ANDROGENS AND ECTOPARASITES AS PROXIMATE FACTORS INFLUENCING GROWTH IN THE SEXUALLY DIMORPHIC LIZARD, *SCELOPORUS UNDULATUS*

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ABSTRACT OF THE DISSERTATION

Androgens and ectoparasites as proximate factors influencing growth in the sexually dimorphic lizard, *Sceloporus undulatus*

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A growing body of evidence indicates that testosterone (T) plays an important role in regulating patterns of growth in lizards. Testosterone has also been found to facilitate the development of male-typical coloration and a suite of male behaviors that increase reproductive success. However, while T promotes male fitness through these characteristics, it appears to hinder fitness through direct molecular inhibition of growth and through indirect potential costs associated with increased parasitism. The relationship between T and ectoparasitism is complicated by seasonal variation in host circulating T levels and ectoparasite life cycles. It is unclear whether sex differences in ectoparasite loads are present year-round, are present only when circulating T is high in males, or are present only when ectoparasite abundances are high. Furthermore, it is often assumed that because ectoparasites feed by taking nutrients and energy from their hosts, then ectoparasites likely impact host growth. Effects of ectoparasitism on host growth may be particularly high in males if they have greater ectoparasite loads than females. This could indirectly lead to slower male growth and smaller overall male body size. To address the lack of information

regarding direct and indirect effects of T on growth, seasonal variation in sex-biased ectoparasite loads, and of the relationship between ectoparasitism and growth, I investigated (1) whether growth inhibition in eastern fence lizards (*Sceloporus undulatus*) is regulated through androgen or estrogen receptors, (2) seasonal correlations of mite loads with environmental mite abundances, and (3) whether sex differences in growth are correlated with sex differences in mite loads. I found that DHT inhibits male growth in *S. undulatus*, suggesting that T inhibits growth through direct androgenic molecular regulation. Furthermore, as indicated by the negative correlation between male growth and mite load, I found that T may also inhibit growth in males indirectly through costs associated with increased mite parasitism. Mite loads on *S. undulatus* varied seasonally, with peak mite loads occurring during months of high environmental mite abundance, coincident with seasonally high circulating T in yearling males and negatively correlated with male growth. This suggests that mites may impose a cost to growth in *S. undulatus* and contribute to male growth-inhibition, sex-specific growth rates, and the development of sexual size dimorphism (SSD).

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LIST OF ABBREVIATIONS

ΔAIC_i	Change in Akaike Information Criterion from Top Model
AIC	Akaike Information Criterion
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
b_{SMA}	Scaling Exponent for Scaled Mass Index
CAST	Castrated Males
CAST+DHT	Castrated Males with DHT Implants
CON	Sham Operated Males with Blank Implants
CON+DHT	Sham Operated Males with DHT Implants
DHT	Dihydrotestosterone
DMSO	Dimethyl Sulfoxide
E_2	Estradiol
FEM	Intact Females with Blank Implants
FEM+DHT	Intact Females with DHT Implants
GH	Growth Hormone
IGF-1	Insulin-like Growth Factor 1
J	Joules
L_i	Snout-Vent Length of Individual i
L_0	Mean Snout-Vent Length of All Lizards
M_i	Body Mass of Individual i
r_s	Spearman Correlation Coefficient

R^2	Correlation
SD	Standard Deviation
SEM	Standard Error of the Mean
SSD	Sexual Size Dimorphism
SVL	Snout-Vent Length
T	Testosterone
w_i	Model Weight
W	Kendall's Coefficient of Concordance
X^2	Chi-Square Value

CHAPTER 1

INTRODUCTION

Across taxa many species exhibit sexual differences in adult body size (sexual size dimorphism; SSD). Female-biased SSD predominates within fishes (Parker 1992; Bisazza 1993), amphibians (Shine 1979; Monnet and Cherry 2002), and snakes, turtles, and crocodilians (Gibbons and Lovich 1990; Shine 1994; Cox et al. 2007a) while male-biased SSD predominates in lizards (Stamps 1983; Cox et al. 2007a), birds (Greenwood and Wheeler 1985; Fairbairn and Shine 1993) and mammals (Ralls 1977; Weckerly 1998). Within taxa, SSD often varies significantly, and it is not uncommon for SSD to range from female-biased to male-biased among species within a single family or even a single genus (Fairbairn 1997; Cox et al. 2007a). Squamate reptiles (lizards and snakes) are a well-suited group of animals to investigate mechanisms influencing growth and the development of SSD. Squamates represent a diverse group of vertebrates with extensive variation in patterns of SSD (Cox et al. 2007a). Even more intriguing is the fact that extensive variation is also present within families, with several closely related species having opposite patterns of SSD (Cox et al. 2009).

As in other vertebrate groups, many studies investigating SSD in squamates have focused on the ultimate selective pressures driving males to become larger as adults in some species while females become larger in others. For example, it is predicted that increases in male aggression and territoriality are correlated with shifts toward male-larger SSD, while evolutionary increases in

traits associated with large clutch size are predicted to be correlated with shifts toward female-larger SSD (Cox et al. 2003). Male aggression, territoriality, and dominance are strongly influenced by body size (Tokarz 1985; Lewis and Saliva 1987; Olsson 1992; Schuett 1997; Cox et al. 2003) and body size, not surprisingly, is heavily influenced by growth. A major pleiotropic regulator of male aggression, territoriality, growth, and a suite of other male life history traits is testosterone (T). Testosterone increases growth rate in several species (Uller and Olsson 2003; Cox and John-Alder 2005; Cox et al. 2009, 2014) and experimentally increased circulating levels of T in adult male lizards increases home range size, daily activity, aggression, and territorial behaviors in multiple species (Marler and Moore 1988; DeNardo and Sinervo 1994; Salvador et al. 1996; Watt et al. 2003; Klukowski et al. 2004; Weiss and Moore 2004). Home range size and access to females increase with body size, and size-assortative mating assures that large males tend to mate with large females (Lewis and Saliva 1987; Olsson 1992; Haley et al. 1994; Luiselli 1996; Gullberg et al.1997; Schuett 1997; Lewis et al. 2000; Miller et al. 2010).

In eastern fence lizards (*Sceloporus undulatus*), similar to several other lizard species, T increases home range size, daily activity, endurance, and territorial behaviors (Klukowski et al. 1998; Smith and John-Alder 1999; Cox et al. 2005a; John-Alder et al. 2009). These characteristics, along with large body size, increase male fitness because large, territorial males have greater access to females than small males (Haenel et al. 2003a) and tend to mate with larger females, which typically have more offspring (Haenel et al. 2003b). Therefore,

the number of offspring sired is positively correlated with male body size (John-Alder et al. 2009). Testosterone also drives the development of male-typical coloration (Cox et al. 2005b), which is likely important in male-male signaling and competition (Smith and John-Alder 1999; Langkilde and Boronow 2010). Despite the fitness advantages of T-induced coloration and behaviors in *S. undulatus*, T paradoxically inhibits male growth and contributes to the development of female-larger SSD. This may occur through direct molecular growth inhibition or through indirect mechanisms of energetic trade-offs and ectoparasitism (Cox et al. 2005a; Klukowski and Nelson 2001).

Testosterone as a Regulator of Direct Growth Inhibiting Mechanisms

An increasing number of recent studies have investigated proximate mechanisms underlying the development of SSD, often involving sex steroid hormones, such as testosterone (T) and estradiol (E_2). Testosterone is commonly regarded as an anabolic steroid because it stimulates growth in numerous vertebrate taxa (Table 1.1). In contrast to the observed anabolic effects of androgens (e.g., T), estrogenic hormones (e.g., E_2) are often considered growth inhibitors, thus leading to slower growth and smaller body size (Table 1.1). However, several studies have reported the reverse, with T suppressing growth and estrogens promoting growth (Table 1.1).

Other studies have investigated the effects of androgens and estrogens on the central endocrine growth axis. The central endocrine growth axis, commonly known as the growth hormone / insulin-like growth factor-1 (GH/IGF-1)

axis, begins with the hypothalamus stimulating the anterior pituitary gland to produce and secrete GH. Growth hormone, in turn, stimulates the liver to synthesize IGF-1, which then goes on to promote cell proliferation and growth in numerous target tissues (Breier 1999; Gatford et al. 1998; Norbeck and Sheridan 2011). Androgens and estrogens can influence the endocrine growth axis centrally at the hypothalamus and pituitary gland or peripherally by regulating the synthesis and bioavailability of IGF-1 (Gatford et al. 1998). Studies investigating effects of androgens and estrogens on the endocrine growth axis have shown that T treatment increases IGF-1 transcription and increases plasma levels of IGF-1. Treatment with E_2, however, decreases IGF-1 transcription and decreases plasma levels of IGF-1 (Table 1.1). In contrast, a few studies have shown E_2 to increase hepatic IGF-1 and GH levels in the pituitary gland and plasma (Table 1.1). Although there is a large body of literature demonstrating the effects of androgens and estrogens on growth and expression of growth regulators, the majority of these studies have been performed in male-larger species. More recently researchers have begun to investigate patterns of growth in female-larger species.

Many studies investigating patterns of growth in female-larger species have involved squamate reptiles because of the extensive variation in SSD present among families, within families, and among species of the same genus (Cox et al. 2007, 2009). Additionally, many squamate species are readily accessible and can be studied in the field and laboratory. Several recent studies investigating the effects of T on growth and the development of SSD in male-

larger and female-larger species have focused on the family Phrynosomatidae, the most species rich and widespread group of lizards in North America (Wiens et al. 2013), which includes tree lizards (*Urosaurus* spp.), horned lizards (*Phrynosoma* spp.), and spiny lizards (*Sceloporus* spp.). This family is a model group to study the development of SSD because it contains several relatively common, closely related species, which exhibit male-biased or female-biased SSD (Fitch 1981; John-Alder et al. 2007). Studies investigating the effects of T on growth in *Sceloporus jarrovii*, a male-larger species, have found that T stimulates growth, leading to higher growth rates in males, and larger male body size (Cox and John-Alder 2005, 2007a). In contrast, in the female-larger species, *Sceloporus virgatus* and *S. undulatus*, T inhibits growth, leading to slower growth rates in males and smaller male body size (Abell 1998; Klukowski et al. 1998; Cox and John-Alder 2005, 2007a; Cox et al. 2005a). In two studies with *Urosaurus ornatus*, a phrynosomatid species that is typically monomorphic or slightly male-larger, castration of males reduced growth rates, but so did T. These perplexing findings are likely due to pharmacological doses of T, as noted by the authors (Hews et al. 1994; Hews and Moore 1995). Regardless, these studies investigating growth in phrynosomatid species exhibiting opposite patterns of SSD suggest that T exhibits bipotentiality for growth depending upon the direction of SSD, where T promotes growth in male-larger species, while T inhibits growth in female-larger species.

The bi-potential effects of T on growth in species of *Sceloporus* with opposite patterns of SSD provides strong support for the ‘bi-potential growth

regulation hypothesis', which states that T exhibits a growth-stimulating effect in male-larger species, but a growth-inhibiting effect in female-larger species (John-Alder et al. 2007; Cox et al. 2009). Further support for this hypothesis comes from studies outside of Phrynosomatidae. Studies on brown anoles (*Anolis sagrei*), a male-larger species in the family Dactyloidae, have shown that T increases growth rate of males and females throughout the time of sexual size divergence (Cox et al. 2014) and that body mass gain is greatly enhanced by T (Cox et al. 2009). Husak et al. (2007) found circulating T levels were positively correlated with body size, head size, dewlap size, and bite force in green anoles (*Anolis carolinensis*).

Recently, it has been suggested that T may not be the key signal of the sexual divergence in growth between male and female lizards. Studies on female-larger (*Aeluroscalabotes felinus*, family: Eublepharidae) and male-larger (*Paroedura picta*, family: Gekkonidae) species of geckos suggest that estrogenic hormones may underlie sex differences in growth and the development of SSD. In *A.* felinus, T treatment decreased growth rates in castrated males and females, thus inducing male-like growth patterns. This is in line with the bi-potential growth regulation hypothesis, but contrary to this is that male castration had no effect on growth (Kubička et al. 2013). More evidence comes from two studies involving P. picta. Kubička et al. (2015) found that while castrated males had lower circulating levels of androgens than intact males, castrated and intact males did not differ in growth. Furthermore, Starostová et al. (2013) reported that castration and T replacement had no effects on growth in males yet interestingly,

T and ovariectomy significantly increased growth and body size of females, preventing the sex differences in growth rate and body size.

Overall, further work is required to investigate whether androgens or estrogens act as the key regulator behind sex differences in growth. Comparison of studies between Gekkota (e.g., Gekkonidae, Eublepharidae) and Iguania (e.g., Phrynosomatidae, Dactyloidae) indicate that T may be a bi-potential regulator of growth in iguanians, but not gekkotans. Within Gekkota, estrogens (e.g., E_2), as opposed to androgens, may be the more important growth regulator. This is possible considering that the evolutionary split between Gekkota and Iguania is basal and occurred approximately 175 million years ago (Wiens and Lambert 2014). While T may not be the major steroidal growth regulator in all squamates, major questions arise from studies investigating the bi-potential effects of T on growth: 1) How can T inhibit growth? 2) Does T have a direct growth suppressing effect regulated through androgen receptors or does T require aromatization to E_2, thus suppressing growth through estrogen receptors?

Energetic Trade-Offs as an Indirect Mechanism Influencing Growth

Energy allocation trade-offs represent an indirect proximate mechanism influencing growth and the development of SSD regardless of the sex-bias. The development of male-larger SSD, for example, may be driven in part through decreased female growth as a result of increased energy allocation and increased metabolic costs associated with the production and maintenance of the clutch or litter (Beuchat and Vleck 1990; Demarco and Guillette 1992; Angilletta

and Sears 2000). Sugg et al. (1995), for example, calculated that, depending upon when females begin to divert energy towards egg production, 63-90% of the size difference between male and female lizards can be accounted for through the energetic costs of egg production. Landwer (1994) decreased the reproductive effort of female tree lizards (*U. ornatus*) through yolkectomy and found that females with 50% reduced clutch sizes grew faster and to a larger body size. Exacerbating the energetic costs of producing and maintaining a clutch is the fact that females may also decrease their energy intake due to locomotor impairment and decreased foraging (Cooper et al. 1990) or a reduction in the frequency and size of meals as a result of the burden of carrying a clutch or litter (Schwarzkopf 1996; Weiss 2001). Although female growth may be constrained by reproduction, the cost of reproduction appears to be insufficient to explain the full magnitude of female-biased SSD in *S. jarrovii*. Cox (2006) showed that differences in growth between reproductive and non-reproductive females are not present until the final month of gestation, by which time SSD has already developed. Furthermore, the growth benefit of experimentally inhibiting reproduction in *S. jarrovii* accounted for just 32% of the natural sex difference in body size.

Female-larger SSD may arise as a result of energetic trade-offs between growth and energetically costly male reproductive behaviors associated with mate acquisition and territory defense. Increased circulating levels of T increases home range size, daily activity, aggression, and territorial behaviors in multiple species (Marler and Moore 1988; Watt et al. 2003; Klukowski et al. 2004; Weiss

and Moore 2004; John-Alder et al. 2009). Male side-blotched lizards (*Uta stansburiana*), for example, increased their daily activity and home range size by 31% and 150%, respectively, when T was experimentally elevated within physiological limits (DeNardo and Sinervo 1994). In *S. undulatus* Cox et al. (2005a) found male lizards with elevated T had increased daily activity periods, increased daily movements, and increased home range size.

Male reproductive behaviors associated with mate acquisition and territory defense, such as increased home range size, daily activity, aggression, and territorial behaviors incur energetic costs, which may trade-off against growth. Field studies on two closely related sympatric species *S. virgatus* (female-larger) and *Sceloporus jarrovii* (male-larger) suggest that if energetic resources are limited during the breeding season when T is high, such as what occurs with *S. virgatus*, then a trade-off occurs where energy is diverted away from growth and towards fueling reproductively beneficial traits by males, such as increased activity period, aggression, and territorial behaviors (Smith and Ballinger 1994; Smith 1996; Cox and John-Alder 2007a). Marler and Moore (1989) found that more aggressive T-implanted male lizards expended more energy by having a longer daily activity period, performing more territorial behaviors and movements, while foraging less than placebo-implanted males. Testosterone-implanted males also had smaller fat bodies (stored energy) and were in lower body condition. In a separate study Marler et al. (1995) estimated energy expenditure to be 31% higher in T-implanted male lizards with increased territorial behaviors and daily activity compared to placebo-implanted males. Estimates by Cox et al. (2005a)

indicate that energetic costs of increased daily activity and territorial behaviors may account for approximately 80% of the growth rate reduction in male *S. undulatus*.

Ectoparasitism as a Proximate Mechanism Influencing Growth

Lizards are common hosts of ectoparasites, such as ticks and mites, in many environments (Wharton and Fuller 1952; Castro and Wright 2007; Klukowski and Nelson 2001). In California, for example, western fence lizards (*Sceloporus occidentalis*) host up to approximately 90% of all larval western black-legged ticks (Ixodes pacificus) at a given time (Talleklint-Eisen and Eisen 1999; Casher et al. 2002). In other regions, such as the New Jersey Pinelands, lizards may not be important hosts for ticks (Rulison et al. 2014), but they are very common hosts for mites. The dynamics of lizard-ectoparasite relationships have been an area of interest for researchers investigating why some individuals harbor more ectoparasites than others. Ectoparasites typically exhibit a pattern of aggregated distribution within a host species where the majority of host individuals in a population have relatively low ectoparasite loads while a relatively few host individuals are heavily parasitized (Anderson and Gordon 1982; Poulin 1993; Shaw et al. 1998; Hughes and Randolph 2001; Poulin 2007; Brunner and Ostfeld 2008).

A particularly strong interest has been placed in why male lizards often have higher ectoparasite loads than female lizards (Schall and Marghoob 1995; Talleklint-Eisen and Eisen 1999; Schall et al. 2000; Eisen et al. 2001; Amo et al.

2005; Salkeld and Schwarzkopf 2005; Cox and John-Alder 2007b; Lumbad et al. 2011; Heredia et al. 2014; Dudek et al. 2016). The majority of studies investigating the mechanisms behind male-biased ectoparasitism have focused on T. In several lizard species ectoparasitism increased with experimentally elevated T (Salvador et al. 1996; Olsson et al. 2000; Klukowski and Nelson 2001; Fuxjager et al. 2011; Pollock et al. 2012). For example, a study on free-ranging male *S. virgatus* showed that castration decreases mite loads while T replacement restores male-typical mite loads (Cox and John-Alder 2007b).

How T can actually lead to increased ectoparasitism is unclear. Currently there are two prevailing hypotheses. First, T has been demonstrated to be immunosuppressive in several vertebrate taxa (Saad et al. 1990; Kamis et al. 1992; Duffy et al. 2000; Casto et al. 2001; Andersson et al. 2004; Roberts et al. 2004; Tripathi and Singh 2014; Foo et al. 2016). Immunosuppression could enhance the survival of ectoparasites, thus resulting in higher ectoparasite loads on male hosts (Olsson et al. 2000; Poiani et al. 2000; Hughes and Randolph 2001). If true, then mite loads would be expected to be higher on males than on females, particularly during seasons and life stages when males experience elevated plasma T. However, some findings are inconsistent with this hypothesis. For example, Oppliger et al. (2004) found that, despite decreased immune function as a result of experimental elevation of T, ectoparasites loads did not increase in wall lizards (*Podarcis muralis*). Saino et al. (1995) reported increased ectoparasite loads in response to exogenous T, but contrary to prediction, immune function was also increased by T. Numerous studies have also failed to

find a suppressive effect of T on immune function altogether (Hasselquist et al. 1999; Bilbo and Nelson 2001; Greenman et al. 2005; Buchanan et al. 2003; Roberts et al. 2004, 2009; Ruiz et al. 2010).

Another potential explanation for how T can increase ectoparasite loads is through T-based increases in movement and home range size (Olsson et al. 2000; Boyer et al. 2010). These behaviors could expose males more often to ectoparasites, especially if they spend a significant amount of time in microhabitats preferred by ectoparasites (Zippel et al. 1996; Curtis and Baird 2008; Bulté et al. 2009; Rubio and Simonetti 2009).

Although T may be important in influencing ectoparasite loads in several species with male-biased patterns of ectoparasitism, the generality of T-based effects on ectoparasitism is unclear since T has not always been shown to have an effect on ectoparasite load in lizards (Salvador et al. 1997; Veiga et al. 1998; Oppliger et al. 2004). Furthermore, sex-biases in ectoparasitism do not always exist (Klukowski 2004; Reardon and Norbury 2004; de Carvalho et al. 2006; Bulté et al. 2009; Davis et al. 2012; Halliday et al. 2014; Dudek et al. 2016) or may be female-biased depending on the time of the year (Cox et al. 2005a; Lumbad et al. 2011). Overall, whether male-biased ectoparasitism is the typical pattern in lizards, whether T increases ectoparasite loads, and how T could increase ectoparasite loads are topics requiring much further research.

The seasonality of ectoparasite life cycles is another important component influencing the ectoparasitism of hosts. The seasonal life cycles of ectoparasites can typically be described by normally distributed seasonal patterns of

abundance for different life stages (Padgett and Lane 2001; Levi et al. 2005; MacDonald and Briggs 2016). Chigger mites in the eastern United States (*Eutrombicula alfreddugesi*), for example, are in low abundance during April and May and peak abundance during June and July, before gradually decline thereafter (Clopton and Gold 1993; Klukowski 2004). Seasonal variation in host infestation is intimately linked to the availability of questing ectoparasites in the environment (Randolph et al. 2002) and ectoparasite abundance and load have both been shown to fluctuate seasonally based on the ectoparasite's life cycle (Eisen et al. 2001, 2002; Godfrey et al. 2008; Lumbad et al. 2011; MacDonald and Briggs 2016). Few studies, however, have investigated the seasonal patterns of ectoparasite abundance and host ectoparasite load in the same study. Klukowski (2004) found that mite loads of *S. undulatus* were low in spring, high throughout summer, and low again in fall, which roughly matched the seasonal variation in environmental mite abundances. Curtis and Baird (2008) found that adult mites were abundant in May, but declined sharply in June shortly after mite larvae began to appear on collared lizards (*Crotophytus collaris*).

The seasonality of mite life cycles suggests that male lizards may not experience the greatest ectoparasitism during months of high T if ectoparasites are not active or abundant during those months. Studies demonstrating an effect of T on ectoparasitism and finding male-biased patterns of ectoparasitism have typically been performed during the breeding season or represent only a snapshot of the overall time period in which the host and ectoparasite species encounter one another. Therefore, the effects of T on ectoparasite load and

seasonality of sex-biases in ectoparasitism have not been thoroughly investigated, although there are a few studies showing a seasonal pattern to male-biased ectoparasite load (Krasnov et al. 2005; Godfrey et al. 2008; Lumbad et al. 2011; Le Coeur et al. 2015; Patterson et al. 2015).

Along with the energetic costs of the reproductively beneficial traits induced by T, ectoparasitism represents another potentially significant factor through which T could indirectly decrease male growth (Møller et al. 1994; Cox and John-Alder 2007a). Ectoparasitism has been shown to be negatively correlated with growth (Vuren 1996; Merino et al. 1999;) and also decrease body condition (Møller 1994; Giorgi et al. 2001; Lourenço and Palmeirim 2007) in various vertebrate species. This is not surprising since ectoparasites directly draw body fluids and energy from their hosts (Nilsson 2003). In the female-larger lizard species, *S. virgatus*, mite loads were higher on male yearling (first full activity season) lizards and were negatively correlated with growth rate (Cox and John-Alder 2007b). Uller and Olsson (2003) showed that exposure to pre-natal T led to increased growth rate in male-larger *Lacerta vivipara*, but growth rate significantly decreased when lizards were exposed to ticks. In contrast, in the male-larger anole, *Norops polylepis*, sexes did not differ in mite load nor was there a correlation between mite load and growth rate (Schlaepfer 2006).

These findings suggest a potential linkage between mite ectoparasitism and growth in lizards, where decreased male growth is indirectly driven in part by increased ectoparasitism during times of significantly elevated T. However, it is unclear whether sexes differ in mite loads, whether mites significantly impact

growth, and whether they play a role in the development of SSD. Both males and females may experience growth costs from increased ectoparasitism, but because males have higher T than females and T has often been shown to increase ectoparasite load (Salvador et al. 1996; Olsson et al. 2000; Klukowski and Nelson 2001; Cox and John-Alder 2007b; Fuxjager et al. 2011; Pollock et al. 2012), ectoparasites would likely decrease growth more in males than females, thus potentially leading to smaller male body size (Potti and Merino 1996; Perez-Orella and Schulte-Hostedde 2005). Mites, however, can contribute to the development of female-biased SSD only if mites have a significant impact on growth and only if there is a male-bias in mite load.

Summary and Specific Aims of this Dissertation

My doctoral dissertation investigates direct and indirect mechanisms by which T may inhibit growth in *S. undulatus* (Fig. 1.1). My central thesis is that T inhibits growth through direct androgenic molecular regulation of the endocrine growth axis (Chapter 2) and also through indirect mechanisms involving growth costs of increased activity (Cox et al. 2005a) and increased susceptibility to ectoparasitism (Chapters 3 and 4).

Hypothesis 1: Male growth-inhibition in a female-larger lizard species is primarily mediated through direct androgenic inhibition of growth, not requiring aromatization of testosterone to estradiol.

Aim 1: To test this hypothesis, I quantified effects of dihydrotestosterone (DHT) on growth in yearling *Sceloporus undulatus*. Dihydrotestosterone is an end

metabolite of T and cannot be converted into E_2 (Fig. 1.2). I quantified color responses to corroborate the efficacy of surgical castration and DHT treatments. I predicted that DHT would inhibit growth and cause the development of male-typical coloration.

Hypothesis 2: Sex-based patterns of ectoparasitism exhibit seasonal variation, such that sex differences in ectoparasite loads do not exist year-round, but when they do, males are more heavily parasitized than females.

Aim 2: To test this hypothesis I quantified mite loads in adult and yearling male and female *Sceloporus undulatus* from May to September of the 2014 and 2015 activity seasons.

Hypothesis 3: Mites contribute indirectly to sex-specific growth rates and the development of sexual size dimorphism in *S. undulatus* because although mites decrease growth in both sexes, males have higher mite loads and, therefore, grow more slowly.

Aim 3: To test this hypothesis I quantified mite loads and calculated growth rates in adult and yearling male and female *Sceloporus undulatus* from May to September of 2014 and 2015 activity seasons.

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Table 1.1: Summary of studies investigating the effects of androgens and estrogens on the expression of growth hormone (GH), insulin-like growth factor-1 (IGF-1), and growth.

Species	Sex Steroid(s)	Variable Measured	Sex Steroid Effects	Authors
Oncorhynchus kisutch	androgens	growth	↑ growth	McBride and Fagerlund 1976
Oncorhynchus mykiss	androgens, estrogens	growth	↑ growth ↓ growth	Cleveland and Weber 2016
Oreochromis mossambicus	androgens	growth	↑ growth	Kuwaye et al. 2003; Sparks et al. 2003
Perca flavescens	estrogens	growth	↑ growth	Malison et al. 1985; Goetz et al. 2009
Anguilla anguilla	estrogens	growth	↑ growth	Degani et al. 1986; Tzchori et al. 2004
Salmo salar	estrogens	growth	↑ growth	Arsenault et al. 2004
Ovis aries	androgens	growth	↑ growth	Arnold et al. 1996
Rattus norvegicus	androgens, estrogens	growth	↑ growth	Borski et al. 1996
Bos primigenius	estrogens	growth	↑ growth	Enright et al. 1990
Meleagris gallopavo	androgens	growth	↑ growth	Fennell & Scanes 1992a
Serinus canaria	androgens	growth	↑ growth	Schwabl 1996
Sialia sialis	androgens	growth	↑ growth	Navara et al. 2005
Gallus gallus	androgens	growth	↓ growth	Fennell and Scanes 1992b; Fennell et al. 1996
Gallus gallus	estrogens	growth	↓ growth	Akiba et al. 1982
Lacerta vivipara	androgens	growth	↑ growth	Uller & Olsson 2003
Sceloporus jarrovii	androgens	growth	↑ growth	Cox and John-Alder 2005
Anolis sagrei	androgens	growth	↑ growth	Cox et al. 2014
Oncorhynchus kisutch	androgens	IGF-1, GH	↑ IGF-1, no GH effect	Larsen et al. 2004
Oncorhynchus mykiss	androgens, estrogens	IGF-1	↑ IGF-1 ↓ IGF-1	Norbeck and Sheridan 2011
Oreochromis mossambicus	estrogens	IGF-1	↓ IGF-1	Riley et al. 2002
Perca flavescens	estrogens	IGF-1	↑ IGF-1	Lynn et al. 2011
Carassius auratus	estrogens	GH	↑ GH	Zou et al. 1997

Figure 1.1: Testosterone (T) as a pleiotropic regulator of various life history traits. Solid arrows indicate direct effects of T and dashed arrows indicate indirect effects of T while “+” and “-” represent increasing and decreasing effects, respectively, on the given characteristic. Focuses of the dissertation chapters are shown in parentheses. Chapter 2 investigates direct androgenic molecular mechanisms by which T inhibits male growth. Chapter 3 investigates sex- and age-based seasonal variation in ectoparasite loads. Chapter 4 investigates ectoparasite load as an indirect mechanism by which T inhibits male growth.

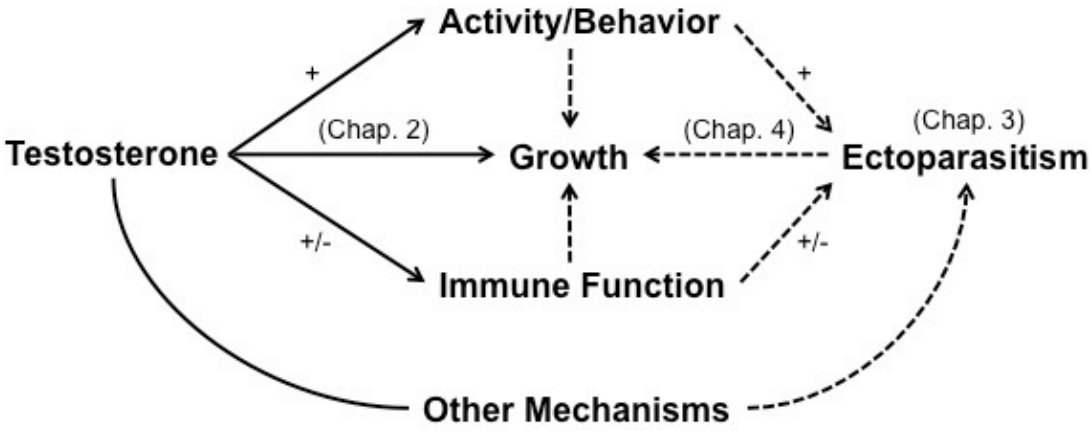
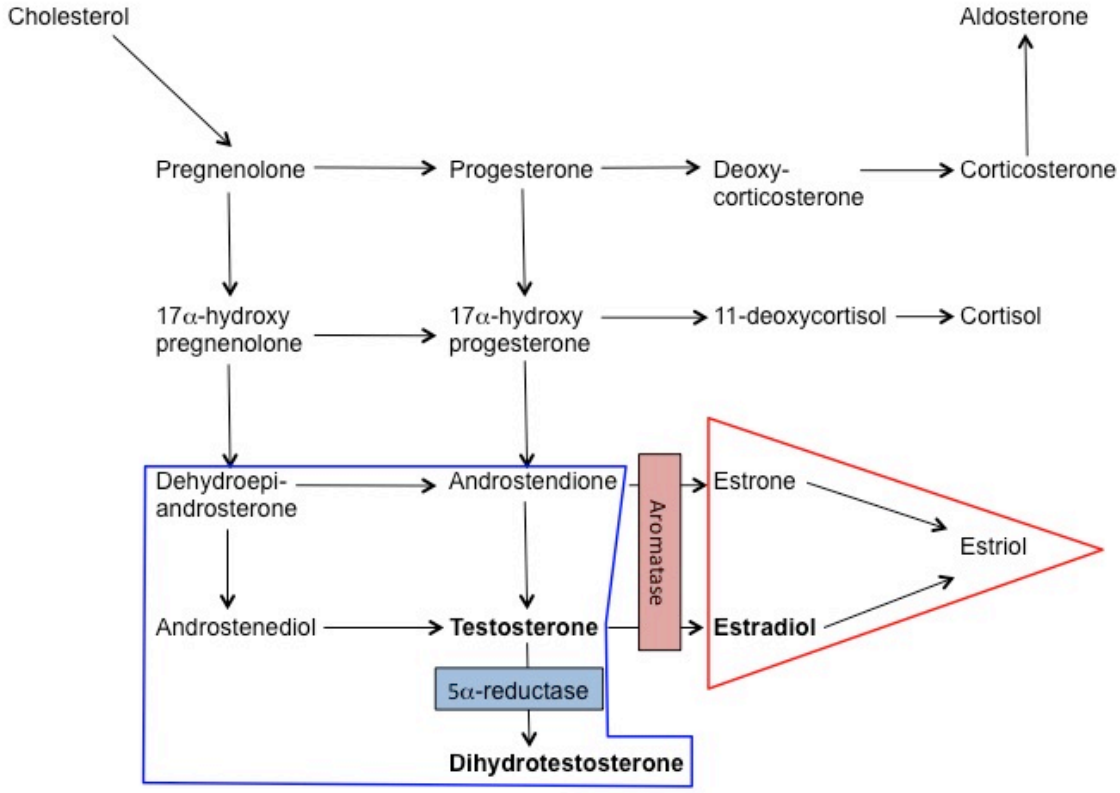


Figure 1.2: Pathways of steroidogenesis with emphasis on the synthesis of testosterone, dihydrotestosterone, and estradiol. Androgens are outlined in blue and estrogens are outlined in red. Testosterone can be reduced into dihydrotestosterone (via 5α-reductase) or aromatized into estradiol (via aromatase). Dihydrotestosterone is a pure androgen and cannot be converted into estradiol.



CHAPTER 2

EFFECTS OF DIHYDROTESTOSTERONE ON GROWTH AND COLOR DEVELOPMENT IN THE SEXUALLY DIMORPHIC LIZARD, *SCELOPORUS UNDULATUS*

Abstract

A growing body of evidence indicates that testosterone (T) can regulate growth and contribute to the development of sexual size dimorphism and sexual dichromatism. However, the underlying mechanism(s) of these effects are conjectural, including possible conversions of T to estradiol (E_2) and dihydrotestosterone (DHT). The present study investigates whether effects of T on growth inhibition and color development in eastern fence lizards (*Sceloporus undulatus*) are mediated by androgen receptors, not requiring aromatization of T to E_2. This study also investigates whether castration alone is sufficient to increase growth and whether females respond to T, which would indicate that the growth regulatory and color development mechanisms are present in both sexes. Experiments were conducted on yearling *S. undulatus*, a female-larger species with striking sexual dichromatism. Silastic tubules containing DHT were implanted into intact females as well as intact and castrated males. Growth rates were calculated and color was quantified for ventral and dorsal surfaces. Dihydrotestosterone decreased growth rate and enhanced ventral coloration in both males and females. The present report also shows that, given adequate time, growth increases in response to castration in males of *S. undulatus* and

that DHT inhibits growth in both males and females of this female-larger species. The results presented here suggest that (1) inhibition of growth by T is mediated by androgen receptors without requiring aromatization of T into E_2 and (2) females possess functional androgen receptors plus downstream pathways required for initiating male-typical inhibition of growth and enhanced coloration in response to androgens.

Introduction

Body size is one of the most important traits of an organism, influencing numerous physiological, life history, and ecological processes (LaBarbera 1989; Blackburn et al. 1999; Blanckenhorn 2000). A particularly widespread phenomenon involving body size is sexual size dimorphism (SSD), in which one sex is larger in adult body size than the other. Within taxa, SSD often varies significantly, and it is not uncommon for SSD to range from female-biased to male-biased among species within a single family or even a single genus (Fairbairn 1997; Cox et al. 2007).

The majority of studies investigating the development of SSD have focused on the ultimate selective pressures driving males to become larger than females in some species while the opposite is true in other species. In male-larger species, sexual selection favors large male body size because of the advantages it confers in territorial behavior and male-male competition for mates (Tokarz 1985; Loison et al. 1999; Cox et al. 2007). In these species, dominance is strongly influenced by body size. Acquisition of home range, access to

females, realized copulations, and number of offspring sired are all facilitated by large male body size (Lewis and Saliva 1987; Olsson 1992; Haley et al. 1994; Luiselli 1996; Gullberg et al. 1997; Schuett 1997; Lewis et al. 2000; Haenel et al. 2003a, b; John-Alder et al. 2009). On the other hand, large female body size is adaptive when species exhibit variable clutch sizes or reproduce infrequently (Fitch 1981; Vitt 1986; Ford and Siegel 1989; Olsson 1993; Sand 1996; Winck and Rocha 2012).

An increasing number of studies have investigated proximate mechanisms influencing growth in sexually dimorphic species. An informed understanding of ultimate adaptive explanations of SSD is strongly dependent upon a thorough understanding of the underlying proximate mechanisms (i.e., ontogenetic, physiological, behavioral mechanisms) influencing growth (Watkins 1996). Many such studies have involved squamate reptiles because they represent a diverse order of vertebrates in which SSD is widespread and closely related species can be male-larger, female-larger, or monomorphic (Cox et al. 2007, 2009). The diversity of patterns of SSD within closely related groups of species is particularly intriguing because the growth-regulatory genome is largely, if not entirely, shared between males and females of a given species (Badyaev 2002; Chenoweth et al. 2008; Cox et al. 2009; Mank 2009). Despite the shared growth-regulatory genome, SSD is unexpectedly evolutionarily malleable, suggesting that the development of SSD is likely driven through epigenomic, sex-specific growth-regulatory mechanisms. One squamate family of particular interest for investigating these growth-regulatory mechanisms has been Phrynosomatidae, a

large family of lizards with several closely related species of differing SSD (Cox et al. 2009). Several recent studies investigating growth in male- versus female-larger phrynosomatid species have focused on testosterone (T) as an important factor influencing sex-specific growth-regulatory mechanisms. Studies on three closely-related *Sceloporus* species in particular, *S. undulatus* and *S. virgatus* (two female-larger species) and *S. jarrovii* (male-larger species), have revealed bi-potential effects of T on growth in species with opposite patterns of SSD.

Studies examining the effects of T on growth in female-larger *S. undulatus* and *S. virgatus* have shown that females grow at faster rates than males during times corresponding with seasonal peaks in male plasma T (Haenel and John-Alder 2002; Cox and John-Alder 2005, 2007). Experimental studies investigating the effects of T on growth in *S. virgatus* and *S. undulatus* confirmed the growth-inhibiting potential of T in these female-larger species (Abell 1998a; Cox and John-Alder 2005; Cox et al. 2005a). In contrast, studies on male-larger *S. jarrovii* have shown that sexual divergence in body size occurs throughout the first full year of activity when males are growing at faster rates than females (Cox and John-Alder 2005, 2007). An experimental study confirmed the growth-promoting potential of T in *S. jarrovii*, showing that castrated males grow more slowly than intact males and that T replacement restores male typical growth rates (Cox and John-Alder 2005).

The bi-potential effects of T on growth in species of *Sceloporus* with opposite patterns of SSD led to the “bi-potential growth regulation hypothesis”, which states that T exhibits a growth-stimulating effect in male-larger species, but

a growth-inhibiting effect in female-larger species (John-Alder et al. 2007; Cox et al. 2009). Further support for this hypothesis comes from experimental studies on female-larger garter snakes (*Thamnophis sirtalis*; Crews et al. 1985; Lerner and Mason 2001) and male-larger brown anoles (*Anolis sagrei*; Cox et al. 2009; Cox et al. 2014) and from a correlational study on green anoles (*Anolis carolinensis*; Husak et al. 2007). While further work is certainly required to investigate the generality of the "bi-potential growth regulation hypothesis", a major question begs an answer. How can T promote growth in some species, but inhibit growth in closely related species?

One potential explanation lies within the central endocrine growth axis, which involves insulin-like growth factor-1 (IGF-1) promoting cell proliferation and growth in numerous target tissues (Breier 1999; Gatford et al. 1998; Norbeck and Sheridan 2011). Studies have shown that androgens (e.g., T) promote growth (Slob et al. 1975; McBride and Fagerlund 1976; Fennell and Scanes 1992a; Kuwaye et al. 1993; Arnold et al. 1996; Borski et al. 1996; Schwabl 1996; Sparks et al. 2003; Uller and Olsson 2003; Cox and John-Alder 2005; Navara et al. 2005; Husak et al. 2007; Cox et al. 2009, 2014; Cleveland and Weber 2016) and increase the expression of IGF-1 (Borski et al. 1996; Riley et al. 2002; Larsen et al. 2004; Norbeck and Sheridan 2011). In contrast to the growth-promoting effects of T, estrogenic hormones (e.g., estradiol; E_2) have been shown to inhibit growth (Borski et al. 1996; Arsenault et al. 2004; Cleveland and Weber 2016) and decrease IGF-1 expression (Borski et al. 1996; Arsenault et al. 2004; Riley et al. 2002; Carnevali et al. 2005; Norbeck and Sheridan 2011).

While these effects of androgens and estrogens on the endocrine growth axis and expression of IGF-1 can be invoked to explain the sex differences in growth in male-larger species, this does not explain how sexes can differ in growth in female-larger species, especially considering the constrained growth-regulatory genomes within males and females of a given species (Badyaev 2002; Chenoweth et al. 2008; Cox et al. 2009; Mank 2009). It is possible that in female-larger species, such as *S. undulatus*, male-growth inhibition is driven through estrogen receptors, similar to what occurs in closely related male-larger species because T can be aromatized into E_2. However, growth inhibition in female-larger species may be through androgen receptors, which would represent a different mechanism of growth inhibition compared to what occurs in male-larger species. Studies have demonstrated growth-suppressing effects of T (Fennell and Scanes 1992b; Fennell et al. 1996; Cox and John-Alder 2005; Cox et al. 2005a) and growth-promoting effects of estrogens (Degani 1986; Malison et al. 1985; Enright et al. 1990; Tzchori et al. 2004; Goetz et al. 2009). Furthermore, estrogens have been reported to increase IGF-1 expression in perch (Goetz et al. 2009; Lynn et al. 2011) and goldfish (Zou et al. 1997) while T has been shown to decrease IGF-1 expression along with growth in *S. undulatus* (Duncan 2011).

The primary aim of the present study was to test the hypothesis that growth inhibition in response to T in *S. undulatus* is mediated by androgen receptors, the alternate being that growth inhibition is mediated by estrogen receptors. I also investigated whether castration alone is sufficient to increase growth and whether females respond to dihydrotestosterone (DHT), which would

indicate that the growth regulatory mechanisms are present in both sexes. To test these hypotheses, I implanted yearling males and females of *S. undulatus* with Silastic tubules containing DHT. Dihydrotestosterone is an end metabolite of T and cannot be converted to E_2 (Frye et al. 2004; Walters et al. 2008; Sartorius et al. 2014). Therefore, DHT is considered a "pure" androgen. Furthermore, T and DHT both bind to the same androgen receptor, although DHT with greater affinity (Fang et al. 2003), while E_2 binds to estrogen receptors (Fang et al. 2000, 2003; Pereira de Jésus-Tran et al. 2006) and has a low binding affinity for androgen receptors (Fang et al. 2003). Thus, the responsiveness to DHT can be interpreted as unambiguous evidence of mediation by androgen receptors (Swerdloff and Wang 1998; Walters et al. 2008).

A secondary aim of the present study was to test if the development of sexual dichromatism (when one sex exhibits distinct coloration from the other; Cooper and Greenberg 1992) is an androgenic effect of T, not requiring aromatization to E_2. This aim also served as corroboration for the efficacy of surgical and androgen treatments. Within sexually dichromatic species researchers have focused on the cellular mechanisms leading to the development of sexual dichromatism (Sherbrooke and Frost 1989; Morrison et al. 1995; Macedonia et al. 2000; Quinn and Hews 2000, 2003; Olsson et al. 2013) and have also attempted to elucidate the proximate endocrinological factors influencing these cellular mechanisms (Hews and Moore 1995; Kodric-Brown 1998; Sinervo et al. 2000; Knapp et al. 2003; Mills et al. 2008). In species where males are the more colorful sex, T induces the development of male-typical

coloration (Cooper et al. 1987; Rand 1992; Hews and Moore 1995; Sinervo et al. 2000). For example, Cox et al. demonstrated in two species of *Sceloporus* (*S. undulatus*, 2005b; *S. jarrovii*, 2008) that castration of juvenile male lizards decreased male-typical blue ventral coloration while exogenous T restored this coloration in castrated males and led to the development of male-typical ventral coloration in females.

In the present study, I help to clarify the androgenic mechanism(s) of bi-potential growth regulation and sexual dichromatism by using the eastern fence lizard (*Sceloporus undulatus*), a female-larger species in which adult males exhibit vivid blue and black ventral and gular patches with indistinct brown chevrons against a reddish-brown dorsal surface. I used DHT to test the hypothesis that male growth-inhibition and color development in this female-larger, sexually dichromatic lizard species is primarily mediated through androgenic mechanisms, not requiring aromatization to E_2. Therefore, treatment with DHT was predicted to decrease growth rate and induce male-typical coloration. A second hypothesis was that females have retained sensitivity to the androgenic growth-inhibiting and color-promoting mechanisms, such that treatment with DHT would decrease growth rate and induce the development of male-typical coloration in females as well as in males.

Materials and Methods

Animals and Experimental Design

I captured 38 (26 male and 12 female) *S. undulatus* yearlings (age ≈10

mo.) from three locations near the Rutgers University Pinelands Research Station in New Lisbon, Burlington County, New Jersey (41°N, 74°35′W) during early June 2012 to investigate the effects of DHT on growth. In a separate experiment during June 2013, I captured 29 (19 male and 10 female) yearling lizards (age ≈10 mo.) to investigate the longer-term effects of castration on growth. All lizards were captured by hand-held noose or by hand. Sex was determined by the presence or absence of enlarged post-cloacal scales and age class was determined by body size, which is absolutely diagnostic for yearlings at this time of year. Individuals were then transported to Rutgers University, New Brunswick, New Jersey in cloth bags where snout-vent length (SVL, to the nearest mm) and body mass (to the nearest 0.1 g) were measured. Animals were housed individually in plastic cages (59.1 x 43.2 x 45.7 cm) with absorbable litter (Kaobed, Marcal Paper Mills Inc., Elmwood Park, NJ) and two bricks that were arranged to form both a basking and shaded site. Opaque barriers were placed between cages to prevent visual interactions. Water was always available in a shallow dish filled with aquarium gravel, and several crickets were offered each day to ensure lizards were fed to satiety. Cages were illuminated under 40-watt fluorescent lighting (Chroma 50, General Electric, Fairfield, CT) for a 13.5L:10.5D photoperiod, and a basking period of 10.5L:14.5D was provided by placing a 45-watt incandescent bulb (Duramax, Philips, Amsterdam, Netherlands) over the basking site.

Experimental Design (2012): Effects of DHT on Growth

Using the initial measurements of SVL and body mass, lizards were assigned to one of six size-matched treatment groups: (1) Castrated males receiving a DHT implant (castrated-DHT males, n = 8); (2) castrated males receiving a placebo implant (castrated-blank males, n = 7); (3) intact males receiving sham surgery and a placebo implant (intact-blank males, n = 7); (4) intact females receiving sham surgery and a DHT implant (intact-DHT females, n = 6); (5) intact females receiving sham surgery and a placebo implant (intact-blank females, n = 5); (6) intact males receiving sham surgery and a DHT implant (intact-DHT males, n = 4). This project was approved by the Rutgers University Animal Care and Use Committee (Protocol # 01-019). Animal capture and housing was approved by the New Jersey Department of Environmental Protection, Division of Fish and Wildlife (Capture Permit #2012043, Housing Permit #2012053).

Experimental Design (2013): Long-Term Effects of Castration on Growth

Using the initial measurements of SVL and body mass, lizards were assigned to one of three size-matched treatment groups: (1) intact males receiving a sham surgery (intact males, n = 9); (2) castrated males (castrated males, n = 10); (3) intact females receiving a sham surgery (females, n = 10). This project was approved by the Rutgers University Animal Care and Use Committee (Protocol # 01-019). Animal capture and housing was approved by the New Jersey Department of Environmental Protection, Division of Fish and Wildlife (Capture Permit #2013110, Housing Permit #2013098).

Surgical Treatments

Prior to surgery, lizards were placed on ice to undergo cold-induced surface anesthesia until they exhibited no foot-withdrawal reflex. For all castrated males, I exposed the testes by making a single ventral incision and bilaterally removed the testes, ligating the spermatic cords with surgical silk. For intact males and all females, I performed sham surgeries in which I performed the same incisions, exposed the testes or ovaries, but left the gonads completely intact. In the 2012 DHT experiment, I then inserted either a DHT implant or a placebo implant into the coelomic cavity. Following all surgeries incisions were closed with polypropylene surgical suture (6-0 Prolene, Ethicon, Somerville, NJ).

Dihydrotestosterone Implants

Tonic-release DHT implants were made from approximately 4 mm pieces of Silastic tubing (Dow Corning, Clarkesville, TN: 1.47 mm inner diameter, 1.96 mm outer diameter) with a DHT chamber of approximately 1 mm in length. I sealed one end of each tubule with Silastic adhesive gel (Dow Corning) and injected 3 µl of a solution of DHT (Sigma-Aldrich, St. Louis, MO) dissolved in dimethyl sulfoxide (DMSO, 50 µg DHT/µl) into the open end. The tubules were then sealed and left for a period of 72 hours to allow the DMSO to diffuse out and evaporate, leaving 150 µg of crystalline DHT in each implant. Placebo implants were constructed in a similar fashion except only DMSO was injected into the tubules, which, following diffusion and evaporation, left empty implants.

Quantification of Growth Rates

All animals were given a 2-week recovery time from the date of surgery. At the conclusion of the recovery period, measurements of SVL and body mass were recorded at approximately weekly intervals over a period of 40 days in order to calculate growth rates (mm/day) for the 2012 experiment. For the 2013 experiment, growth rates were calculated over a period of 113 days. Feeding rates (crickets/day) and body conditions (scaled mass index; Peig and Green 2009) were also calculated. The scaled mass index for each lizard was calculated using the formula, $M_i \times (L_0/L_i)^{bSMA}$, where M_i and L_i are the body mass and SVL of individual i, respectively, and L_0 is the mean SVL of all lizards. The scaling exponent (b_{SMA}) is calculated by regressing $\log_{10}$ body mass against $\log_{10}$ SVL for each lizard and dividing the slope from this regression by Pearson's correlation coefficient (r). The scaled mass index was used instead of the residual index (uses residuals from regression of $\log_{10}$ body mass and $\log_{10}$ SVL) for calculation of body conditions because recent analyses by Peig and Green (2009) showed the scaled mass index to be a better overall indicator of the relative size of fat and protein reserves in several vertebrate species (5 small mammals, 1 bird, 1 snake). Another reason why the scaled mass index was used rather than the residual index is because measures of body condition should be independent of sex- and age-based variation in body size in order to be an accurate assessment of individual body condition. Peig and Green (2010) compared the scaled mass index to six other conventional body condition indices

(including the residual index) and found that, unlike the scaled mass index, all six conventional methods failed to account for sex- and age-based variation in body size.

Quantification of Coloration and Patch Size

Because I did not have an established DHT assay I used measures of dorsal and ventral coloration to corroborate treatment efficacies for the 2012 DHT experiment. Dorsal and ventral surfaces of lizards were scanned at 600 dpi using an Epson Perfection V500 digital photo scanner (Epson America Inc., Long Beach, CA) prior to surgeries and again at the termination of the experiment (mean 53 days post-treatment). I used Adobe Photoshop version CS6 (Adobe Systems Inc., San Jose, CA) to estimate the hue, saturation, and brightness of each animal's dorsal chevrons, dorsolateral areas, gular patches, and ventral patches in the scanned images (Fig. 2.1). For the chevrons, I selected the lowest dark portion of the third chevron from the head. This was repeated for the left and right sides of the body and I used these measures as representative of overall chevron coloration. For the dorsolateral areas, I selected the area between the second and third chevrons from the head on the left and right sides of the body and used these measures as representative of overall dorsolateral region coloration. For the gular and ventral patches, only the areas of blue were selected. I used the Elliptical Marquee tool to capture the area of interest and then used the Histogram tool to obtain mean red, green, and blue color values of all the pixels within the selected area. I used the Color Picker tool to convert

these values into corresponding measures of hue (color reflected; measured on a standard 360º color wheel), saturation (purity of the color; 0% = gray, 100% = fully saturated), and brightness (relative lightness of the color; 0% = black, 100% = white).

To assess measurement precision I calculated Pearson correlation coefficients for left and right measurements of ventral and dorsal coloration. Correlations between left and right color measurements were higher for ventral surfaces (0.85–0.99) than for dorsal surfaces (0.49–0.92; Table 2.1), suggesting lower measurement precision for the dorsal surfaces. For all subsequent analyses I used the mean values of the animals' left and right halves.

I also used the digital images to measure the area (mm^2) of blue and black gular and ventral patches at the termination of the experiment for each lizard. Using ImageJ version 1.46r software (National Institutes of Health, USA) I set the scale of each image to 11.5 pixels/mm and used the Freehand Selection tool to outline the left and right gular and ventral patches at 200x magnification. When defining patches, only scales with greater than 50% blue or black were included. All subsequent analyses of patch sizes were done using mean values of the animals' left and right halves.

In order to assess accuracy of the color measurements I repeated the described methodology two times for each lizard. To assess accuracy of the patch size measurements I repeated the described methodology for the gular and ventral patch areas four separate times per image for a subset (3 images/treatment group, n = 18) of lizards. I performed analyses of variance

(ANOVA) on hue, saturation, and brightness values of chevrons, dorsolateral regions, gular patches, and ventral patches and on gular and ventral patch sizes. I measured repeatability as the ratio of variance within individuals to total variance (within individuals + across individuals). Repeatability was extremely high (> 0.95) for all measures of color and patch size (Table 2.1). Although this does not measure potential variation within individuals over time, this does show that measurements of coloration from any single image are highly repeatable relative to the typical variation across individuals and treatment groups.

Statistical Analyses

All color values were log-transformed in order to meet the assumptions of parametric analyses. Due to the absence of ovariectomized females in the 2012 DHT study, I carried out separate analyses involving intact males and females and intact and castrated males. To analyze growth rates, I used analysis of covariance (ANCOVA) with initial SVL as a covariate and with DHT and sex as main effects in analyses of intact males and females. I used ANCOVA with initial SVL as a covariate and with DHT and gonad (presence/absence) as main effects in analyses of intact and castrated males. I also performed a repeated-measures ANOVA to test if SVL changed over the course of the 2012 DHT experiment, regardless of treatment group. I used two-way ANOVA to analyze 12 post-treatment measures of color, patch sizes, feeding rates, and body conditions, with DHT and sex as main effects in analyses of intact males and females and DHT and gonad (presence/absence) as main effects in analyses of intact and

castrated males. To test the effect of body size on patch sizes I used ANCOVA with SVL as the covariate. The homogeneity of slopes assumption of each ANCOVA was met.

For the 2013 castration study I used ANCOVA with initial SVL as a covariate to analyze feeding rates, body conditions, and growth rates over the 113-day experimental period, with sex as the main effect in analyses of females and intact males and castration as the main effect in analyses of castrated and intact males. All p-values were considered significant at the $\alpha = 0.05$ level. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

Growth (2012): Analyses of Intact Males and Females

All lizards increased in SVL over the course of the experiment ($F_{3,28} = 172.54$, $P < 0.001$; Table 2.2). Intact males and females treated with DHT grew at a significantly slower rate compared to placebo-implanted individuals ($F_{3,18} = 20.77$, $P < 0.001$; Fig. 2.2), but intact males and females grew at similar rates ($F_{3,18} = 3.16$, $P = 0.092$; Fig. 2.2). Body condition ($F_{3,18} = 0.00$, $P = 0.957$) and feeding rate ($F_{3,18} = 2.64$, $P = 0.122$) were not affected by DHT. Body condition ($F_{3,17} = 0.04$, $P = 0.849$) and feeding rate ($F_{3,17} = 4.07$, $P = 0.059$) did not differ between intact males and females.

Growth (2012): Analyses of Intact and Castrated Males

Intact and castrated males treated with DHT grew at a significantly slower rate compared to placebo-implanted individuals ($F_{3,17}$ = 14.40, P = 0.001; Fig. 2.2). Castration of males did not have a significant effect on growth rate ($F_{3,17}$ = 0.00, P = 0.970; Fig. 2.2). Body condition ($F_{3,17}$ = 1.30, P = 0.270) and feeding rate ($F_{3,17}$ = 2.15, P = 0.161) were not affected by DHT. Castration of males had no significant effect on body condition ($F_{3,18}$ = 0.06, P = 0.809) or feeding rate ($F_{3,18}$ = 0.04, P = 0.837).

Growth (2013): Long-Term Effects of Castration

After 113 days, growth rate was significantly faster in castrated males than intact males ($F_{2,13}$ = 4.76, P = 0.048; Fig. 2.3). Neither body condition nor feeding rate was significantly different between castrated and intact males (body condition: $F_{2,13}$ = 2.53, P = 0.136; feeding rate: $F_{2,13}$ = 0.41, P = 0.535). Females grew at significantly faster rates than intact males ($F_{2,16}$ = 52.83, P < 0.001; Fig. 2.3). Body condition did not differ between males and females ($F_{2,16}$ = 0.13, P = 0.728). However, feeding rate was higher in females than in intact males ($F_{2,16}$ = 26.06, P < 0.001).

Ventral and Gular Coloration (2012): Analyses of Intact Males and Females

Ventral coloration was significantly more vivid in intact males than females (saturation: $F_{3,18}$ = 14.01, P = 0.002; brightness: $F_{3,18}$ = 8.26, P = 0.010; Fig. 2.4A). These males also had significantly more vivid gular patches (saturation: $F_{3,18}$ = 12.53, P = 0.002; brightness: $F_{3,18}$ = 6.56, P = 0.020; Fig. 2.4B) compared

to females. Hue, however, did not differ between sexes (ventral: $F_{3,18} = 0.95$, $P = 0.343$; gular: $F_{3,18} = 1.24$, $P = 0.280$).

Treatment with DHT made ventral patches significantly more pronounced in both sexes by increasing saturation ($F_{3,18} = 9.25$, $P = 0.007$; Fig. 2.4A) and decreasing brightness ($F_{3,18} = 46.12$, $P < 0.001$; Fig. 2.4A). Ventral hue was not significantly affected by DHT treatment ($F_{3,18} = 3.99$, $P = 0.061$).
Treatment with DHT made gular patches more pronounced by increasing saturation and decreasing brightness (saturation: $F_{3,18} = 17.03$, $P < 0.001$; brightness: $F_{3,18} = 7.42$, $P = 0.014$; Fig. 2.4B) without significantly impacting hue ($F_{3,18} = 0.92$, $P = 0.349$).

Ventral and Gular Coloration (2012): Analyses of Intact and Castrated Males

Treatment with DHT made ventral patches significantly more pronounced in intact and castrated males by increasing saturation ($F_{2,18} = 8.37$, $P = 0.009$; Fig. 2.4A) and decreasing brightness ($F_{2,18} = 32.80$, $P < 0.001$; Fig. 2.4A). Ventral hue was not significantly affected by DHT treatment ($F_{2,18} = 0.53$, $P = 0.477$). Treatment with DHT led to more pronounced gular patches by increasing saturation ($F_{2,18} = 11.49$, $P = 0.003$; Fig. 2.4B) and shifting hue from blue towards green ($F_{2,18} = 7.92$, $P = 0.012$), but not influencing gular brightness ($F_{2,18} = 0.15$, $P = 0.704$; Fig. 2.4B).

Castration did not influence ventral patch hue ($F_{2,18} = 0.06$, $P = 0.817$), saturation ($F_{2,18} = 0.14$, $P = 0.714$; Fig. 2.4A), or brightness ($F_{2,18} = 1.53$, $P = 0.232$; Fig. 2.4A). Furthermore, castration did not influence gular patch saturation

($F_{2,18}$ = 0.02, P = 0.903; Fig. 2.4B), brightness ($F_{2,18}$ = 0.27, P = 0.607; Fig. 2.4B), or hue ($F_{2,18}$ = 1.34, P = 0.263).

Dorsal Coloration (2012): Analyses of Intact Males and Females

Females had darker and more conspicuous chevrons (hue: $F_{3,18}$ = 6.57, P = 0.020; brightness: $F_{3,18}$ = 5.64, P = 0.029) than intact males. Chevron saturation ($F_{3,18}$ = 1.13, P = 0.302) was not significantly different between intact males and females. Treatment with DHT made chevrons less pronounced by decreasing hue ($F_{3,18}$ = 5.85, P = 0.026) without affecting saturation ($F_{3,18}$ = 0.20, P = 0.659) and brightness ($F_{3,18}$ = 2.78, P = 0.113). Dorsolateral coloration was not different between the sexes (hue: $F_{3,18}$ = 0.21, P = 0.649; saturation: $F_{3,18}$ = 0.51, P = 0.484; brightness: $F_{3,18}$ = 2.48, P = 0.133) and was not affected by DHT treatment (hue: $F_{3,18}$ = 0.01, P = 0.928; saturation: $F_{3,18}$ = 0.84, P = 0.370; brightness: $F_{3,18}$ = 2.12, P = 0.162). All dorsal coloration data is shown in Table 2.3.

Dorsal Coloration (2012): Analyses of Intact and Castrated Males

Treatment with DHT made chevrons less pronounced by decreasing hue ($F_{2,18}$ = 4.70, P = 0.044) without affecting saturation ($F_{2,18}$ = 0.27, P = 0.608) and brightness ($F_{2,18}$ = 0.58, P = 0.457) in intact and castrated males. Castration had no significant effect on any measure of chevron coloration (hue: $F_{2,18}$ = 0.83, P = 0.374; saturation: $F_{2,18}$ = 1.97, P = 0.177; brightness: $F_{2,18}$ = 1.82, P = 0.194). Dorsolateral coloration of intact and castrated males was not affected by DHT

treatment (hue: $F_{2,18} = 0.02$, $P = 0.885$; saturation: $F_{2,18} = 2.13$, $P = 0.161$; brightness: $F_{2,18} = 1.11$, $P = 0.306$). However, while castrated males were similar to intact males for dorsolateral hue ($F_{2,18} = 1.70$, $P = 0.209$) and saturation ($F_{2,18} = 1.89$, $P = 0.186$), intact males had brighter dorsolateral coloration ($F_{2,18} = 5.28$, $P = 0.034$). All dorsal coloration data is shown in Table 2.3.

Patch Sizes (2012): Analyses of Intact Males and Females

Snout-vent length did not correlate with patch sizes (gular: $F_{6,25} = 1.02$, $P = 0.323$; ventral: $F_{6,25} = 1.25$, $P = 0.274$) in any of the treatment groups. Intact male lizards had larger ventral patches than female lizards (blue: $F_{3,18} = 6.65$, $P = 0.019$; black: $F_{3,18} = 6.03$, $P = 0.024$). Blue gular patch sizes were not significantly different between females and intact males ($F_{3,18} = 1.96$, $P = 0.179$), but intact males had significantly larger black gular patches ($F_{3,18} = 9.00$, $P = 0.008$).

As indicated by a significant sex-by-treatment interaction, DHT increased the size of blue ventral patches in females, but decreased blue ventral patches in intact males ($F_{3,18} = 61.83$, $P < 0.001$). Ventral black patch size, however, was significantly increased by DHT in both intact males and females $F_{3,18} = 22.56$, $P < 0.001$). Treatment with DHT had no effect on the size of blue gular patches ($F_{3,18} = 1.11$, $P = 0.306$), but significantly increased the size of black gular patches in intact males and females ($F_{3,18} = 45.76$, $P < 0.001$). All patch size data is shown in Table 2.4.

Patch Sizes (2012): Analyses of Intact and Castrated Males

As indicated by a significant castration-by-treatment interaction, DHT increased the size of blue ventral patches in castrated males, but decreased blue ventral patches in intact males ($F_{3,17}$ = 24.02, P < 0.001). Ventral black patch size was significantly increased by DHT ($F_{3,17}$ = 25.40, P < 0.001) and decreased by castration ($F_{3,17}$ = 8.39, P = 0.010) in intact and castrated males. Treatment with DHT and castration had no effect on the size of blue gular patches in intact and castrated males (DHT: $F_{3,17}$ = 0.01, P = 0.914; castration: $F_{3,17}$ = 0.17, P = 0.686). However, DHT increased the size of black gular patches ($F_{3,17}$ = 35.30, P < 0.001) while castration decreased black gular patch size ($F_{3,17}$ = 4.63, P = 0.046). All patch size data is shown in Table 2.4.

Discussion

The present study shows that DHT inhibits growth in both males and females of the female-larger lizard species, *S. undulatus*. The observed reductions in growth rate induced by DHT treatment are unlikely to be attributed to body condition or feeding rate since there were no significant effects of DHT treatment on body condition or feeding rate in any of the treatment groups. These results strongly suggest that the inhibitory effect of T on growth is mediated by androgen receptors, exclusive of estrogen receptors, because of two main reasons. First, the purity of the DHT I used in my implants was very high (>98%; Steraloids, Inc., Newport, RI). Second, DHT has very low binding affinity to estrogen receptors (relative binding affinity = 0.03 for DHT versus 100.00 for E_2;

Fang et al. 2000; Matthews et al. 2000) so it is unlikely that the effects I have seen are due to DHT binding to estrogen receptors. One caveat to the present study is that I did not directly measure circulating DHT and, therefore, it is possible that the observed effects are the result of pharmacological doses of DHT. However, I introduced precise amounts of DHT dissolved in DMSO, and Hews and Moore (1994, 1995) used similar implants of T and DHT in hatchling tree lizards (*Urosaurus ornatus*) and reported physiologically relevant levels of both androgens.

Effects of DHT on Growth

A few other studies have demonstrated a growth-inhibiting effect of T in species with female-larger SSD (Crews et al. 1985; Cox and John-Alder 2005; Cox et al. 2005a), but studies examining the effects of DHT on growth have been limited (Hews and Moore 1995). Therefore, the results here help clarify the androgenic mechanism(s) regulating bi-potential growth in female-larger lizards and further raises the question: how does T inhibit growth in some species, but not others?

One potential explanation for bi-potential growth regulation by T and DHT lies within the somatotropic axis (endocrine growth axis). In most cases, previous studies have found that T and other androgenic hormones stimulate production of GH and IGF-1 (Jansson et al. 1985; Hall et al. 1986; Borski et al. 1996; Vendola et al. 1999; Huggard et al. 2003; Riley et al. 2002, 2004; Larsen et al. 2004). However, the majority of the studies examining the effects of T on the

endocrine growth axis have been in male-larger species of mammals and teleost fishes. It is plausible that within female-larger species the pattern is reversed, with GH and IGF-1 production decreasing in response to androgenic hormones, thus leading to slower male growth (John-Alder et al. 2007). While my experiments here did not examine effects of T or DHT on GH and IGF-1 production, an earlier study by Duncan (2011) demonstrated that T decreased hepatic IGF-1 expression in juvenile male and female *S. undulatus* and that this was correlated with decreased growth rate. Castration of yearling males led to a 3-fold increase in hepatic IGF-1 expression. The inhibitory effect of T on IGF-1 expression is contrary to what is observed in male-larger species of other taxa (Larsen et al. 2004; Norbeck and Sheridan 2011; Reindl and Sheridan 2012). In a study by Vaughan et al. (1994) on Syrian hamsters (*Mesocricetus auratus*), a female-larger species, surgical castration elevated IGF-1 levels compared to intact control and T-replaced males. While these findings are promising they are nonetheless preliminary and thus require further investigation. Future studies should further examine the relationships between androgens, GH, and IGF-1 in female-larger species in order to elucidate the interplay between T and the somatotropic axis in regulating growth-inhibition and driving the development of SSD.

Female *S. undulatus* administered DHT exhibited phenotypic responses (e.g., decreased growth rate and development of male-typical ventral coloration) that were similar or greater in magnitude than those observed in males. This indicates that despite pronounced differences in body size and color between

males and females of *S. undulatus*, females have retained functional androgen receptors capable of initiating male-typical growth and color development. While female lizards have functional androgen receptors, females lack the necessary androgenic signal (e.g., DHT) to initiate the development of male-typical characteristics. Therefore, the evolution of androgen mediated sexual dimorphisms in *S. undulatus* is likely through the linkage of trait expression with activation of the androgen receptor and females have not lost this linkage. Cox et al. (2014) reported similar findings in brown anoles (*A. sagrei*) and concluded that sex differences in growth and color development between males and females are likely more through sex differences in circulating androgens and less so through tissue responsiveness to androgens. Additionally, Golinski (2013) concluded that the percent of androgen receptors were not different among males and females in two species of geckos, but treatment with exogenous T induced male sexual behaviors and the development of hemipenes in females (Golinski et al. 2011, 2014). In *S. undulatus*, sex differences in growth and color development between males and females are likely through sex differences in circulating androgens, similar to what occurs in *A. sagrei*, but sexual dimorphisms may also arise in part through sex differences in androgen receptor densities. Hews et al. (2012) and Moga et al. (2000), for example, reported that while both male and female *S. undulatus* had androgen receptors present in regions of the brain, males had higher receptor densities and higher circulating T than their female counterparts.

I failed to detect a significant effect of castration on male growth over the short-term, but demonstrated a long-term growth-promoting effect of castration on male growth. Cox et al. (2005a) reported a similar pattern with *S. undulatus* in a large field enclosure in which castrated and intact males both had low levels of circulating T and grew at similar rates during the 45- and 56-day experimental time periods. However, following recapture 418 days later, castrated males had grown larger in body size compared to intact males. Taken together, the long-term effects of castration on male growth observed in the present study and the study by Cox et al. (2005a) demonstrate that, given adequate time, castration alone can promote growth in *S. undulatus*. It is unclear why an effect of castration on male growth is apparent only after an extended time. However, one possibility for the delayed castration effect is that all male lizards, regardless of gonad presence, had low circulating levels of androgens due to being held under experimental conditions and only after acclimation to laboratory conditions did circulating androgen levels increase in intact males. Circulating T levels have been shown to be atypically low in *S. undulatus* removed from natural home ranges and confined in field and laboratory enclosures (Cox et al. 2005a,b). In tree lizards (*Urosaurus ornatus*), circulating T levels decreased significantly when housed in individual cages in the laboratory (Moore et al. 1991). Another possibility is that all males had low circulating androgen levels because they had not yet reached physiological maturity. Therefore, there would not have been a significant source of androgens to remove via castration and only following

attainment of physiological maturity would differences in circulating androgen levels and growth be observed in intact versus castrated males.

Recently it has been suggested that T may not be the key mechanism behind the sexual divergence in growth between male and female lizards. Studies on female-larger (*Aeluroscalabotes felinus*, family: Eublepharidae) and male-larger (*P. picta*, family: Gekkonidae) species of geckos suggest that estrogenic hormones, rather than androgenic hormones, underlie sex differences in growth and the development of SSD. In *A. felinus*, T treatment decreased growth rates in castrated males and females, thus inducing male-like growth patterns. This is in line with the "bi-potential growth regulation hypothesis", but contrary to this is that male castration had no effect on growth (Kubička et al. 2013). More convincing evidence comes from two studies involving *P. picta*. Kubička et al. (2015) found that while castrated and intact males differed in circulating androgens, they did not differ in growth. Furthermore, Starostová et al. (2013) reported that castration and T replacement had no effects on growth in males yet interestingly, T and ovariectomy significantly increased growth and body size of females, preventing the sex differences in growth rate and body size. Comparison of these studies within Gekkota (e.g., Gekkonidae, Eublepharidae) and studies within Iguania (e.g., Phrynosomatidae, Dactyloidae) suggest that T may be a bi-potential regulator of growth in iguanians, but not gekkotans. Within Gekkota, estrogens (e.g., E_2), as opposed to androgens (e.g., T, DHT), may be the more important growth regulator. This is an interesting possibility, considering that the evolutionary split between Gekkota and Iguania is

basal and occurred approximately 175 million years ago (Wiens and Lambert 2014).

Effects of DHT on Color Development

I found that ventral coloration was made more vivid by administration of DHT and less vivid by castration. The effect of DHT was especially pronounced in females, which normally do not express significant amounts of ventral coloration. Furthermore, DHT increased ventral patch sizes in both males and females. These findings indicate that sexual dichromatism in males of *S. undulatus* is mediated via androgen receptors and that females possess functional androgen receptors and have retained sensitivity to androgens capable of initiating the development of male-typical coloration. Several previous studies have reported that T stimulates color development in lizards (Kimball and Erpino 1971; Cooper et al. 1987; Rand 1992; Sinervo et al. 2000; Knapp et al. 2003; Calisi and Hews 2007; Cox et al. 2005b, 2008; Mills et al. 2008), but only Hews and Moore (1995) reported effects of DHT on color development in phrynosomatid lizards. They found that in tree lizards (*U. ornatus*) DHT had a greater effect than T on the development of ventral and gular patch color in both hatchling and adult females. The effects of T and DHT on the development of male-typical blue and black ventral coloration likely involve the distribution and density of various pigment containing cells, such as iridophores and melanophores. Iridophores contain light reflecting platelets that give rise to blue colors based on platelet shape, size, number, and orientation (Morrison et al.

1995). Melanophores are light absorbing cells and influence the brightness of colors (Kuriyama et al. 2006). Blue coloration develops when blue wavelengths are reflected by iridophores and other wavelengths are absorbed by the melanophores. Black coloration, on the other hand, develops when iridophores are absent and all wavelengths of light are absorbed by melanophores (Quinn and Hews 2003; Kuriyama et al. 2006).

My study is the first to provide direct experimental evidence of the effects of DHT on dorsal coloration in *S. undulatus*, although the results are not as resoundingly significant as those for ventral coloration because left and right color measurements are less highly correlated for dorsal than for ventral measurements. My results show that DHT made dorsal chevrons lighter and less vivid in females (more male-like), and castration made chevrons darker and more vivid in males (more female-like). The effects of DHT were not observed on the dorsolateral regions, although dorsolateral regions trended darker (more female-like) incastrated than in intact males. When examining the effects of T on measures of dorsal coloration in *S. undulatus* Cox et al. (2005b) found a similar pattern, where castrated males had darker, more vivid chevrons and darker dorsolateral regions than intact males. Testosterone has been shown to influence dorsal coloration in several other species as well (Cooper and Ferguson 1972; Cooper et al. 1987; Cooper and Crews 1987; Rand 1992). The effects of T and DHT on dorsal coloration may be indirect, involving activation of the sympathetic nervous system (John-Alder et al. 2009). For example, melanophore migration leading to aggregation (darkening) or dispersion (lightening) is tied with beta-

adrenergic and alpha-adrenergic receptors stimulation, respectively (Hadley and Oldman 1969; Cooper and Greenberg 1992).

Conclusions

In summary, results in the present study clearly show that exogenous DHT inhibits growth and stimulates male-typical color development in both males and females of the sexually dichromatic, female-larger phrynosomatid lizard, *Sceloporus undulatus*. These results strongly suggest that effects of T on growth and color development are mediated through androgen receptors. My results help to clarify how androgens can inhibit growth in males of female-larger lizards, but promote growth in males of male-larger lizards. However, we still lack a complete understanding on how T and DHT directly affect the physiological and molecular mechanisms regulating color development and how they can have differential effects on growth depending on the direction of SSD. Because body size and color in species exhibiting SSD and sexual dichromatism may also be correlated with several other traits, such as dominance, immunocompetence, and age, it is possible for future studies in this area to become incorporated into a larger ecological and evolutionary framework of behavior, physiology, morphology, and life history.

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Table 2.1: Repeatability (upper panel) and precision (lower panel) of color measurements (hue, saturation, and brightness) and gular and ventral patch sizes. Repeatability (ratio of variance within individuals to total variance) was extremely high (> 0.95) for all measures of color and patch size. Precision (correlation between left and right body sides) was higher for ventral (gular and ventral patches) color measurements compared to dorsal (chevrons and dorsolateral areas) color measurements. Details of analyses are presented in the text.

Area of Measure	Hue Repeatability	Saturation Repeatability	Brightness Repeatability	Patch Size Repeatability
Gular Patches	0.994	0.999	0.994	0.969
Ventral Patches	0.999	0.999	0.999	0.994
Chevrons	0.988	0.998	0.999	.
Dorsolateral Areas	0.961	0.996	0.956	.

Area of Measure	Hue Precision	Saturation Precision	Brightness Precision	
Gular Patches	0.996	0.947	0.848	.
Ventral Patches	0.895	0.945	0.980	.
Chevrons	0.496	0.695	0.922	.
Dorsolateral Areas	0.568	0.827	0.769	.

Table 2.2: Mean (± 1 SEM) initial and final SVL measurements and growth rates for each treatment group for the 2012 DHT-growth study. FEM = intact-placebo females, CON = intact-placebo males, CAST = castrated-placebo males. All treatment groups increased in SVL ($P < 0.001$) over the course of the experiment and DHT treatment decreased growth rate in all treatment groups ($P < 0.001$). Details of analyses are presented in the text.

Treatment Group	Initial SVL (mm; 29 June 2012)	Final SVL (mm; 8 August 2012)	Growth Rate (mm/day)
FEM	44.0 ± 0.9	53.6 ± 1.1	0.208 ± 0.028
FEM+DHT	43.3 ± 1.4	51.0 ± 1.3	0.133 ± 0.014
CON	44.0 ± 1.5	53.4 ± 1.1	0.184 ± 0.015
CON+DHT	41.3 ± 0.8	47.6 ± 1.2	0.097 ± 0.018
CAST	47.0 ± 0.7	53.8 ± 1.3	0.175 ± 0.035
CAST+DHT	49.8 ± 1.2	53.7 ± 1.5	0.098 ± 0.010

Table 2.3: Mean (± 1 SEM) color values of dorsal chevrons and dorsolateral areas for all treatment groups. FEM = intact-placebo females, CON = intact-placebo males, CAST = castrated-placebo males. Intact females had significantly more vivid (higher hue, lower brightness; $P < 0.05$) chevrons compared to intact males. Treatment with DHT made chevrons less conspicuous by decreasing hue in all treatment groups ($P < 0.05$). The only significant difference in dorsolateral color was between castrated and intact males, where castration decreased brightness ($P < 0.05$). Details of analyses are presented in the text.

	CHEVRON			DORSOLATERAL		
Treatment Group	**Hue (Degrees)**	**Saturation (%)**	**Brightness (%)**	**Hue (Degrees)**	**Saturation (%)**	**Brightness (%)**
FEM	41.5 ± 4.7	26.3 ± 8.0	13.5 ± 5.4	39.0 ± 3.2	22.6 ± 3.5	39.5 ± 1.0
FEM+DHT	34.0 ± 1.7	27.3 ± 4.1	27.2 ± 4.3	38.8 ± 1.6	26.6 ± 3.7	37.1 ± 2.4
CON	33.6 ± 1.5	30.9 ± 3.1	30.5 ± 2.8	38.1 ± 2.4	26.7 ± 4.6	43.2 ± 1.5
CON+DHT	28.4 ± 2.0	34.6 ± 11.7	29.4 ± 8.5	37.4 ± 1.2	30.0 ± 3.2	40.0 ± 2.9
CAST	33.8 ± 2.3	36.9 ± 5.8	20.3 ± 5.9	35.3 ± 3.1	29.3 ± 1.8	37.6 ± 3.2
CAST+DHT	31.5 ± 0.8	42.6 ± 4.2	27.9 ± 1.5	34.7 ± 1.5	38.5 ± 3.1	35.7 ± 1.6

Table 2.4: Mean (± 1 SEM) sizes of blue and black ventral and gular patches for all treatment groups. FEM = intact-placebo females, CON = intact-placebo males, CAST = castrated-placebo males. Intact males had larger blue and black ventral patches and larger black gular patches compared to intact females ($P < 0.05$). Treatment with DHT increased black ventral and gular patch sizes ($P < 0.001$). Blue ventral patch size was decreased by DHT in intact males, but increased by DHT in females and castrated males ($P < 0.001$). Details of analyses are presented in the text.

Treatment Group	Ventral Blue (mm^2)	Ventral Black (mm^2)	Gular Blue (mm^2)	Gular Black (mm^2)
FEM	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 1.4	0.0 ± 0.0
FEM+DHT	58.81 ± 4.8	24.5 ± 6.3	8.0 ± 1.1	26.4 ± 2.7
CON	78.0 ± 9.1	8.8 ± 3.5	8.6 ± 1.7	12.5 ± 2.4
CON+DHT	19.3 ± 9.6	49.4 ± 15.1	7.4 ± 1.7	22.7 ± 4.3
CAST	49.0 ± 9.5	0.0 ± 0.0	7.8 ± 0.9	4.5 ± 2.2
CAST+DHT	77.0 ± 3.8	22.2 ± 1.6	9.4 ± 0.8	26.2 ± 1.6

Figure 2.1: Digital scans of the dorsal (top panels) and ventral (bottom panels) surfaces of individual *S. undulatus*, illustrating the typical coloration of each treatment group. Lettered ellipses indicate the areas sampled for analyses of hue, saturation, and brightness: (A) dorsal chevrons, (B) dorsolateral areas, (C) blue gular patches, (D) blue ventral patches.

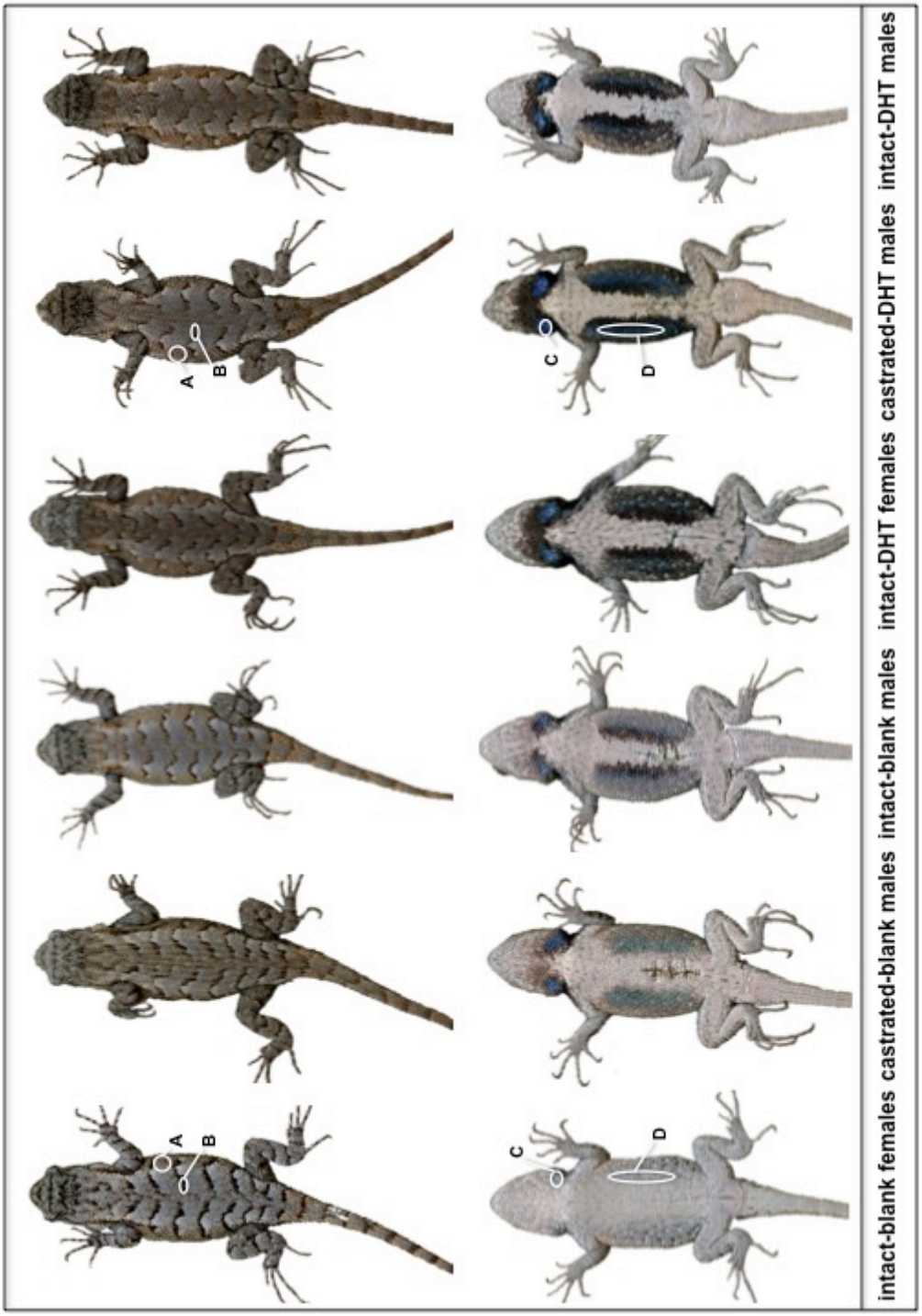


Figure 2.2: Mean (± 1 SEM) growth rate of each treatment group for the 2012 DHT-growth study. FEM = intact-placebo females, CON = intact-placebo males, CAST = castrated-placebo males. DHT treatment decreased growth rate. Letters denote significant differences ($P < 0.001$) between treatment groups in separate analyses of intact males versus females (a_1, b_1) and intact versus castrated males (a_2, b_2). Details of analyses are presented in the text.

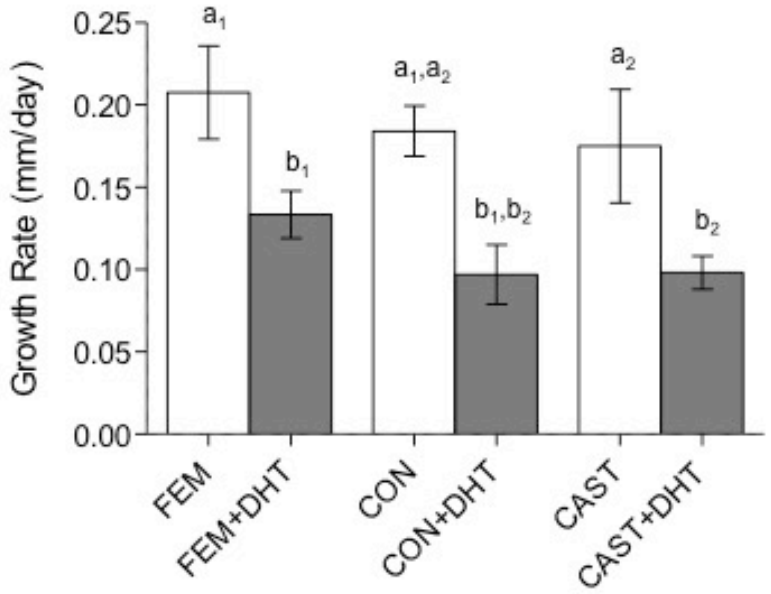


Figure 2.3: Mean (± 1 SEM) growth rate of each treatment group for the 2013 castration-growth study. FEM = intact females, CAST = castrated males, CON = intact males. After 113 days, females grew faster than males ($P < 0.001$) and castrated males grew significantly faster than intact males ($P < 0.05$). Letters denote significant differences ($P < 0.05$) between treatment groups in separate analyses of intact males and females (a_1, b_1) and intact and castrated males (a_2, b_2) for the 113-day time period. Details of analyses are presented in the text.

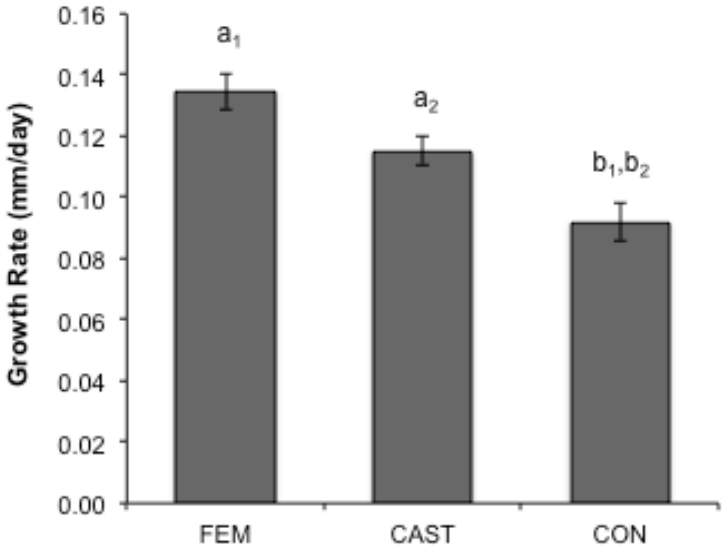
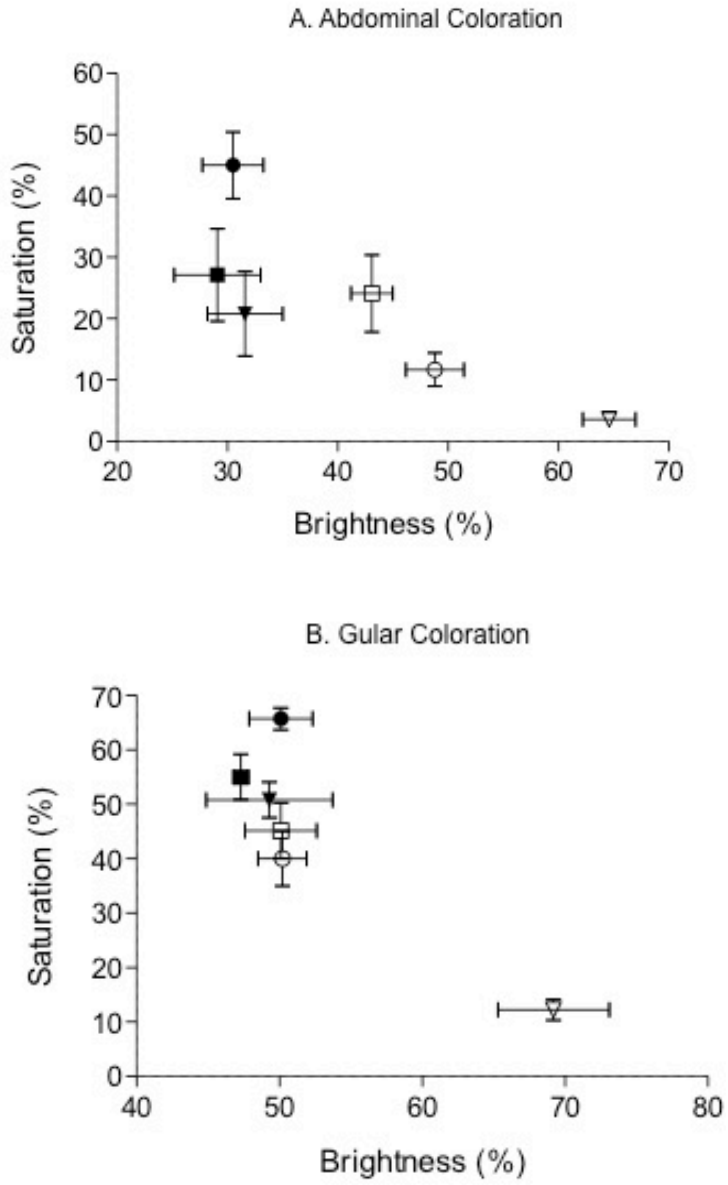


Figure 2.4: Mean (± 1 SEM) color values of ventral (A) and gular (B) patch saturation and brightness for intact-placebo females (▽), intact-DHT females (▼), castrated-placebo males (○), castrated-DHT males (●), intact-placebo males (□), and intact-DHT males (■). Intact males had more vivid (higher saturation and lower brightness) ventral and gular patches compared to females ($P < 0.05$). Treatment with DHT in both sexes made ventral patches more vivid by increasing saturation and decreasing brightness ($P < 0.001$). Gular patches were made more vivid in females by increasing saturation and decreasing brightness ($P < 0.05$). In males, DHT made gular patches more vivid by increasing saturation ($P < 0.001$). Details of analyses are presented in the text.



CHAPTER 3

SEASONALITY OF SEX- AND AGE-SPECIFIC PATTERNS OF MITE PARASITISM IN *SCELOPORUS UNDULATUS* AND CORRELATIONS WITH ENVIRONMENTAL MITE ABUNDANCE

Abstract

In the eastern United States, chigger mites (*Eutrombicula alfreddugesi*) frequently parasitize eastern fence lizards (*Sceloporus undulatus*). The natural histories of these species are intertwined. Mites, which extract matter and energy from host lizards, may induce an immune response and negatively affect growth and other host life history traits. I investigated the seasonality of environmental mite abundance and of sex- and age-specific patterns of mite parasitism in a New Jersey population of *S. undulatus*. Mite loads on adult and yearling lizards were recorded at monthly intervals from May through September in 2014 and 2015. Environmental mite abundances were measured monthly at each lizard capture location during 2015. Environmental mite abundances were lowest in May and September and highest in June and July. Mite loads were lowest in May, increased in June, peaked in July, and gradually decreased thereafter. From July to September, yearling males had higher mite loads than yearling females. In adults, however, females had higher mite loads than males during June and July. From June to September, yearling males had higher mite loads than adult males, and during August and September, yearling females had higher mite loads than adult females. These findings indicate that the abundance of

mites in the environment is a prime determinant of mite loads on *S. undulatus* and that testosterone may increase mite loads in males, but only when mites are abundant in the environment. High mite loads in yearling males from July to September, coinciding with high circulating levels of testosterone and growth inhibition, suggests that mites could contribute to greater growth inhibition in males during these months.

Introduction

Parasites can inflict numerous costs on hosts, including altered behavioral patterns, decreased growth, decreased reproduction, decreased health (i.e., body condition and hematocrit), and decreased survival (Schall and Dearing 1987; Merino and Potti 1995; Salvador et al. 1996; Vuren 1996; Klein 2003; Uller and Olsson 2003; Marzal et al. 2005; Hawlena et al. 2006; Ujvari and Madsen 2006; Gooderham and Schulte-Hostedde 2011). Several factors influence parasite load in a given host individual, including host body size, age, sex, hormone concentrations, and reproductive effort (Veiga et al. 1998; Christe et al. 2000; Amo et al. 2004; Krasnov et al. 2005; Rocha et al. 2008; Fuxjager et al. 2011; Pollock et al. 2012; Halliday et al. 2014; Dudek et al. 2016). In particular, host sex appears to be a major determinant of parasite load. When parasite loads differ between males and females of a host species, then any significant effects of parasitism may result in differences in the development of various host life history traits. For example, if males are more heavily parasitized than females, and the parasite species decreases growth and survival, then this could lead to slower growth and increased mortality in males compared to females.

Male animals tend to have higher parasite loads than females in diverse taxa, includiing rodents (Morand et al. 2004; Krasnov et al. 2005; Perez-Orella and Schulte-Hostedde 2005; Colombo et al. 2015; LeCoeur et al. 2015), birds (Zuk 1990; Tschirren et al. 2003), fish (Appleby and Mo 1997; Reimchen and Nosil 2001), salamanders (Anthony et al. 1994), snakes (Aubret et al. 2005), and lizards (Schall and Marghoob 1995; Talleklint-Eisen and Eisen 1999; Salkeld and Schwarzkopf 2005; Heredia et al. 2014; Dudek et al. 2016). The majority of studies investigating the mechanisms behind male-biased parasitism have focused on the androgenic steroid hormone, testosterone (T), which has been commonly implicated with higher male parasite loads simply because males have higher circulating levels of this hormone. Studies on free-ranging male lizards have shown that castration decreases parasite loads while T replacement restores male-typical parasite loads (Cox and John-Alder 2007). Other studies provide further support, finding males with experimentally elevated T have higher parasite loads than control males (Salvador et al. 1996; Olsson et al. 2000; Klukowski and Nelson 2001; Cox et al. 2005; Pollock et al. 2012).

Currently there are two prevailing hypotheses for how T may increase male parasite loads. First, T has been shown to be immunosuppressive (Saad et al. 1990; Duffy et al. 2000; Casto et al. 2001; Andersson et al. 2004; Roberts et al. 2004; Foo et al. 2016), which could allow more ectoparasites to feed and survive on males (Olsson et al. 2000; Poiani et al. 2000; Hughes and Randolph 2001). Another potential explanation for how T can increase parasite loads is through T-induced behaviors, including increases in movement and home range

size (Olsson et al. 2000; Boyer et al. 2010), which could increase the ecological exposure of males to ectoparasites, especially if they spend a significant amount of time in microhabitats abundant in parasites (Zippel et al. 1996; Curtis and Baird 2008; Bulté et al. 2009; Rubio and Simonetti 2009).

While males are more heavily parasitized than females in many species, this is not always the case. Female-biased parasitism has been documented in some taxa, including bats (Christe et al. 2007), frogs (McAlpine 1997), and snakes (Pandit et al. 2011), and sex biases in parasitism have not been found in several species of lizard (Klukowski 2004; Reardon and Norbury 2004; de Carvalho et al. 2006; Davis et al. 2012; Halliday et al. 2014). Complicating the situation are discrepancies between studies on a single species. The presence or absence of sex-biased parasitism within a species could very well depend upon when and where the study was conducted. In eastern fence lizards (*Sceloporus undulatus*), for example, Klukowski and Nelson (2001) found male-biased patterns of chigger mites in Tennessee during July 1997, but Cox et al. (2005) found female-biased patterns of chigger mites on lizards within a field enclosure in New Jersey during July 2002. Klukowski (2004) failed to find a sex difference altogether from May to October 2002. The presence or absence of sex-biased parasitism within a species could also depend upon the species of ectoparasite and when the study was carried out. For example, Lumbad et al. (2011) found that tick loads were higher in males than females of western fence lizards (*Sceloporus occidentalis*) during the spring breeding months, but that chigger mite loads were higher in females than males during the fall months.

In many lizard species, such as those of the *Sceloporus* genus, circulating T levels vary seasonally (John-Alder et al. 2007; Lind et al. 2010; Eikenaar et al. 2012) and studies finding an effect of T on parasitism and finding male-biased parasitism have typically been performed during times of year when T is high in adults or juveniles (Salvador et al. 1996; Veiga et al. 1998; Olsson et al. 2000; Klukowski and Nelson 2001; Cox and John-Alder 2007; Pollock et al. 2012). Therefore, these studies represent only a snapshot of the overall time period in which the host and parasite species encounter one another, and fail to fully describe how parasite loads may vary in relation to circulating T. Furthermore, the timing of seasonal variation in circulating T can also depend upon age. For example, adult male *S. undulatus* have high circulating levels of T upon emergence from hibernation and during the breeding months of April and early May. Testosterone levels decline to a low level in June before increasing to intermediate levels in late July, August, and September (John-Alder et al. 2009). Yearling (first full activity season) males, on the other hand, have high circulating levels of T during June, July, and August (Cox et al. 2005). Because of the age-specific seasonal variation in T and the findings linking T with parasitism, it is plausible that sex and age differences in parasite loads vary depending upon the time of the year. I predicted adult males would have more parasites than females and yearling males during the breeding months when their T levels are high and I predicted yearling males would have more parasites than females and adult males later in the year when their T levels are high. During times when male T is

not at a seasonal high and significantly elevated compared to females, I predicted sex differences in parasite loads to be absent altogether.

To further understand temporal, spatial, and inter-individual variation in parasite loads, studies on the natural history of host-parasite relationships are needed. Ticks and mites are common and widespread ectoparasites that attach to the integument of terrestrial vertebrates. In many habitats, squamate reptiles, particularly lizards, serve as major hosts for larval and nymphal forms of ticks and chigger mites (Tuegel and Wrenn 1998; Eisen et al. 2004). The family Trombiculidae (chigger mites) is cosmopolitan, with numerous species occurring on every continent as far north as Alaska and as far south as New Zealand (Wharton and Fuller 1952). Across North and South America and the Caribbean, chigger mites are common ectoparasites of vertebrate hosts (including humans) with chigger mites of the genus *Eutrombicula* parasitizing several species of lizards throughout the United States (Goldberg and Bursey 1993; Klukowski 2004; Curtis and Baird 2008; Pollock et al. 2012), Central America and the Caribbean (Zippel et al. 1996; Schlaepfer 2006), and South America (Cunha-Barros et al. 2003; Rubio and Simonetti 2009). Throughout the eastern United States an important host for eutrombiculid chigger mites (*Eutrombicula alfreddugesi*; US Department of Agriculture, Forest Service) is the eastern fence lizard (*S. undulatus*).

The seasonality of ectoparasite life cycles and the environmental abundance of ectoparasites are likely important components influencing the ectoparasitism of hosts and patterns of sex-biased ectoparasitism (Roberts et al.

2004; Krasnov et al. 2005; Patterson et al. 2015). The seasonal life cycles of ectoparasites can typically be described by normally distributed seasonal patterns of abundance for different life stages (Padgett and Lane 2001; Levi et al. 2015; MacDonald and Briggs 2016). Chigger mites exhibit a 4-stage life cycle consisting of egg, larval, nymphal, and adult stages, with ecdysis occurring during the transition from one stage to the next (Sasa 1961). As larvae, chiggers are parasitic and feed on digested, liquefied skin until they fully engorge, drop off, and molt into the nymphal stage. As nymphs and adults, they are free-living and no longer parasitic (Sasa 1961). Relatively low abundances of parasitic larvae occur during April and May, peak during June and July, and gradually decline thereafter (Clopton and Gold 1993; Klukowski 2004). I predicted that mite abundance in the environment varies seasonally in the New Jersey Pinelands, and that this seasonal variation in environmental mite abundance is a primary factor influencing mite loads of lizard hosts.

Materials and Methods

Seasonal Variation in Mite Loads

This study was carried out during 5-month periods (May–September) of 2014 and 2015 at Colliers Mills Wildlife Management Area in New Egypt, Burlington County, NJ (40°07’N, 74°43′W) in the New Jersey Pinelands. Colliers Mills is heavily managed and characterized by extensive edge habitat between open fields and forested tracts of oak and pine. Its forests tend to have a relatively open canopy, sparse understory, and a forest floor with an abundance

of fallen trees and logs. I calculated the density of adult and yearling (first full activity season) *S. undulatus* within my sampling area to be approximately 10-11 adult female, 11-12 adult male, 16-17 yearling female, and 20-21 yearling male lizards per hectare.

Adult and yearling lizards were captured at monthly intervals by noosing or by hand. Upon capture, SVL (to the nearest 0.5 mm) and body mass (to the nearest 0.1 g) were measured using a ruler and Pesola scale, and sex was determined by the presence or absence of enlarged post-cloacal scales. Mite load was measured by counting the number of chigger mites using a 10x hand lens. Repeatability of mite counts was measured by performing 5 repeated counts of mite load for 10 different lizards (mite loads ranging from 10 to 129). Repeatability of mite counts was extremely high (correlation = 0.996, SD = 2.10). Mites were most commonly found in closely packed clusters in the neck pockets ("mite pockets"), dorsal and ventral surfaces of the head and neck, and axillary pits. Mites occasionally occurred around the cloaca and, in heavily infested lizards, the eyes. Each lizard was marked and identified with a unique toe-clip number, which allowed for multiple recaptures and quantification of individual variation in ectoparasitism across months. Lizards were also marked using a paint pen to allow identification from a distance and prevent multiple recaptures during a single month. Animal capture was approved by the New Jersey Department of Environmental Protection, Division of Fish and Wildlife (Permit #2014086, #2015090).

Seasonal Variation in Environmental Mite Abundance

To quantify environmental mite abundance, I used a variation of the plate method first described by Williams (1946) and later modified by Klukowski (2004) and Schöler et al. (2006). Sampling locations were chosen on the basis of lizard capture locations during the 2015 field season. Sampling periods were between the hours of 08:00 and 12:40 as these were within the range of hours commonly used in previous studies (Reed 1977; Klukowski 2004) and before high early afternoon temperatures reduce chigger activity (Clopton and Gold 1993). At each sampling location, 9 black ceramic bathroom tiles (15 x 15 cm; Daltile, Cranbury, NJ) were firmly placed on the substrate in a 3 x 3 grid format with approximately 1 cm between tiles. For a period of 90 seconds, mites were counted and removed with a paintbrush as they crossed over the tiles. This methodology was carried out once per month at each known lizard capture location.

Consistency of Mite Load Rankings Among Lizards

To investigate the consistency of mite load rankings among individuals, adult and yearling lizards were recaptured at weekly intervals from 14-June to 14-July 2016 (5 weeks total). The aim of this study was to determine if individuals with high mite loads consistently have high mite loads week to week and if individuals with low mite loads consistently have low mite loads week to week. Mite load was measured by counting the number of chigger mites using a 10x hand lens. Each lizard was marked and identified with a unique toe-clip number, and also with a unique paint marking to allow identification from a distance.

Statistical Analyses

All mite load data from Colliers Mills 2014 and 2015 were combined, and mite loads were transformed by taking the square root to meet the assumptions of parametric analyses. To evaluate overall seasonal patterns of mite load and environmental mite abundance, I combined all lizard mite loads together and all environmental mite abundance together for each month. I then calculated the monthly mean mite load and monthly mean environmental mite abundance for each month (May to September). Comparisons of mite load and environmental mite abundance across months were then performed using ANOVA with Ryan-Einot-Gabriel-Welsch post-hoc pair-wise comparisons. Spearman correlation (r_s) was used to determine if individual mite loads were correlated with individual environmental mite abundances. Specifically, I used individual lizard mite load versus environmental mite abundance at the site of lizard capture.

A general linear model approach was used to generate models evaluating the effects of season (Julian Day), Sex, Age, and interactions. I then utilized Akaike Information Criterion (AIC; Burnham and Anderson 2002), which measures the goodness-of-fit of a model to the data set using maximum likelihood while taking into account the number parameters in the model (Akaike 1978; Burnham and Anderson 2002). I chose the top model by evaluating the relative likelihood of each model (w_i), but present all close competitors to the top model (models ≤2 ΔAIC from the top model; Burnham and Anderson 2002) as well. Using AIC, I also calculated the relative importance (how important a

variable is as an explanatory variable relative to the other variable in the model) and model-averaged estimates (β) of each variable given the data set. To analyze the consistency of mite load rankings among individual lizards captured during 2016, I calculated Kendall's coefficient of concordance. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) with p-values being considered significant at the $\alpha = 0.05$ level.

Results

Seasonal Variation in Environmental Mite Abundance and Host Mite Loads

Environmental mite abundance and mite loads exhibited significant seasonal variation (mite abundance: $F_{4,737} = 33.82$, $P < 0.001$; mite loads: $F_{4,1403} = 473.85$; $P < 0.001$; Table 3.1). Environmental mite abundance was low in May, high in June and July, and low again in August and September (Table 3.1). Mite loads were low in May, increased in June, peaked in July, and gradually decreased thereafter (Table 3.1; Fig. 3.1; Fig. 3.2).

Mite loads on individual lizards in July were positively correlated with individual environmental mite abundances in June ($n = 105$, $r_s = 0.24$; $P = 0.015$) and July ($n = 209$, $r_s = 0.27$; $P < 0.001$). Mite loads on individual lizards in August were positively correlated with individual environmental mite abundances in July ($n = 120$, $r_s = 0.18$; $P = 0.050$) and August ($n = 202$, $r_s = 0.14$; $P = 0.047$).

Sex- and Age-Based Seasonal Variation in Mite Loads

Mite loads were best approximated by a model including main effects of Julian Day and Age and interaction effects of Julian Day x Age, Julian Day x Sex, and Sex x Age (w_i = 0.2978; Table 3.2). Sex differences in mite load were heavily influenced by age (Sex x Age relative importance = 0.9934; P < 0.001; Table 3.3) and moderately influenced by time of the year (Julian Day x Sex relative importance = 0.5848; P = 0.035; Table 3.3). As adults, females had higher average mite loads than males in June and July (Fig. 3.1A), but as yearlings, males had higher average mite loads than females during July, August, and September (Fig. 3.1B).

Age differences in mite load were influenced by time of year (Julian Day x Age relative importance = 0.8645; P < 0.001; Table 3.3). In females, average mite loads were higher on yearlings than adults in August and September (Fig. 3.2A), but in males, average mite loads were higher on yearlings than adults from June to September (Fig. 3.2B).

Regardless of sex and age, individual rankings of mite load exhibited consistent patterns (df = 44, Kendall's W = 0.4851, X^2 = 106.72, P < 0.001). Lizards with high mite loads during week 1 were likely to have high mite loads the following weeks. In contrast, lizards with low mite loads were likely to have low mite loads throughout the following weeks. In other words, lizards that ranked first, second, third, and so on in mite load during week 1 were likely to rank first, second, third, and so on in mite load in the subsequent weeks, even if mite loads increased in all lizards.

Discussion

In the present study, I investigated the seasonal abundance of ectoparasites in the environment together with seasonality of sex- and age-specific patterns of ectoparasitism in the host-parasite system of eastern fence lizards (*S. undulatus*) and chigger mites (*E. alfreddugesi*). Previous studies have documented seasonal patterns of host ectoparasite loads (Talleklint-Eisen and Eisen 1999; Godfrey et al. 2008; Lumbad et al. 2011) or seasonal patterns of environmental ectoparasite abundances (Clopton and Gold 1993; MacDonald and Briggs 2016). However, my study is one of the few studies to investigate seasonal variation in ectoparasite loads and environmental ectoparasite abundance in the same study. I found a strong temporal association between lizard mite loads and environmental mite abundance across months. Furthermore, mite loads on individual lizards in July were correlated with environmental mite abundances measured at the specific sites where the lizards were captured in June and July. Similarly, mite loads on individual lizards in August were correlated with environmental mite abundances where the lizards were captured in July and August. These findings indicate that the abundance of mites in the environment is a prime determinant of host lizard mite loads.

Two other studies to date have investigated seasonal variation in ectoparasite loads and environmental ectoparasite abundance in the same study. Curtis and Baird (2008) investigated the seasonality of mite loads and environmental mite abundance using collared lizards (*Crotophytus collaris*) and found that there was seasonality to mite parasitism and environmental

abundance, but lizard mite loads and environmental mite abundance were temporally dissociated. This temporal dissociation between lizard mite loads and environmental mite abundance is likely due to the fact that the abundance of non-parasitic adult mites was monitored, not the abundance of the parasitic larval stage. Curtis and Baird (2008) do make note of this, however, and report that larval mites parasitize lizards after adult mites disappear in early June. This observation suggests that adult mites descend into the soil to oviposit in late May, after which parasitic larvae emerge in June to search for and feed on lizard hosts. This could similarly explain the low mite loads and low environmental mite abundance observed during May in this study. The parasitic larvae present in the soil had not emerged and it requires increased air temperatures in spring to stimulate egg development and larval hatching. As a result, the large increases in mite loads and environmental mite abundance observed in June could be the result of newly emerged larval mites. The seasonal pattern of mite development and timing of different life stages are still unclear, however, and require further investigation (Shatrov and Kudryashova 2006).

The other study to date to investigate seasonal patterns of ectoparasite loads and environmental ectoparasite abundances reported results similar to those presented here. Klukowski (2004) found that *S. undulatus* in Tennessee had low mite loads in May and August, but high mite loads in June and July. Lizards in that study, however, obtained peak mite loads in June, approximately a month earlier than what occurs in New Jersey. Environmental mite abundance in that study also remained high for a longer period of time, extending into August

before gradually decreasing into October (Klukowski 2004). The differences in timing of mite loads and environmental mite abundances between my study in New Jersey and the study by Klukowski (2004) in Tennessee suggest that patterns of mite loads vary depending upon geography. Schall et al. (2000) reported that mite loads on *S. occidentalis* increased from spring to summer in northern California (summer to autumn changes not reported) and Goldberg and Bursey (1991) reported that mite loads declined dramatically from June to September on *Uta stansburiana* in southern California. However, mite loads on *U.* stansburiana were similar in early spring and early summer, which is in contrast to what occurs w*ith S. undulatus in New Jersey and Tennessee (Klukowski 2004) and S. occidentalis* in northern California (Schall et al. 2000). In contrast to these studies and the present study, Lumbad et al. (2011) reported that mite loads on *S. occidentalis* in central California were high in spring, decreased in summer, and remained low in autumn. In south Texas, Mather (1979) reported relatively high mite loads and 100% infestation prevalence on *S. undulatus* in late autumn. This is in sharp contrast to what has been found in California (Goldberg and Bursey 1991; Schall et al. 2000; Lumbad 2011), Tennessee (Klukowski 2004), and New Jersey studies.

The geographic variation in patterns of seasonal mite loads is likely the result of climatic differences between the regions and subsequent impacts on the abundance of mites in the environment. Studies have demonstrated strong effects of temperature and humidity on environmental mite abundance. Mite activity is confined to the warmer times of the year (Sasa 1961) and, as a result,

mites are active for longer periods of time in warmer climates and produce more generations per year (Wharton and Fuller 1952). Environmental mite abundances tend to be highest in areas with high humidity and moderate temperatures, and mite activity is determined by a combination of temperature and humidity, rather than by either of these variables alone (Jenkins 1948; Clopton and Gold 1993). Rubio and Simonetti (2009) reported chigger mite prevalence and loads on lizards across habitats of varying temperature and humidity combinations. All lizards were parasitized by mites, but lizards in habitats characterized by lower temperatures and higher humidity had almost twice as many mites compared to lizards found in habitats characterized by higher temperatures and lower humidity. In another study Zippel et al. (1996) found that *Anolis* lizards inhabiting mesic habitats with high moisture and moderate temperatures were more heavily infested than lizards in more xeric habitats.

Based on the commonly observed relationship between T and parasitism, I predicted that mite loads would be higher in males than females and that this sex difference would be most prominent in adults during the spring breeding season (the time of year when circulating T is highest in males; John-Alder et al. 2009). Further, I predicted that mite loads would be highest during the mid- to late-summer months in yearling males (the time of year when circulating T is high; Cox et al. 2005). My predictions were only partially supported by my findings. In agreement with my predictions, I found no clear differences in mite load between male and female yearlings until July, when environmental mite abundance is high and and circulating T is high in yearling male *S. undulatus*.

Circulating levels of T remain high into August and early September (Cox et al. 2005) and, coinciding with these months, yearling males had higher mite loads than yearling females. This male-biased pattern of parasitism is in accordance with several other studies on lizards (Schall and Marghoob 1995; Talleklint-Eisen and Eisen 1999; Klukowski and Nelson 2001; Salkeld and Schwarzkopf 2005; Heredia et al. 2014; Dudek et al. 2016). In contrast to my predictions, I failed to find a male-bias in mite load in adults. Rather, I found that adult females had higher mite loads than adult males in June and July, months in which circulating T is lower in adult males than at any other time during the activity season (John-Alder et al. 2009).

The male-bias in yearling mite loads from July to September may be a result of increased behavioral exposure to chigger mites. In May and June, yearling males have low circulating levels of T and small home range sizes (Skelly and John-Alder 2002; Cox et al. 2005; John-Alder, unpublished). Although yearling males have low T during these months, their T levels are higher than yearling females. Despite this, however, mite loads do not differ between males and females, which can probably be attributed to the observation that environmental mite abundance was very low until June. From July to September yearling males had higher mite loads than yearling females. This sex difference in mite load coincides with a time in which mite abundance in the environment is high and, similar to what occurs in other lizard species, the seasonal elevation in T increases the frequency of territorial behaviors (Marler and Moore 1989; Klukowski and Nelson 1998; Skelly and John-Alder 2002;

Weiss and Moore 2004), home-range size (DeNardo and Sinervo 1994; Skelly and John-Alder 2002; Cox et al. 2005), daily activity (Marler and Moore 1989; DeNardo and Sinervo 1994; Klukowski et al. 2004; Cox et al. 2005), and movement (Marler and Moore 1989; Olsson et al. 2000; Cox et al. 2005), all of which likely increase exposure to questing mites and could lead to higher male mite loads. Ectoparasites, such as ticks and mites, exhibit preferences for particular habitats, and their abundances are under strong influence from temperature, humidity, and precipitation (Clopton and Gold 1993; Eisen et al. 2002). Therefore, male yearlings could become increasingly infested with mites if they were to increase their movements through preferred microhabitats abundant in mites (Zippel et al. 1996; Schlaepfer 2006; Curtis and Baird 2008; Bulté et al. 2009; Rubio and Simonetti 2009).

Another way in which yearling male lizards could become more heavily infested with chigger mites is through T-based immunosuppression, allowing more mites to feed and survive (Veiga et al. 1998; Olsson et al. 2000; Poiani et al. 2000; Hughes and Randolph 2001). Increased movement, daily activity, home range size, and male-typical behaviors, which increase in yearling males during July to September, are regulated by T and are energetically expensive (Marler and Moore 1988; Marler et al. 1995; Haenel et al. 2003; Cox et al. 2005). These behaviors likely come at a cost to other physiological processes, such as immune function, when resources are limiting (French and Moore 2008; Ruiz et al. 2010). Several studies have shown an immunosuppressive effect of T (Saad et al. 1990; Kamis et al. 1992; Hillgarth and Wingfield 1997; Veiga et al. 1998; Duffy et al.

2000; Casto et al. 2001; Hughes and Randolph 2001; Andersson et al. 2004; Belliure et al. 2004; Roberts et al. 2004; Tripathi and Singh 2014), and the immune system can be energetically expensive, not necessarily to maintain, but to activate (Lochmiller and Deerenberg 2000; Ots et al. 2001; Bonneaud et al. 2003; Brace et al. 2015). These findings suggest that during times when circulating T is high in yearling male lizards, yearling males are exhibiting energetically expensive characteristics, such as increased daily activity, and home range size, at the cost of immune function. This is still a tentative concept, however, since numerous studies have failed to find a suppressive effect of T alone on immune function (Hasselquist et al. 1999; Bilbo and Nelson 2001; Greenman et al. 2005; Buchanan et al. 2003; Roberts et al. 2004, 2009; Ruiz et al. 2010). Further studies are required to examine the interplay between T and the immune response with ectoparasites of reptiles.

For male lizards, T may very well increase susceptibility to mites, whether through behavioral, immunosuppressive, or other mechanisms. Adult males would, therefore, be expected to have higher mite loads in May when their circulating T levels are high and yearling males would be expected to have higher mite loads from June to August when their T levels are high (Cox et al. 2005; John-Alder et al. 2007). This prediction was partly met in that yearling male lizards did indeed exhibit higher mite loads from June to August when their T levels are high and T increases home range size, daily activity, and movements while decreasing growth (Skelly and John-Alder 2002; Cox et al. 2005). Adult males, despite their elevated T levels during the spring breeding months of April

and May (John-Alder et al.2009), did not significantly differ in mite loads from females in May. This can be attributed to the fact that all lizard mite loads and the abundance of mites in the environment were extremely low during this month. Testosterone may very well increase susceptibility to ectoparasites, but if the ectoparasites are absent or rare during that time of year, then a sex difference in ectoparasitism would fail to occur. Overall, the timing of peak mite loads and growth inhibition in yearling males suggests that mites may contribute to sex-specific growth rates by decreasing growth in yearling males.

The female-bias in adult mite loads during June and July in the present study may be the result of immunosuppression, which could be through an energetic trade-off with reproduction, allowing more mites to feed and survive (Veiga et al. 1998; Olsson et al. 2000; Poiani et al. 2000; Hughes and Randolph 2001). Vitellogenesis and gravidity are energetically expensive and account for the high reproductive effort in females (Tinkle 1969; Tinkle and Hadley 1975; Beuchat and Vleck 1990; Sugg et al. 1995). For example, gravid *S. undulatus* females had metabolisms elevated by 122% compared to non-gravid females (Angilletta and Sears 2000). Studies on *Plestiodon laticeps* and *Sceloporus virgatus* have shown that gravid females become less active, forage less for food, and take smaller meals, thus exacerbating the energetic costs of reproduction (Cooper et al. 1990; Weiss 2001). It is possible that adult female *S. undulatus* show a similar decrease in foraging and meal size during gravidity.

Peak vitellogenesis for *S. undulatus* occurs in May and females are gravid until egg deposition in approximately early-June (Phillips and Klukowski 2008;

John-Alder et al. 2009). Timing of vitellogenesis and gravidity in *S. undulatus* coincides with times of low environmental mite abundance. However, the energetic costs of vitellogenesis and gravidity likely extend beyond their duration since following egg deposition, adult females are significantly lower in body condition (John-Alder, unpublished) and, in several other species, adult females exhibit decreased sprint speed following egg deposition (de la Cruz 1988; Sinervo et al. 1991; Miles et al. 2000; Weiss 2001). Therefore, when mites become abundant in the environment (mid-June to July), adult females are in low body condition and perhaps immunosuppressed, thus potentially allowing more mites to feed and survive on adult females than adult males. Decreased body condition has been shown to be correlated with decreased immune function (Alonso-Alvarez and Tella 2001; Navarro et al. 2003; Amo et al. 2006; Ujvari and Madsen 2006) and studies investigating the inter-relationships between female reproduction and immune function have found that increased reproductive effort comes with a decrease in immune function and vice versa (Uller et al. 2006; French et al. 2007a; Harshman and Zera 2007; French and Moore 2008). However, this immune-reproductive trade-off occurs only under conditions in which animals are food-limited (French et al. 2007b; Ruiz et al. 2011). Further studies are required, to examine the interplay between female reproductive effort, immune function, and parasite loads. Furthermore, high parasite loads in adult females may not be through immunosuppression exclusively, but may be influenced by other mechanisms as well, such as behavior and body size.

Additional studies are needed to explore these possibilities and to clarify mechanisms driving high parasite loads in females.

The majority of studies investigating the effects of age on parasitism in reptiles have found higher parasite loads on older individuals than younger individuals (Amo et al. 2004; Reardon and Norbury 2004; Dudek et al. 2016). This strong age effect could be the result of body size, since reptiles, as in other ectothermic taxa, exhibit indeterminate growth where older individuals are typically larger in body size than younger individuals (Halliday and Verrell 1988). Larger body size, and therefore older age, means an individual has more space for parasites to colonize and likely more resources for parasites to acquire (Pacala and Dobson 1988; Rocha et al. 2008; Halliday et al. 2014). Larger body size has been shown to be correlated with higher parasite loads in several lizard species (Rocha et al. 2008; Rubio and Simonetti 2009; Halliday et al. 2014; Dudek et al. 2016). Larger body size may also increase parasite loads through relationships with movement and home range size, both of which tend to increase with age and increased body size in lizards (Schoener and Schoener 1982; Rocha 1999; Perry and Garland Jr. 2002). In adult male *S. undulatus*, home range size increases with SVL, and older, larger males move greater distances than younger, smaller males (Haenel et al. 2003), both of which would presumably increase exposure to mites.

Interestingly, older, larger lizards did not have higher mite loads in the present study. Rather, just the opposite was seen, with yearling males having higher mite loads than adult males in all months except May, and yearling

females having higher mite loads than adult females in August and September. Within males, yearlings could have higher mite loads from June onwards because mites are abundant in the environment during those months and because circulating T levels are elevated (Cox et al. 2005), which, regardless of the mechanism, could increase mite loads. It is possible that adult males would have had higher mite loads than yearling males in May (when adult male T is high; John-Alder et al. 2009) had mites been abundant during this month. Within females, yearlings exhibit high growth rates from July to September (John-Alder et al. 2007) and could have higher mite loads in August and September because of energetic trade-offs between growth and immune function. Both of these processes are energetically expensive (Peterson et al. 1999; Bonneaud et al. 2003; Martin et al. 2003; Cox et al. 2008; Brace et al. 2015) and could trade-off under limiting resources, thus allowing more mites to feed and survive on yearling females.

In summary, sex-biases in mite parasitism are dependent on age and time of the year in *S. undulatus*. Age-biases in mite parasitism are also present, and are dependent upon sex and time of the year. The overriding determinant of mite loads, however, in both yearlings and adults, is seasonal variation in environmental mite abundance. Future studies should focus on determining the physiological and behavioral mechanisms that give rise to seasonal patterns of female- and male-biased ectoparasite loads. Studies should also investigate the direct and indirect effects of T and reproductive effort on immune function and the influence of T and reproductive effort on activities and behaviors, which may

increase exposure to ectoparasites. Lastly, studies are needed to describe the local population dynamics of chigger mites and the abiotic factors mediating local environmental mite abundance and mite prevalence on hosts.

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Table 3.1: Mean (± 1 SEM) environmental mite abundances (# of mites), mite loads (# of mites), and mite prevalence (% of lizards with at least 1 mite) for the 2014 and 2015 activity seasons at Colliers Mills. Sample size (n) is the number of mite abundance sampling locations or the number of lizards in which mite loads were measured. Environmental mite abundances were low in May, high in June and July, and low again in August and September. Mite loads were low in May, increased in June, peaked in July, and gradually decreased thereafter. A low percentage of lizards were infested with mites in May, but thereafter a high percentage of lizards were infested with mites.

	May	**June**	**July**	**August**	**September**
Mite Abundance	1.14 ± 0.21 (112)	10.15 ± 1.06 (160)	9.16 ± 0.98 (160)	1.86 ± 0.34 (189)	0.12 ± 0.04 (121)
Mite Load	0.8 ± 0.14 (235)	28.9 ± 1.63 (376)	94.9 ± 3.28 (316)	68.3 ± 2.54 (310)	20.4 ± 1.33 (171)
Mite Prevalence	26.8	90.5	99.4	99.0	98.2

Table 3.2: Model selection results using Akaike Information Criteria (AIC) evaluating the impacts of season (Julian Day), Sex, Age, and interactions on host lizard mite loads. Only models with ≤2 ΔAIC (substantial evidence for the model; Burnham and Anderson 2002) from the top model are shown. All models included an intercept (α). The best-supported model is shown in bold. w_i = model weight, indicating the likelihood that the model is best given the data.

Model	Model Variables	# of Parameters	AIC	ΔAIC_i	w_i	R^2
1[a]	**Julian Day, Age, Julian Day x Age, Julian Day x Sex, Sex x Age**	**6**	**3571.9937**	**0.0000**	**0.2978**	**0.2069**
2[b]	Julian Day, Age, Sex, Julian Day x Age, Sex x Age	6	3571.9993	0.0059	0.2969	0.2069
3[c]	Julian Day, Age, Sex, Julian Day x Age, Julian Day x Sex, Sex x Age	7	3573.9404	1.9406	0.1125	0.2069

[a] Model 1: Mite Load = $\alpha + \beta_1$Day + β_2Age + β_3Day•Age + β_4Day•Sex + β_5Sex•Age
[b] Model 2: Mite Load = $\alpha + \beta_1$Day + β_2Age + β_3 Sex + β_4Day•Age + β_5Sex•Age
[c] Model 3: Mite Load = $\alpha + \beta_1$Day + β_2Age + β_3 Sex + β_4Day•Age + β_5Day•Sex + β_6Sex•Age

Table 3.3: Relative importance, model averaged estimates, and ANOVA statistics for variables included in the top model evaluating the impacts of season (Julian Day), Sex, Age, and interactions on host lizard mite loads. The larger the relative importance value, the more important that variable is as a predictor given the data. Details of analyses are described in the text.

Model Variable	Model-Averaged Estimates	Relative Importance	F-Statistic (ANOVA)	P-Value (ANOVA)
Sex x Age	1.053 ± 0.267	0.9939	15.63	< 0.001
Julian Day	2.671 ± 0.947	0.9387	73.26	< 0.001
Age	-0.882 ± 1.051	0.8659	56.95	< 0.001
Julian Day x Age	-0.768 ± 1.364	0.8645	2.83	< 0.001
Julian Day x Sex	-0.013 ± 1.164	0.5848	1.48	0.035

Figure 3.1: Mite loads recorded on adult (A: solid red = females, solid blue = males) and yearling (B: open blue: males, open red = females) lizards during the 2014 and 2015 activity seasons at Colliers Mills. Circles represent mite loads of individual lizards. Horizontal bars represent mean monthly mite loads on adults (A: blue = males, red = females) and yearlings (B: blue = males, red = females). Mite loads followed a seasonal pattern with peak ectoparasitism occurring in July. As adults, females had higher mite loads than males in June and July. As yearlings, males had higher mite loads than females from July to September.

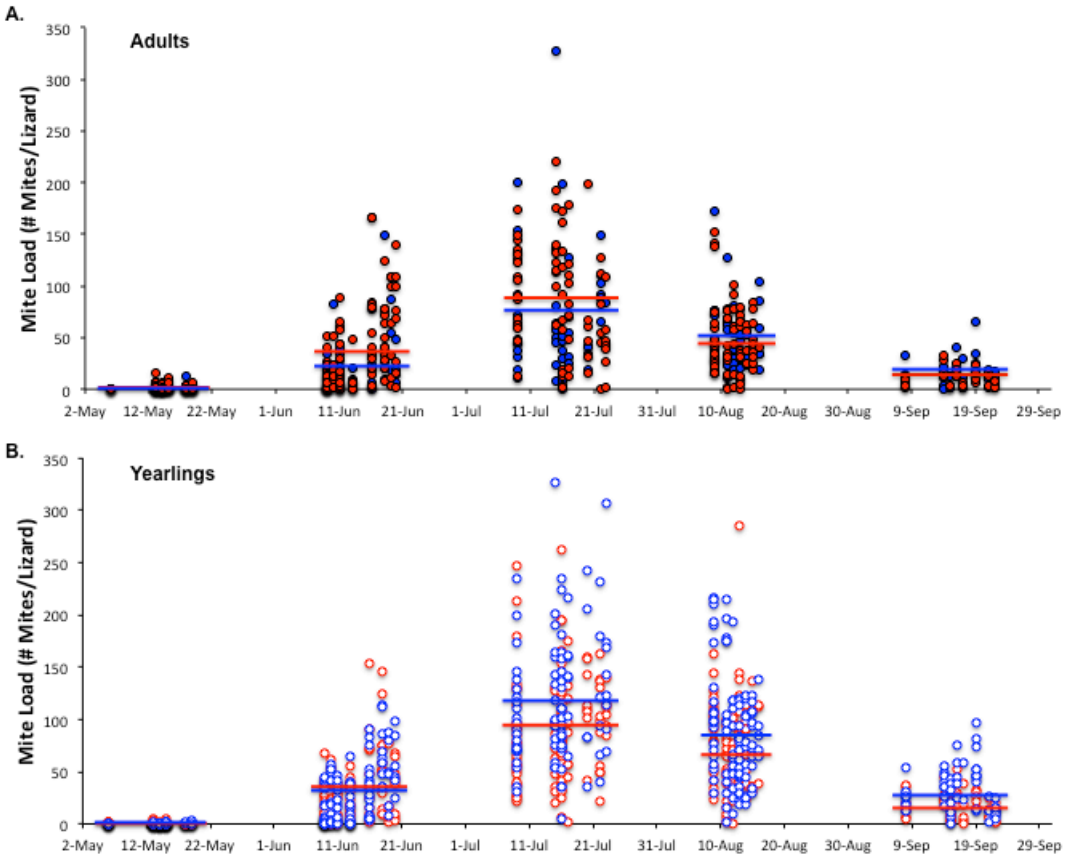
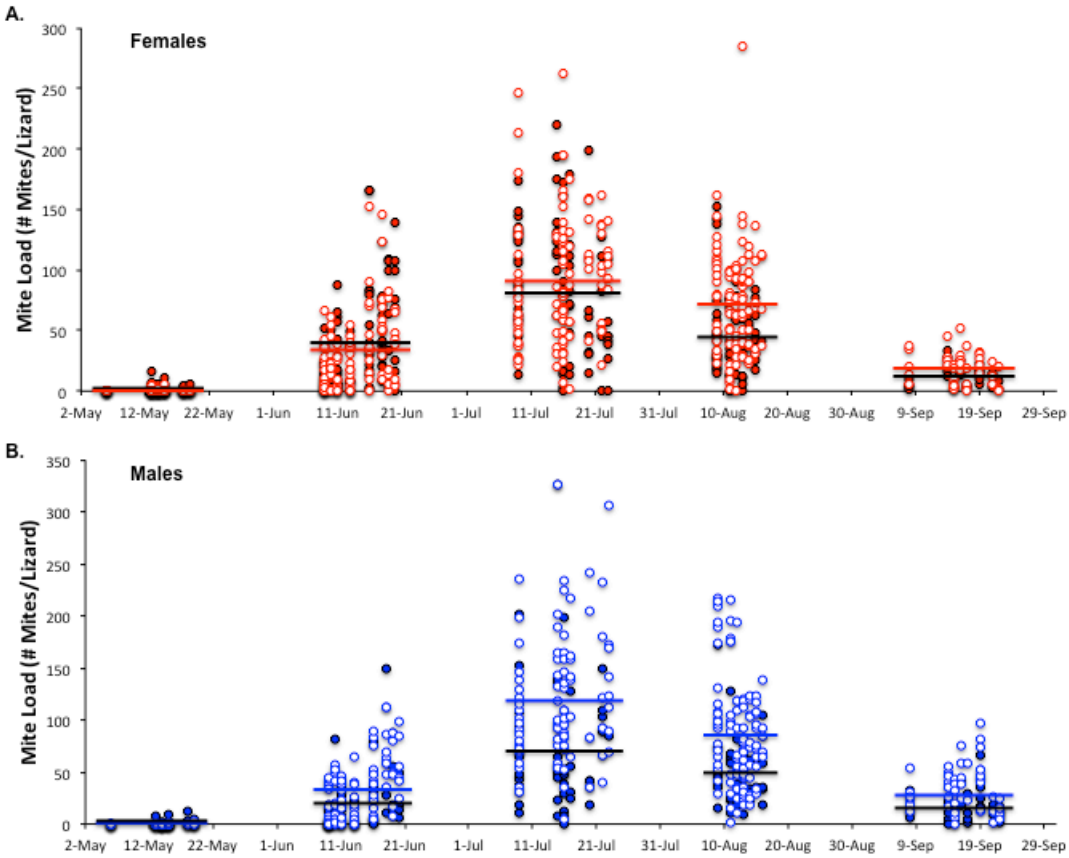


Figure 3.2: Mite loads recorded on female (A: solid red = adults, open red = yearlings) and male (B: solid blue = adults, open blue = yearlings) lizards during the 2014 and 2015 activity seasons at Colliers Mills. Circles represent mite loads of individual lizards. Horizontal bars represent mean monthly mite loads on females (A: black = adults, red = yearlings) and males (B: black = adults, blue = yearlings). Mite loads followed a seasonal pattern with peak ectoparasitism occurring in July. Yearling females had higher mite loads than adult females in August and September while yearling males had higher mite loads from June to September.



CHAPTER 4

ECTOPARASITISM AND GROWTH IN THE FEMALE-LARGER LIZARD, *SCELOPORUS UNDULATUS*

Abstract

Testosterone (T) is an important regulator of growth across vertebrate taxa. In lizards, T has been shown to regulate sex-specific growth rates, promoting male growth in male-larger species, while inhibiting male growth in female-larger species. In eastern fence lizards (*Sceloporus undulatus*), a female-larger species, T inhibits male growth directly through androgenic molecular mechanisms. However, T may also inhibit male growth through indirect mechanisms, including increased ectoparasitism. In the eastern United States, yearling *S. undulatus* can be heavily infested with chigger mites (family: Trombiculidae) at the same time that growth rate is highest during the first activity season. The purpose of the present study was to investigate the relationship between growth and mite parasitism in *S. undulatus* to test two hypotheses: (1) that mites decrease growth in yearling lizards, and (2) that costs to growth are particularly high for males because they have higher mite loads. Mite loads and growth rates were quantified in yearling lizards during the 2014 and 2015 activity seasons in the New Jersey Pinelands. Growth rate decreased with increased mite load in males from June to August, coinciding with a time of maximal growth inhibition. A correlation between growth and mite load was not observed in females. These findings suggest that mites may impose a greater cost in yearling

males than in yearling females, and that mites may contribute to growth inhibition in yearling males and the development of sexual size dimorphism.

Introduction

Testosterone (T) has been shown to be a major coordinator for the sexual and seasonal development of male secondary sex characteristics, which contribute to male reproductive success. These characteristics include increased activity, endurance, territorial behaviors, and home range size (Moore 1986; Marler and Moore 1989; Sinervo et al. 2000; Weiss and Moore 2004). These characteristics have been linked to higher male reproductive success through increased access to females, increased copulations, and a greater number of sired offspring (Lewis and Saliva 1987; Olsson 1992; Olsson et al. 2000; Bajer et al. 2012). Testosterone is also typically considered a promoter of growth, leading to increased male growth and larger male body size (Kuwaye et al. 1993; Borski et al. 1996; Navara et al. 2005; Husak et al. 2007; Cox and John-Alder 2005; Cox et al. 2009; 2014). In return, larger body size in males has been linked to greater male reproductive success (Olsson and Shine 1996; Gullberg et al. 1997; Lewis et al. 2000; Stapley and Keogh 2006).

Despite the mating advantages of large body size (Tokarz 1985; Olsson 1992; Luiselli 1996; Gullberg et al. 1997; Stapley and Keogh 2006; John-Alder et al. 2009; Bajer et al. 2011) and the positive effects of T on morphological and behavioral traits that increase reproductive success (Olsson et al. 2000; Cox et al. 2005a,b; Cox et al. 2009), T inhibits growth in some female-larger lizard

species, contrary to what occurs in male-larger species (Cox and John-Alder 2005; Cox et al. 2005a). Growth inhibition by T in eastern fence lizards (*Sceloporus undulatus*) is regulated through direct androgenic mechanisms (e.g., androgen receptors; see Chapter 2) involving decreased expression of insulin-like growth factor-1 (IGF-1; Duncan 2011). This is contrary to what occurs in male-larger species of other taxa (Larsen et al. 2004; Norbeck and Sheridan 2011). Along with the direct effects of T on molecular mechanisms of growth inhibition in male lizards, inhibition of growth may also reflect an energetic trade-off resulting from increased activity and ectoparasitism (Cox et al. 2005a).

In reptiles, males often have higher parasite loads than females (Tälleklint-Eisen and Eisen 1999; Amo et al. 2005; Aubret et al. 2005; Salkeld and Schwarzkopf 2005; Vaclav et al. 2007; Dudek et al. 2016), and male-biased parasitism is hypothesized to be linked to higher circulating T in males than in females (Folstad and Karter 1992; Shalk and Forbes 1997; Poulin and Forbes 2012). Many studies of the relationships between T and parasitism have involved lizards and ectoparasites (e.g., ticks and mites), and the positive correlation between T and ectoparasites is strong according to a meta-analysis by Roberts et al. (2004). Experimental studies have shown that T increases parasite loads in several species (Salvador et al. 1996; Olsson et al. 2000; Klukowski and Nelson 2001; Cox et al. 2005a; Cox and John-Alder 2007; Pollock et al. 2012). In *S. undulatus*, yearling males have higher mite loads than yearling females in July, August, and September (see Chapter 3), coinciding with higher circulating levels of T and slower growth in males compared to females, and to the onset of sex-

specific growth rates and sexual divergence in body size (Haenel and John-Alder 2002; Cox et al. 2005a). This suggests that high mite parasitism may act as a potential T-induced indirect growth inhibitor in yearling males and may contribute to sex-specific growth rates and the development of sexual size dimorphism (SSD) in *S. undulatus*.

Ectoparasitism is associated with decreased growth and body condition in several mammalian and avian species (Vuren 1996; Merino and Potti 1995; Saino et al. 1998; Merino et al. 1999; Fitze et al. 2004; Devevey et al. 2008; Brommer et al. 2011). Studies involving reptiles have focused on lizards and acarine ectoparasites (ticks and mites). Higher mite loads were correlated with increased mortality, lower growth rate, and lower body mass in *Lacerta vivipara* (Sorci and Clobert 1995; Clobert et al. 2000). Uller and Olsson (2003) found that prenatal T led to increased growth rate in male-larger *L. vivipara*, but growth rate significantly decreased when exposed to ticks. In the female-larger species, *Sceloporus virgatus*, mite loads were positively correlated with levels of T and negatively correlated with growth rates (Cox and John-Alder 2007). Ectoparasitism, however, may not always be negatively correlated with growth and body condition of their lizard hosts (Schlaepfer 2006). In the present study, I investigated the relationships between mite load, body size, and growth in a New Jersey population of *S. undulatus* to test two hypotheses: (1) that mites decrease growth in yearling lizards, and (2) that costs to growth are particularly high for males because they have higher mite loads.

Materials and Methods

Field Data Collection

This study was carried out during 5-month periods (May–September) of 2014 and 2015 at two different locations (37 km apart) in the New Jersey Pinelands: Colliers Mills Wildlife Management Area (40°07'N, -74°43'W) and the Rutgers University Pinelands Research Station (39°91'N, -74°59'W). These two locations are both dominated by oak-pine forests, but Colliers Mills is heavily managed with extensive edge habitat between open fields and forested tracts and its forests tend to have more open canopy and a forest floor with a sparser understory. In 2015, this study was conducted only at Colliers Mills due to the low abundance of lizards at the Rutgers Pinelands Research Station.

Yearling (first full activity season) lizards were captured once a month by noosing or by hand. Upon capture, SVL (to the nearest 0.5 mm) and body mass (to the nearest 0.1 g) were measured using a ruler and Pesola scale, and sex was determined by the presence or absence of enlarged post-anal scales. Mite load was measured by counting the number of chigger mites using a 10x hand lens. Mites were found most commonly in the neck pockets ("mite pockets"), dorsal and ventral surfaces of the head and neck, and axillary pits. Mites occasionally occurred around the cloaca and, in heavily infested lizards, around eyes. Repeatability of mite counts was measured by performing 5 repeated counts of mite load for 10 different lizards (mite loads ranging from 10 to 129). Repeatability of mite counts was extremely high (correlation = 0.996, SD = 2.10). Each lizard was marked and identified with a unique toe-clip number, which

allowed for multiple recaptures and quantification of individual variation in ectoparasitism across months. Lizards were also marked using a paint pen to identify lizards from a distance and prevent multiple recaptures during a single month. Animal capture was approved by the New Jersey Department of Environmental Protection, Division of Fish and Wildlife (Permit #2014086, #2015090).

Quantification of Growth Rates

To examine the relationship between ectoparasitism and growth of yearling lizards, growth rates were calculated and analyzed with respect to mite load. Growth rates (mm/day) were assumed to be linear and were calculated using monthly measurements of SVL from recaptured lizards. Specifically, growth rates were calculated for lizards captured during the May to June, June to July, July to August, and August to September time periods.

Statistical Analyses

Mite loads and growth rates from 2014 and 2015 were combined for all analyses. To analyze the relationships between body size (SVL) and growth, and between body size and mite load, I performed analysis of variance (ANOVA) on log-transformed data. To analyze the correlation between mites and growth, I first removed the variance associated with body size by calculating the residuals of lizard growth rates and mite loads based on mean values for each sex/age class for June to July and July to August. I selected these time periods because they

encompass the months with significant mite loads and when yearling lizards are growing the most. The 'size corrected' residuals of growth rate and mite load were then analyzed using ANCOVA for yearling males and yearling females. To compare the growth rates of males and females, I analyzed 'size corrected' residuals of growth rates using ANCOVA. For each ANCOVA, initial SVL was used as a covariate and the homogeneity of slopes assumption was verified. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) with p-values being significant at $\alpha = 0.05$.

Results

Growth rate decreased with increased SVL in both yearling males and yearling females (males: $F_{1,80} = 53.40$, $P < 0.001$; females: $F_{1,106} = 73.05$, $P < 0.001$; Fig. 4.1). Mite loads increased with SVL in both sexes (males: $F_{1,80} = 4.26$, $P = 0.042$, Fig. 4.2A; females: $F_{1,106} = 8.09$, $P = 0.005$, Fig. 4.2B), but for any given mite load, yearling females grew significantly faster than yearling males ($F_{3,186} = 87.08$, $P < 0.001$; Fig. 4.3). Regarding mites and growth, residual growth rate was negatively correlated with residual mite load in yearling males ($F_{1,80} = 4.74$, $P = 0.033$; Fig. 4.3), but there was no significant correlation between residual mite load and residual growth rate in yearling females ($F_{1,106} = 0.43$, $P = 0.514$; Fig. 4.3).

Discussion

Sceloporus undulatus is a female-larger species of lizard in which sexual divergence in body size and the development of SSD becomes significant by July of the first full activity season (≈ 10 months of age; Haenel and John-Alder 2002; John-Alder et al. 2007). By September (≈ 13 months of age) females are approximately 12% larger than males as measured by SVL, and this SSD continues into adulthood (Haenel and John-Alder 2002). I found that yearling females grew significantly faster than yearling males, regardless of mite load, and that both sexes decreased in growth with increased body size, which has been reported in other studies (Ricklefs et al. 1998; Cox et al. 2006, 2009). The sex difference in growth was greatest from June to August. The sexual divergence in body size from June to August of the first full activity season coincides with the months of highest mite loads on lizards. Yearling males, which grow less quickly than females, have the highest mite loads of any sex/age class during these months (see Chapter 3). The association of suppressed male growth and higher mite loads (compared to females) suggests that mite parasitism may decrease growth rate in males.

In the present study, I found a negative correlation between mite load and growth rate in yearling male *S. undulatus*, suggesting a growth cost of mite parasitism from June to August. Mite load was not correlated with growth rate in yearling females. Parasites can reduce growth and body size of their host (Vuren 1996; Saino et al. 1998; Merino et al. 1999; Uller and Olsson 2003; Brommer et al. 2011). Previous studies with lizards have focused primarily on the effects of ectoparasites on growth in males, and several of these have demonstrated a

negative correlation of ectoparasites on growth. Cox and John-Alder (2007), for example, found a negative relationship between residual measures of mite load and growth rate in male *S. virgatus*, suggesting a growth cost to mite ectoparasitism. A study by Schlaepfer (2006), however, failed to find a correlation between chigger mites and growth of male and female anoles (*Norops polylepis*). The lack of a correlation may have been the result of adults being included in analyses of growth and mite load, even though they had reached their asymptotic growth curves, and the fact that lizards had very low mite loads altogether (juvenile mean: 0.7 mites; adult mean: 6.0 mites; range: 0-37 mites). In an experiment investigating the relationship between T and mite loads in *S. undulatus*, Klukowski and Nelson (2001) found that male lizards with the most mites experienced the smallest gains in body mass from July to August.

During June and July, yearling male *S. undulatus* are experiencing a seasonal peak in circulating T and are beginning to expand their home range size, daily activity, and endurance (Klukowski et al. 1998; Haenel et al. 2003; Cox et al. 2005a; John-Alder et al. 2009). Increases in these male traits result in increased energetic requirements (Marler and Moore 1989; Marler et al. 1995; Klukowski et al. 2004) and potentially increased exposure to questing mites. At the same time, growth rate in males is only about half the rate of females (Haenel and John-Alder 2002), possibly due to direct androgenic inhibition of IGF-1 expression (see Chapter 2) and indirect androgenic effects on energy allocation (Cox et al. 2005a; Duncan 2011; Duncan et al. 2015). Cox et al. (2005a) estimated the total energetic cost of growth to be approximately 130 Joules/Day

for *S. undulatus*. Further estimates put the energetic costs of T-induced increases in daily movement and daily activity of yearling males at approximately 6–22 J/Day and 102 J/Day, respectively (Cox et al. 2005a). This equates to roughly 84–91% of the reduction in yearling male growth during June and July.

While mites may not be the primary cause of male growth inhibition in *S. undulatus*, high mite loads from June to August (see Chapter 3) may exacerbate the situation since ectoparasites, such as mites, feed by acquiring nutrients and energy directly from the host (Price 1980; Hogue 1983; Møller et al. 1994). Mites, then, may add to the energetic stresses experienced by males during the months of growth inhibition. Using estimates of chigger mite body size (body length = .4 mm, body mass = 9μg; Johnson and Strong 2000) and the standard metabolic scaling exponent (0.75; West et al. 2002), I estimated the energy requirement of a single chigger mite to be approximately 0.004 J/Day. Taking the mean mite loads of yearling male lizards in June (29 mites), July (117 mites), and August (90 mites), and assuming all energy consumed by mites is deducted directly from the energy budget of the host lizard, this equates to a 0.116 J/Day, 0.468 J/Day, and 0.360 J/Day energetic cost to a host yearling male lizard. This is roughly 0.1%, 0.4%, and 0.3% of the energetic growth cost of 130 J/Day experienced by yearling male *S. undulatus* (Cox et al. 2005a), during June, July, and August, respectively.

These estimates indicate that the energetic needs of mites are not likely to have much of a direct impact on the energy budgets of host lizards. However, these estimates do not account for indirect energetic costs of mite parasitism,

including potential costs of an immune response and a stress response. The energetic cost of mounting an immune response against feeding mites may be significant (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Bonneaud et al. 2003; Martin et al. 2008; Brace et al. 2015). An inflammatory immune response occurs in lizards with chigger mites (Goldberg and Bursey 1991; Goldberg and Holshuh 1992), and, although the energetic costs of an inflammatory response in lizards have yet to be investigated, a similar inflammatory response initiated by phytohaemagglutinin in house sparrows resulted in a 29% increase in resting metabolic rate (approximate cost of 4.20 kJ/day; Martin et al. 2003). Similarly, an inflammatory response in rats increased the resting metabolic rate by 28% (Cooper et al. 1994). High mite loads may also indirectly exert an energetic cost to host lizards through a stress response involving corticosterone. Corticosterone, which is the major glucocorticoid in reptiles, is an energy-mobilizing hormone (French et al. 2007; Robert et al. 2009) and has been found to be significantly elevated in heavily parasitized individuals (Fast et al. 2006; Raouf et al. 2006; Pedersen and Greives 2008; Mougeot et al. 2010). Therefore, lizards that are heavily parasitized by mites may have high circulating levels of corticosterone and, therefore, be experiencing a significant indirect energetic cost of high mite loads. Furthermore, corticosterone itself may inhibit growth (Weil et al. 2001; Wada and Breuner 2008; Uller et al. 2009). Overall, it appears plausible that slower male growth rates of *S. undulatus* from June to August are driven by a combination of internal physiological factors, such

as molecular mechanisms, energy balance, and hormones, and also external ecological factors, such as mites.

In summary, growth can be influenced by several factors, including perhaps ectoparasites, which draw nutrients and energy away from the host and also induce a host immune response, which may add to the energetic costs. In *S. undulatus*, mite loads were negatively correlated with growth rate in yearling males from June to August. In this female-larger species, yearling males grow significantly slower than yearling females from June to August, which is a time when they have high circulating T, increased home range sizes, and high mite loads. The combination of all these factors plays an important role in decreasing male growth and body size, which suggests that mites may be, at least in part, important in contributing to sex-specific growth rates and the development of SSD. Future studies are required to investigate how much of an energetic cost ectoparasites can exact on their host and how this relates to costs to growth and the development of SSD. Studies should also be carried out to further examine growth costs of ectoparasitism in other species.

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Figure 4.1: Growth rate versus body size (SVL) for yearling male (blue) and yearling female (pink) lizards captured June to August during the 2014 and 2015 activity seasons. Growth rate decreased with increasing body size in both sexes ($P < 0.001$).

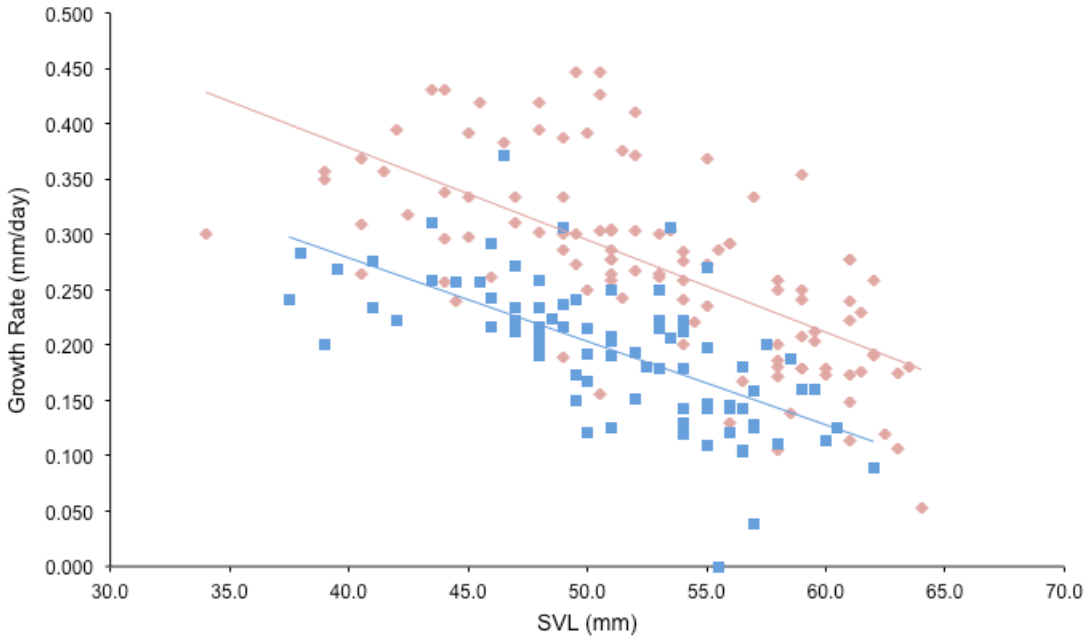

Figure 4.2: Log mite load versus log SVL (body size) for yearling male (A) and yearling female (B) lizards captured June to August during the 2014 and 2015 activity seasons. Mite loads increased with increasing body size in both sexes. F-statistics and P-values are shown. Shaded blue regions = 95% confidence intervals. Dashed lines = 95% prediction limits.

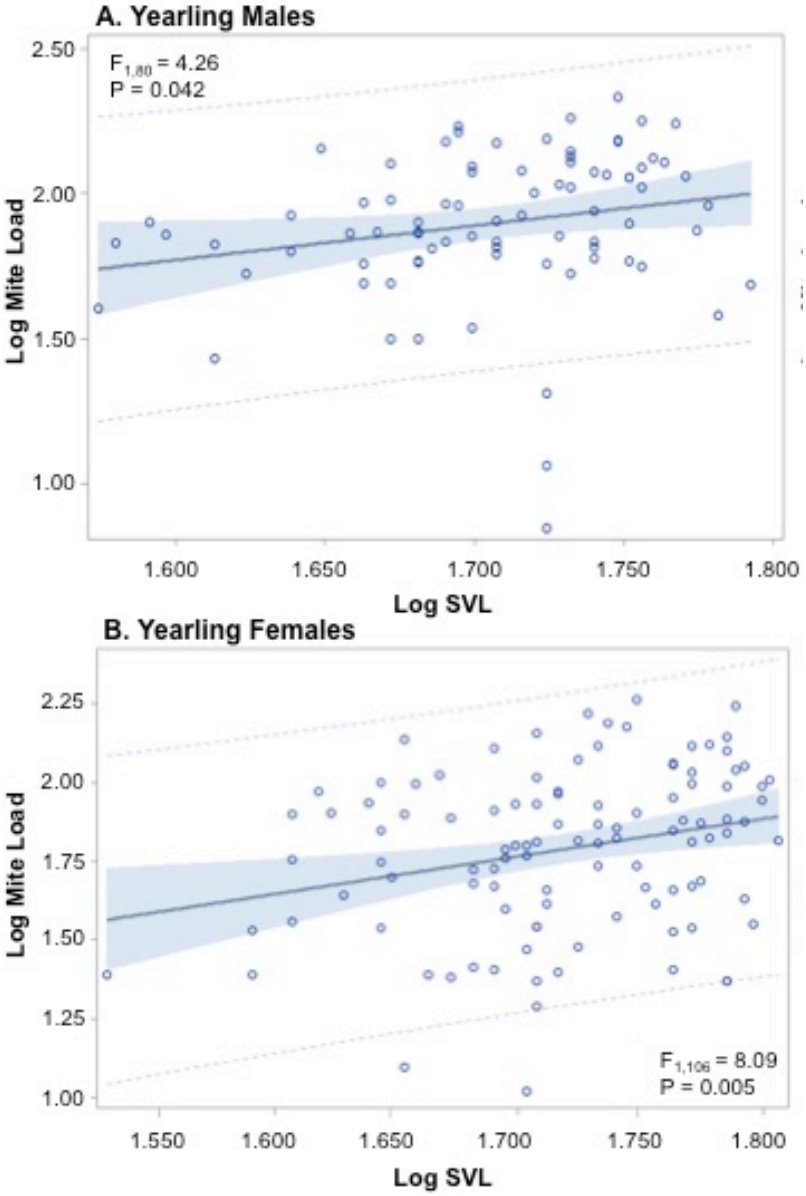

Figure 4.3: Size corrected residual growth rates versus residual mite loads for yearling male (blue) yearling female (red) lizards captured June to August during the 2014 and 2015 activity seasons. Growth rate decreased with increasing mite load in yearling males, but not in yearling females. Additionally, yearling females grew faster than yearling males for any given mite load ($P < 0.001$). F-statistics and P-values for correlations between residual growth rate and residual mite load are shown. Shaded blue regions = 95% confidence intervals. Dashed lines = 95% prediction limits.