage by C₃ species, I predict that spatial overlap and trophic similarity should increase among the morphs. In addition, variation in diet may arise from selection of prey from various trophic levels among sites. Consequently, variation in vegetation affects arthropod prey availability as well as the degree of morph spatial segregation. These are nuances in trophic variation not captured by focusing on habitat selectivity alone.

Here I adopt an integrative approach that capitalizes on habitat variation and stable isotopic methods to test whether male color morphs of the tree lizard, *Urosaurus ornatus* differ in trophic niche. I monitored *U. ornatus* populations at two study sites in the semi-arid grasslands of southeastern Arizona that differ in habitat structure and resource availability due to prescribed burning. Variation in fire frequency represents an excellent model for quantifying diet divergence. Grasslands were historically sustained
by periodic wildfires ignited by lightning strikes. However, prolonged fire suppression favored tree and shrub encroachment. Recent management practices reinstated burns to mimic historical processes and reduce recruitment of tree and shrubby vegetation (C3) in favor of grass (C4) propagation (Collins et al. 1998). As a result, sites differing in burn history also vary predictably in structural complexity and patterns of vegetation dominance. My study sites differ in the relative dominance of C3:C4 vegetation; therefore, I should be able to determine whether a trophic polymorphism occurs among the male color morphs.

Data used to quantify trophic polymorphisms are often point estimates derived from prey items identified by stomach flushing or behavioral observations. Such methods provide only short-term estimates that characterize prey consumption of an individual over a period of hours. I suggest that a better estimate is that of the trophic niche, which can be characterized using methods based on stable isotopes (Newsome et al. 2009, Paull et al. 2012). Stable isotope data provide information on the variation in base-resources of a food web (carbon) and trophic level of a consumer (nitrogen) assimilated over time scales varying from weeks to years (Newsome et al. 2009). Thus, isotopic data represent a long-term integration of diet and are more informative from an ecological standpoint than diet variation gleaned from point estimates. The use of isotopic data also facilitates a direct comparison between differences in the availability of different base-resource functional groups (C3 to C4) among treatment sites with any observed variation in trophic niche among color morphs.
I compared the trophic niche characteristics of male morphs in *Urosaurus ornatus* between two sites differing in burn history. Higher burn frequencies favor C₄ domination, whereas lower-frequency burns favor greater heterogeneity in vegetation resources (more C₃ vegetation). Male *U. ornatus* color morphs differ in degree of territoriality, and thus should also differ in their ability to access higher quality prey types, especially in the more-burned site. I address the following questions. Do sites varying in vegetation cover differ in arthropod diversity (e.g. Engle et al. 2008)? Do lizard morphs vary in their ability to access food resources or are all lizards non-selective with respect to prey use? And finally, if morphs differ in their trophic niche, are these differences greater in the more-burned site?

II. Materials and Methods

*A. Study System and Data Collection*

The ornate tree lizard (*U. ornatus*) is a common and widespread species throughout Arizona. At the study site, *U. ornatus* establish home ranges in oaks (*Quercus emoryi* and *Q. arizonica*), mesquite (*Prosopis* sp.), and acacia (*Acacia* sp.). Males of this species exhibit a polymorphism in throat coloration associated with alternative mating strategies (Thompson and Moore 1991a, b). In this study I focused on the three common morphs that have orange, yellow, or blue coloration on the throat fans. Although other morphs occur at the site (e.g. orange/blue, yellow/blue, orange/yellow), their low frequency (n < 5 per site) precluded them from inclusion in the statistical analyses.
I obtained data for quantifying the trophic niche using stable isotope analysis during an 8-week period (June – August 2009) at the Appleton-Whittell Research Ranch in Santa Cruz County, Arizona (AWRR, 31° 35' N, 110° 30' W). I sampled at two sites that varied in burn frequency. One site was burned once during 2002 (Low Burn [LB] site), whereas a second site was burned in both 1980 and 2002 (High Burn [HB] site). Apart from differences in burn frequency, both sites are similar in elevation, aspect, slope and soil characteristics.

I established two 1-ha (100 m × 100 m block) census plots per site. I conducted daily surveys for lizards between 0800 and 1800 h, but ensured each was randomly sampled over the duration of the study. I geo-referenced all capture points using a portable GPS unit (Garmin GPSMAP 60CSx, Garmin Ltd., USA).

The coverage of C3-C4 plants may influence the potential prey base for lizards. Therefore I measured vegetation coverage at each site that may influence diet selection of *U. ornatus*. I evaluated whether male morphs selected microhabitat characteristics that differed from what was available by sampling vegetation at the capture point of each lizard and a random point within the same census area. I sampled vegetation within 100 m² (~5.6 m radius) of the point of capture because the mean home range of *U. ornatus* is less than or equal to this size (Zucker 1989). Random points sampling vegetation at points along a grid aligned to each 1-ha survey plot.

I placed four 1-m² frames within each 100 m² plot. I quantified habitat structure by determining percent cover of bare ground, rocks (> 2 cm diameter), woody debris, and leaf litter. I estimated the percent cover of four vegetative types (grass, forb, shrub, and
tree). I also recorded the distances to the two nearest trees from the plot to the nearest 0.1 meter. I measured all percent-cover variables to the nearest 1.0%. I collected vegetation samples (leaf, stems, and flowers) within these plots and stored these samples in separate plastic bags within a freezer bag.

I determined prey availability at each site by systematically sweep netting each study site over a two-day period for four hours per site. This method captured representative arthropods from every major group identified by Asplund (1964), including some groups not included in that study that are difficult to sample (e.g. Orthoptera, Dunham 1980). I placed each arthropod into a separate 2 ml plastic vial and immediately placed the samples on ice. I identified all arthropods to the family level at the end of each collection day.

For all lizards captured, I recorded morph as well as mass (g), snout-vent length (SVL, mm), head width (HW) and jaw length (JL). I recorded mass to the nearest 0.1 g. I measured SVL to the nearest 1.0 mm with a ruler and HW and JL to the nearest 0.1 mm using calipers. I also clipped two claws from each lizard for stable isotope analysis.

**B. Stable Isotope Protocol**

I identified a total of 25 and 30 separate arthropod families in the HB and LB sites, respectively. I kept all tissue samples (vegetation, arthropod, and claw) in a laboratory freezer at -20°C until the end of the study. I subsequently oven-dried all tissue samples at 60°C until they reached a constant mass (approximately 48 h). I then ground and homogenized the dried samples separately.
I weighed and transferred all dried samples into separate 5 x 8 mm tin capsules (Costech Inc., Valencia, CA, USA). I pooled some arthropod samples by family, because of their small mass, to generate enough tissue for isotopic processing (e.g. ants, leafhoppers). I analyzed all samples for δ\(^{13}\)C and δ\(^{15}\)N using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Crewe, Cheshire, UK) at the University of California Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu). The error deviation for δ\(^{13}\)C was 0.2‰ and 0.3‰ for δ\(^{15}\)N. All isotopic values generated by this analysis are expressed in standard per-mil delta (δ) notation,

\[
\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000\%
\]

where X = δ\(^{13}\)C or δ\(^{15}\)N, and \(R = ^{13}\text{C}/^{12}\text{C}\) or \(^{15}\text{N}/^{14}\text{N}\), respectively. My experimental design facilitated calculation of δ\(^{15}\)N and δ\(^{13}\)C values from 8-16 replicate samples of each vegetation type and arthropod functional group per site.

C. Statistical Analysis

I conducted all statistical analyses within the R 2.15 software environment (R Development Core Team 2010). I compared habitat use (capture points) and availability (grid points) at each site with a non-parametric multivariate analysis of variance (MANOVA) based on distance matrices derived from a Bray-Curtis dissimilarity metric (function ‘adonis’ in the VEGAN package, see also Anderson 2001). I compared site and sample type (capture or grid point) as factors. My analyses are based on 4000 permutations limited to randomizations within-sites only. I determined significant habitat variables driving this variation by first generating separate non-metric multidimensional
scaling (NMDS) ordinations for structural and resource use data. To determine whether any variable(s) explained variation in the NMDS ordination, I applied Pearson’s product-moment correlations (r) between all input variables and the first two axes generated by these NMDS ordinations. Because there are currently no post-hoc tests for permutation procedures such as ‘adonis,’ I utilized this NMDS ordination for each factor with k > 2 groups to scan for potential pairwise differences. For each factor group on this plot, I outlined an ellipse representing the standard deviation of the weighted averages from its centroid. I interpret significant differences between pairs of factor groups in which these ellipses do not overlap.

I applied Sorenson’s D coefficient to presence-absence data of arthropod families to determine the dissimilarity between sites in arthropod composition (Legendre and Legendre 1998). The value of D ranges from 0 – 1, with greater values indicating greater dissimilarity. I compared overall arthropod family diversity (number of families) among the sites using the Chi-square statistic.

I used a multinomial test to compare variation in morph frequencies among the sites. I assessed morphological variation among the male morphs at each site using a multiple analysis of covariance (MANCOVA) with morph and site as fixed factors, SVL as a covariate, and HW and JL as dependent variables. I analyzed variation across sites in the degree of morph segregation by applying a separate MANCOVA for each site with morph as a fixed factor, distance to the nearest tree as a covariate, and normalized latitude and longitude of each lizard capture point as dependent variables. Normalization refers to centering of data so that the mean becomes 0 and the standard deviation set to 1.
I included distance between trees as a covariate because the distribution of this species may be influenced by tree density. Prior to running these MANCOVAs, I first ensured that all slopes were homogeneous (morphology, both interactions $P > 0.4$; spatial segregation, interaction $P > 0.7$).

Differences within the vegetation groups (grass, shrub, forb, and tree) were low, but shrub and tree groups overlap in isotopic space. I thus separated vegetation into one of three functional groups: grasses, forbs, or shrubs. I divided arthropods into one of four functional groups (most common types in parenthesis): $C_3$ herbivores (grasshoppers, leafhoppers, and stick insects), $C_4$ herbivores (grasshoppers), non-spider predators (some ants, beetles, and true bugs), and spiders, following Gratton and Denno (2006). I tested for differences in the carbon and nitrogen isotopic delta-values of each of these groups among the two sites using separate ANOVAs with site and group as factors and $\delta^{13}C$ or $\delta^{15}N$ as response variables.

I estimated trophic linkages between vegetation, arthropods, and lizard color morphs using separate mixing models for each site, implemented with the function ‘siarmemcdirichletv4’ (package SIAR, Parnell et al. 2010). This function uses a Bayesian approach to predict the most likely contribution scenarios between resource and consumer end-members based on their isotopic values and the expected change between them (i.e. fractionation). The mean likelihood of contribution between each resource and a consumer can therefore be retained as an estimate of the linkage between paired end-members. In a concurrent diet-switch experiment, I validated the use of carbon and nitrogen isotopes as estimators of the trophic niche for $U. ornatus$ from claw tissue
(Chapter 2). I therefore used the fractionation data I obtained during that experiment 
\( \Delta ^{13}C = 1.2 \pm 0.4\%o \) and \( \Delta ^{15}N = 0.7 \pm 0.3\%o \) as parameters in my models. I used 
published fractionation coefficients for the four arthropod groups in these models as well 
(Table 4).

Table 4

Trophic inputs and fractionations for the four mixing models I implemented in the SIAR 4.0.2 statistical package (Parnell et al. 2010) in R. Fractionation (\( \Delta \)) values are estimates of the expected change in isotopic delta-values between resources and a consumer type (Tieszen et al. 1983). Sources for these data are provided in the last column. Some studies did not report standard deviation (SD). I adopted the maximum SD reported among all groups I analyzed (i.e. 0.4) as a conservative estimate of SD for those cases (indicated by an *).

<table>
<thead>
<tr>
<th>Mixing Model</th>
<th>Resource end-members</th>
<th>Consumer end-member(s)</th>
<th>( \Delta ^{13}C ) (‰)</th>
<th>( \Delta ^{15}N ) (‰)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary arthropod consumers (C\textsubscript{3} and C\textsubscript{4} Herbivores)</td>
<td>Grass, forb, and shrub vegetation</td>
<td>( C_3 ) herbivores, ( C_4 ) herbivores</td>
<td>3.7, 0.3</td>
<td>3.1, 0.3</td>
<td>Webb et al. (1998)</td>
</tr>
<tr>
<td>Secondary arthropod consumers (non-spider predators)</td>
<td>( C_3 ) herbivores, ( C_4 ) herbivores</td>
<td>non-spider predators</td>
<td>-0.2, 0.4*</td>
<td>2.9, 0.4*</td>
<td>Ostrom et al. (1997)</td>
</tr>
<tr>
<td>Tertiary arthropod consumers (spiders)</td>
<td>( C_3 ) herbivores, ( C_4 ) herbivores, Non-spider predators</td>
<td>spiders</td>
<td>0, 0.4*</td>
<td>1.5, 0.4*</td>
<td>Gratton and Denno (2006)</td>
</tr>
<tr>
<td>Lizards (\textit{Urosaurus ornatus})</td>
<td>All arthropod types</td>
<td>yellow, blue, and orange morphs</td>
<td>1.2, 0.4</td>
<td>0.7, 0.3</td>
<td>Chapter 2</td>
</tr>
</tbody>
</table>
I tested for differences in the trophic position of each morph using a MANCOVA containing site and morph as fixed factors, SVL as a covariate, and isotopic delta-value ($\delta^{13}$C and $\delta^{15}$N) as response variables. A homogeneity of slopes test found no significant interactions in the model (all $P > 0.4$). I applied correlations to head shape and isotopic nitrogen data to explore relationships between morphology and diet. I only report post-hoc pairwise comparisons that remain significant after Bonferroni-adjustment. Unless otherwise noted, I present all isotopic means as $\pm 1.0$ standard deviation (SD).

III. Results

A. Resource Availability and Use

The LB and HB sites were largely characterized by variation in structural habitat (NMDS, axis 1: leaf litter, $r = 0.897, P < 0.001$; tree distances, $r = -0.5, P < 0.001$; rocks, $r = -0.563, P < 0.001$; bare ground, $r = -0.511, P < 0.001$) and vegetation cover (NMDS, axis 2: grass, $r = 0.936, P < 0.001$; forbs, $r = -0.567, P < 0.001$; Figure S1). Overall the LB and HB site differed in habitat structure and vegetation cover (adonis, $F_{1,100} = 8.17, P = 0.001$). The HB site was grassier and had more leaf litter cover, but also had less bare ground and fewer rocks and forbs than the LB site (Figure S1). The structural habitat and vegetation cover at male capture points differed from gridded points across the sites (adonis, type: $F_{1,100} = 26.15, P = 0.001$), but these differences were not consistent across the sites (adonis, site $\times$ type: $F_{1,100} = 1.59, P = 0.178$). Specifically, although males used similar microhabitats in both sites, the relative differences between those points and grid points increased in the HB site (Figure S2). I detected no differences in the microhabitats
used among the three male *U. ornatus* morphs (adonis, all $P > 0.4$). Specifically, male microhabitats were relatively central in habitat space, characterized by closer trees, greater amounts of leaf litter, fewer rocks, and greater forb cover than gridded points (Figure S2).

I collected a greater diversity of arthropods at the LB site (by family: Sorenson’s $D = 0.83; \chi^2 = 7.57, df = 1, P = 0.006$). In particular, the species richness of non-spider predator and spider groups were greater at the LB compared to the HB site (14 versus 8 and 5 versus 3 families identified, respectively).

### B. Isotopic Variation in Trophic Resources

Isotopic carbon signatures of C$_4$ (grasses, mean = $-13.5 \pm 0.7\%$) differed from C$_3$ (forbs, mean = $-27.5 \pm 1.6\%$; shrubs, mean = $-26.3 \pm 4\%$) at the sites. The vegetation at the LB site is more depleted in $\delta^{13}C$ relative to the HB site (site means are $-24.3 \pm 5.9\%$ versus $-19.6 \pm 7.2\%$, respectively). This is supported by the higher proportional cover of C$_3$ vegetation at the LB site (forb: $\chi^2 = 12.48, df = 1, P < 0.001$; shrub: $\chi^2 = 1.93, df = 1, P = 0.165$) compared to grass cover at the HB site (grass: $\chi^2 = 11.3, df = 1, P < 0.001$). All four arthropod groups were enriched in both $\delta^{13}C$ and $\delta^{15}N$ in the HB site, although this trend was not always significant (ANOVA, site $\times$ group, carbon: $F_{7,147} = 19.81, P < 0.001$; nitrogen: $F_{7,147} = 31.69, P < 0.001$; Table 5).

### C. Spatial Variation of *U. ornatus* Color Morphs

Morphs occur in similar frequencies at both sites (multinomial test, $\chi^2 = 4.41, df = 2, P = 0.11$), although blue males were less common at the HB site (Figure 12). I only detected spatial segregation among these morphs at the LB site (MANCOVA, LB site:
Male lizards collected in the HB site were similar in body size (SVL) to their LB counterparts (mean: HB: 47 ± 2.4 mm, LB: 45 ± 2 mm). The patterns of head shape variation among morphs however differed among sites (MANCOVA, site: $F_{2,32} = 3.23, P = 0.052$; morph: $F_{4,66} = 3.03, P = 0.024$; site × morph: $F_{4,66} = 3.52, P = 0.011$). These effects were driven primarily by variation in jaw length: yellow males exhibited shorter jaws than blue males overall, but the jaws of yellow males in the HB were longer than those of yellow males in the LB site (pairwise comparisons, $P = 0.009$ and 0.015, respectively).

Table 5

Relationships of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic delta-values of each arthropod group identified at the two sites. Sites are delineated by historical disturbance frequency: low- or high-burn. P-values are from post-hoc comparisons of isotopic delta-values within each category by location (Low- or High-Burn site, respectively). Significant P-values are italicized.

<table>
<thead>
<tr>
<th>Category</th>
<th>Low-Burn</th>
<th></th>
<th>High-Burn</th>
<th></th>
<th>N</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{15}N$</td>
<td>$\delta^{13}C$</td>
<td>$\delta^{15}N$</td>
<td>$\delta^{13}C$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₄ herbivores</td>
<td>2.9 ± 0.2</td>
<td>-16.6 ± 0.5</td>
<td>2.7 ± 0.3</td>
<td>-14.7 ± 0.3</td>
<td>0.508</td>
<td>0.002</td>
</tr>
<tr>
<td>C₃ herbivores</td>
<td>2 ± 0.5</td>
<td>-24.5 ± 0.5</td>
<td>3.7 ± 0.6</td>
<td>-23.5 ± 1</td>
<td>0.033</td>
<td>0.392</td>
</tr>
<tr>
<td>Non-spider predators</td>
<td>7.1 ± 0.4</td>
<td>-18.7 ± 0.9</td>
<td>7.9 ± 0.8</td>
<td>-20.4 ± 1.1</td>
<td>0.35</td>
<td>0.243</td>
</tr>
<tr>
<td>Spiders</td>
<td>5.9 ± 0.3</td>
<td>-18.1 ± 0.5</td>
<td>7.9 ± 0.4</td>
<td>-17.5 ± 0.5</td>
<td>0.002</td>
<td>0.387</td>
</tr>
</tbody>
</table>

D. Trophic Niche Variation

Resources varied significantly in isotopic delta-space at both sites (Figure 13), and mixing models suggested variation in trophic linkages across the sites (Figure S3). C₃ herbivores in the LB site primarily exploited forb plants. However, their counterparts in
the HB relied evenly on shrubs and forbs (44% and 52%, respectively). C4 herbivores
were somewhat more consistent in resource use across sites (>60% reliance on grasses).
Non-spider predators in the HB site relied more on C3 than C4 herbivores (69% versus
31% at the LB). Spiders on the other hand likely maintain consistent trophic positions
across sites that favor C4 herbivores and non-spider predators over C3 herbivores (~15%
reliance on C3 herbivores at both sites). Blue and orange *U. ornatus* at the LB site occupy
a similar trophic niche, with higher consumption of spiders (43 and 41%, respectively)
than yellow males. Yellow *U. ornatus* males however appear to evenly consume the
available prey types (19-30% for each source). At the HB site, trophic niche relationships
among the morphs differed such that yellow males converged with blue males in trophic
niche, favoring non-spider predators and spiders over other sources (> 24% for each type,
69 – 77.8% total). Orange *U. ornatus*, however, relied more heavily on C4 and C3
herbivores (61% total use).

The diets of lizards captured at the HB site are more enriched in both δ^{15}N and
δ^{13}C delta-values compared to the diet of lizards captured at the LB (Figure 14). Morphs
differed in trophic position among the two sites (MANCOVA, site: $F_{2,32} = 7.26, P =
0.003$; morph: $F_{4,66} = 7.3, P < 0.001$; site × morph: $F_{4,66} = 1.63, P = 0.177$). Specifically,
yellow and blue males consumed prey from higher trophic levels than orange males
(pairwise comparisons, both $P < 0.001$). The lack of interaction between site and morph
is due to the high δ^{13}C overlap of prey sources at both sites. This diet shift by yellow
males at the HB site correlated with their shift in jaw length ($r = 0.54, P = 0.02$).
Figure 12. The frequency of each male *U. ornatus* color morph at the a) Low-burn and b) High-burn site. Sections are colored by morph: yellow, orange, or blue.

Figure 13. Carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotopic bi-plots of vegetation and arthropod resources entered into mixing models for estimating trophic linkages at the a) Low-burn and b) High-burn site. These resources were entered into four separate mixing models to quantify trophic linkages to my focal species, *U. ornatus*. Groups are defined in Material and methods: vegetation - grass, forb, or shrub; arthropod - C$_4$ herbivores, C$_3$ herbivores, non-spider predators, and spiders). Points represent mean values and bars are ± 1.0 standard error (SE).
IV. Discussion

Apart from behavioral differences associated with mating strategies, discrete morphs are assumed ecologically equivalent. However, behavioral differences should affect how morphs interact with their environment, including their diet selection. In this paper I characterized a trophic niche polymorphism among male color morphs of the tree lizard, *U. ornatus*. My results provide support for the hypothesis that habitat variation results in an interaction between prey availability (namely, changes in the trophic milieu at each site) and morph spatial segregation to shape trophic patterns among *U. ornatus*.

*Figure 14.* Carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotopic bi-plot of the trophic niche of *U. ornatus* male color morphs in the a) Low-burn and b) High-burn site. Points are colored by morph: yellow, orange, or blue. Values represent mean carbon and nitrogen delta-values (‰) and bars are ± 1.0 standard error (SE). Note that the scales of both axes differ.
male color morphs. Variation in diet among morphs has long-been considered to play a key role in speciation through ecological divergence (Smith and Skúlason 1996). Divergence in trophic niche among morphs varying in behavior may enhance this process. Here I not only document diet variation, my data suggest that discrete color morphs occupy different trophic niches. To my knowledge, this is the first study to quantify such variation in a color polymorphic species, in addition to its ties to environmental variation.

Current hypotheses suggest that a dietary polymorphism will evolve 1) in sympatry when morphs vary in their ability to exploit different prey types (Skúlason and Smith 1995), or 2) when morphs exploit different microhabitats (Karpestam and Forsman 2011). I combine elements of these two non-exclusive hypotheses by proposing that habitat variation may mechanistically drive the interaction between these parameters, thereby contributing to the expression (or, possibly, the masking) of a trophic polymorphism. For example, consistent sympatric dietary differences across sites should only be expected to occur if those differences are an outcome of similar mechanisms operating within each morph (e.g. selectivity). If morphs differ in their degree of prey selectivity, then habitat variation may mask diet differences among morphs (e.g. habitats where all prey types are available and preferred microhabitat resources are abundant). In addition, an assumption underlying allopatric morph diet differences is that prey distributions are coincident with morph spatial distributions. This suggests predators may be capable of tracking favorable prey (Ujvari et al. 2011). However, this may not hold for species like U. ornatus that have small home ranges, do not disperse long distances, and
are territorial (e.g. Zucker 1989). Whereas the first hypothesis ignores the influences of habitat variation and prey availability on diet, the second hypothesis ignores variation in diet selectivity. My hypothesis integrates trophic and ecological data to overcome these limitations. Consideration of the links between habitat variation and its effects on prey availability and morph spatial segregation are essential for documenting trophic niche variation among discrete morphs.

Although trophic niche variation is an appealing consequence of species with discrete morphs (Forsman and Åberg 2008), few studies have detected an associated resource polymorphism (Roulin 2004, Anthony et al. 2008). Color morphs differ in physiology (Sinervo et al. 2000, Knapp et al. 2003), behavior (Calsbeek et al. 2002), and morphology (Huyghe et al. 2007), consequently, they should also differ in ecology, given the ecological consequences of these differences (Forsman et al. 2008). Here I also suggest that the various elements associated with each morph should promote variation in their trophic niche, specifically the mechanisms underlying their foraging behavior (i.e. selectivity versus plasticity or generalism). I also demonstrate that stable isotope analysis facilitates the detection of this trophic niche segregation that may not be immediately apparent using other methods (e.g. stomach-flushing).

Essentially, I identified diet and morphological trends among the morphs at my study sites that coincide with their behavioral attributes. Among males at the LB, it appears both blue and orange males forage primarily on predator arthropods, whereas yellow males consume relatively even proportions of every arthropod type. The increased availability of preferred structural habitat and greater predatory arthropod diversity is
common at sites burned less-frequently (Peterson and Reich 2001, Engle et al. 2008). This high availability of resources likely allows morphs to spatially segregate and access diverse resources. Yellow coloration indicates a satellite behavioral strategy in this species (Thompson and Moore 1991a). By feeding more on other prey types at the LB site, yellow males may be exhibiting a foraging strategy which minimizes competition with the other morphs. In contrast, the HB site had fewer trees and shrubs, the preferred microhabitats for *U. ornatus*, and males spatially overlapped in habitat use. I observed greater trophic niche segregation at this site. Blue and yellow males foraged primarily on predatory arthropods. Previous studies have shown that orange males are nomadic (Thompson and Moore 1991b), and they foraged in this site as expected given that strategy (*i.e.* generalist tracking prey availability). The obvious incongruence between these site-specific conclusions prompts attention to my prediction that habitat variation may play a key but indirect role in how trophic niche variation manifests. If I combine results from both sites it becomes clear that the dominant blue males are specialist (similar diet among sites), orange males are generalist (no relative change, diet tracks availability among sites), and yellow males are plastic (change in diet among sites unrelated to prey availability). Trophic niche variation was also tied to head shape morphology: blue males had longer heads than the other morphs, and yellow males in the HB site had longer heads than yellow males in the LB site. The shift in diet by yellow males is also likely associated with their satellite strategy as well as the decrease in frequency of dominant blue males at the HB site. These results demonstrate that *U. ornatus* male color morphs also exhibit, in addition to mating strategies (Knapp et al.}
2003), distinct ecological strategies. Moreover, my results suggest that color polymorphisms may also enhance morphological diversity. Altogether, these findings provide evidence that additional factors may contribute to the maintenance and evolution of discrete morphs within a species.

A. Conclusions

Color polymorphic species have been studied primarily at the behavioral level in relation to mating strategies. In contrast it has been suggested that polymorphism species are prone to utilization of more-diverse resources and habitat types (e.g. Forsman and Åberg 2008), and predicted to be less vulnerable to environmental change than monomorphic species (Forsman et al. 2008). Variation in diet has been described as an important player in the speciation process (Smith and Skúlason 1996), and therefore morph trophic differences could be maintained by divergent natural selection. These differences may be enhanced in environments differing in prey availability and resource characteristics. My results demonstrate that morphs differing in behavior and resource utilization can also differ in morphology and their trophic niche. Although trophic polymorphisms are driven by divergent natural selection, color polymorphisms are typically associated with elevated levels of socially-mediated speciation (Sinervo et al. 2001). Jointly, these differences may thus serve to increase speciation rates in polymorphic species.

Given these considerations, the consequences of environmental change ought to manifest differentially for discrete morphs maintained by selection. For instance, whereas all *U. ornatus* consume arthropods, morphs that are capable of exploiting available prey
or are plastic in diet selection may be favored by repeated disturbances over morphs that are selective for certain prey types. In *U. ornatus*, these effects are likely compounded by differences in preferred microhabitat availability following each burn (Peterson and Reich 2001). The relative (although non-significant) increase in frequency of yellow and orange males, and concomitant decrease in blue males, at the HB site provide some support for this prediction. Consequently, while it is appealing to infer that polymorphic species are less vulnerable to environmental change than monomorphic species (Forsman *et al.* 2008), not all morphs may be equally capable of persistence. For *U. ornatus*, the lower frequency of blue morph males in the HB site suggests that this morph may be negatively affected by environmental changes that result in a reduction in both preferred microhabitat and prey types (Gilroy and Sutherland 2007). Decline or loss of a color morph may have profound effects on the strength of selection on behavioral and ecological (microhabitat use, diet) traits contributing to the maintenance of the color polymorphism in *U. ornatus* across its range (Sinervo *et al.* 2001).
I. Introduction

Anthropogenic disturbance is a major environmental stressor and its role in the extinction of animal populations or entire species has received considerable attention. However, its effects on members of populations remain poorly understood. In particular, disturbance may affect microhabitat use, which may then feedback into phenotypic traits and how those traits and microhabitats interact. Any changes should influence the ability of individuals to optimally exploit environmental resources in disturbed areas, which will affect fitness (e.g. survivorship). The resulting fitness variation will then contribute to whether the population as a whole persists or goes extinct. Given the pervasive and global nature of many types of anthropogenic disturbance (e.g. climate change, prescribed burning), and their well-documented effects on biodiversity (Lindo et al. 2012), consideration of these individual responses is critical.

Disturbances have profound impacts on the habitat structure and resource distributions that characterize an environment which may affect patterns of microhabitat use in a population. Shifts in the vegetation composition, forest cover, and thermal profile of disturbed environments have perhaps the most critical impacts on animal populations (Lindenmayer et al. 2008, Sinervo et al. 2010, Clavero et al. 2011). These changes interfere with the ability of individual organisms to successfully exploit disturbed
environments. Changes in microhabitat use may thus be favored when habitat quality is reduced (e.g. use of larger areas, Calsbeek and Sinervo 2002). Alternatively, if microhabitat use is conserved, divergence in other traits may occur in resource-limited habitats (e.g. Chapter 3). In either case, the environmental variation induced by a disturbance will affect the ability of individual organisms to exploit resources, thereby favoring phenotypic reorganization in affected populations. For example, patterns of phenotypic integration, behavioral repertoires, and/or shifts in overall phenotypic trait optima have been found to differ in populations that persist in the disturbed environment relative to those in undisturbed regions (Wright and Zamudio 2002, Bonte and Van Dyck 2009, Crispo et al. 2010). Species that exhibit greater phenotypic variation may be better equipped to withstand environmental change (Forsman and Aberg 2008, Forsman et al. 2008). That is, some phenotypic variation may be present within populations in undisturbed habitats that might favor their persistence in disturbed habitats. Altogether, these changes suggest that selective regimes differ between disturbed and undisturbed habitats.

If selective regimes differ across habitats, then the interactions between individual phenotypes and their microhabitats may also differ. The degree to which individual phenotypes match their microhabitats is an important consideration to this end (i.e. local adaptation, Roulin and Wink 2004). Members of a population that exhibit variation in their phenotypic traits that parallels environmental variation may have a selective advantage in disturbed habitats over others. Selection may either act to push those that match poorly closer towards more-optimal trait combinations or, alternatively, eliminate
them entirely from the gene pool (e.g. Mouillot et al. 2012). These effects should vary depending on how disturbance affects the environment. Weaker-effect regimes should tend to occur in structurally heterogeneous habitats where environmental variability is high and thus multiple trait optima might have equal (or near-equal) selective value. However, in more homogenous habitats such as those generated by some forms of disturbance (e.g. prescribed burning, D'Antonio and Vitousek 1992), selective processes may instead act to constrain the number of trait optima that are favored. If some phenotypic combinations are favored over others (i.e. are a better match to their environment), then those phenotypes should be more likely to persist in disturbed environments. The relative ability of each member of a species to match its environment is therefore informative of the evolutionary trajectory of the species following disturbance.

The phenotypic variation present among alternative morphs of a polymorphic species that allows them to successfully exploit a wide range of habitat types might also buffer them against broad-scale habitat changes induced by disturbance (Forsman and Aberg 2008, Forsman et al. 2008). The suites of phenotypic differences among morphs, analogous to multiple adaptive trait optima, are maintained in large part by correlational selection (Gray and McKinnon 2007, Forsman et al. 2008). In effect, these multiple optima represent multiple opportunities for a polymorphic species to match the novel selective regime present in a disturbed habitat. Therefore, depending on the severity or frequency of a disturbance, some morphs may therefore be better equipped to persist than others. To my knowledge, this possibility has not been explored.
Lizards are excellent models for these types of inquiries because much of their phenotypic variation is tightly linked with environmental parameters (Goodman, Miles and Schwarzkopf 2008). Several species exhibit discrete color morphs (Thompson and Moore 1991a, Sinervo and Lively 1996, Huyghe et al. 2007), and numerous studies indicate that these color differences are associated with alternative mating tactics and divergence in other phenotypic traits (Knapp and Moore 1997, Sinervo, Bleay and Adamopoulou 2001, Healey, Uller and Olsson 2007, Huyghe et al. 2009). Adult tree lizards (*Urosaurus ornatus*) in particular exhibit several sex-specific color morphs that are inferred to represent alternative mating strategies ranging from territorial to nomadic (Thompson and Moore 1991a, b, Knapp et al. 2003). Because these morphs also differ in behavior, aggression, and physiology, it is likely that each morph should respond differently to environmental perturbations.

In this study I characterize the responses of adult *U. ornatus* lizards to a common anthropogenic disturbance, prescribed burning. The effects of burning on biodiversity and ecosystem functioning are well-documented and include changes in habitat invasibility, nutrient cycling, and plant community diversity (D'Antonio and Vitousek 1992, Collins et al. 1998). In grasslands of the arid southwestern US, fire generates a mosaic of habitat types varying in habitat heterogeneity, depending largely on burn frequency. The disturbance gradient present among these study sites thus provides an excellent opportunity to study the selective links between environmental variation and phenotypic expression.
I monitored these sites, as well as the lizard populations within them, for a period of three years. I compared microhabitat availability and use by *U. ornatus* in order to determine whether lizards were selecting for specific microhabitat characteristics and, if so, do those characteristics differ among disturbed and undisturbed sites. Given that disturbance may also affect their phenotypic traits, I compared phenotypic variation among lizards at my study sites. I then explored the relative degree to which disturbance may favor increased matching between those traits and microhabitats, and whether variation in matching was morph-specific. I also combined matching and phenotypic distribution data to gain insight on whether the ability to match disturbed habitats is associated with average or unique (tails) phenotypic trait combinations. Finally, to estimate the fitness consequences of variation in how individuals interact with their microhabitat, I generated robust estimates of survival for males and females at each site using capture-mark-recapture data and empirically-derived matching scores as covariates in these models. By addressing how disturbed sites alter microhabitat use and phenotypic traits, and then linking their interaction to estimates of survival, I am able to provide significant insight into the ecological and evolutionary mechanisms that contribute to species loss or persistence in altered landscapes.

II. Materials and Methods

* A. Study Sites

I conducted surveys for lizards during their breeding season (ca. June – August of 2010 – 2012) at the Appleton-Whittell Research Ranch in Santa Cruz County, Arizona. I
surveyed three treatment sites varying in disturbance history: a non-burned site (hereafter Non-Burned [NB]), a site burned once in 2002 (hereafter Low-frequency Burn [LB]), and a site burned in 1980 and again in 2002 (hereafter High-frequency Burn [HB]). The same burn affected both LB and HB treatment sites in 2002. The NB site is located at the southernmost region of the ranch which has been protected from burning and wildfire since at least 1970. All three sites share similar topography and soil characteristics. As a result of differences in burn history the sites should vary in structural complexity and patterns of vegetation dominance (Grant et al. 2010).

**B. Characterization of Resource Use by Tree Lizards**

Collection of resource and structural data follow Chapter 3. Briefly, I established two 1-ha census areas within each site to search for lizards. Each area began at a random coordinate and paired areas were maintained at a minimum distance of 50 m apart at the nearest edge. To monitor resource availability I positioned 16 points along a stratified grid within each census area (thus a total of 32 points/site). I centered a 100 m² plot on each of those points and recorded habitat data at four 1-m² frames within that plot. Frame positions were as follows: one central, and three peripheral beginning at a random distance and starting angle (relative to north), placed evenly at 120° angles from each other. I chose 100 m² plots to accommodate comparisons between these availability points and the same data recorded at lizard capture points (see below), but most importantly because it encompasses the maximum home range size recorded for adult *U. ornatus* (Zucker 1989, Lattanzio unpublished data).
I monitored resources within each plot by recording the number of species and percent cover of each vegetative type (grass, forb, shrub, and tree as defined by the USDA Plants database, plants.usda.gov/). Structural cover variables monitored were percent cover of bare ground (including rocks <2 cm diameter), rocks (>2 cm diameter), woody debris, and leaf litter. I also recorded the distances to the two nearest trees from the center of each plot. I recorded all percent cover variables to the nearest 1.0% and all tree distances to the nearest 0.1 m.

C. Capture Methods

I collected adult lizards by hand or noose and marked all capture points using a handheld GPS unit (Garmin GPSMAP 60CSx, Garmin Ltd., USA). Capture points were determined to be at the point of initial observation of the lizard. At my study site, *U. ornatus* males exhibit six throat color morphs: yellow, blue, orange, yellow-blue, orange-blue, or orange-yellow (hereafter Y, B, O, YB, OB, or OY). Female morphs exhibit yellow, orange, or white coloration (hereafter Y, O, or W). These color morphs are associated with alternative social and mating strategies (Thompson and Moore 1991b, Knapp et al. 2003). All morphs frequent scrub oak trees (*Quercus emoryi* and *Q. arizonica*) and mesquite and acacia shrubs (*Prosopis* and *Acacia* spp.).

D. Morphology

I transported all lizards to field laboratory to complete morphological and performance measurements. I housed individual lizards in separate in 5.7 liter aquaria (27.9 cm L x 17.8 cm W x 12.7 cm H, Frey Scientific, Nashua, NH, USA). All lizards were provided water *ad libitum*. For each lizard I recorded snout-vent length (SVL), fore-
and hind-limb lengths, head width (distance between external sides of head at widest point), and jaw length (distance between snout tip and base of jaw on ventral side) to the nearest 0.1 mm using digital calipers. I recorded the mass of each lizard to the nearest 0.1 g. I assessed whether a female was gravid by palpation of the abdomen. Prior to release, I uniquely marked each lizard with a paint spot and toe-clip combination. I returned all lizards to their point of capture. No lizards were held in captivity longer than 48 h.

**E. Stamina Measurements**

I allowed lizards to acclimate to lab conditions for a minimum of 24 h post capture prior to estimating performance. My method of obtaining stamina involved chasing a lizard around a circular racetrack. The racetrack has an external and internal diameter of 100 and 60 cm, respectively, and a track width of 20 cm. A 1 cm deep layer of fine-grained sand served as a substrate. I encouraged each lizard to run by lightly tapping on its tail (Clobert *et al.* 2000). During each trial I recorded the duration (sec), total distance (m), and number of escape attempts. All lizards were raced at 32 °C (air and substrate). I raced each lizard only once and one observer (MSL) conducted all races to avoid inter-observer bias. My measure of stamina was the time until a lizard became fatigued. I used the loss of a righting response as my measure of fatigue as done in past studies (Clobert *et al.* 2000, Robson and Miles 2000).

**F. Survival Estimation**

I estimated survival probabilities for males and females for each site separately using the program M (version 6, White and Burnham 1999). I included morphological matching scores as covariates to determine whether or not they had an effect on survival
at each site. My capture history included nine sampling occasions (three times per year, once during June, July, and August of each year over three years). I adjusted for the uneven time intervals between August of each year and June of the following year by calculating the time that passed between each interval and dividing that by the longest duration between sampling occasions (following Rabosky et al. 2012).

G. Statistical Analysis

I analyzed adult male and female U. ornatus separately because the color morphs are sex-specific. I transformed all morphological variables to a log10 scale in order to satisfy normality assumptions. I compared morph frequencies across the sites using multinomial tests. I compared frequencies of gravid females across sites by summing frequencies from each study year separately. I pooled these sums among four two-week periods: early and late June and July, respectively. I conducted all statistical analyses within the R software environment version 2.15 (R Development Core Team 2012).

i. Environmental Variation over Time

I applied principal response curves (PRC) to investigate the environmental response (i.e. habitat differences among sites) to burning. This method is useful because it allows researchers to contrast time series data on treatment sites with that of a reference site (Van den Brink and Ter Braak 1999). This procedure provides a convenient way to visualize the direction and magnitude of changes in patterns of habitat selection occurring as a result of disturbance. I thus compared the environmental data from the LB and HB sites to that of the NB site over the duration of the study using this analysis. I applied
PRC methods using the ‘pre’ function in the VEGAN package in R. I tested the significance of the PRC results using an analysis of variance (ANOVA).

ii. Lizard Habitat Use and Selectivity

I compared the microhabitat characteristics of lizard capture sites to that of the random, available (grid) points at the NB, LB, and HB using a nonparametric analysis of variance based on distance matrices (function ‘adonis’ in the VEGAN package in R, see also Anderson 2001) with type (random or capture plot), site, and year as factors and ran the analysis for 4000 permutations (recommended by Anderson 2001). I used the Gower distance (function ‘vegdist’) for this analysis because this metric is robust to heterogeneity in the ranges for each variable and multiple zeroes. If burning reduces the availability of preferred habitats and increases habitat selectivity, then the multivariate distance between data collected at lizard capture sites in treatment sites should be greater than that of the control site.

iii. Morphological and Performance Variation among Male and Female U. ornatus

I entered male and female morphological data into separate principal component analyses (PCAs) to reduce the dimensionality of the data. Recaptured lizards were not included in this analysis. I retained the first two PC-axes for each sex (accounting for 90.9 and 85.4% of the total variation in male and female morphology, respectively). I then applied a multivariate analysis of variance (MANOVA) to determine the degree to which treatment, morph, and year predict variation in morphology (PC1 and PC2) for each sex. I used Pearson’s correlation coefficient ($r$) to determine what morphological
variables contributed significantly to the variation along each independent PC-axis. I only report those variables with correlation coefficients greater than 0.7 ($P << 0.05$).

I analyzed variation in stamina with the following procedure. I first generated a Gower distance matrix of the stamina dataset (distance, time until exhaustion, and number of escape attempts) separately for each sex. I applied the ‘adonis’ function to that distance matrix with the same factors (treatment, morph, and year) as with the morphological data. For female lizards, I also included reproductive state as a factor (gravid or not-gravid). Because there are currently no post-hoc tests for permutation procedures such as ‘adonis,’ I utilized graphical representations of the data for each factor with $k > 2$ groups to scan for potential pairwise differences. For these graphs, I plotted the first two axes of a non-metric multidimensional scaling ordination (using Gower distances) for each sex. For each group on these plots, I outlined its convex hull (e.g. Y, O, and W female morphs), and an ellipse representing the standard deviation of the weighted averages from its centroid. I thus interpret significant differences between pairs of factor groups in which these ellipses do not overlap.

iv. Multivariate Trait-Environment Matching by Male and Female U. ornatus

Comparisons of changes in the trait-environment relationship following perturbations have been accomplished using three-matrix methods (e.g. Lindo et al. 2012). However, matrix superimposition methods may be a superior approach for numerous reasons. Superimposition methods allow for the calculation of residual differences between patterns of variation in two multivariate matrices (e.g. morphological and environmental datasets). Residuals in this case therefore represent a matching index
among these two datasets. The variation in these residuals among groups of interest can be explored using a general linear model approach.

I monitored trait-environment relationships across my study sites with the following procedure. First, for each sex I extracted those variables that significantly correlated with the first two axes of the microhabitat and morphology ordinations. For female lizards, habitat variables included the distance to the two nearest trees, and forb and leaf litter cover. Male lizards were influenced by the percent cover of woody debris in addition to those variables. Morphological variables describing female lizard variation included SVL, HW, and JL. In addition to those variables, male lizard variation also included FLL. I then applied separate non-metric multidimensional scaling (NMDS) ordinations on the Euclidean distances for these 1) ecological and 2) morphological data for each sex at each site, respectively. I thus ran six total NMDS ordinations (two per site for each sex). For each ordination I retained the first two NMDS axes. Next, I applied a Procrustes superimposition of the morphological axes onto the ecological axes for each sex using the function ‘procrustes’ in the VEGAN package. This procedure generates a new rotated morphological matrix which maximizes the similarity between the two initial configurations. The residuals of the superimposed matrix can then be retained as an index of the degree an individual’s position in the morphology ordination matches its position in the ecological ordination. I refer to these residuals as matching scores: residuals closer to zero indicate close morphology-environment matching. I conducted this procedure separately for each site. I then applied separate ANOVAs to the resulting residuals for each sex-site combination to test for morph and year differences in degree of matching. I
applied a Tukey’s Honestly Significant Difference post-hoc test following each ANOVA to all significant factors with greater than two levels.

v. Relationship between Phenotypic Variation and Matching

Because disturbed environments may influence patterns of phenotypic integration and thus favor different phenotypes compared to undisturbed areas, I determined 1) the changes in phenotypic variation among study sites and 2) the phenotypes that best matched the HB site. I used the function ‘betadisper’ to calculate phenotypic dispersions (distances to group centroids) based on morphological data for each sex-site combination. For these data, dispersion values near zero represent phenotypically average individuals whereas larger values indicate individuals at the tails of the phenotypic distribution. I applied an ANOVA to this model to determine whether dispersions, and overall phenotypic variation, were similar among the study sites. I then applied a linear regression to compare the degree to which distance to centroid predicts morphological matching scores. For this analysis, a relationship between centroid distance and matching would provide evidence of either disruptive (negative slope) or stabilizing (positive slope) selection in burned areas.

vi. Survival Models

Inferences into the selective regimes operating on organisms among variable environments are greatly strengthened if they are explicitly linked to estimates of fitness for those organisms in those same environments. I analyzed survivorship of male and female lizards separately for each site. I ran all models in MARK with the logit link function, 2ndPart variation estimation, and individual covariate design matrix options. In
these models I included morphological matching scores (residuals) as covariates. This procedure resulted in two models per sex per site (Table S2). Following Rabosky et al. (2012), I computed weighted-averages of both resulting survival ($\phi$) parameters to obtain robust estimates of survival (see Table S2). I used the program CONTRAST (Hines and Sauer 1989) to compare the estimates of survivorship separately for males and females among the three sites. I did not conduct any goodness-of-fit procedures (e.g. Likelihood-ratio tests) because they are not amenable to individual covariate datasets (White and Burnham 1999).

I set all statistical procedures at a threshold for significance of $\alpha=0.05$. I present all means as value ±1.0 standard deviation (SD) unless otherwise noted.

III: Results

I captured most *U. ornatus* lizards on living oak (*Quercus* sp.) trees, followed by tree snags, mesquite (*Prosopis* sp.), and large rocks. Male morph frequencies did not fluctuate over time at any site (NB: $\chi^2 = 7.62$, df = 10, $P = 0.665$; LB: $\chi^2 = 6.2$, df = 10, $P = 0.798$; HB: $\chi^2 = 13.26$, df = 10, $P = 0.21$). Female Y morph frequencies increased over time at the LB site, but no changes in morph frequency were observed at the NB or HB sites over time (NB: $\chi^2 = 9.32$, df = 4, $P = 0.054$; LB: $\chi^2 = 10.58$, df = 4, $P = 0.032$; NB: $\chi^2 = 6.11$, df = 4, $P = 0.191$). Female reproductive phenology (based on frequency of gravidity) was similar over time among the three study sites (Figure 15).
Figure 15. Percent of gravid female *U. ornatus* lizards encountered from June through July of 2010-2012 across three study sites varying in disturbance history in southeastern Arizona. Study sites are coded as follows: NB (non-burned control), LB (low-frequency burned), and HB (high-frequency burned). Percent data are divided among two-week intervals to aid in interpretation.

### A. Patterns in Habitat Availability and Use by *U. ornatus*

Both low- and high-frequency burning had a significant effect on the habitat variation over time ($F_{6,279} = 17.66, P = 0.005$). Specifically, grasses average over 80% of the vegetation at the HB site, and 70% at the LB site, compared to only 48% at the NB site. Grass, leaf, and woody debris cover increased significantly in both treatment sites with respect to the control site (Figure 16). In addition, shrub and forb vegetation cover decreased over time. I observed similar trends in the site-specific environmental response to burning: both sites peaked in response in 2011 and decreased thereafter (see Figure 16). The vegetation characteristics at lizard capture points differed significantly from random points (adonis, type: $F_{1,455} = 40.31, P = 0.01$; Table 6). Selected microhabitats
matched the available environment at the NB site relative to the two treatment sites. The analysis resulted in a significant interaction between treatment, plot type, and year (adonis, treatment × type × year: $F_{4,455} = 2.13, P = 0.02$). Thus, although lizard habitat associations were consistent across sites, the degree that the characteristics of these microhabitats differed from those of random points varied across the sites.

For male lizards, variables significantly driving variation in habitat use were the distances to the two nearest trees ($r = 0.956$ and 0.92, respectively, $P < 0.001$ for both cases), as well as the cover of forbs ($r = 0.16$, $P = 0.022$), leaf litter ($r = 0.19$, $P = 0.005$), and woody debris ($r = 0.24$, $P < 0.001$) in the plot. For female lizards, these variables included the distances to the two nearest trees ($r = 0.95$ and 0.92, respectively, $P < 0.001$ for both cases), as well as the cover of forbs ($r = -0.37$, $P < 0.001$) and leaf litter ($r = 0.4$, $P < 0.001$).
Figure 16. Results of a principal response curve (PRC) analysis comparing variation in environmental characteristics among two treatment sites with that of a control site. Site designations are as follows: NB (non-burned control, solid grey line), LB (low-frequency burned, dashed black line), and HB (high-frequency burned, solid black line). This analysis zeroes the control site and examines variation in response variables in treatment sites relative to that control over time (in this case, 2010 – 2012). Significant variables are plotted on the right-hand axis: those falling above the zero line (NB site) are greater in treatment sites and those falling below it are more common in the control.
Table 6

Results of non-parametric multivariate analysis of variance comparing vegetation characteristics among three sites (treatment) that were recorded at stratified grid and lizard capture points (types) from 2010 – 2012 (year). Significance is based on 4000 permutations with randomizations limited to within specific plot types only.

<table>
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<tr>
<td>residuals</td>
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</table>

B. Morphological Variation in U. ornatus

Male morphs differed in their external morphology among the sites over the three years of study ($F_{46,173} = 3.07$, $P < 0.001$; Table 7). Most male differences were more site-rather than morph-specific (Table 7). The morphological variation among males was consistent over time (Homogeneity of dispersions test, $F_{2,217} = 2.34$, $P = 0.09$). The majority of variation among males was attributable to differences in body size, head shape, and limb length among individual lizards (SVL: Pearson’s $r = 0.668$, $P < 0.001$; HW: Pearson’s $r = 0.492$, $P < 0.001$; JL: Pearson’s $r = 0.762$, $P < 0.001$; FLL: Pearson’s $r = 0.679$, $P < 0.001$).

Female lizard morphs differed in morphology among the sites ($F_{27,129} = 11.45$, $P < 0.001$; Table 7). In particular, female morphological variation increased regardless of morph identity in 2011 and 2012 compared to 2010 (Homogeneity of dispersions test,
Most of this variation in morphology was attributable to body size and head shape differences (SVL: $r = -0.958$, $P < 0.001$; HW: $r = -0.541$, $P < 0.001$; JL: $r = 0.651$, $P < 0.001$).

Table 7

Results of multivariate analysis of variance of male (top) and female (bottom) tree lizard (*Urosaurus ornatus*) morphological scores. Morphological scores were calculated as the first and second principal component scores from separate principal component analyses on each sex. Cumulatively, these scores accounted for 90.9 and 85.4% of the total variation in male and female morphology in this study, respectively.

<table>
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</tr>
</tbody>
</table>

*C. Stamina Variation in U. ornatus*

Performance capacity differed between male and female lizards. Male *U. ornatus* exhibited greater stamina over time (adonis, $F_{2,170} = 18.532$, $P = 0.001$; Figure S4). Body size however explained a significant amount of variation in male performance capacity (adonis, $F_{1,170} = 4.406$, $P = 0.018$). In particular, larger males attempted escape more
frequently than smaller males (*Spearman’s rho* = 0.323, *P* < 0.001; all else *P* > 0.3). Male morphs did not differ in performance capacity (adonis, *F*$_{5,170}$ = 1.037, *P* = 0.43). I detected an interaction between year and site (adonis, *F*$_{4,127}$ = 2.293, *P* = 0.027; see Figures S4-S5 for main factor plots of year and site). Gravid females exhibited lower stamina than non-gravid females (adonis, *F*$_{1,127}$ = 20.113, *P* < 0.001; Figure S6). Female stamina differed among the morphs (adonis, *F*$_{2,127}$ = 3.11, *P* = 0.023). Yellow females had greater stamina and fewer escape attempts compared to orange females (Figure S7). The performance of females varied among years: females exhibited greater stamina in 2011 and 2012 compared to 2010 (adonis, *F*$_{2,127}$ = 17.894, *P* = 0.001; Figure S8). I also detected an interaction between these factors (adonis, *F*$_{4,127}$ = 2.341, *P* = 0.027; all else *P* > 0.1), suggesting that year-year patterns among the morphs varied (see Figures S7-S8 for main factor plots of morph and year).

**D. Microhabitat Matching**

Male and female *U. ornatus* differed in the correspondence between morphology and environmental variation at their capture sites. Male lizards exhibited no pattern in morphology-environment matching at the NB or LB site among morphs, years, or their interaction (NB: ANOVAs, all *P* > 0.06; LB: ANOVAs, all *P* > 0.1). However, male morphs varied in the morphology-environment matching at the HB site (ANOVA, *F*$_{5,61}$ = 3.135, *P* = 0.014; Table 8). Specifically, Y and B morphs matched their environment better than the O morphs (*i.e.* smaller residuals; Figure 17). Female lizards did not exhibit any variation in matching with respect to morph or year in the NB or LB sites (NB: ANOVAs, all *P* > 0.5; LB: ANOVAs, all *P* > 0.2), but there was an interaction between
morph and year in the HB site (ANOVA, $F_{4,35} = 3.193, P = 0.025$; Table 8). Specifically, O females matched the environment better than W females in 2012 (i.e. smaller residuals; Figure 18).

Table 8

Results of an Analysis of variance (ANOVA) comparing matching scores among male lizard morphs (six morphs) and years of study (three) for all tree lizards (Urosaurus ornatus) at the High-frequency Burn (HB) site. Matching scores represent the residual variation extracted from a Procrustes superimposition of the morphological distance matrix over the habitat matrix for the High-Burn site. Thus, residuals represent degree that the multivariate position of each individual lizard in morphological space matches its position in habitat space.

<table>
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<th>$F$-ratio</th>
<th>$P$-value</th>
</tr>
</thead>
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</tr>
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<td>residuals</td>
<td>61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E. Relationship between Morphological Variation and Matching Scores

Male lizards exhibited similar degrees of phenotypic variation among the study sites with respect to the morphological variables measured (ANOVA, $F_{2,217} = 0.436, P = 0.647$). I found a significant negative relationship between morphological dispersion and matching scores for males at the HB. Males diverging in morphological characteristics with respect to mean values matched their environments better than those males that are phenotypically average ($F_{1,74} = 4.512, R^2 = 0.06, P = 0.037$; Figure 19). Female lizards however exhibited no such relationship ($F_{1,42} = 0.001, R^2 = 0, P = 0.997$).
F. Survival Estimates

Male survival was greater in the HB site than either the LB or NB sites ($\chi^2 = 10.448$, df = 2, $P = 0.005$; Figure 20a). Females did not differ overall in survival probability by site ($\chi^2 = 1.271$, df = 2, $P = 0.53$; Figure 20b). Males with higher matching scores at the HB site had higher survival (Figure 21a). I detected no evidence of a relationship between female matching scores and survival (Figure 21b).

Figure 17. Residuals of a Procrustes superimposition of a matrix containing morphological data of male *U. ornatus* over a matrix describing their microhabitat (capture site) characteristics. Residuals are separated by morph: Blue (B), Yellow (Y), Orange (O), Yellow-blue (YB), Orange-yellow (OY), and Orange-blue (OB). Values are mean and bars represent $\pm 1.0$ S.E. Letters denote significant differences based on a Tukey’s HSD post-hoc test.
Figure 18. Residuals of a Procrustes superimposition of a matrix containing morphological data of female *U. ornatus* over a matrix describing their microhabitat (capture site) characteristics. Residuals are separated by morph: Yellow (Y), Orange (O), and White (W). Values are mean and bars represent ± 1.0 S.E. Letters denote significant differences based on a Tukey’s HSD post-hoc test.

Figure 19. Correlation between multivariate dispersions of morphological data (variance) and residuals extracted from Procrustes superimposition for male *U. ornatus* lizards at the HB (High-frequency burned) site. Correlation is significant at the 0.05 level.
Figure 20. Robust estimates of apparent survival across three study sites varying in disturbance history for a) male and b) female *U. ornatus* lizards. Study sites are coded as follows, based on prescribed burn history: NB (non-burned site), LB (low-frequency burned), and HB (high-frequency burned). Survival estimates are generated using the program MARK and differences were evaluated using the program CONTRAST (see Methods). Points represent mean survival estimates and bars are ±1.0 S.E.
Figure 21. Relationship between matching scores (residuals of Procrustes superimposition of morphological data over microhabitat data) of a) male and b) female *U. ornatus* lizards with respect to their predicted apparent survivorship from models implemented in the program MARK (see Methods for model details). Dashed lines represent ±1.0 S.E.
IV. Discussion

Disturbances the availability and distribution of resources in the environment that are essential for individual organisms to meet life-history demands and maintain homeostasis. Organisms may thus react by exhibiting a plastic or adaptive response. In this study I characterize these responses. In particular, I show that both male and female lizards were consistent in their microhabitat selection, yet the similarity of those microhabitats to random points at each site differed. The availability of these microhabitats however decreases when moving from the NB to the LB and HB sites. My results suggest that this habitat change has contrasting effects for each sex. For males, the greater environmental perturbation (relative to the NB site) is associated with increased morphological divergence among the male morphs, particularly in head shape and limb length. Yet despite these morph differences, all males were similarly larger at the HB site, and exhibited greater stamina there as well. Males, and in particular territorial yellow and blue males, matched their habitats better than other males in the HB site. In this site, I also detected greater survival among males, especially those that matched their habitat better. For females, morph head shape and body size differences were consistent throughout the study and these differences only increased over time. Unlike males, female differences in stamina mirrored their morph differences. In addition, female survival was consistent across sites and not associated with their variation in matching scores or phenotypic dispersion.

In this paper I combined information on environmental variation with data on morphology, performance, and survival to ascertain the responses of a species to one
facet of anthropogenic disturbance, burning. Prescribed burning is implemented to restore grassland ecosystems to a more natural state. However, an assumption underlying this practice is that the environmental changes do not negatively impact wildlife. At the burned sites, I detected significant shifts in environmental heterogeneity which match the findings of other studies regarding the effects of fire on grassland ecosystems (D'Antonio and Vitousek 1992, Keeley, Lubin and Fotheringham 2003, Grant et al. 2010). In particular, I documented significant loss of trees, shrubs, and forbs in favor of grassy vegetation. These changes also reduced the structural heterogeneity at these burned sites relative the unburned control site. For tree lizards in particular, these changes incur an ecological cost, namely, loss of preferred microhabitat.

Previous studies have noted that male *U. ornatus* morphs vary in reproductive strategies, including territorial behavior (Thompson and Moore 1991a,b). Thus, I predicted that they should exhibit a strong response to these habitat changes. My results however indicate that this is not supported. Preferred microhabitats for all lizards in this study included oak and mesquite trees and a greater amount of cover of shrubs, forbs, rocks, and woody debris. These preferences are consistent with those reported for *U. ornatus* elsewhere (e.g. shrubs and rocks, Herrel, Meyers and Vanhooydonck 2001). Both males and females inhabited similar microhabitats, with the exception that female capture sites were more open. The availability of these microhabitats is low in burned sites, and lowest at the HB site in particular. Yet, lizard capture points were consistent in vegetation and structural characteristics across the study sites. This suggests that lizards may become more selective at disturbed sites. Another consequence of reduction of
preferred microhabitat is an increase in the spatial overlap among individuals and/or morphs. Indeed, results from a previous study I conducted in 2009 at the LB and HB sites suggest that male *U. ornatus* morphs overlap spatially in the HB site, but are segregated in the LB site (Chapter 3).

The relative consistency among all lizards in their habitat use across sites suggests that lizards in habitat-limited burned sites may experience greater competition for resources than lizards in non-burned sites. Resource competition may then favor divergence in other aspects of their ecology and life history. Indeed, male morph differences in body shape were only distinguishable in the HB site, where differences in vegetation cover were largest compared to the NB site. Males at the HB site had shorter fore- and hind-limbs than their conspecifics at the NB site. Indeed, *U. ornatus* in other forested areas generally exhibit longer limbs, a condition beneficial for the biomechanics of a primarily arboreal lifestyle (Herrel, Meyers and Vanhooydonck 2001). Males at the HB site were also larger than males at other sites. Because the extent of overall male phenotypic variation was similar among all sites, this suggests that the trait optima of male morphs have shifted in phenotypic space. Moreover, the matching and dispersion data show that territorial males occupy the periphery of the multivariate distribution at the HB site. One hypothesis is that disruptive selection might generate this shift, as a consequence of discrete morphs competing for access to similar resources. Resource limitation should favor spatial restructuring of a population according to competitive ability (Fretwell 1972). Over time, this restructuring should enhance morphological differences among the male morphs, especially if it favors use of alternative resources.
(e.g. Chapter 3). If morphological differences increase fitness, then this may feedback into morphology (Huey 1991), potentially enhancing morph differences. Prey selection in particular will influence head shape, and in contexts where prey-use differences are amplified, head shape differences should be amplified as well (Chapter 3). If lizards prefer different prey types in burned sites, but overlap in diet in unburned sites, then perhaps this may explain in part their variation in head shape. The results of a previous study support that male morphs differ in their trophic niche at the HB site (Chapter 3). Morph differentiation would be advantageous if it reduced competition (e.g. Bolnick et al. 2003), especially given the reduction in preferred microhabitats at the HB site.

Another consequence of reduced competition, ecological release, might also result in selection for further variation in morphology (Losos and De Queiroz 1997). Whether resource competition structures *U. ornatus* populations at any of these sites is subject of a concurrent study (Chapter 5).

Male lizards at the burned sites exhibited shorter limbs but larger body sizes than those in the non-burned control. Similarly, Herrel et al. (2001) found the same trends among *U. ornatus* lizards in open habitats. However, others have suggested that more-wooded areas like the NB site should support more resources and thus, larger lizards (e.g. Smith 1996). Differences in body size of individuals across different sites might reflect differences in habitat quality as inferred from these studies. Or it may be a result of competition for access to similar resources, given the links between body size and male competitive ability in lizards (Calsbeek and Sinervo 2002). Yet it may also involve variation in other factors such as reproductive phenology or predation risk. If females
from one site deposit eggs earlier in the season than females at other sites, then those offspring will have more time to grow. This could explain the occurrence of larger body sizes in male *U. ornatus* at the HB site. However, females from all three sites are gravid at equal frequencies throughout the season. Instead, differences in body size across sites may be related to differences in resource availability among the sites. Alternatively my estimates of apparent survival suggest that survivorship is in fact greatest for male lizards in the high-burned site. This suggests that predation risk may be lower at this site than the LB and NB sites. Greater survivorship may also explain the larger male body sizes at the HB site. Indeed, the high recapture rates of adult lizards (see Table S2), given my equal search effort among all three sites.

Male and female color morphs exhibited different patterns of trait-environment matching with the exception of 2012 when both sexes exhibited morph-specific matching variation in morphology. The polymorphism in *U. ornatus* may include up to six male and three female morphs, and tends to vary microgeographically (Thompson and Moore 1991a). Grassland habitats appear to support extensive morph variation. In my study, blue and yellow morph males, as well as yellow morph females in 2012, matched their habitat better than the other morphs at the HB site. For male lizards, the best matchers also included YB morphs which are aggressive at this (Lattanzio, unpublished data) and other sites (Hover 1985, Thompson and Moore 1991a, b) compared to O morphs. For female lizards, O morphs matched significantly better than, and Y somewhat better than, W morphs, but only in 2012. Little is known regarding the function of the female color morphs in *U. ornatus* (Zucker 1989, Zucker and Boecklen 1990, Mahrt 1998). Previous
results suggest that Y and O coloration provides information on reproductive status and may be a conditional trait rather than a fixed genetic trait (Zucker and Boecklen 1990). However, gravid individuals of all three morphs were observed in this study. I also demonstrate that the three female morphs exhibit discrete morphological differences, namely in body size and limb lengths. In addition, all females may be territorial, especially when defending potential nest sites (Mahrt 1998). Female territories often exhibit a high degree of overlap with male territories. I show that male and female capture sites in this study share some microhabitat characteristics, including proximity to trees and a preference for greater forb cover. Less data are available with respect to the reproductive strategies of W morph females. My survival and matching data suggest however that these females may exhibit a wide-ranging, nomadic strategy like that of O males. My results also indicate there are differences in the degree each female morph is able to match its microhabitat. For males at least, the best matchers were typically more-aggressive, territorial morphs. Given that females vary in morphology and possibly territoriality as well, the three female morphs may be fixed at my study sites. Indeed, Zucker and Boecklen (1990) also suggest that female coloration may be fixed in some populations.

Territorial behavior may be beneficial for male lizards in burned areas, where the preferred microhabitats of all morphs are lacking due to the homogenizing effect of fire in this ecosystem. My survival models support this claim: in the HB site, the best model included matching score as a covariate. The relationship between apparent survival and matching clearly indicate that those individuals that are phenotypically a better match
(i.e. better adapted) to their microhabitat characteristics are likely to experience a boost in survival relative to those that match more poorly (e.g. nomadic O males, see Figures 17 and 21).

The patterns of variation among morphs in the HB showed that B, YB, and Y morphs not only were more likely to have higher survival they were also more phenotypically extreme. Frequent burning may promote these shifts towards favoring more extreme phenotypic trait optima. Longer-term studies on these populations are necessary however to determine if, over time, the novel selective regimes in burned areas result in loss of phenotypic diversity in *U. ornatus*, or other species. My results provide evidence that anthropogenic disturbance does indirectly favor divergence in this polymorphic species. Moreover, my results suggest that disturbances may not favor all morphs equally. For *U. ornatus*, blue and yellow males (and their intermediates) may be more likely to persist in burned habitats. Interestingly, to my knowledge, monomorphic populations of *U. ornatus* are always composed of blue morph males (e.g. Zucker 1989). Further study is required to determine whether the mechanisms proposed in this study contribute to divergence in other polymorphic species is necessary.
CHAPTER 5: ECOLOGICAL CONSEQUENCES OF VARIATION IN RESOURCE
AVAILABILITY FOR MALE TREE LIZARD COLOR MORPHS

I. Introduction

Success in male contests is primarily governed by asymmetry in resource holding potential (RHP, Parker 1974). Variation in RHP among males may ultimately affect dominance status, territory size, and reproductive success. Although numerous studies emphasize variation in RHP in structuring social interactions among individuals, asymmetry in resource value may override any differences in male fighting ability in some contexts. Moreover, RHP and resource value may covary and mutually influence contest outcomes (Kelly, 2008). Both RHP and resource value are partly dependent upon external factors, including resource availability. Spatial variation in the availability of resources influences their value to organisms. That is, morphology, behavior, and life-history traits may be influenced by resource quality and availability (Justino et al., 2012, Radford, 2012, Van Buskirk, 2011, Wright et al., 2013). Phenotypic variation among males in particular will influence their relative fighting ability, and thus their RHP. Resource availability thus directly (resource value) and indirectly (RHP, through its influence on contestant phenotypes) affects the factors that determine agonistic interactions among males and dominance patterns.

The ecological consequences of competitive differences among males remain poorly understood (but see Calsbeek and Sinervo, 2002a, b). Recent theory aimed at characterizing the social structure of populations may however serve to bridge this
knowledge gap (e.g. social network theory, Krause et al., 2007, Wasserman and Faust, 1994, Wey et al. 2008). In particular, the collective outcomes of all male contests (i.e. dominance hierarchies) should structure male spacing patterns, including territories and home ranges. Fretwell (1972) described social structuring as an ideal despotic distribution. The overall pattern of these spatial relationships between individual organisms represents the social network of that population (Wey et al., 2008). The properties of this network will have important consequences for population fitness (e.g. Hamede et al., 2009). For instance, spatial proximity of individuals in social networks has been associated with variation in individual parasite load (Fenner et al., 2011) and nonlethal injuries arising from contests (Cañon Jones et al., 2010). These factors may contribute to variation in male survival and reproductive success. The structure of a network will also be influenced by environmental variation (Webster et al., 2013, Wey et al., 2008). Changes to network structure will likely be mediated through the effects of environmental variation (i.e. changes in resource availability) on male resource value and RHP outlined above. Therefore, social networks should differ in structure among different environments. Since network structure may also affect fitness, the fitness consequences (e.g. injury or parasite load, Fenner et al. 2011) for lizards in each network should also differ.

Here I use data collected on male ornate tree lizards (Urosaurus ornatus) in order to explicitly address the effects of environmental variation on social network structure. Male U. ornatus are polymorphic in throat coloration, and most populations exhibit three fixed throat colors: blue, yellow, and orange (Thompson and Moore 1991a, b). These
color differences are under genetic and hormonal control (Thompson et al., 1993, Hews et al., 1994). Morphs are fixed by attainment of sexual maturity and there is no evidence of ontogenetic variation in throat color (Carpenter 1995a). Male throat coloration is associated with alternative mating tactics (Moore et al., 1998). Blue males exhibit a fixed territorial behavior, orange males are nomadic, and yellow males are typically satellite morphs. Blue males tend to use display behavior to settle contests, although there is a positive relationship between size of the blue throat patch and aggression (Thompson and Moore, 1991b). The orange male tactic varies with environmental conditions (e.g. drought) and resource availability: males switch to defending territories when resources are abundant (Knapp et al., 2003). Much less is understood regarding the tactic of yellow males, other than their apparent satellite behavior with respect to other male territories. Initial data from staged contests suggests that yellow males are aggressive and field data show they are capable of usurping blue male territories (Lattanzio, unpublished data). These data suggest that yellow males may play an escalate tactic in response to the blue male display tactic (e.g. Smith and Parker, 1976). The conditions favoring usurpation over satellite behavior are not known.

The behavioral differences among these morphs reflect variation in RHP. I used staged contests to characterize patterns of display and escalation behavior among the morphs. In these contests, I removed confounding effects of ownership by simultaneously introducing males into the arena each trial. I controlled for the influence of body size by size-matching males and all contests were for the same resource (heated central perch). I paired males based on social context: contests occur either between territorial males, or
between territorial and non-territorial males. I then use male capture data at each site to quantify social network parameters (e.g. Fenner et al., 2011, Wasserman and Faust, 1994), and combine those data with parasite load and injury (bite mark) counts to infer the possible effects of network structure on population fitness.

I make four predictions based on data from three study sites varying in resource availability as a consequence of prescribed burning. Specifically, prescribed burning reduces the availability of trees and shrubs, preferred resources by *U. ornatus* males (e.g. Herrel et al. 2001, Chapter 4). First, I predict that blue males will consistently defend higher-quality resources (e.g. trees and shrubs), regardless of site, because of their aggressive behavior and expectations of ideal despotic theory (Fretwell, 1972). Second, male body size should be larger in the more-burned site where available territories (resources) are limiting and competition likely greater (Calsbeek et al., 2007). Third, if body size is larger in burned sites, males in these sites may escalate contests more frequently due to a correlation between body size and fighting ability in asymmetric contests (Smith and Parker, 1976). Finally, because of the likely costs of persistent aggressive interactions (e.g. injury or mortality), I predict that aggressive blue males do not neighbor each other in resource-limited burned sites.

II. Material and Methods

*A. Study System and Capture Methods*

I captured adult male blue (B), orange (O), and yellow (Y) morph *U. ornatus* at the Appleton-Whittell Research Ranch in Santa Cruz County, Arizona from June – July
2012. At this stage in the breeding season, blue morph *U. ornatus* have established territories and their spacing patterns with other males (yellow and orange morphs) have stabilized. I surveyed three sites that vary in vegetation cover and heterogeneity as a consequence of variation in history of prescribed burning. These sites include: a non-burned site (since 1970, hereafter Non-Burned [NB]), a site burned once in 2002 (hereafter Low-frequency Burn [LB]), and a site burned in 1980 and again in 2002 (hereafter High-frequency Burn [HB]). The same burn affected both LB and HB sites in 2002. All study sites are at least 3 km apart each, which is significantly farther than any documented *U. ornatus* dispersal distance (e.g. Zucker, 1989). All three sites share similar soil and topographic characteristics. I collected all lizards by noose-pole or hand and georeferenced all capture points using a hand-held GPS unit (Garmin GPSMAP 60CSx, Garmin Ltd., USA). I refer to capture points as the geographic point of initial observation for each lizard. Based on the average territory and/or range size of *U. ornatus* (~100 m², Zucker, 1989, Lattanzio, unpublished data), their preference for and primary use of a central tree, I assume that capture points represent the center (or near-center) of each male’s territory or home range. I recorded the perch substrate (tree/shrub, snag, rock) for each lizard upon capture. I transported all lizards to a lab facility in a climate-controlled vehicle after capture. I housed individual lizards overnight in separate in 5.7 liter aquaria (27.9 cm L × 17.8 cm W × 12.7 cm H, Frey Scientific, Nashua, NH, USA). I provided lizards water *ad libitum*. I thoroughly washed and dried all aquaria prior to reuse.
B. Characterization of Social Dominance

I size-matched pairs of males ($\Delta_{SVL} \leq 2.0$ mm) and subjected those males to contests for access to a resource (following Garland Jr et al., 1990, Robson and Miles 2000). I randomly assigned males to one of three possible male morph pairings for each trial: B-B, B-O, or B-Y. Contests took place in a small, circular plastic tank (0.2 $\times$ 1.2 m, depth $\times$ diameter), with sand as a substrate and a central perch (raised branch). I suspended a heat source (100 W light) ~0.4 m above this perch. This generated a thermal gradient in the arena ranging from about 22 °C at the periphery to approximately 42 °C on and under the perch. This gradient encourages lizards to move towards the central perch to thermoregulate, thus encountering each other and initiating a competition for this limiting resource (Garland Jr et al., 1990, Robson and Miles 2000). I placed competing males at opposite ends of the arena, underneath opaque plastic cups. I initially used these overturned containers to obscure each lizard’s view of the arena until the start of a trial. I initiated each trial by lifting these containers simultaneously, exposing the lizards to the experimental arena. I recorded the behavior of each male beginning at this point and for a total of 30 minutes from behind a blind.

I recorded the frequency of each of the following behaviors: aggressive - head-bob, lateral flattening, full-show, chase, bite, and submissive - flee, and flatten. These behaviors are described elsewhere (e.g. Carpenter, 1995b, Martins, 1993). I assigned scores to each male based on these behaviors, following Garland Jr et al. (1990). I also determined a subjective winner for each trial based on which lizard occupied the central perch for the longest duration.
C. Quantification of Parasite Load and Injuries

Parasite load may also vary in different environments and is influenced by individual spacing patterns (e.g. Fenner et al. 2011). At my study sites, lizards vary in their degree of infection by ectoparasitic mites. I recorded parasite load as both the total number of distinct body regions (e.g. separate joints, skin folds) where ectoparasitic mites were detected and the rank abundance of total mites on the individual lizard. Rank abundance was scaled 0-6: 0 mites (0), 1-5 mites (1), 6-10 (2), 10-15 (3), 16-20 (4), 20-25 (5), 25+ (6).

Some male interactions will result in escalation to biting, and these interactions may be expected to increase in resource-limited habitats where competition for resources may be high. Lizard bite marks have a characteristic U or V-shape, and the small size of tree lizards make their bite marks readily identifiable as residual scars on the ventral surface of a male. I thus recorded the number of bite marks (injuries) on each lizard. I only counted marks that were clearly from other U. ornatus to avoid confounding contest escalation with other factors (e.g. failed predation attempts).

D. Social Network Characterization

The microhabitat preferences of adult U. ornatus lizards at these sites have been described in an earlier study (Chapter 4), thus I only describe those results in brief here. The microhabitats of male lizards are characterized by their proximity to trees as well as relative cover of non-grassy forbs, leaf litter, and woody debris. The results of that study, as well as others (M’Closkey et al. 1987, Herrel et al., 2001, Thompson and Moore, 1991a), suggest that trees and shrubs represent preferred (and higher-quality) territories
for male *U. ornatus*. Therefore lizards on trees or shrubs should occupy better territories than those on snags (fallen trees) or other substrates (*e.g.* rocks).

I calculated social networks within each site based on georeferenced capture locations of each lizard in each study site. In these networks, links are based on the geographic distances (nearest 0.1 m) between all lizard capture points. I assume that the spatial proximity among these points represents an index of territorial overlap among the males. I used these networks to test my predictions regarding how the spatial structuring of *U. ornatus* males varies across the study sites. I ranked the connectedness in these networks by modifying a protocol outlined by Fenner *et al.* (2011) to fit my study species (Table 9). I used a maximum distance cut-off of 50 m because *U. ornatus* male territories rarely approach that size. As with Fenner *et al.* (2011), I assigned weights from 0 to 5 to each edge (connection between adjacent males) depending on proximity of each pair of adjacent males (see Table 9).

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>Edge weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 50</td>
<td>0</td>
</tr>
<tr>
<td>40 - 50</td>
<td>1</td>
</tr>
<tr>
<td>30 - 40</td>
<td>2</td>
</tr>
<tr>
<td>20 - 30</td>
<td>3</td>
</tr>
<tr>
<td>10 - 20</td>
<td>4</td>
</tr>
<tr>
<td>0 - 10</td>
<td>5</td>
</tr>
</tbody>
</table>
Social networks may be characterized by several properties which provide information on connectedness among individuals in the network (Wasserman and Faust, 1994). I retained two measures of connectedness for this study: degree and eigenvector centrality. Degree centrality refers to the number of edges connecting to each individual. Eigenvector centrality provides an index of how connected each individual is to the entire network (Wasserman and Faust, 1994). Those individuals that are more connected (more edges between them and other individuals which also have more edges) will have larger values. I determined node strengths by summing the edge weights that connected to each individual (i.e. node). I retained the average weight (mean strength) of all edges connected to each individual as well. I retain both estimates because they provide quantitatively different information about a network. Whereas strength provides an estimate of how many neighbors each individual has, mean strength provides an estimate of the average intensity of those associations (Fenner et al., 2011). Thus, a large value for strength could indicate either several far edges or few close edges. Overall, individuals with higher mean weights are more closely connected to their neighbors within a given social network.

E. Statistical Analysis

I conducted all statistical procedures using the R software environment (R Development Core Team, 2010). All post-hoc tests were interpreted using Bonferroni-adjusted $P$-values. I compared the frequencies of male morphs among the three sites using a multinomial test. I used Chi-Square tests to compare microhabitat use differences across the sites for each male morph. I used permutation $F$-tests to compare variation in
male body size (log-transformed SVL), with morph and site as factors. For the number of injuries (bite marks, count data), I applied Kruskal-Wallis tests with those same factors. I compared estimates of parasite load (number of affected areas, rank-total number of mites) by first transforming these data into a distance matrix using the Gower distance metric (Gower, 1971). I then used non-parametric multivariate analysis of variance (function ‘adonis’ in the vegan package, see Anderson, 2001). In all applications of this function, I set the number of permutations to 4000 and limited randomizations to within-sites only.

I compared variation in male agonistic behavior during paired-male contests using NMDS plots on Gower distances derived from frequencies of each of the following behaviors for each male (gular shudder, full-show, lateral flattening, chase, and bite). I used convex hulls and 95% confidence limit ellipses of the standard error of the median, weighted by the appropriate Chi-square value with 2 degrees of freedom, to visualize behavioral differences. I applied the function ‘adonis’ to explore differences in these behaviors across the sites, by morph, and by male dyad (i.e. B-B, B-O, or B-Y).

I explored lizard connectivity (degree and eigenvector centrality) using non-metric multidimensional scaling (NMDS) plots from distance matrices generated using the Gower distance metric on connectivity data. For all uses of NMDS, I applied Spearman’s rho correlations to determine which variables contributed significantly to each ordination axis. I also used the function ‘adonis’ to determine differences in connectivity among the morphs and/or sites. I compared strength (number of edges connecting to a node) and mean strength (average number of all edges directly or
indirectly connected to a node) using separate permutation \( F \)-tests. In these tests, I also included edge type (i.e. B-B, B-O, or B-Y) as a factor in addition to morph and site.

III. Results

A. Variation in Morph Frequencies, Morphology, and Parasite Load

All lizards were captured on or near a central tree (living or dead). The relative frequencies of B, O, and Y males were similar across the study sites (multinomial test, \( \chi^2 = 2.79, \text{df} = 4, P = 0.6 \)), although I observed a decline in B males and increase in Y males from the NB to the HB site (Figure 22). Overall, B males consistently used similar microhabitats across the sites, preferring trees or shrubs over snags (\( \chi^2 = 18.69, \text{df} = 1, P < 0.001 \)). Yellow males shifted their microhabitat use among the sites. Yellow males were more likely to be found on trees or shrubs in the HB site than at the NB site (\( \chi^2 = 7.89, \text{df} = 2, P = 0.019 \); all other pairwise \( P > 0.1 \)). The body size of male lizards differed among the sites, regardless of morph (permutation test, site: \( F_{2,79} = 2.64, P = 0.04 \); morph: \( F_{2,79} = 0.2, P = 0.674 \); site \( \times \) morph: \( F_{4,79} = 0.82, P = 0.459 \)). These differences were driven by a trend for males to be larger in the HB site compared to the NB site (Tukey’s HSD, \( P = 0.09 \); Figure 23). Lizards varied in their number of injuries (bite marks) across the sites (Kruskal-Wallis, site: \( H = 2.45, \text{df} = 2, P = 0.032 \); all else \( P > 0.29 \)), with HB lizards exhibiting greater number of injuries than lizards from the NB site (pairwise comparisons, \( P = 0.048 \)).
Figure 22. Relative frequencies of blue, orange, and yellow morph male *U. ornatus* at the three sites monitored during this study. Sites are as follows: non-burned control (a), low-frequency burn (b), and high-frequency burn (c) site.
Figure 23. Body size (snout-vent length, mm) of all male *U. ornatus* at the three sites monitored during this study. Sites are coded as follows: non-burned control (NB), low-frequency burn (LB), and high-frequency burn (HB) site. Points are means and bars are ±1.0 SE.

Parasite load varied among the sites but did not differ by morph (adonis, site: \( F_{2,79} = 6.56, P = 0.003 \); morph: \( F_{2,79} = 1.14, P = 0.314 \); site × morph: \( F_{4,79} = 0.77, P = 0.541 \)). In particular, HB site lizards had fewer mites in fewer areas than lizards at the NB site (pairwise comparisons, \( P < 0.001 \) and \( P = 0.024 \), respectively).

**B. Social Dominance**

Variation in male behavior across sites was mostly attributed to variation in display behavior (NMDS, x-axis: pushups, \( r_s = -0.799, P < 0.001 \); head-bobs, \( r_s = -0.73, P < 0.001 \); lateral flattening, \( r_s = -0.898, P < 0.001 \)) and escalation behavior (NMDS, y-
axis: chase, $r_s = -0.212, P = 0.033$; bite, $r_s = -0.495, P < 0.001$) frequencies. Subjective winners of dominance contests exhibited more display and escalation behaviors than losers (adonis, $F_{2,84} = 2.14, P = 0.002$; Figure 24). I detected a near-significant trend in behavior differences by males across the sites (adonis, $F_{2,84} = 2.14, P = 0.074$). Whereas males at the NB site tended to exhibit behavioral displays more often, males at the HB site more frequently escalated contests to chasing and/or biting (Figure 25). Males at the LB site were relatively central to the other lizards in behavioral space. Although I did not detect differences in behavioral variation among the dyads (adonis, $F_{2,84} = 0.72, P = 0.57$), I was able to detect an interaction between site and dyad (adonis, $F_{4,84} = 2.18, P = 0.032$). Specifically, there was a greater frequency of display behaviors in B-B dyads at the NB and LB sites than B-O dyads at the LB site.

**Figure 24.** Behavioral scores of subjective winner and loser male *U. ornatus* lizards during paired male contests. Scores represent total frequency of dominant behaviors (push-up, head-bob, lateral flattening, chase, and bite) minus the frequency of submissive behaviors (flee, flatten). Bars are means +1.0 SE.
Figure 25. Non-metric multidimensional scaling plot of male *U. ornatus* behaviors during paired male contests. Males primarily differed along two axes, based on their relative variation in display (push-ups, head-bobs, and lateral flattening) and escalation (chase and bite) behaviors (see Results). Points and ellipses are by site: non-burned control (NB, circles, solid line), low-frequency burned (LB, triangles, larger dashed line), and high-frequency burned (HB, crosses, small dashed line) sites. Ellipses refer to 95% confidence limits about the median for each site, weighted by the appropriate Chi-Square value at 2 degrees of freedom.

C. Social Network Properties

Male social networks differed across the sites (Table 10). These social networks had an overall mean density of 0.21 and thus accounted for 21% of all possible edges at each site.
Table 10

Properties of male *Urosaurus ornatus* social networks at three sites: a non-burned control (NB), low-frequency burned (LB), and a high-frequency burned (HB) site. Overall values are presented and values divided by male color morph: blue (B), orange (O), or yellow (Y). Variables are defined in Materials and Methods. Values for degree and eigenvector centrality data are mean (range). Values for strength and mean strength data are mean ± 1.0 SE and refer to connections originating from B males to each of the three male morphs (*i.e.* B-B, B-O, and B-Y).

<table>
<thead>
<tr>
<th></th>
<th>Degree centrality</th>
<th>Eigenvector centrality</th>
<th>Strength</th>
<th>Mean strength</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NB site (overall)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morph</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4.6 (1, 9)</td>
<td>0.34 (0, 1)</td>
<td>5.4 ± 0.7</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>O</td>
<td>4.5 (2, 8)</td>
<td>0.28 (0.05, 0.82)</td>
<td>3.2 ± 0.6</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Y</td>
<td>4.3 (1, 8)</td>
<td>0.16 (0, 0.74)</td>
<td>4.6 ± 1.3</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td><strong>LB site (overall)</strong></td>
<td>2.3 (0, 5)</td>
<td>0.16 (0, 1)</td>
<td>3.2 ± 0.5</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Morph</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.9 (1, 5)</td>
<td>0.21 (0, 1)</td>
<td>4.3 ± 0.9</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>O</td>
<td>2 (1, 3)</td>
<td>0.12 (0, 0.48)</td>
<td>1.4 ± 0.4</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Y</td>
<td>1.6 (0, 5)</td>
<td>0.1 (0, 1)</td>
<td>2.6 ± 0.6</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td><strong>HB site (overall)</strong></td>
<td>3.5 (0, 9)</td>
<td>0.2 (0, 1)</td>
<td>5.6 ± 0.9</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>Morph</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.6 (0, 6)</td>
<td>0.17 (0, 0.4)</td>
<td>2.5 ± 0.9</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>O</td>
<td>3 (1, 5)</td>
<td>0.05 (0, 0.3)</td>
<td>4.7 ± 0.3</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Y</td>
<td>4.5 (2, 9)</td>
<td>0.35 (0, 1)</td>
<td>7.5 ± 1.3</td>
<td>3.2 ± 0.5</td>
</tr>
</tbody>
</table>

**i. Network Connectedness**

The first axis of the NMDS plot of lizard connectivity correlated most strongly with individual eigenvector centrality (*Spearman’s rho* = -0.788, *P* < 0.001), and the second axis with degree (*Spearman’s rho* = -0.923, *P* < 0.001). I was only able to detect significant differences in connectivity among lizards in each of the study sites (adonis,
site: $F_{2,78} = 8.36, P = 0.001$; all else $P > 0.18$). This result was primarily driven by individual lizards in the NB site having more edges (connections) than lizards in the LB site (pairwise comparisons, $P < 0.001$). Individual lizards within all three sites largely overlapped in eigenvector centrality (pairwise comparisons, $P > 0.5$). In other words, all morphs were similarly integrated into the network, regardless of site.

**ii. Edge Strength**

The strength of the three edge types examined in this study varied among the sites (permutation test, $F_{2,67} = 2.97, P = 0.048$; Figure 26), with lizards in general exhibiting a higher strength in the HB site relative to the LB site (Tukey’s HSD, $P = 0.05$). I also detected an interaction between treatment and edge type (permutation test, $F_{4,67} = 2.68, P = 0.016$): specifically, BY edges were significantly stronger than BB edges in the HB site, and BY edges in the HB site were stronger than BO edges in the NB site (Tukey’s HSD, $P = 0.05$ and 0.01, respectively). I detected no difference in strength by edge type alone (permutation test, $F_{2,67} = 1.83, P = 0.112$).
Figure 26. Connectedness of male *U. ornatus* at each study site. Units are number of edges and data points are strength (open circles, solid line) and mean strength (gray diamonds, dashed line) between all pairs of male *U. ornatus* lizards < 50 m apart at the three study sites. Strength refers to the number of edges connecting to a given male. Mean strength refers to the average of all edges (direct and indirect) connecting an individual lizard to all other lizards in a particular population. Sites are coded as follows: non-burned control (NB), low-frequency burn (LB), and high-frequency burn (HB) site. Points are means and bars are ±1.0 SE.

I detected similar patterns regarding mean strength across the sites (permutation test, $F_{2,67} = 9.87, P < 0.001$; Figure 26). In particular, individual lizard associations were stronger in the HB site relative to either the LB or NB site (Tukey’s HSD, $P < 0.001$ and $P = 0.003$, respectively). I did not detect any differences among the male color morphs (permutation test, $F_{2,67} = 1.68, P = 0.141$), but the interaction between site and morph was significant (permutation test, $F_{4,67} = 3.46, P = 0.007$). Additionally, BY edges at the
HB site had a higher mean strength than either BB or BO edges at the LB site (Tukey’s HSD, \( P = 0.015 \) and 0.026, respectively). Finally, BO edges at the HB site exhibited a greater average strength than BB or BO edges in the LB site (Tukey’s HSD, \( P = 0.001 \) for both cases), as well as BB or BY edges at the NB site (Tukey’s HSD, \( P = 0.008 \) and 0.004, respectively).

IV. Discussion

An integrative framework has been called for to understand how environmental variation mediates the relationship between individual behavior and population structuring (Krause et al., 2007). Social networks are largely structured by species interactions, including male territorial contests. The outcome of male social contests depends on asymmetries in RHP (fighting ability) and resource value among contestants (Smith and Parker, 1976). Both of these factors however are clearly influenced by environmental variation, mediated by changes in social network structure and/or male behavior. Therefore, resource-poor environments should affect the behavior and social structure of animal populations.

Studies of the effects of environmental change on animal populations have taken many forms, but typically demonstrate significant consequences for population-wide phenomena including habitat use, phenology, and trophic ecology (Kovach-Orr and Fussmann, 2013, Salido et al., 2012). In this study I demonstrate that the behavior and spatial segregation of morphs are influenced by environmental variation. These spacing patterns are useful for describing the social network of a population (Wasserman and
Faust, 1994). I explored social networks across three grassland sites that vary in prescribed burn history, and thus also the availability of trees and shrubs (Grant et al., 2010). These are preferred microhabitats for *U. ornatus*, and thus I expected differences in microhabitat to affect male social networks across sites. I demonstrate that male social networks do indeed vary across the sites and that network differences match my predictions. Male behaviors also vary across the sites. Moreover, I also identify some of the fitness-relevant consequences of environmental variation for social network shifts: changes in parasite load and injury frequency. By incorporating analyses from both social network and game theory, I am able to provide significant insight into the consequences of environmental variation for male *U. ornatus* in the wild.

These patterns in my results match the successional histories of my study sites. The majority of variation I detected occurred between the NB and HB site, two habitats which differ substantially in vegetation cover. In almost every analysis, the LB site was central to the NB and HB sites, especially with respect to male behavior and measures of network connectedness (*e.g.* degree centrality). The LB site is also central with respect to burn history. Whereas fire (prescribed or wild) was prevented in the NB site, the LB site was burned once in the past 15 years, and the HB site was burned twice during that same period. Fire promotes propagation of C4 plants, especially grasses (D'Antonio and Vitousek, 1992, Grant et al., 2010).

Homogenization of the habitat towards grass-dominance is associated with reduced availability and diversity of C3 shrubs, forbs and trees (Grant et al., 2010). In general, C3 vegetation is a significantly better quality resource than C4 vegetation, based
on differences in protein and carbohydrate content (Barbehenn et al., 2004). Studies suggest that *U. ornatus* lizards prefer trees and shrubs (Herrel et al., 2001, Thompson and Moore, 1991a). This preference should confer thermal advantages given the heterogeneity of habitat structure afforded by trees and shrubs compared to homogeneous grassy microhabitats. The use of tree and shrub microhabitats should also confer trophic advantages given the better quality of C$_3$ vegetation. In particular, a greater availability of C$_3$ vegetation may support a greater diversity of arthropod types (e.g. Engle et al., 2008). Ecosystem-level shifts towards grass dominance should therefore have cascading effects on trophic linkages, community diversity, and, with respect to tree lizards, the availability of preferred microhabitats. I have demonstrated several of these effects and their consequences in previous work (Chapters 3 and 4) as well as the current study.

Predation and competition are two major density-dependent selective mechanisms which may contribute to resource use variation among male lizards (or in general, Calsbeek et al., 2007). Moreover, clustering as a result of increased competition should influence rates of parasitic infection among males (Garrido and Pérez-Mellado, 2013). Both predation and parasitism may be significant sources of mortality, but the relative importance of any of these mechanisms appears to vary by site. Evidence suggests that predation intensity may in fact be greatest at the NB site where resources are abundant, based on estimates of apparent survival (Chapter 4). Those estimates also show that, of all three sites, males at the HB site exhibit the highest survivorship. Limited resource availability should also favor increases in body size (and RHP) among males if they compete for resources, given that body size predicts fighting ability (and thus RHP) in
lizards (e.g. Calsbeek and Sinervo, 2002b). Concordant with my predictions, males in the HB site were larger and more closely connected than males at the NB or LB sites. In other words, this population was denser than populations at the other sites. Males at this site also exhibited more bite marks from other males, clear evidence of increased rates of direct combat. Population clustering around limited resources is common, and resource competition alone may promote adaptive divergence (e.g. Grant and Grant, 2006).

Altogether, these results suggest that competition, mediated by differences in morph behavior and the availability of preferred resources, may be the primary selective driver of variation in diet selection and spatial segregation patterns across the sites. Both of the more-aggressive male phenotypes (blue and yellow) occupied preferred habitats in the HB site, whereas nomadic orange males were non-selective in microhabitat use. Male U. ornatus spatial relationships in the HB site therefore conform to predictions of the ideal despotic distribution (Fretwell, 1972). Surprisingly, despite clustering and evidence which suggests increased rates of male-male contact (bite marks), lizards in the HB site exhibited the lowest degree of infection, both in terms of number of infected body regions and in overall number of ectoparasitic mites. Mites and other parasites can pose serious risks to a population, including disease spread and mortality, and these effects are typically density-dependent (e.g. Garrido and Pérez-Mellado, 2013). I did not observe any differences in infection among the morphs, suggesting they are all equally susceptible to mites. The changes to male lizard social networks are therefore not likely to act to reduce infection rates in lizards at the HB site. Rather, it is possible that either the environmental conditions in the NB site are more suitable for the mites, or that simply
male lizards at that site are able to tolerate higher infection rates (Bouma et al., 2007).
Regardless, the reduced infection intensity should benefit HB site males by reducing their
stress and enabling them to focus energy into growth or reproduction instead of
maintenance. To this end, the greater mite loads on lizards in the NB site may contribute
to their low survivorship.

In this study I assume that the value of preferred microhabitats should be greater
in the HB site where they are most limiting. If this is the case, males that occupy those
resources (live trees or shrubs) may have an ecological or life-history advantage over
males that occupy poorer microhabitats (snags or rocks). Blue males consistently
occupied preferred tree and shrub microhabitats, regardless of site differences. I also
demonstrate that yellow males shifted their microhabitat use to match blue males in the
HB site only. My results from a previous study suggest that the microhabitat use overlap
among yellow and blue males affords one key advantage: preferred prey access (Chapter
3). Although morph diets differed, yellow males exhibited a distinct trophic shift that
accompanied their increased proximity with blue males in the HB site. This access may
directly contribute to the greater survivorship of yellow and blue morph male lizards in
the HB site (Chapter 4). Moreover, yellow and blue males also morphologically match
these microhabitats better than orange males match their microhabitats. I suggest that the
increased value of tree and shrub microhabitats for male U. ornatus may be partly
attributable to the ability of these microhabitats to support diverse arthropod assemblages,
including the preferred prey types of U. ornatus. Greater competition for tree and shrub
microhabitats between yellow and blue morphs may drive these phenomena, and would
also explain the greater number of bite marks on males at this site. The fitness advantages of defending these microhabitats may outweigh the costs of injuries associated with escalated combat. These advantages could then act as a feedback mechanism to further enhance divergence in RHP or resource value between yellow and blue *U. ornatus* males.

It is therefore likely that resource limitation may drive the observed adaptive divergence among these two morphs in trophic space (*i.e.* Chapter 3). Trophic niche differentiation should enhance rates of ecological divergence in polymorphic species like *U. ornatus* (Skulason and Smith, 1995). Moreover, the close proximity of blue and yellow males in the HB site would certainly explain their greater overlap in prey use at this site (Chapter 3). The costs associated with an increase in contest frequency may therefore be offset by the advantages provided through access to higher-quality prey. This suggests that environmental variation may influence trophic niche variation among the morphs, mediated by spatial changes to social networks in disturbed environments. Changes to social networks among polygynous males should also affect access to other crucial resources, including females and thermal refugia. By framing male contest strategies with respect to environmental variation and their fitness-relevant consequences, I have demonstrated that the outcomes of male contests have far-reaching consequences for the ecology of *U. ornatus*, as well as other species whose populations span an environmental gradient.

The clear dependence of male spacing patterns on resource availability suggests that concepts like the ideal free and ideal despotic distribution (Fretwell, 1972) be re-evaluated. In particular, these distributions are extremes of a continuum of population
social network patterns associated with variation in resource availability. Populations of species that span a gradient of environmental variation like *U. ornatus* may thus vary in their degree of conformity to predictions of either distribution. In environments where resources availability is high (*e.g.* the NB site), individuals may all be able to acquire preferred resources, regardless of differences in competitive ability. However, in environments where resources are limiting (*e.g.* the HB site), spacing patterns may conform to (or favor the evolution of) differences in competitive ability among individuals. Both types of environments will therefore influence on the ecology and evolution of animal populations.


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APPENDIX A. SUPPORTING TABLES

Table S1

Arthropods captured using sweep sampling and included in our analyses for estimating the diet of *Urosaurus ornatus* lizards at the Appleton-Whittell Research Ranch. Arthropods were assigned to one of four consumer types: C₄ herbivores, C₃ herbivores, Non-spider predators, and Spiders. I determined the consumer groups for each species based on literature descriptions for the study region. Most taxa could be categorized into a single group. However, some species in families within the Lepidoptera, Coleoptera, and Orthoptera included two consumer groups. Most of the dual categorizations involved families that had species whose diet included either C₃ or C₄ vegetation, or both. In these cases I used capture location (*i.e.* shrub, forb, tree, or grass) to assign individuals to either the C₄ or C₃ herbivore group. Thus, individuals in the same family are assigned to different consumer groups. Non-spider predators also include some taxa (*e.g.* Muscidae) that consume animal fluids (*e.g.* exudates, sweat). Number processed refers to total number of individuals processed for δ¹³C and δ¹⁵N for each family. However, some smaller arthropods within a consumer group had to be pooled together to meet the minimal amount of tissue necessary for stable isotope analysis (~ 2 mg tissue). For these cases, I pooled individuals belonging to the same family.

<table>
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<th>Order</th>
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<th>Consumer group</th>
<th>Number processed</th>
</tr>
</thead>
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<td>C₃ herbivores, Non-spider predators</td>
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<td>C₃ herbivores</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sphingidae</td>
<td>C₃ herbivores</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Tortricidae</td>
<td>C₃ herbivores</td>
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</tr>
<tr>
<td><strong>Coleoptera</strong></td>
<td>Chrysomelidae</td>
<td>C₃ herbivores</td>
<td>5</td>
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<td>Cincindelida</td>
<td>Non-spider predators</td>
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<td></td>
<td>Scarabaeida</td>
<td>C₃ herbivores, C₄ herbivores</td>
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<tr>
<td></td>
<td>Meloidae</td>
<td>C₃ herbivores</td>
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<tr>
<td></td>
<td>Elateridae</td>
<td>C₃ herbivores</td>
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<tr>
<td></td>
<td>Cerambycidae</td>
<td>C₃ herbivores</td>
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</tr>
<tr>
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<td>Cryptophagida</td>
<td>C₃ herbivores</td>
<td>2</td>
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<td>Salticidae</td>
<td>Spiders</td>
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<td>Herbivory Types</td>
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<td>Non-spider predators</td>
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<td>Non-spider predators</td>
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Table S2

Survival models as implemented in the program Mark (White and Burnham 1999). Model specifications are reported in Materials and Methods. Weighted survival (phi) estimates were based on AIC scores. I assumed constant survival because preliminary analyses in MARK indicated that estimates did not differ over time.

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Figure S1. Non-metric multidimensional scaling plot depicting environmental variation between the study sites. Ellipses represent 95% confidence intervals around the centroid for the low-frequency burned site (LB, dashed line) and high-frequency burned site (HB, solid line). Vectors denote relative direction and magnitude of variation for each environmental variable that had a strong ($P < 0.001$) fit to the ordination configuration.
Figure S2. Non-metric multidimensional scaling plot depicting environmental variation between vegetation and structural cover measured at lizard capture (dashed lines) and random (solid lines) points in each study site. Ellipses represent 95% confidence intervals around the centroid for the low-frequency burned site (LB, grey lines) and high-frequency burned site (HB, black lines). Vectors denote relative direction and magnitude of variation for each environmental variable that had a strong \((P < 0.001)\) fit to the ordination configuration.
Figure S3. Results of Bayesian mixing models of contributions (%) of each resource to each consumer at the A) Low-Burn and B) High-burn sites. Arthropod mixing models are illustrated as paths (arrows), weighted by the contribution of each resource to each consumer. Estimated contributions are also provided along each path. Grey broken paths denote the mixing model from the four arthropod groups to male tree lizards (*U. ornatus*). Estimated contributions of these four consumer types to each male color morph (Yellow, Orange, or Blue) are provided as a stacked bar graph for each site. Section colors of each bar correspond to the text color of that consumer type in the arthropod mixing models. Arthropod consumer types are defined in Material and Methods and model parameters are provided in Table 1. Mixing models were implemented in R using the function ‘siarmcdirichletv4’ within the package siar (Parnell *et al.* 2010).
Figure S4. Non-metric multidimensional scaling plot depicting variation in male *U. ornatus* performance over time. Performance includes stamina (time until exhaustion), distance run, and number of escape attempts exhibited by each lizard during performance experiments. Distances are based on the Gower dissimilarity metric. Convex hull and ellipsis properties are defined in Materials and Methods. Shape borders are by year: 2010 (solid line), 2011 (dashed line), and 2012 (dotted line).
Figure S5. Non-metric multidimensional scaling plot depicting variation in male *U. ornatus* performance among three sites varying in prescribed burn history. Performance includes stamina (time until exhaustion), distance run, and number of escape attempts exhibited by each lizard during performance experiments. Distances are based on the Gower dissimilarity metric. Convex hull and ellipsis properties are defined in Materials and Methods. Shape borders are by site: Non-burned control (solid line), Low-frequency burned (dashed line), and High-frequency burned (dotted line).
Figure S6. Non-metric multidimensional scaling plot depicting variation in female *U. ornatus* performance, based on reproductive state: gravid or non-gravid. Performance includes stamina (time until exhaustion), distance run, and number of escape attempts exhibited by each lizard during performance experiments. Distances are based on the Gower dissimilarity metric. Convex hull and ellipsis properties are defined in Materials and Methods. Shape borders are by reproductive state: gravid (solid line) or not-gravid (dashed line).
Figure S7. Non-metric multidimensional scaling plot depicting differences in female *U. ornatus* performance, by throat color morph. Performance includes stamina (time until exhaustion), distance run, and number of escape attempts exhibited by each lizard during performance experiments. Distances are based on the Gower dissimilarity metric. Convex hull and ellipsis properties are defined in Materials and Methods. Shape borders and colors are by morph: Yellow (solid line, yellow fill), Orange (dashed line, orange fill), and White (dotted line, grey fill).
Figure S8. Non-metric multidimensional scaling plot depicting variation in female *U. ornatus* performance over time. Performance includes stamina (time until exhaustion), distance run, and number of escape attempts exhibited by each lizard during performance experiments. Distances are based on the Gower dissimilarity metric. Convex hull and ellipsis properties are defined in Materials and Methods. Shape borders are by year: 2010 (solid line), 2011 (dashed line), and 2012 (dotted line).