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UNIVERSITY OF MIAMI

LEAFWING POPULATION DYNAMICS (GENUS *ANAEA*, LEPIDOPTERA; NYMPHALIDAE) AND VIABILITY OF THE ENDANGERED FLORIDA LEAFWING

By

Robert M. McElderry

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LEAFWING POPULATION DYNAMICS (GENUS *ANAEA*, LEPIDOPTERA; NYMPHALIDAE) AND VIABILITY OF THE ENDANGERED FLORIDA LEAFWING

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Leafwing Population Dynamics (May 2015)
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and Viability of the Endangered Florida Leafwing

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Butterfly populations are in decline in Florida. An example is the Florida leafwing, *Anaea troglodyta floridalis* (Nymphalidae), a species endemic to the pine rocklands in South Florida. I used viability analysis of population dynamics and extinction risk to evaluate the sensitivity of *A. t. floridalis* to disturbances such as fire. Using an annual population density index estimated from monthly count data over 15 years, I estimated a time series of relative population growth rates. To these relative growth rates I fit density independent and density dependent population dynamics models and estimated both intrinsic growth rate (r_0) and carrying capacity (K) in response to fire.

Projecting density independent and density dependent population models into the future by simulations indicated that the density dependent model was the more appropriate and also provided a more optimistic viability prediction. Sensitivity of relative growth rate to density increased in the absence of fire, but this sensitivity decreased when fires occurred due to post-fire increase in carrying capacity.

While my analysis suggested that improving carrying capacity would reduce the extinction risk for this endangered endemic butterfly, I cannot say for sure how to improve carrying capacity. Carrying capacity in butterflies is generally a function of either predator or host plant abundance, and I addressed this issue using two common leafwings, *A. aidea* and *A. andria*, as proxies for the endangered leafwing.

In an unusual application of mark-recapture methods, I repeatedly surveyed 510 *A. aidea* caterpillars in their natural environment for one month in the spring. I asked how the probability of surviving and growing varied across that month, and tested for the effect of temperature on growth and the effect density on survival.

In mark-recapture, the demographic process is partially “hidden” due to imperfect detection, so I modeled caterpillar demography as a hidden Markov process using Bayesian procedures. With data on survival and development of individuals during each 3-day time step, I modeled the entire process of juvenile development as a column stochastic Markov process. The stage of each individual alive at time *t* identifies a particular column, which contains all possible fates (dead or alive and which stage they would be in) for individuals in that stage 3 days later. These columns together describe the entire juvenile demographic process (from egg to pupa) as a stage-structured matrix model that contains the probabilities of surviving and growing for each stage after a 3-day time step. Using two multinomial probability functions, I estimated the joint likelihood of being alive and observed in a particular stage at each time step given the demographic matrix, the probability of being observed, and the individual’s stage at the previous time step.

During this survey, caterpillar densities increased to a maximum then declined as individuals either died or pupated. I observed both live and dead caterpillars with high probability, and was able to determine the cause of death for most corpses encountered. The abundance of caterpillars attracted multiple insect and arachnid predators, and survival declined as per capita predation rate increased over time. Survival of fifth instars improved at high density, with density scaled by the size of all caterpillars on a host plant, but this is arguably an artifact resulting from the time lag between predator and prey abundance. The most likely model showed that survival of fifth instars declined over the growing season while early instars (first to fourth) had constant high survival. Similarly, the most likely model supported time-invariant stage-specific growth rates, despite a compelling relationship between growth rates and temperatures.

This stage-structured matrix model represented the recruitment process as a progression of individuals from egg to pupa through all juvenile stages, which are absent in most studies of butterfly demography. This work not only demonstrated that predator abundance affected *Anaea* population dynamics, but it also showed that predation may play a role in density dependence.

Insect herbivores commonly feed on multiple plants throughout their geographic range, but feed locally on only one or a few plants. I asked how specialized were the diets of *Anaea aidea* (tropical) and *Anaea andria* (temperate). Rearing almost 300 caterpillars in the lab on controlled diets of one of five *Croton* host plants, I evaluated the performance of caterpillars in terms of survival to pupation, development time, pupa mass, and adult size.

Both leafwings showed a moderate ability to feed on multiple *Croton*, but tended to perform best on their *local host*. Even though each leafwing was capable of surviving to pupation on *non-local hosts* and *local non-hosts*, either growth or development time was less than ideal compared with the *local host*. The tropical leafwing performed well on all but one *Croton* in this experiment and performed better on its *local host* compared with the temperate leafwing on its *local host*. The temperate leafwing's *local host*, *C. argyranthemus* was a poor host for both leafwings, and *C. monanthogynus* (*non-local host* for the temperate leafwing and *local non-host* for the tropical leafwing) was a suitable host for both species.

The results of this experiment and my experience with this system indicated to me that the endangered Florida leafwing might be restricted to a single host only because there are no suitable *Croton* nearby. I argue that the Florida leafwing could potentially feed successfully on other species of *Croton*, particularly those known as hosts of other *Anaea* in the Caribbean. Further experimental work would be needed to test this hypothesis and to determine if there would be management implications of finding alternative hosts.

Surviving inhospitable periods or seasons may greatly affect fitness. Evidence of this exists in the prevalence of dormant stages in the life cycles of most insects. Here I focused on butterflies with distinct seasonal morphological types in which one morphological type, or form, delays reproduction until favorable conditions return, while the other form develops in an environment that favors direct reproduction.

For two butterflies, *Anaea aidea* and *A. andria*, I tested the hypothesis that the development of each seasonal form involves a differential allocation of resources to survival at eclosion. I assayed differences in adult longevity among summer and winter forms in either a warm, active environment or a cool, calm environment.

Winter form adults lived 40 times longer than summer form but only in calm, cool conditions. The magnitude of this difference provided compelling evidence that the winter form body plan and metabolic strategy favor long term survival. Neither adult feeding nor reserve size appeared to be the main cause, leaving resource conservatism as the primary explanation. This research suggests that winter form adults maintain lowered metabolic rate, a common feature of diapause, to conserve resources and delay senescence while overwintering.

Seasonal climatic fluctuations affect the life history of most organisms. Leafwing butterflies have a complex life cycle that is characterized by there being multiple generations within a year, distinct seasonal forms of adults, and relatively long-lived adults who feed on nitrogen-rich sources. During a single year, the stage structure of the population changes seasonally as do the probabilities of survival and growth and the amount of reproduction.

This section addresses the question: when the fates of individuals depend on both life stage and season, how do life stage transitions within each season contribute to population dynamics evaluated over the entire seasonal cycle?

I combined demographic rates estimated for the tropical leafwing in the field and in the laboratory to construct a periodic stage-structured matrix model that encapsulates the progression of the population through each seasonal phase along with the demography within each phase. I used a megamatrix form of the periodic matrix model. This approach is distinct from compiling a series of annual matrices as the particular products of the single phase matrices, which is the more commonly utilized approach. I show that these two approaches to the problem are equivalent and that the analytical properties of the megamatrix provide the seasonal population dynamic properties of interest.

My results show that stable stage structure, reproductive value, and elasticity all changed seasonally, highlighting the importance to annual dynamics of different life stages in different seasons. Using elasticity of the annual growth rate to demographic rates for each stage and season, I found that overwinter survival of adults has the largest overall effect on average annual dynamics. During the breeding season, from spring to fall, reproduction and growth have the largest effect.

This research is the first application of a periodic megamatrix to model seasonal butterfly population dynamics. With this approach I demonstrated that the importance of each life stage depends on season, and my results suggest which selection pressures maintain two distinct seasonal forms and life histories in leafwing butterflies, *Anaea*.

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Chapter I

Introduction: Population viability models for an endangered endemic subtropical butterfly: effects of density and fire on population dynamics and risk of extinction[1]

Overview

Population viability analyses for butterflies typically use metapopulation models, but for endemic species with few source populations, we need to understand local population dynamics. However, little is known about the sensitivity of butterfly population vital rates and viability to common disturbances such as fire. We fit quadratic models to monthly butterfly count data (1999-2014) to estimate an annual population density index that represents density during peak abundance each year. Relative population growth rate was estimated using a time series of the population density index, and population dynamics parameters r_0 and K were estimated by fitting relative growth rates to density independent and dependent models that include the effects of fire. Population models were simulated 20 and 100 years into the future to evaluate the sensitivity of extinction probability to density dependent dynamics and fire. Although the density independent model had the highest relative likelihood, density dependent models produced population trajectories with behavior more congruent with data from the *A. t. floridalis* population. The absence of fire increased sensitivity of relative growth rate to density, and the occurrence of fire buffered this sensitivity by increasing carrying capacity. Extinction risk was most sensitive to the inclusion of density dependent dynamics. Density dependent models provided a more optimistic outlook relative to density independent models (8% vs 66% probability of extinction in 20 years). Our

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[1] McElderry, R. M., M. H. Salvato, and C. C. Horvitz. 2015. Population viability models for an endangered endemic subtropical butterfly: effects of density and fire on population dynamics and risk of extinction. Biodiversity & Conservation:1-20

simulations suggest that improving carrying capacity would provide the best buffer to extinction for this endangered endemic butterfly.

Background

The effects of fire on insect populations are not well known (Swengel 2001). For species dependent on plants in fire-maintained communities, populations may respond positively to frequent fire despite temporary mortality and displacement from burned areas (Swengel 2001, Swengel and Swengel 2007). Prescribed fire both in prairie communities (Vogel et al. 2007) and in coniferous forests (Huntzinger 2003) has been shown to increase butterfly diversity and abundance. Resources are renewed following fire, with gap size increasing in forests (Huntzinger 2003), and the percent cover of forbs increasing in grasslands (Vogel et al. 2007), both of which are positive for most butterflies. The scale of the disturbance however is critical in its effect. Cleary and Genner (2004) documented reduced species richness and diversity persisting several years following three years of fires that burned millions of hectares in Borneo. However, rainforests are not fire prone communities, therefore rainforest inhabitants are not expected to be fire adapted. It is not surprising that butterflies endemic to Borneo were not found after these catastrophic fires (Cleary and Genner 2004). Species endemic to fire prone habitats are expected to be resilient to periodic fire, but endemic species are mostly threatened or endangered and may currently exist in only one or few locales. For rare endemics, impacts from single or combined threats may result in extinction when there are no available source populations from which to re-colonize locally extinct patches. Although the positive effects of fire have been demonstrated for some endangered butterflies such as Fender's blue, an endemic to fire-prone prairie communities (Schultz and Crone 1998), the authors point out that only a third of the habitat should be burned with prescribed fire. While we know fire is critical in maintaining fire prone habitats, we know relatively little about how altered fire regimes may threaten species endemic to these habitats and thus the basis for fire management recommendations in specific cases.

For endangered species in general, little is known about how various natural and anthropogenic factors interact in their effects on the population (Wilcove et al. 1998, Lawler et al. 2002). Population viability analyses (PVA) project demographic processes into the future and thereby provide quantitative measures by which conservation status can be evaluated (IUCN 2013, Pe'er et al. 2013, Regan et al. 2013). Whether or not PVA's have predictive accuracy has been debated, for (Brook et al. 1997, Brook et al. 2000) and against (Coulson et al. 2001), with agreement that accuracy is more likely when two criteria are met: (1) data are sufficiently high quality, and (2) future vital rates remain similar to those observed and used to parameterize models. These criteria will seldom be met for endangered species, but accuracy is thought to be less important than sensitivity when comparing among management options or potential causal factors affecting population dynamics (Beissinger and Westphal 1998, Coulson et al. 2001, Pe'er et al. 2013). Evaluating the sensitivity of population parameters and extinction risk to environmental factors improves our understanding of a population’s expected viability and also informs management decisions (Drechsler and Burgman 2004). Estimating the probability of extinction and evaluating the relative importance of natural and anthropogenic factors are most relevant for prioritizing management options (Pe'er et al. 2013).

Density dependent population models that estimate carrying capacity from time series data have been shown to provide more realistic population projections compared with density independent models (Brook et al. 1997, Brook and Bradshaw 2006), and have been encouraged in PVAs (Pe'er et al. 2013). While evidence for density dependence in butterfly population dynamics has been criticized in the past (e.g., Dempster 1983), more recent, carefully executed studies support density as a key factor determining butterfly dynamics (Schtickzelle et al. 2005, Baguette and Schtickzelle 2006, Nowicki et al. 2009). Natural enemies and intraspecific competition for resources are the commonly cited players having a density dependent effect on late instar larval stages specifically (Dempster 1983, Nowicki et al. 2009). Resource dependence clearly affects population dynamics when resources are limiting, but the proper data to document this phenomenon are difficult to obtain. With a unique dataset documenting the phenology and abundance of both butterflies and their preferred nectar plants over three decades, Boggs and Inouye (2012) demonstrate both direct density independent effects of climate, and indirect density dependent effects of climate transmitted via the per capita abundance of nectar plants, which ultimately affect egg production. Similarly, both adult and juvenile resource density were shown to affect population dynamics of the rare Fender’s blue butterfly (Schultz and Dlugosch 1999). Nowicki et al. (2009) argue that density dependent dynamics have been hidden both by the inaccuracy of transect counts and environmental stochasticity, and demonstrate that density dependent dynamics become apparent by modeling this stochasticity explicitly.

We use field data from 16 years of an ongoing, long-term study to evaluate the population dynamics and extinction risk for an endangered endemic subtropical butterfly, *Anaea troglodyta floridalis* F. Johnson & Comstock (Nymphalidae). We hypothesized that frequent fire increased the abundance and quality of available habitat and its host plant, a low shrub, and is therefore beneficial to the persistence of this endemic butterfly population. Because these positive effects depend on the frequency of fire, we asked how sensitive is extinction risk to changes in fire frequency, and in which direction does extinction risk change in response to increasing fire frequency? We also explored the interaction between fire frequency and density dependence and predicted that frequent fire would increase either the intrinsic rate of growth (r_0) or carrying capacity (K) while decreasing extinction risk.

In a disturbance-maintained ecosystem, periodic disturbance likely has positive indirect effects on locally adapted inhabitants despite potential negative direct effects. The Florida leafwing appears to have persisted throughout the Everglades pine rocklands, via colonization, following local extinction within the larger historic landscape (Salvato and Salvato 2010a). In its current landscape, *A. t. floridalis* has no source population for re-colonization following local extinction, and managers aim to balance the needs of the ecosystem (i.e., fire management) and the needs of the Florida leafwing. A viability analysis can evaluate the positive and negative impacts of disturbance in this declining habitat and can determine if the marginal risk posed by fire is outweighed by the benefit of restoring habitat quality by increasing prescribed burning.

Methods

STUDY POPULATION – *Anaea t. floridalis* (Florida leafwing) is a federally endangered species and has historically occurred with its host plant, *C. cascarilla* (L.) L. (=*C. linearis* Jacq.; Van Ee and Berry 2009) (Euphorbiaceae) throughout the pine rocklands of southern Florida and the lower Florida Key (Smith et al. 1994, Salvato and Hennessey 2003). The pine rockland ecosystem, where *A. t. floridalis* is endemic, is critically imperiled globally (FNAI 2012), and approximately 90 percent of all historic pine rockland habitat has been lost to development (O'Brien 1998). Due to this extensive habitat loss, *A. t. floridalis* is now restricted to the Long Pine Key region of Everglades National Park (Miami-Dade County, Florida, USA) (Salvato and Salvato 2010a). Additionally, remnant habitat is extremely fragmented and degraded, namely from natural fire suppression with limited prescribed burns. In this current state, disturbances (fire, hurricanes) and natural enemies (parasitism, predation) may have greater influence on the extant population, than when its distribution was more widespread (Salvato and Salvato 2010b, a). Unlike other *Anaea*, late instar *A. t. floridalis* does not typically roll single leaves or tie leaves together with silk to form a nest or shelter in order to evade parasitism and predation (Smith et al. 1994). Although late instar *A. t. floridalis* have been observed to construct shelters (Cech and Tudor 2005, Salvato et al. 2015), this behavior appears to be rare and less obligatory.

The role and frequency of natural disturbances, including fire and hurricanes, remains an important factor influencing pine rockland habitat and *A. t. floridalis* (Salvato and Salvato 2010a). Pine rocklands occur on limestone outcrops and are savanna-like with an open canopy and a low understory that includes a number of endemic forbs and shrubs (Slocum et al. 2003, FNAI 2010). These endemic plants only persist with frequent fire and rapid, rigorous re-sprouting after fire is presumably beneficial for insect herbivores. In the absence of fire, open pine savannas transition over time to a closed canopy tropical dry forest. As early as seven years after a fire, tree saplings dominate and shade out typical pine rockland species resulting in a loss of habitat for insect herbivores specialized on these endemic plants.

Historically, lightning induced fire mostly at the onset of the wet season (Slocum et al. 2003). Managers adapted prescribed burn protocols towards the end of the twentieth century to mimic the timing of natural fires (Salvato and Salvato 2010a). Early in our study, Everglades land managers administered prescribed burns in Long Pine Key in its entirety over a 3-year window (Salvato and Salvato 2010a). These methods attempted to burn adjacent pine rocklands alternately, as well as provide refugia (i.e., unburned areas of host plant) within occupied *A. t. floridalis* habitat. Leafwing have strong flight abilities and can disperse to adjacent unburned patches and quickly re-colonize burned areas where host plant resurgence is rapid. Salvato and Salvato (2010a) encountered similar leafwing densities within 6 months pre- and post-burn throughout their 10-year study, and suggest that alternating prescribed burn treatments among patches allows this movement and re-colonization. Leafwing densities increased following prescribed burns; however these gains were of short duration. Additionally, prescribed burns have largely ceased in recent years, leaving only wild fires since 2009.

ESTIMATING POPULATION DENSITY FROM BUTTERFLY COUNTS – *Anaea t. floridalis* is multivoltine with continuous broods throughout the year (Salvato 1999) and completes its life cycle in about 60 days (Hennessey and Habeck 1991). Although adult *A. t. floridalis* can be observed in every month, abundance peaks early in the dry season between December and January. To represent abundance each year, we used data on the number of butterflies observed in a 0.2 ha area each month at as many as 5 sites (see description of field methods in Salvato and Salvato (2010a)) to model the monthly abundance and identify the month of peak butterfly density for each year. Data collected between 1999 and 2010 are summarized in Salvato and Salvato (2010a), and data for 2010 to 2014 were included for the analyses presented here.

Sites were on average 4.6 km apart with the closest sites 1.9 km and the furthest sites 9.2 km apart. We counted individuals only once per survey and assume that butterflies observed at each site are different individuals than butterflies observed at other sites. These rare butterflies fly long distances, so an observation of zero butterflies in a month does not mean there are no butterflies present. Therefore we used the sum of the number of observed butterflies over all sites for each month.

Monthly count data, C_{ij}, were modeled with a Poisson distribution with a single parameter, N_{ij}, which represents a population density index and is estimated for each month j and each year i. To mimic the characteristic rise and fall of butterfly density in each year, population density is modeled via a quadratic function arranged in vertex form using Bayesian procedures.

$$C_{ij} \sim \text{Poisson}\left(N_{ij}\right) \tag{1}$$

$$\log\left(N_{ij}\right) = -a_i * \left(month_j - h_i\right)^2 + v_i * \sum_j C_{ij} \tag{2}$$

In equation 2, the vertex of the density parabola each year is proportional, v_i, to the sum of counts for that year, and is centered at h_i for each year i, which represents the time of peak *A. t. floridalis* abundance. The parameter a_i modifies the speed of increase in density and is always positive to ensure a concave down parabola. Density is log transformed to ensure positivity. Parameters a_i and v_i are lognormal variables, h_i is a normal variable, and all three are random among years. Mean a and v were given uninformative lognormal prior distributions, mean *h* was given an uninformative normal prior, and the precision for each random parameter was given an uninformative gamma prior.

The posterior distributions for all parameters were estimated in a Bayesian format using conventional Markov chain Monte Carlo methods. A conservative 'burn in' of 600,000 iterations allowed for convergence, after which 20,000 iterations were used to evaluate standard convergence diagnostics and to estimate posterior distributions. These procedures were performed using R (version 3.1.0 (2014-04-10), R Core Team 2014) and JAGS (Plummer 2003) via the R package, rjags (Plummer and Stukalov 2014), and are identical for all statistical models described below.

Only one site (gate 4) had data every year. The other 4 sites had data only for some years. Data from all sites were pooled to improve the fit of the density model, because even though observations of zero counts were common we do not think these counts were indicative of zero density. A second model was employed to estimate the proportion of yearly counts that came from each site (using a multinomial distribution with the conjugate Dirichlet distribution for uninformative priors). Density estimates were then weighted by the proportion of counts that came from gate 4. This weighted density index represents the number of butterflies in 0.2 ha at gate 4. This estimate is only an index of population density. We cannot estimate encounter probability from these data, which would be required to transform the index into an estimate of true density. With uncertainty in the exact relationship between our density index and true density, we do not attempt to scale up our index in our analyses (but see Accuracy and the extinction threshold in the Discussion section below). We performed all analyses with the simplifying assumption that the probability of encounter is constant across years.

POPULATION MODEL – The Ricker equation is a discrete time population model that includes density dependence and is commonly applied to butterfly populations (Morris and Doak 2002, Schtickzelle et al. 2005, Boggs and Inouye 2012). Butterfly populations in temperate regions typically have discrete breeding seasons making discrete time models appropriate. Although the subtropical *A. t. floridalis* is intermediate between truly continuous and discrete breeding, we treat the cyclic peaks in abundance as the breeding season and employ the Ricker equation to describe population growth between peaks each year.

The Ricker equation states the density at time $t+1$, N_{t+1}, is obtained by multiplying the density at time t, N_t, by a factor that changes with density.

$$N_{t+1} = N_t exp[r_0(1 - (N_t/K)) + \varepsilon] \tag{3}$$

The particular form of density dependence is one in which the $ln[N_{t+1}/N_t]$, the instantaneous per capita growth rate of the population at time t, also called the "relative growth rate", depends linearly upon density. The slope and intercept of the line respectively are r_0/K and $r_0 + \varepsilon$. Note that this equation models unexplained variance in the intercept rather than in the slope. Density-dependence is encapsulated by the ratio of population density, N_t, to the carrying capacity, K. K must be nonzero and positive; it represents the population density at which growth rate becomes zero and population density remains stable. At very low density, i.e., N_t approaching 0, this model reduces to the exponential model. Hence, r_0 represents a theoretical instantaneous per capita growth rate at zero density. When N_t is smaller than K the population will grow, and when N_t is larger than K the population will decline (Morris and Doak 2002).

EFFECTS OF FIRE ON POPULATION DYNAMICS – We test for the effects of fire by adding a term representing fire to the Ricker function. We created a binary variable indicating the occurrence of fire in the vicinity of gate 4 in year *i*, using fire records obtained from National Park Service staff. Six fires were recorded in this area of the Everglades within the 16 years of this study. Overall fire frequency appeared to decrease during the survey period, with no fires occurring after 2009. We allowed the additive effect of fire, f_r or f_K, to either r_0 or K, e.g., $r_0 = r + f_r{*}fire_i$. Stochastic population models such as equation 1 include random variability, ε, due to unknown environmental factors. Adding fire into this equation will reduce ε and allows us to modify the frequency of fire in evaluating its effect on population dynamics and extinction risk.

STATISTICAL ANALYSIS – We rearranged equation 3 such that the relative growth rate, $RGR = ln[N_{t+1}/N_t]$, is a linear function of r_0, K, and N_t.

$$RGR_t \sim \mathrm{Normal}(rgr_t, \tau) \tag{4}$$

$$rgr_t = r_0[1 - N_t/K] \tag{5}$$

Relative growth rate is normally distributed with mean rgr_t and precision τ. Uninformative normal priors were given to both r_0 and K, and restricted to biologically reasonable intervals; -3 to 3 for r_0 and 0 to 5 for K. An uninformative gamma prior distribution was used for the precision, which is the inverse of variance. Residual standard error, σ_E, which estimates random variation, ε, due to unknown factors, is thus estimated by the inverse square of τ.

Candidate models tested include the density dependent model displayed above, density dependence with either r_0 or K including the effect of fire, density independence

($rgr_t = r_0$), and density independence with r_0 including fire effects. Nonlinear density dependence was also tested with a theta logistic model (replacing N_t/K with $(N_t/K)^\theta$), but this model performed poorly (Table 1.1) and results are not further discussed here. Akaike information criteria corrected for small sample size (AICc) and relative model likelihood, also known as model weight, were consulted to evaluate the support for each model. As a rule of thumb, models with a ΔAICc less than two have substantial support, and the relative likelihood allows for comparisons of the level of support for each model relative to all models tested (Burnham and Anderson 2002). Rather than selecting a single 'best' model, we simulate results from multiple models that had substantial support.

SIMULATION OF POPULATION TRAJECTORIES – We simulated select models as recursion equations (similar to equation 3) to visualize and analyze the character of population trajectories predicted by these models. Stochastic projections of $T = 100$ populations (sample paths) were simulated 100 years into the future, for density independent (models 1 and 6; Table 1.1) and dependent models (models 2 and 3) with and without the effect of fire. For each sample path at each time step, a value representing random variation (ε in equation 3) was drawn from a standard normal distribution using the model standard error, $Normal(0,\sigma_E)$. Initial population density (N_0=1 adult per 0.2 hectare) in all simulations was set to match the last survey. When population density dropped below the extinction threshold ($N_x=0.1$) within a sample path, population density was set to zero and the population was considered to have gone extinct.

Selection of this quasi-extinction threshold is somewhat arbitrary, but represents an extremely low density. We selected $N_x = 0.1$ to represent a density of about 1 adult in

2 ha, which if scaled up to the 1,068 ha of croton-bearing pinelands in the Long Pine Key area (Salvato 1999) would estimate a total of only 530 butterflies. This calculation likely represents an upper bound for a population size estimate for two reasons. First, our density index likely overestimates density, because *A. t. floridalis* flies long distances relative to the sampling scheme. Observed butterflies occur in a larger area than we sampled. Second, we are using a density index for a site that occurs at the core of the available habitat. Butterflies were consistently observed at higher densities at this site relative to others, so assuming this density throughout the available habitat is a best case scenario. Nonetheless, we explored the sensitivity of extinction risk to this threshold and discussed this below.

The occurrence of fire was simulated via a binomial distribution with the probability of occurrence equal to observed frequency, 6/16. For visualization of population trajectories, sample paths did not constitute independent samples of the disturbance environment. They all experienced synchronous disturbances (the same temporal sequence of fires) so patterns in population trajectories could be identified. What differed among sample paths was only the error term (ε) each year of the simulation, which represented random environmental variation. Simulations were performed using MATLAB version 7.12.0 (R2011a, The MathWorks, Natick, Massachusetts) using code modified from Morris and Doak (2002).

POPULATION VIABILITY – Population viability was quantified by simulating select models and calculating the cumulative extinction probability. For viability analysis, we simulated $T = 100{,}000$ random populations (sample paths) over 20 years, with $N_0 = 1$ and $N_x = 0.1$, and random environmental variation included in each projection at each time step by a normally distributed random value, *Normal(0,* σ_E*)*. The proportion of populations that had gone extinct before or at each time represented the cumulative probability of extinction at that time (Morris and Doak 2002). Density independence (model 1; Table 1.1) and density dependence (model 2) were simulated to explore the sensitivity of the 20-year extinction risk at each time to density dependence, and models with (model 3) and without (model 6) density dependence were simulated that include the effect of fire.

For the PVA, occurrence of fire was not synchronized among sample paths, in contrast to the Simulation of population trajectory section above. The occurrence of fire was simulated as a binomially distributed random variable, with all time steps in all sample paths independent of one another. Sensitivity to fire frequency was tested over a range of 0 to 1 fire per year.

The possibility of mitigating or increasing the potential decline in habitat quality was evaluated by varying K in a series of simulations. Declining habitat quality is suggested in the decline in population density over the 16 year study period, and managers may need to consider this angle despite a lack of the data required to validate a declining habitat.

Bootstrapped confidence intervals were estimated for density independence and dependence with and without fire effects using 1,000 of the 20,000 iterations used to estimate parameter distributions. Parameter values are coupled in each iteration and incorporate the correlation structure between parameters while exploring each parameter's distribution. Quantiles are reported to represent the median and 95% confidence interval given the strong skew in the distributions of extinction probabilities.

Simulations were extended to 100 years and we report the 100-year extinction risk, which has been requested for all endangered species (IUCN 2013).

Results

BUTTERFLY COUNTS – A pronounced peak in butterfly abundance was apparent during the dry season (November to May) in each year when data from all sites were pooled (Figure 1.1). Our sites were regularly spaced in a line from southeast to northwest across the pine rocklands and the proportion of butterfly sightings at each site appeared to trail off with increasing distance from the central pinelands (data not shown). Abundance at gate 4, our most consistently sampled site and occurring in the central pinelands, comprised 69% of the total butterfly sightings between 2004 and 2009, but only 44% of the butterfly sightings between 2010 and 2014 after a new site was added to the survey. During the period of this study, the number of butterfly sightings and our estimated population density index showed a negative secular trend (Figure 1.2). The oscillatory pattern of both butterfly sightings and the density index over time suggested density dependence. Synchronous oscillations among sites support our decision to consider the leafwing at Long Pine Key in Everglades National Park as a single population (data not shown). High density in general was associated with negative growth and low density with positive growth (Figure 1.2). The occurrence of both fire and hurricane disturbances are both indicated in Figure 2 for reference.

POPULATION DYNAMICS AND FIRE – Density independence (model 1) was equally supported by the data compared with density dependent models (2 and 3), and models that included fire were less supported than those that excluded fire, according to AICc

(Table 1.1). We selected models 1, 2, and 3 to simulate population trajectories because they received substantial model support, (relative likelihoods = 43%, 25%, and 10%), and because they compare and contrast the effects of density and fire. To balance these comparisons, we also simulated model 6 despite less model support, because it includes fire with density independence. Density independent models estimated a negative intrinsic growth rate, r, while density dependent models estimated a positive intrinsic zero density growth rate, r_0, and a low carrying capacity. Fire either decreased the intrinsic growth rate or increased carrying capacity, which effectively reduced the effect of density on the relative growth rate (Figure 1.3). Conversely, the absence of fire either increased the intrinsic growth rate or decreased carrying capacity (Table 1.1), effectively increasing the decline in relative growth rate in response to increasing density. To further evaluate how appropriate density dependence is in our models, we tested for environmental autocorrelation in each model by examining correlations between model residuals at time t and model residuals at time $t+1$ (Morris and Doak 2002). There was no evidence of autocorrelation in density dependent models, but density independent models did have significantly negative autocorrelation, providing additional support for density dependence.

SIMULATION OF POPULATION TRAJECTORIES – When we simulated 100 population trajectories under the assumption of density independence (model 1), we obtained populations that either grew or declined rapidly and sporadically (Appendix A Figure 1.7A). At the end of 100 years, extant populations (<10%) either remained small or had become unrealistically large with a density index exceeding 30 (Figure 1.7C). In this scenario, primarily those populations that reached unrealistically high density avoided extinction. When we simulated 100 population trajectories under the assumption of density dependence (model 2), we obtained populations that mostly oscillated around the carrying capacity (Figure 1.7B). At the end of 100 years, a majority of the simulated populations had become moderately sized, very few were unrealistically large, and only about 20% had gone extinct (Figure 1.7D). Density dependence in our models buffered against both extreme population densities and extinction.

When we simulated 100 density dependent population trajectories that included the stochastic occurrence of fire (model 3), we obtained populations that exhibited decreased oscillation amplitude and extinction (Appendix A Figure 1.8C). Disturbance generally increased relative growth rate in response to increased carrying capacity. This slight increase in *K* lessened the decrease in relative growth rates at high density while maintaining high relative growth at low densities (Figure 1.8B). Periodic fire dampened population oscillations (Figure 1.8C) and decreased extinction risk (Figure 1.8D).

POPULATION VIABILITY – Overall, the cumulative probability of extinction varied considerably depending on the extinction threshold, and this relationship was dramatically different between density independent and dependent models (models 1 and 2, Figure 1.4). Extinction probability increased dramatically for the density independent model in response to the extinction threshold only at low threshold densities. At moderate threshold densities, extinction probability for both models increased linearly with extinction threshold. We selected a moderate extinction threshold density well below the minimum density index (0.5) estimated in our data.

Using an extinction threshold of 0.1, extinction probability in 20 years increased rapidly over time for the density independent model (model 1), indicating significant threat to Florida leafwing viability in the near future (Figure 1.5A, black line). Including fire at the observed frequency with density independence (model 6) did not significantly affect this extinction probability (Figure 1.5A, red line). The density dependent model (model 2) predicted a slow and steady increase in the cumulative probability of extinction (Figure 1.5B, black line), and an 8.3% (0-74%) probability of extinction in 20 years, which increases to 36% (0-96%) in 100 years. Including fire at the observed frequency with carrying capacity (model 3) decreased the probability of extinction to 5.7% (0-80%) in 20 years and 28% (0.1-96%) in 100 years (Figure 1.5B, red line). The confidence limits for these projections are relatively wide and increase with projected time. Density dependent models had narrower confidence limits compared with density independent models, and the confidence limits for models with fire were wider than for models without. The rapid increase in the upper limit for extinction probability indicates significant risk for the Florida leafwing in the near future. An increase in either carrying capacity or in fire frequency would result in reduced probability of extinction (Figure 1.6). Extinction probability is more sensitive to changes in carrying capacity than to changes in fire frequency, but in the range of estimated carrying capacities, extinction probability appears most sensitive to whether or not fire is included in the model (Figure 1.6).

Discussion

Populations of this endangered endemic butterfly have declined (Salvato and Salvato 2010a, b), and our population viability analysis indicates that extinction risk is sensitive to carrying capacity and fire frequency. Density dependent population dynamics for *A. t. floridalis* are supported over density independent dynamics, despite substantial AIC model support for the density independent model. Similar to what has been found in other studies (Brook et al. 1997, Brook and Bradshaw 2006), our simulations of density dependent models better resembled the oscillating butterfly densities we observed in the field compared to simulations from density independent models. Oscillating densities are typical of density dependent dynamics, with populations growing at low densities and shrinking at high densities. This phenomenon is demonstrated in our analyses by the negative autocorrelation of residuals from the density independent model, and violates an assumption of population viability analysis (Morris and Doak 2002). The lack of resolution between density dependence and independence according to AIC is likely due to high variance in the time series of relative population growth rates. Estimating density dependence has been difficult for butterflies (Dempster 1983), but it has been argued that the lack of precision in transect count data and an inability to model environmental stochasticity are the main contributors to this difficulty (Nowicki et al. 2009). In general, this inability to distinguish between observation (i.e., sampling) and process error means that all population density variation is treated as environmental variation and results in more pessimistic estimates of extinction probability (Morris and Doak 2002, Regan et al. 2013). We use transect counts and attempted to model some environmental variance with the occurrence of fire, but we could not tease apart observation and process error with these data. In addition to observation error, our data likely do not reflect equilibrium conditions, which are implicitly assumed in the models described here. A number of perturbations have affected this population, e.g., habitat loss, loss of population redundancy, declining habitat quality, and variable fire management protocol, and the resulting transience of this system means increasing uncertainty in our estimates of demographic parameters and extinction probability the further we project into the future. Notwithstanding these difficulties and the inability to rule out density independence, density dependent dynamics provide a more realistic representation of leafwing population dynamics compared with density independent models, and predict a low probability of extinction with a wide confidence interval.

POPULATION VIABILITY AND CARRYING CAPACITY – Our predictions for population viability of *A. t. floridalis* depend strongly on our assumptions about which factors influence population dynamics. Namely, whether or not this population exhibits density dependent dynamics greatly influences the estimated probability of extinction in 20 years. Populations simulated with density independent processes avoid extinction only by growing to unrealistically high density populations. Despite AIC support for the density independent model, sample path trajectories bear little resemblance to the observed population trajectory. This result, along with generalization that density dependent dynamics generally improve population viability estimates (Brook et al. 1997) lends credibility to population viability estimates from the density dependent models. The oscillating population trajectories exhibited both in simulations and the observed *A. t. floridalis* population show some population regulation mechanism characteristic of density dependence. Without this regulation in our models, extinction risk is likely overestimated. In our density dependent models, sample paths oscillated around *K* and were buffered against extremely dense populations and extinction. All extinctions in our simulations were in populations that were extremely small, not populations exceeding carrying capacity, then crashing.

Simulating a decrease or increase in carrying capacity to represent declining conditions or improving conditions through habitat restoration indicates that increasing carrying capacity would restore resilience to this system by allowing population trajectories to oscillate around a carrying capacity further from the extinction threshold. The secular decline in the *A. t. floridalis* population trajectory over the period of this study may indicate a decline in carrying capacity, although this cannot be tested statistically with these data. Declining or variable carrying capacity not due to the direct effects of fire could account for the lack of overwhelming support for density dependence in our analyses. While we do not know for sure what aspects of the habitat contribute to carrying capacity, our results indicate its presence, and we propose that natural disturbances, host plant abundance and quality, and natural enemies may interact and contribute to carrying capacity.

The distribution and abundance of both quality host plants and predators/parasitoids over the landscape are likely to contribute to *A. t. floridalis* carrying capacity and viability. The quality of *C. cascarilla* from the caterpillar's perspective is likely defined by its nutritional qualities and its potential to provide refuge. Following fire *C. cascarilla* re-sprouts from the base, generally with denser foliage and softer leaves compared with unburned *C. cascarilla*. Soft new foliage is easier to consume and digest and therefore likely to be more nutritious. Dense foliage offers the opportunity for caterpillars to tie multiple leaves together into leaf shelters where predators and occasional near freezing temperatures may be avoided. The rarity or ephemeral use of leaf shelter construction behavior by *A. t. floridalis* may be a result of either the inability to roll the slender leaves of *C. cascarilla*, or inability to web multiple leaves together due to the sparse leaf arrangement characteristic of unburned *C. cascarilla*. The leaves of C*roton* hosts used by other *Anaea* larvae are generally large enough to roll a shelter, or are arranged densely enough to allow multiple leaves to be webbed together (pers. obser.). Leaf shelters do provide crypsis and protection from insects and arachnid predators that attack the soft caterpillar body (pers. obser.). Without the protection of a leaf shelter, late instar *A. t. floridalis* rest in exposed perches, unlike other *Anaea* larvae, and a number of predators and parasites have been documented for *A. t. floridalis* (see U.S. Fish and Wildlife Service (2014) for detailed summary). Predators, parasites, and cold exposure were the most common cause of mortality in the field for an average mortality rate of 70% for immature stages (Salvato and Salvato 2012). We argue that caterpillars developing on burned and re-sprouting *C. cascarilla* will have a more nutritious diet, and will be better protected from natural enemies and cold conditions. Nevertheless, investigating the impacts of bottom-up and top-down biotic factors on *A. t. floridalis* has become increasingly difficult with the decline in this population. Regardless of our knowledge gaps, any effort to improve the abundance and quality of host plants while reducing predators should be an aim of conservation efforts and doing so is likely related to restoring the natural disturbance regime.

NATURAL DISTURBANCE – Natural disturbances maintain the open pine savannas where *C. cascarilla* and *A. t. floridalis* coexist, and our results indicate that fire is indeed positive for *A. t. floridalis* population growth. Including the stochastic occurrence of fire in our models appeared to buffer sample path oscillations with theoretical populations fluctuating closer to carrying capacity (Figure 1.8C) compared with the density dependent model without fire (Figure 1.7). Simulating an increase in fire frequency decreased the probability of extinction, which essentially followed from increasing the number of years with an increased carrying capacity. We should caution misinterpreting these results however. The full effects of fire were not analyzed here, and our results do not suggest burning the pine rocklands every year. However, they do suggest that the National Park Service should resume a frequent prescribed burning protocol. Suspension of prescribed burns in recent years has resulted in a predominance of hardwood saplings in the pine rockland vegetation in the Everglades (Rick Anderson, pers. comm.) and may have contributed, in part, to the decline in numbers of *A. t. floridalis*. We were unable to test for long term fire effects with our data, but fire certainly has lasting effects. Our inability to test for lasting fire effects means we are underestimating the true impact of fire on *A.t. floridalis* and the ecosystem. Our results support a relationship between fire and carrying capacity, but only through fire's direct effect in the year when fire occurred. Frequent fire with a return interval of 3 to 7 years is known to maintain habitat quality (Slocum et al. 2003, FNAI 2010, Saha et al. 2011), which suggests that fire likely maintains carrying capacity for more than a single year and that frequent fire should also be beneficial for *A. t. floridalis*.

While the effects of hurricanes were not analyzed in our study, a single hurricane of significant magnitude could wipe out the last Florida leafwing population. Hurricanes, like fire, may be considered generally beneficial for pine rocklands, because they create openings for understory herbs and shrubs. However, the frequency of hurricanes is too low to maintain pine rockland habitat. Additionally, strong hurricanes cause flooding (storm surge), which either floods low lying areas with sea water or contaminates fresh groundwater and has a lasting negative effect on terrestrial vegetation (Saha et al. 2011). In 2005, Hurricane Wilma caused substantial damage to the pine rocklands of the Florida Keys, due in part to storm surge (Saha et al. 2011), and may have been the final cause of leafwing extinction in the Florida Keys (Salvato and Salvato 2010b). The pine rocklands where the remaining *A. t. floridalis* occur in Everglades National Park are currently far enough from the coast that storm surge is less of a threat, but rising sea level may bring the coast closer and increase the threat posed by each hurricane.

HABITAT CONNECTEDNESS – We were not able to test for spatial patterns in *A. t. floridalis* population dynamics, but we argue that connectedness among habitats may reduce extinction risk. Although synchrony among patches within a metapopulation increases extinction risk (Hinrichsen 2009), clumping of patches has been shown to improve metapopulation persistence (Adler and Nuernberger 1994). Pine rocklands are very heterogeneous, with hydrology and fire interacting with micro-topographic features to create dynamic vegetation communities that are patchy in space and time (Slocum et al. 2003). *Croton cascarilla* is sparsely distributed with an estimated 1,068 ha of croton-bearing pineland within 8,029 ha of pine rockland in the Everglades (Salvato 1999) and occurs in relatively small patches within pine savannas and ecotones between marl prairie and pine savanna (McElderry, unpublished data). Butterflies move essentially unhindered between patches due to the relative openness of this wilderness matrix consisting of either pine savanna, marl prairie, hardwood hammock, or some transitional or successional ecotone between these three main community types. With strong flight abilities noted for this butterfly (Salvato and Salvato 2010a), it is likely that patches are highly connected. This could explain the observed synchrony in population trajectories measured at each study site. In addition to dispersal, synchrony may also result either from regional environmental drivers, or through interactions with other synchronous species (Liebhold et al. 2004). Regional environmental variation certainly affects the entire pine rockland ecosystem equally, but the host species may not be synchronous within the entire habitat. Variability in the occurrence of fire occurs both among fire management blocks and within blocks due to micro-topography and hydrology in the Everglades, thus creating a mosaic of burned and unburned areas. This is in fact a positive feature, in that large fires burning evenly over the entire pine rockland would be catastrophic for many of its inhabitants. Uneven burning allows unburned refugia within the landscape where animals can escape from fire and from which they can then disperse back into the recently burned and rejuvenated area.

Uneven burning, rejuvenated host plant growth, and dispersal of *A.t. floridalis* and its natural enemies likely interact and affect *A.t. floridalis* population viability. Rapid re-colonization of burned areas is more likely from small fires with patchy burn patterns. Re-colonizing before natural enemies return would allow at least brief predator free recruitment on re-sprouting *C. cascarilla* that may be more nutritious and a better refuge. Insect predators may re-colonize on a similar timescale as *A. t. floridalis*, but avian predators likely have a much longer return time. Fire kills the above ground stems of shrubs and tree saplings, effectively removing avian forage habitat until woody vegetation returns (about 3 to 7 years). *Anaea t. floridalis* in burned patches likely avoid more avian predators, than those in unburned patches with abundant shrubbery.

CONCLUSION – Our work is one of very few PVAs that links butterfly population dynamics to stochastic disturbances (e.g., Schultz and Crone 1998). Frequent fire maintains pine rockland habitat quality, and our work suggests fire increases carrying capacity and stabilizes *A. t. floridalis* populations over time. Broad confidence intervals around our estimates characterize the uncertainty in otherwise optimistic extinction probability estimates. Our work does not refute the general opinion held by local land managers and butterfly enthusiasts that the Florida leafwing is significantly threatened with extinction in the near future. What we contribute is a perspective of this dynamic system. Returning to frequent prescribed fires in the pine rocklands in order to improve the pine rockland ecosystem should be made the first priority in the recovery plans for all suitable habitats.

Table 1.1 Model selection criteria and parameter estimates among competing models predicting relative growth rates of *A. t. floridalis*. Relative growth rates (RGR) of the population were modeled as functions *r*, *K*, *θ*, and *σ* (equations 4 and 5). The intrinsic growth rate (*r*), and carrying capacity (*K*) were each modeled as functions of the occurrence of fire, *r(fire)* and *K(fire)*. Fire represents the additional component added to either *r* or *K* in a year with fire. Nonlinear density dependent dynamics were tested with *θ*, and the residual standard error of each model is represented by *σ*. The lowest Akaike Information Criteria corrected for small sample size (AICc = 30.45) is subtracted from each model AICc to obtain the ΔAICc, which is then used to calculate the relative likelihood (Weight) of each model. Models 1, 2, 3, and 6 were the only models used in simulations.

	RGR = f( )	Par	ΔAICc	Weight	*r*	*K*	*θ*	*fire*	*σ*
1	*r*	1	0	0.43	-0.115	--	--	--	0.61
2	*r, K*	2	1.07	0.25	0.251	2.311	--	--	0.58
3	*r, K(fire)*	3	2.88	0.1	0.340	1.652	--	1.770	0.55
4	*r(fire), K*	3	3.4	0.08	0.513	1.994	--	-0.377	0.56
5	*r, K, θ*	3	3.44	0.08	0.853	1.897	0.271	--	0.56
6	*r(fire)*	2	3.87	0.06	-0.104	--	--	-0.027	0.64

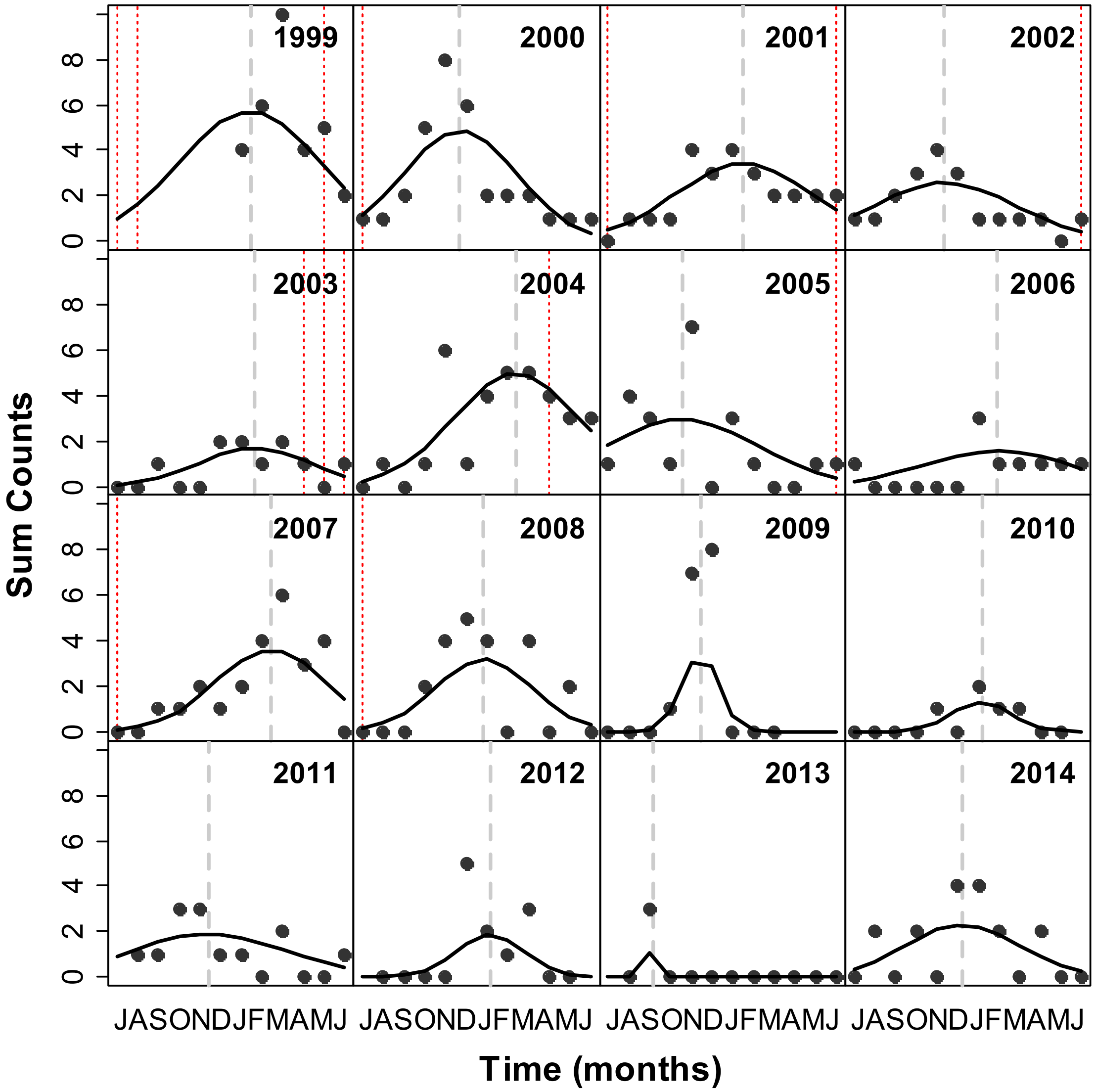

Figure 1.1 Scatterplots of number of adult *A. t. floridalis* observed in the field. Solid lines represent the population density, i.e., the fit of equations 1 and 2. Dashed gray lines mark the month of peak abundance each year. Dotted red lines indicate the timing of fire occurring in the vicinity of at least one site.

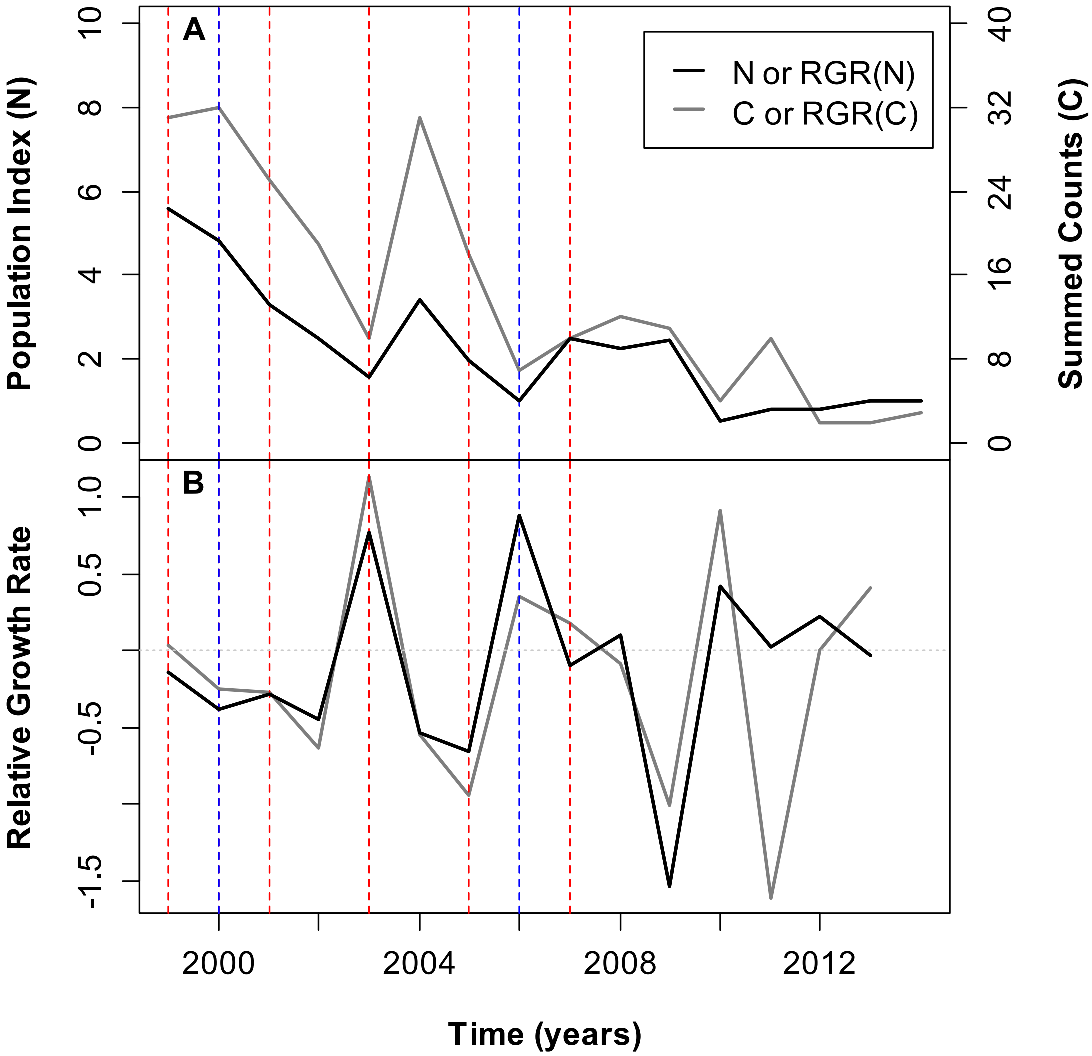

Figure 1.2 Line graphs contrasting *A.t. floridalis* population density indices (peak density for each year), with the sum of butterfly sightings in each year (A), and the relative growth rates for each abundance measure (B). Dotted red and blue lines indicate years when fire or hurricanes occurred.

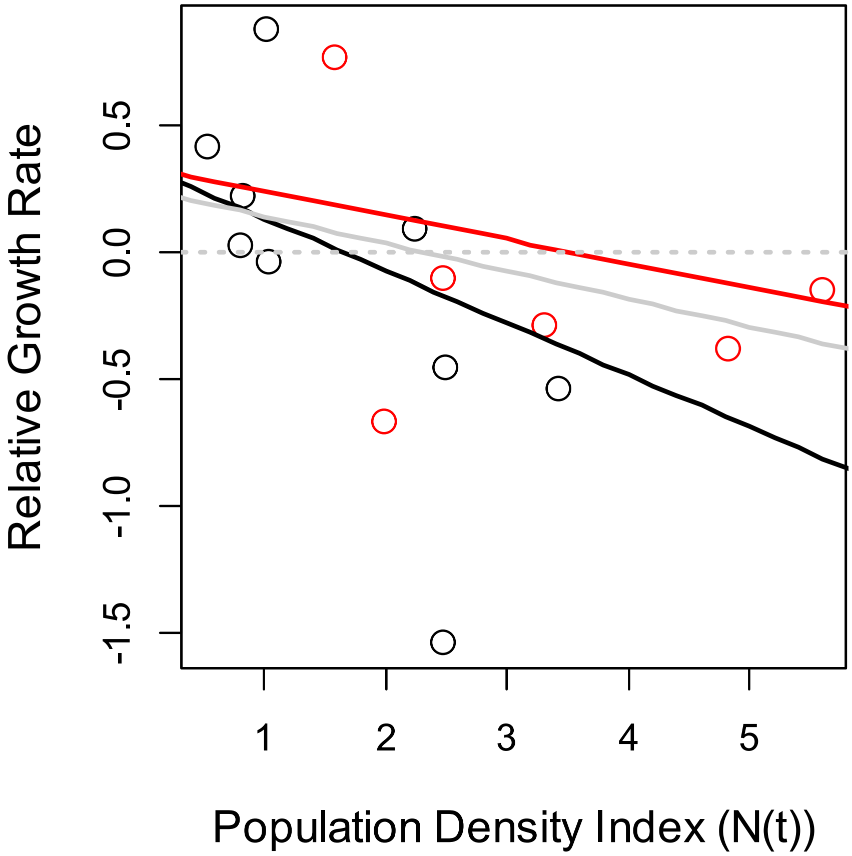

Figure 1.3 Scatterplot of *A. t. floridalis* relative population growth rate, $log[N_{t+1}/N_t]$, as a function of density N_t. The gray line represents the density dependent model without fire effects. Black and red lines represent the density dependent model with fire affecting carrying capacity. The black line represents the relationship in years without fire, while red lines represent years with fire. Fire frequency was simulated at the observed frequency, 6/16

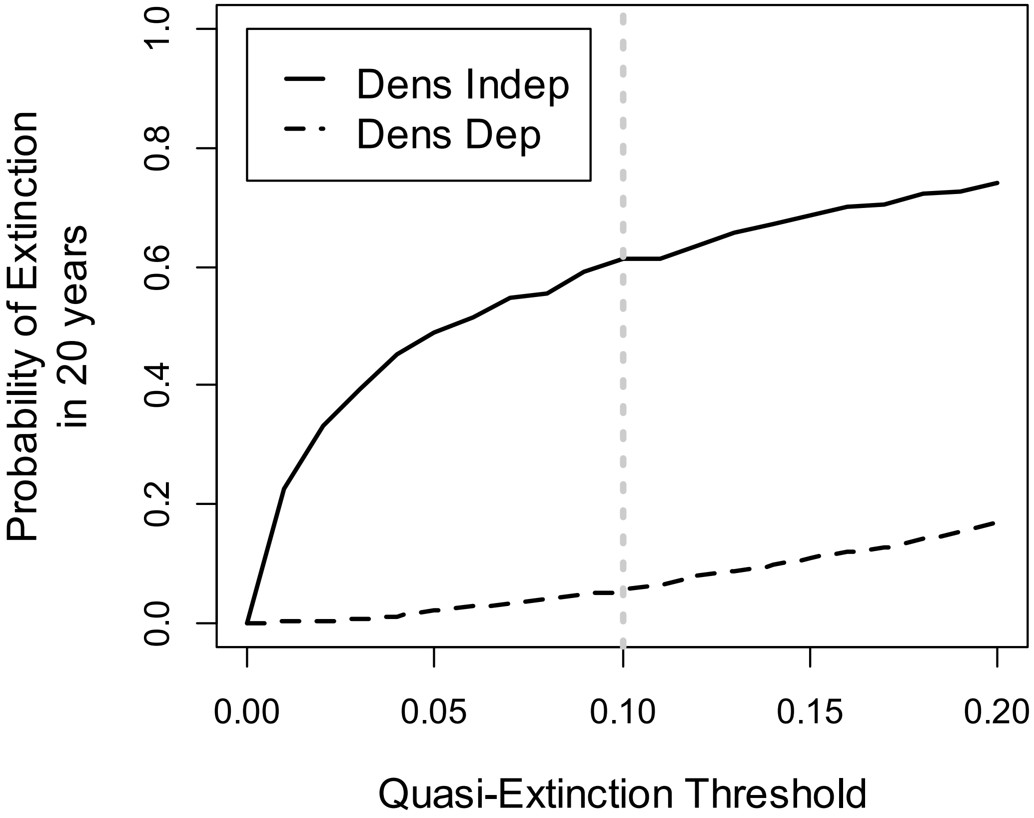

Figure 1.4 Line graph displaying the probability of extinction in 20 years in response to increasing the extinction threshold for *A.t. floridalis*. The solid line is the density independent model (model 1, Table 1.1) and the dashed line is the density dependent model (model 2). The vertical dotted line represents the extinction threshold used in all subsequent simulations.

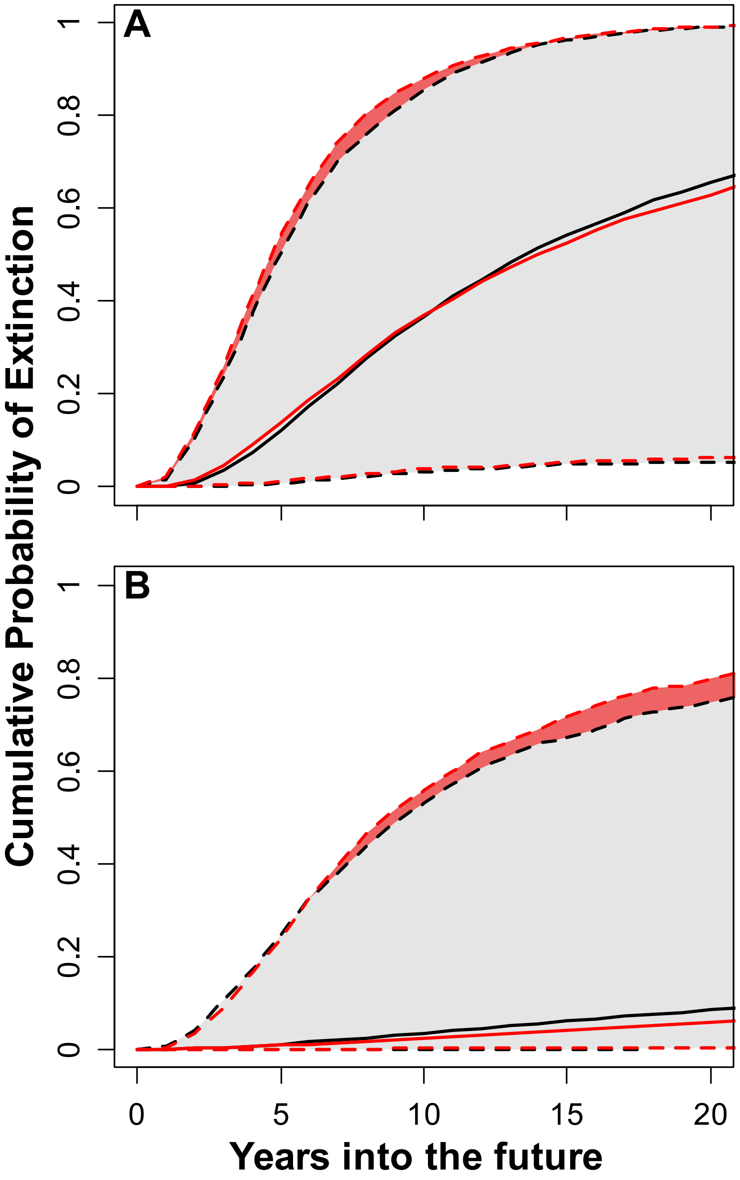

Figure 1.5 Line graph displaying the cumulative probability of extinction projected 20 years into the future under four scenarios for *A. t. floridalis*. Density independent models (A) are plotted without fire in the model (r = -0.115) in black and with fire in the model (r_{nofire} = -0.104, r_{fire} = -0.131) occurring stochastically in our simulations shown in red. Density dependent models (B) are plotted without fire in the model (r = 0.251, K = 2.311) in black and with fire in the model (r = 0.340, K_{nofire} = 1.652, K_{fire} = 3.422) occurring stochastically in our simulations shown in red. Dotted lines represent the bootstrapped 95% confidence limits for the cumulative extinction probability. Black dotted lines enclose the confidence envelope in gray for models without fire, and red lines enclose the confidence envelope for models with fire occurring stochastically. Fire frequency was simulated at the observed frequency, 6/16.

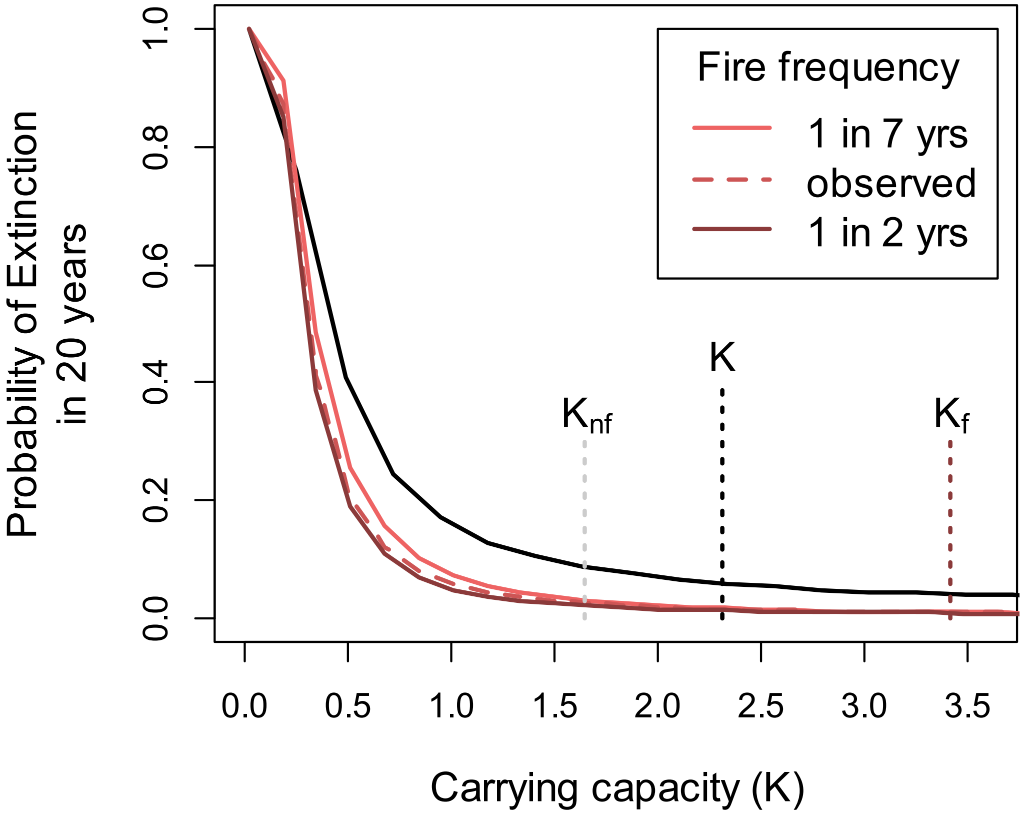

Figure 1.6 Line graph displaying the sensitivity of the probability of extinction within 20 years in response to changes in carrying capacity and fire frequency. Model 2 ($r = 0.251$, $K = 2.311$), shown in black, and model 3 ($r = 0.340$, $K_{nofire} = 1.652$, $K_{fire} = 3.422$), shown in red were simulated for T=10,000 sample paths, each over a range of carrying capacities. Three fire frequencies were simulated representing the minimum fire return interval aimed for by Everglades National Park staff (1 fire every seven years), the observed frequency (6 fires in 16 years), and very frequent fire (1 fire every two years). Three carrying capacity estimates are displayed for reference; K is the estimate from model 1, and K_{nf} and K_f are the K's estimated for no fire and fire years in model 3

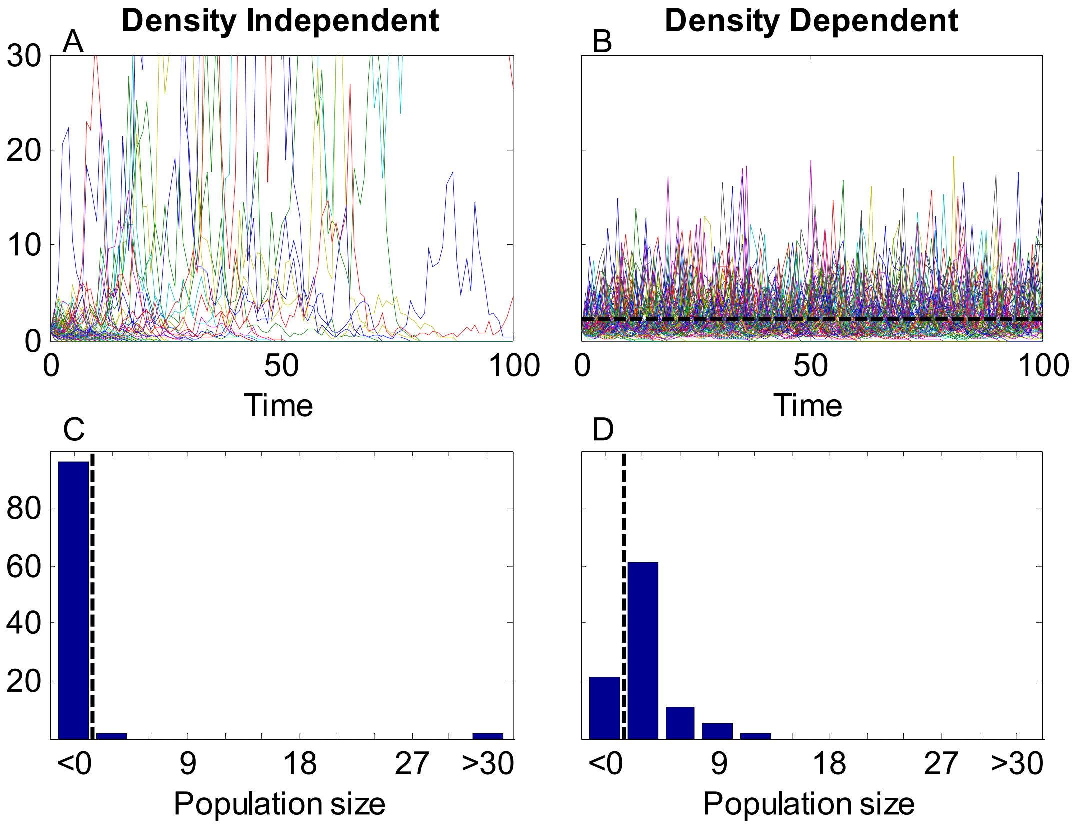

Figure 1.7 Simulation detail showing population trajectories for T = 100 sample paths (A-B) and population density frequency distribution at the end of the 100 year simulation (C-D) for the density independent model (A,C) and density dependent model (B, D).

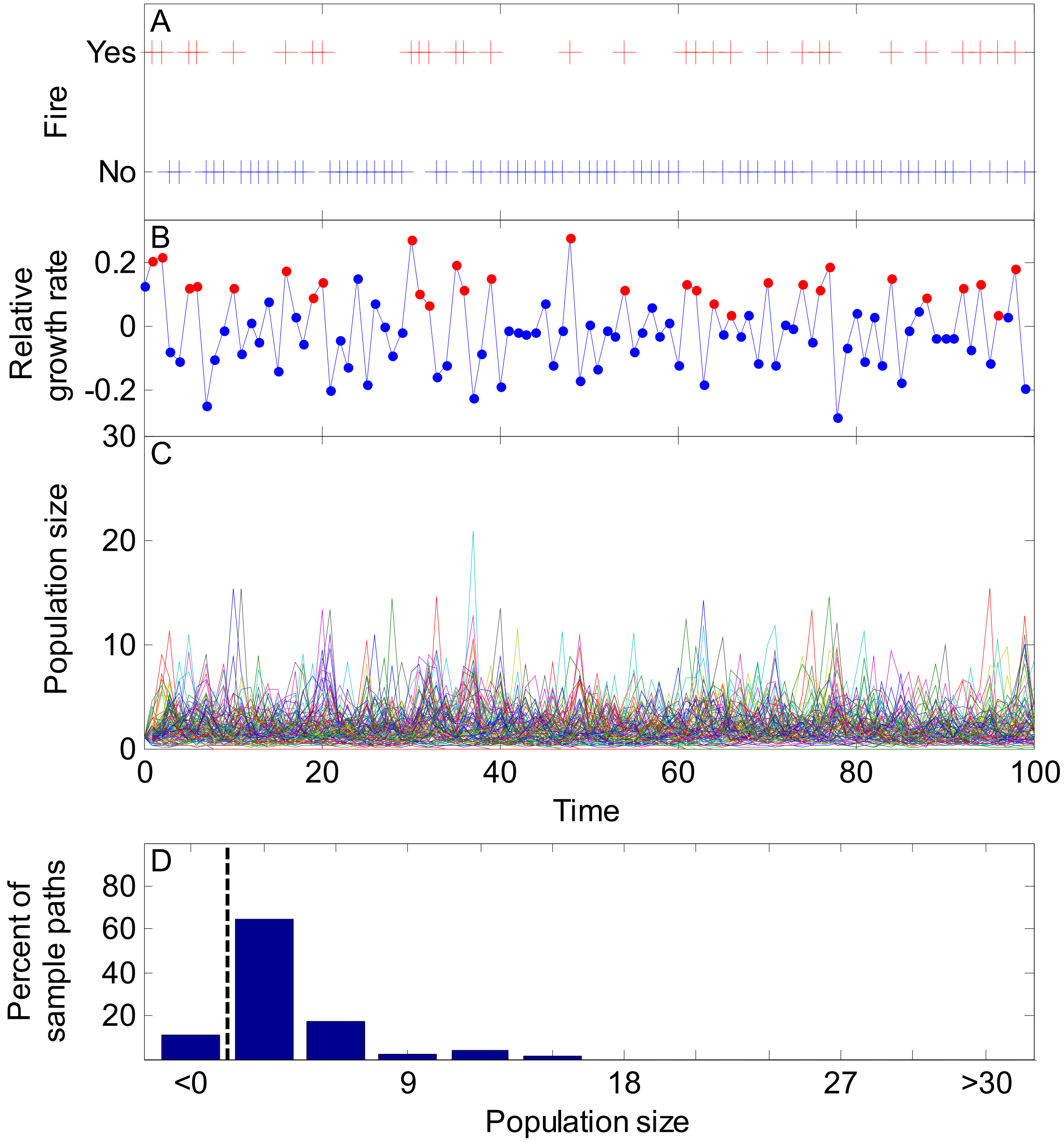

Figure 1.8 Simulation detail indicating the occurrence of fire (A), the mean relative growth rate for all sample paths at each time step (B), population trajectories for each sample path (C), and the population density frequency distribution at the end of the 100 year simulation (D).

Chapter II

Estimating stage-specific demographic rates using a hidden Markov model for juvenile stages in a nymphalid butterfly

Overview

In butterflies, little is known about the dynamics of demographic rates (survival and growth) among juveniles in nature. Using mark-recapture methods, I repeatedly surveyed 510 caterpillars of a common butterfly of Mexico and Central America, *Anaea aidea* (Nymphalidae), in their natural environment for one month in the spring. I asked how the probability of surviving and growing varies across that month, testing for effects of temperature and density.

In mark-recapture, the demographic process is partially “hidden” due to imperfect detection, so I modeled caterpillar demography as a hidden Markov process using Bayesian procedures. With data on survival and development of individuals during each 3-day time step, I modeled the entire process of juvenile development as a column stochastic Markov process. The stage of each individual alive at time *t* identifies a particular column, which contains all possible fates (dead or alive and which stage they would be in) for individuals in that stage 3 days later. These columns together describe the entire juvenile demographic process (from egg to pupa) as a stage-structured matrix model that contains the probabilities of surviving and growing for each stage after a 3-day time step. Using two multinomial probability functions, I estimated the joint likelihood of being alive and observed in a particular stage at each time step given the demographic matrix, the probability of being observed, and the individual’s stage at the previous time step.

During this survey, caterpillar densities increased to a maximum then declined as individuals either died or pupated. I observed both live and dead caterpillars with high probability, and was able to determine the cause of death for most corpses encountered. The abundance of caterpillars attracted multiple insect and arachnid predators, and survival declined as per capita predation rate increased over time. Survival of fifth instars improved at high density, with density scaled by the size of all caterpillars on a host plant, but this is arguably an artifact resulting from the time lag between predator and prey abundance. The most likely model showed that survival of fifth instars declined over the growing season while early instars (first to fourth) had constant high survival. Similarly, the most likely model supported time-invariant stage-specific growth rates, despite a compelling relationship between growth rates and temperatures.

This stage-structured matrix model represents the recruitment process as a progression of individuals from egg to pupa through all juvenile stages, absent in most studies of butterfly demography, and will contribute to our understanding of *Anaea* population dynamics including the endangered *Anaea troglodyta floridalis*.

Background

State-space population models allow us to evaluate plant and animal life histories and population dynamics. Age and stage are standard measures of the state of a population, but attempts to survey population state over time are characterized by observation error due to missing individuals. A rich history of statistical procedures exists to address this issue and at the core of all procedures is the evaluation of the joint likelihood of individuals surviving and being detected (Cormack 1964, Jolly 1965, Seber 1965, Lebreton et al. 1992). Building from this core, models have become increasingly sophisticated to include age structure (Pollock et al. 1990), both live and dead encounters (Burnham 1993), and multiple states as either movement among geographic areas (Brownie et al. 1993) or among discrete developmental stages (Nichols et al. 1992). Multi-state models were initially developed to describe animal movement (Arnason 1973) and remained relatively simple models due to the tedious calculations required to describe complex encounter histories for individuals moving through multiple states. Nichols et al. (1992) extended multi-state mark-recapture models to stage-structured matrix models, a common tool in population biology used to represent organisms with discrete life stages. This link is important because a variety of analytical tools exist to extract demographic (e.g., population growth rate, stable stage structure, and sensitivity of the growth rate to perturbation) and life history properties (e.g., passage times and life expectancy) from age or stage-structured matrices (Caswell 2001). To further integrate multi-state mark-recapture analysis and stage-structured population matrix models, Fujiwara and Caswell (2002) rearranged the typical mark-recapture parameterization into matrix notation that simplifies the likelihood function and flows directly into conventional population matrix model analyses (Caswell and Fujiwara 2004). Gimenez and colleagues updated these statistical procedures using a Bayesian approach and treat the state-space model as a hidden process (Gimenez et al. 2007, Gimenez et al. 2012). The joint likelihood of survival and detection is represented as two parallel time series consisting of the true state dynamics and the observed state dynamics (Gimenez et al. 2012). With these new innovations in multi-state mark-recapture analyses, we can directly estimate all the elements of a stage-structured transition matrix in a single statistical model while accounting for imperfect detection, and all transition and detection probabilities can be defined as functions of time-varying environmental components.

Using methods from Gimenez et al. (2012), I present an analysis of mark-recapture data for caterpillars to estimate a stage-structured matrix model representing the probabilities of surviving and developing from egg to pupation through 5 developmental stages called instars. Lepidoptera (butterflies and moths), like all insects, undergo a series of discrete developmental stages making a stage-based model appropriate. Newborns hatch from eggs, develop through 5 instars, and pupate before emerging as winged adults. Adult butterflies are more often the focus of scientific studies, because juvenile stages (egg – pupa) are cryptic and small. However, recruitment is largely defined by processes affecting survival and growth of juvenile stages, which is a major link between recruitment and the environment. For endangered species, such as *Anaea troglodyta floridalis* (Florida leafwing) with juvenile mortality exceeding 70% (Salvato and Salvato 2012), identifying the environmental factors influencing juvenile dynamics is crucial to mitigating threats and cultivating persistence (Schultz and Crone 1998, Saastamoinen et al. 2013). There are a number of population studies that do include the juvenile stage, but these have typically focused on cohort survival between two sample dates (Young and Moffett 1979, Schultz and Crone 1998, Harrison et al. 2011). Juvenile stage dynamics, such as the timing of stage transitions and stage-specific survival rates, are important in predicting an individual's adult fitness and the overall population dynamics (Saastamoinen et al. 2013).

Anaea aidea (Lepidoptera; Nymphalidae) was selected for this study due to its phylogenetic and ecological similarity to the endangered *A. t. floridalis* (Smith et al.

1994). *Anaea t. floridalis* is endemic to the pine rocklands, which is an endangered ecosystem of southern Florida (Smith et al. 1994), and is at significant risk of extinction especially with continued alteration of disturbance regimes (McElderry et al. 2015). However, few data exist describing *A. t. floridalis* life history and sensitivities of demographic rates to disturbance and the environment. To represent the leafwing life cycle, I developed a periodic stage-structured matrix model of population dynamics that projects the population through multiple *Anaea* generations over one year in 3-day time steps (see Coll et al. 2012 for model description). The periodic model consists of a stage-structured matrix that describes all transitions from one time step to the next. The work described here estimates the demographic parameters for juvenile stages in these matrices. The construct of this periodic model fits my understanding of the ecology and life history of *Anaea* in general, and I will use demographic rates estimated from *A. aidea* as reference points in modifying this model to represent *A. t. floridalis*. Developing a model for *A. t. floridalis* using *A. aidea* as a proxy will provide more insight into the life history and population dynamics of the threatened butterfly than current data allow.

Within a demographic matrix representing all survival and growth probabilities among juvenile stages from time t to $t + 1$ in 3-day increments, I formulated a series of statistical models to test a set of hypotheses describing how survival and development change over time during one month of spring, the month that covers the time between the appearance of eggs and the emergence of adults. Each statistical model included an effect of caterpillar density and an effect of warming spring temperatures, or an unspecified temporal effect that replaced one or both of these covariates in the model. I tested survival for the effects of stage and either caterpillar density or an unspecified temporal effect. I hypothesized that smaller stages would be more susceptible to environmental stress, and that survival of all stages would increase during the recruitment period as warming spring temperatures reduce the chance of death due to cold and frost. Alternatively, density-dependent effects could dictate survival (Nowicki et al. 2009). High density could mean intraspecific competition for food or it may attract more predators. Both scenarios would result in lower survival at high density. Conversely, there may be higher survival at high density, which could result from safety in numbers, predator satiation or dilution, or a time lag between prey and predator densities. I also tested transition rates (i.e., growth from stage to stage vs. stasis within a stage) for the effects of stage, temporal effect, and temperature dependence. I hypothesized that early compared with late instars would have higher probability of growing to the next stage, or perhaps two stages, within 3 days, given that early instars are generally shorter in duration than late instars. I also hypothesized that growth rates of these exothermic insects would increase over time as temperatures warmed throughout the month of April. Both time and temperature were tested independently to see which might better predict changes in growth rates.

Methods

Anaea aidea (tropical leafwing) is distributed throughout Central America and Mexico in tropical dry forest (Scott 1992), but a population has persisted many years in Austin, Texas, U.S.A., (Larry Gilbert, personal communication), which is the northernmost occurrence of *A. aidea*. *Anaea troglodyta floridalis* (Florida leafwing) is endemic to South Florida, where it feeds exclusively on a small shrub, *Croton cascarilla*, and is the northernmost representative of a Caribbean lineage of leafwings, *A. troglodyta*

(Smith et al. 1994). I surveyed *A. aidea* and its local larval host plant, *Croton fruticulosus*, as a proxy for the endangered *A. t. floridalis – C. cascarilla* system. Field work was conducted at the University of Texas Brackenridge Field Laboratory (BFL), which comprises 82 acres along the Colorado River in Austin (30°17’00”N, 97°46’44”W) within a habitat characterized by oak-cedar woodland on limestone upslope from riparian hardwood forest and meadows on alluvial terraces. The limestone outcrop at BFL was once the site of a quarry, and *C. fruticulosus* occurred both in highly disturbed quarry sites and on relatively undisturbed limestone outcrop similar in appearance to habitats were *C. fruticulosus* occurs in larger, less-disturbed natural areas west of Austin. This community differs from the pine savanna were *A. t. floridalis* occurs in that it is more shaded, and associated with canyons. The subtropical pine savannas of South Florida are also on exposed limestone bedrock, but are flat upland features just above the low Everglades wetland. *Croton fruticulosus* is more leafy with wider, softer leaves compared with the narrow tough leaves of *C. cascarilla*.

Mark-recapture of caterpillars is seldom performed (see Young and Moffett 1979, Weseloh 1985) since they shed their skins when transitioning from one stage to another, but particular features of the biology of *A. aidea* provide the opportunity for mark-recapture studies (see Caldas 1995). *Anaea aidea* at the study site feeds exclusively on *C. fruticulosus*, which is a small shrub, and strong host fidelity and characteristic construction behavior in these caterpillars facilitated re-sightings of caterpillars in marked locations on tagged host plants. Caterpillars largely remain on a single host plant (with sufficient foliage) for the duration of all juvenile stages. Similar to other *Anaea* and closely related genera, e.g., *Memphis* and *Consul*, early instars web their feces, called frass, together into a chain that extends from the leaf tip (Muyshondt 1974b, a, Ramos 1984, Caldas 1994). This frass chain serves as a perching spot that dangles from the leaf, and has been hypothesized to reduce predation risk via camouflage and/or predator free space (DeVries 1987). As caterpillars develop into later and larger instars, the frass chain is abandoned and caterpillars form leaf shelters by either webbing leaves together or rolling single leaves (Smith et al. 1994). When not feeding, caterpillars position themselves with their soft bodies protected inside the rolled leaf and their hard head capsules facing out (pers. obser.). *Anaea t. floridalis* is the only *Anaea* that does not consistently roll leaves (Smith et al. 1994, Salvato et al. 2015). Completing larval development, fifth instar caterpillars of the spring brood leave the host plant in search of a pupation site on a non-*Croton* host. This behavior was not observed in the fall brood, when pupation occurs on the same host plant where an individual developed (pers. obser.).

FIELD METHODS – A map of suitable habitat, defined as oak-cedar woodland on limestone outcrop, was delineated and overlaid with a grid composed of 25-m × 25-m cells. Of 16 randomly selected grid points, 14 (22% of 71 total grid points) identified sites containing *C. fruticulosus* that were used for this study. Only one of these sites occurred on natural outcrop, so I added one additional *C. fruticulosus* patch on limestone outcrop for a total of 15 survey sites. At the base of each *C. fruticulosus* shrub within a contiguous patch at each site, I tied an aluminum tag with number unique to each individual, and each individual *C. fruticulosus* was then searched for *A. aidea* caterpillars at each of nine survey dates. For each caterpillar found, I marked the leaf with the caterpillar with a letter unique within an individual plant, identifying the individual caterpillar. Leaves were marked by writing directly on the upper leaf surface with a permanent marker. Surveys began March 28th and continued every day until April 23rd. Three days were required to survey all *C. fruticulosus* in all sites, so I resurveyed sites every three days. All *A. aidea* caterpillars found on marked plants were included in the survey, and the developmental stage (egg, 1 of 5 instars, or pupa), length, perch type (frass chain or leaf roll), and status (live or dead) were recorded for each individual at each survey date.

For each survey date, I recorded the developmental stage of the live or dead caterpillar, and arranged the string of observations for each caterpillar over all dates as an encounter history. Dead caterpillars were removed from the survey after being observed dead. To estimate each caterpillar's size, I measured their length, and the lengths of all caterpillars on an individual plant were summed to represent size-scaled density as a potential factor affecting caterpillar survival. Caterpillar length should scale with biomass, even though length measurements lack a high degree of precision because caterpillars can shorten or lengthen their bodies. Density on a single host plant is the relevant metric of density for this study because juvenile stages generally stay on one individual host plant throughout their development in this species. Size-scaling is relevant since large individuals consume more resources than small ones.

MODEL DESCRIPTION – I represented all possible states (live, dead, removed) and stage (egg, first to fifth instar, or pupa) in a column stochastic model ($\boldsymbol{P_X}$) that describes all possible transitions among states and stages (Fujiwara and Caswell 2002, Gimenez et al. 2012).

$$\boldsymbol{P_X} = \begin{bmatrix} \boldsymbol{J} & \boldsymbol{Z} \\ \boldsymbol{M} & \boldsymbol{U} \end{bmatrix}$$

The matrix, $\boldsymbol{P_X}$, is a block matrix consisting of matrices ***J, M***, ***Z***, and ***U*** and contains the transition rules for a Markov process whereby an individual's current state and stage predict its future state and stage. Matrix ***J*** represents probabilities of fates of juvenile stages that remain alive, the elements are a product of survival and growth.

The life cycle graph for the juvenile portion of the *A. aidea* life cycle proceeds linearly from egg to pupa via discrete and identifiable developmental stages (Figure 2.1). Adults lay eggs individually, from which hatchlings emerge and progress through 5 instars before pupation and adulthood. In 3-day time steps, there is a stage-specific probability of surviving, ϕ_i, and a stage-specific probability of growing from stage i to stage j, ψ_{ji}, independent of survival. Individuals can either remain in the same stage, $j=i$ (called stasis), grow to the next stage, $j=i+1$, or early instars may grow two stages, $j=i+2$.

I used elements of the life cycle graph for the juvenile portion of the life cycle in the current model, and arranged only these probabilities for the juvenile stages in a matrix (***J***);

$$\boldsymbol{J} = \begin{bmatrix} \phi_0\psi_{00} & 0 & 0 & 0 & 0 & 0 & 0 \\ \phi_0\psi_{10} & \phi_1\psi_{11} & 0 & 0 & 0 & 0 & 0 \\ \phi_0\psi_{20} & \phi_1\psi_{21} & \phi_2\psi_{22} & 0 & 0 & 0 & 0 \\ 0 & \phi_1\psi_{31} & \phi_2\psi_{32} & \phi_3\psi_{33} & 0 & 0 & 0 \\ 0 & 0 & \phi_2\psi_{42} & \phi_3\psi_{43} & \phi_4\psi_{44} & 0 & 0 \\ 0 & 0 & 0 & \phi_3\psi_{53} & \phi_4\psi_{54} & \phi_5\psi_{55} & 0 \\ 0 & 0 & 0 & 0 & 0 & \phi_5\psi_{65} & \phi_6 \end{bmatrix}$$

each column represents a particular stage at time t, each row represents the stage at time $t+1$, and each element j_{ji} represents the probability of survival and growth from stage i to stage *j*. Matrix ***J*** only represents transitions among living juvenile stages, but I also observed a large number of dead caterpillars.

Matrix ***M*** describes transitions from the live state to the dead state, and utilizes the same stage-specific survival and ϕ μ ψ growth probabilities. Each element is a product of the probability of dying, $\mu_i = 1 - \phi_i$, and the probability of stage transitioning independent of the probability of dying, ψ_{ji}.

$$\boldsymbol{M} = \begin{bmatrix} \mu_0\psi_{10} & \mu_1\psi_{11} & 0 & 0 & 0 & 0 & 0 \\ \mu_0\psi_{20} & \mu_1\psi_{21} & \mu_2\psi_{22} & 0 & 0 & 0 & 0 \\ 0 & \mu_1\psi_{31} & \mu_2\psi_{32} & \mu_3\psi_{33} & 0 & 0 & 0 \\ 0 & 0 & \mu_2\psi_{42} & \mu_3\psi_{43} & \mu_4\psi_{44} & 0 & 0 \\ 0 & 0 & 0 & \mu_3\psi_{53} & \mu_4\psi_{54} & \mu_5 & 0 \\ \mu_0\psi_{00} & 0 & 0 & 0 & 0 & 0 & \mu_6 \end{bmatrix}$$

The probability of being dead and in stage *i* at time $t+1$ is given by the complement of survival given a caterpillar is in stage *i* at time *t* $(1-\phi_i)$ multiplied by the probability of growing from stage *i* to *j* (ψ_{ji}). Matrix ***M*** represents all possible transitions from live stages to dead stages, i.e., transitions independent of survival. Distinguishing the developmental stage of dead caterpillars allows this model to account for growth occurring between the last observation and death. Here the probability of growing from a fifth instar to a pupa then dying is equal to zero (element $\boldsymbol{M}_{66}$).

For both matrices, ***M*** and ***J***, the probability of growing from a pupa to an adult was set to zero. Pupae were rarely observed in the field and I was unable to collect data on pupa survival and the pupa to adult transition. The last row in matrix ***M*** represents individuals leaving the study, which is the absorbing state I called 'removed'. In matrix ***M***, individuals enter the absorbing state by dying in a stage that left no visible remains, i.e., neither eggs nor pupae were observed in the dead state during this study.

The remaining two matrices within $\boldsymbol{P_X}$, $\boldsymbol{Z}$ and $\boldsymbol{U}$, serve to enforce the absorbing state. Matrix $\boldsymbol{Z}$ is has dimensions 7 × 6 (row × column) with all elements set zero, which represents the impossibility of transitions from the dead state to the live state. Matrix $\boldsymbol{U}$ has dimensions 6 × 6 and represents stage transitions among dead stages, which are similarly impossible. All elements of $\boldsymbol{U}$ are set to zero except for the last row, which represents individuals in the dead state transitioning to the absorbing state. This model classifies dead individuals by developmental stage, and the dead state itself is not absorbing. When an individual dies, it moves to the dead state for one time step so that it may be observed dead. In the next time step, dead individuals move to the absorbing state, removed, indicating it has left the study.

While the block matrix $\boldsymbol{P_X}$ describes the underlying state-space process of interest, matrix $\boldsymbol{P_O}$ describes the parallel process by which we observe individuals.

$$\boldsymbol{P_O} = \begin{bmatrix} 1-p_0 & 1-p_1 & 1-p_2 & \cdots & 1-p_{10} & 1-p_{11} & 1 \\ p_0 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & p_1 & 0 & \cdots & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 0 & 0 & \cdots & p_{10} & 0 & 0 \\ 0 & 0 & 0 & \cdots & 0 & p_{11} & 0 \end{bmatrix}$$

Matrix $\boldsymbol{P_O}$ contains all state and stage specific probabilities of being observed (p_i). The columns of $\boldsymbol{P_O}$ correspond to the 13 state × stage categories [live(Egg, 1st, 2nd, 3rd, 4th, 5th, Pupa), dead(1st, 2nd, 3rd, 4th, 5th), removed] at time *t*. The rows of $\boldsymbol{P_O}$ represent the possible states and stages an individual can be observed as at time *t* [not observed, live(Egg, 1st, 2nd, 3rd, 4th, 5th, Pupa), dead(1st, 2nd, 3rd, 4th, 5th)]. The probability of not being observed is represented in the top row and is the complement of each state- and stage-specific probability of being observed, i.e., $1-p_i$. The probability that an individual is observed in stage i is equal to p_i, and stage identification error was not included. These probabilities are arranged along the sub-diagonal of $\boldsymbol{P_O}$. All other elements of $\boldsymbol{P_O}$ are equal to zero, because they represent being observed in a stage i when the individual is in fact not in stage i.

DEMOGRAPHIC RATES AND COVARIATES – Survival in each stage i, ϕ_i, was either constant through time, modeled as a function of time, or modeled as a function of caterpillar density. The logit link was used to equate survival probability with a linear function of x, $logit(\phi_i) = b_{0k} + b_{1i}*x$, where x was either time (an unspecified temporal effect) or caterpillar density. Reducing this model by setting b_{1i} to zero I obtained the constant survival model. Similarly, the probability of growing from stage i to stage j, ψ_{ji}, was either constant through time, modeled as a function of time, or modeled as a function of local air temperature. Growth probabilities were modeled using a multinomial logit to allow for transitions to multiple stages while requiring that the sum of all possible transitions from i to any stage j sum to one.

$$\psi_{ii} = 1/[1 + \exp(\alpha_{1i}) + \exp(\alpha_{2i})]$$

$$\psi_{i+1\,i} = \exp(\alpha_{1i})/[1 + \exp(\alpha_{1i}) + \exp(\alpha_{2i})]$$

$$\psi_{i+2\,i} = \exp(\alpha_{2i})/[1 + \exp(\alpha_{1i}) + \exp(\alpha_{2i})]$$

The parameters α_{1i} and α_{2i} for each stage i are equal to the log odds of growing one or two stages, respectively, with respect to remaining in stage i. These two parameters estimate the three transition probabilities (growth one stage, growth two stages, and stasis), given the restriction that the probabilities sum to one ($\psi_{ii} = 1 - \psi_{i+1\,i} - \psi_{i+2\,i}$). To test for environmental effects, I formulated each parameter α_{1i} and α_{2i} as a linear function of x,

$$\alpha_{1i} = a_{1i} + a_{xi} * x$$

$$\alpha_{2i} = a_{2i} + a_{xi} * x$$

where x was either time or air temperature, and a_{xi} describes the slope of the relationship between α and x, which is equal for both α_{1i} and α_{2i}. Setting a_{xi} to zero reduces this model so that growth probabilities are constant. For certain transitions (fourth to fifth and fifth to pupa), the probability of growing two stages is zero, so the binomial logit was used for these probabilities with only two possible outcomes.

Caterpillar density was measured in two ways; one representing the number of caterpillars per plant, and the other representing density scaled by the size of all caterpillars on a plant, a proxy for caterpillar biomass. I expected the number of caterpillars to change over time due to the number of births and deaths, both of which should vary through time, and presumed that most births occurred early in the season and declined over time. The number of individuals should increase while eggs are being laid, but then will decrease as egg laying slows and population numbers change only with death. Despite the predicted decline in the number of individuals, I expected the size-scaled density over the whole study area and on individual plants to increase throughout the early spring, as caterpillars fed and accumulated biomass over time. To track changes in the size-scaled density, I measured the length of each caterpillar using a dial caliper, and summed the lengths of all caterpillars for each plant at each survey. These two density metrics correspond to two mechanisms by which density dependence may be realized. Numbers of caterpillars may be an attractor for foraging predators, or could provide safety in numbers, given a constant number of predators. Increasing caterpillar biomass may indicate decreasing food, because larger late instar caterpillars are capable of consuming many more leaves than early instar caterpillars.

Temperature is known to affect development time in insects, with increasing temperature generally increasing metabolic feeding rates resulting in quicker development (Wagner et al. 1984). I obtained air temperature data collected every hour for the Austin area from the National Weather Service webpage (NOAA NWS 2011) and averaged hourly temperatures over three day intervals corresponding to the survey intervals for each site.

MODEL LIKELIHOOD – The likelihood of this hidden process model is composed of two equations, one a state equation, and the other an observation equation. The state equation uses matrix $\boldsymbol{P_X}$ to describe the state of an individual at time *t* given its state at time *t-1*. The observation equation uses matrix $\boldsymbol{P_O}$ to describe the probability of being observed at time *t* given the state of an individual at time *t*.

$$X_{k,t} | X_{k,t-1} \sim Categorical\left(\boldsymbol{P_X} * X_{k,t-1}\right)$$

$$Y_{k,t} | X_{k,t} \sim Categorical\left(\boldsymbol{P_O} * X_{k,t}\right)$$

Each individual *k* at each time *t* was represented by a matrix $\boldsymbol{X_{k,t}}$ of zeros with a one in the row and column indicating the state of the individual at time *t*. The state of an individual at time *t* depends on its state at time *t-1*. Multiplying $\boldsymbol{P_X}$ by $X_{k,t-1}$ gives a vector of probabilities that predict state at time *t* via the categorical distribution, which is the multinomial distribution for a single sample. Similarly, the observed state of individual *i* was contained in matrix $\boldsymbol{Y_k}$. Observations of all individuals over all time steps were modeled with the categorical distribution to estimate the observation matrix, $\boldsymbol{P_O}$.

I used Bayesian estimation via Markov chain Monte Carlo (MCMC) simulations to fit the equations above to capture histories of marked caterpillars surveyed in the field. For linear model parameters, e.g., b_{0i}, a_{1i}, etc. describing survival and growth probabilities, I used uninformative normal prior distributions, and I used uninformative uniform distributions between zero and one for observation probabilities. Demographic parameters for eggs and pupae, including the fifth instar to pupa transition, were rarely observed but necessary for model stability, so I estimated informative prior distributions using data from lab-reared larvae. The posterior distributions for all parameters were estimated using 10,000 MCMC iterations following 120,000 iterations of 'burn in', which according to standard convergence diagnostics was sufficient for model convergence. I performed all procedures using R (version 3.1.0 (2014-04-10), R Core Team 2014) and JAGS (Plummer 2003) via the R package, rjags (Plummer and Stukalov 2014).

Constructing 18 competing models I used Akaike's information criteria (AIC) to evaluate the relative support of candidate models (Burnham and Anderson 2002). Specifically, I investigated the effects of date and density and their interactions on survival and the effects of date and temperature and their interaction on growth. Within this factorial design I also tested for reduced stage structure in survival, grouping first to fourth instars as distinct from fifth instars. In all models the probability of observation was stage-dependent and constant over time.

It is important to note that I did not independently evaluate each hypothesis concerning the relationship between survival, growth and the environmental covariates.

Instead, each candidate model contained a specific combination of factors and represented a competing hypothesis for the system's dynamics. I evaluated the fit of the entire of the model, which was penalized for the number of parameters used. With this approach, the relationship between one demographic rate and the environment was evaluated in the context of all other demographic rates. Using model selection in this case was appropriate, because AIC helps select the model with the best fit using the fewest parameters and is not typically used for testing null hypotheses (Burnham and Anderson 2002). I evaluated the relative support of each model using AIC, and selected the best model for future predictions, but I also identified models with a reasonable fit and model support and described their relevance to the biology of *Anaea*.

Results

Caterpillar abundance increased during this study to a maximum in mid-April, and then decreased as new individuals appeared less often and individuals either died, left the host plant to pupate, or left the host plant and the study area (Figure 2.2C). Over 27 survey dates (9 surveys per plot), I observed 510 individuals in a total of 2,183 observations. I observed both live (n = 2,062 observations) and dead (n = 107) caterpillars with relatively high probability (Figure 2.3A), but I seldom observed eggs and pupae (n = 14). The probabilities of observing live individuals varied by stage, with third and fourth instars observed with a lower probability compared with first, second, and fifth instars, which may be due to the shift in perch construction behavior from frass chain to leaf roll during these intermediate instars. The probabilities of observing dead individuals were relatively high, but these probabilities dropped significantly for fourth instars and more so for fifth instars (Figure 2.3A). Caterpillar corpses encountered most commonly showed signs of predation, i.e., a blackened and somewhat shriveled body at a single feeding point, but there were a few corpses found that died during the molting process. Failed molting was indicated by a persistent head capsule from the previous instar stuck over the mouth parts of the new instar head capsule.

Survival of fifth instar larvae decreased over time, and relative model likelihood favored a simple model (Table 2.1) with early instars sharing one constant survival rate (Figure 2.2A). Survival started high for all stages, but steadily declined through the growing season for fifth instar larvae (Figure 2.4). Survival did indeed appear linked to density, but survival increased rather than decreased with high density (Figure 2.2A). Models that used the size-scaled density (the summed length of caterpillars per plant) outperformed models that used the number of caterpillars per plant. For simplicity, only models with size-scaled density were used for model evaluation (Table 2.1). However, density models in general did not receive significant support relative to other models. Mean caterpillar length, increased almost 5 fold during larval development from first to fifth instars (1st = 4.1, 2nd = 6.4, 3rd = 9.4, 4th = 13.3, 5th = 19.9 mm). Total size-scaled density lagged a few days behind the total number of caterpillars as each of these metrics increased to a maximum midway through the brood then decreased (Figure 2.2C). Over this time period, stage structure progressed from early instar predominance to late instar predominance.

Per capita predation rate, measured by Poisson regression of the number of corpses encountered over time, increased by 11% per day during the month of April ($z=7.32$, $p<0.001$). Individuals late in the brood therefore had higher risk of predation than individuals early in the brood (Figure 2.2). Most dead caterpillars looked as if they had been attacked by one of several insect or arachnid predators (i.e., the corpses were left clinging to the plant and exhibited evidence of feeding damage). I observed numerous hemolymph-sucking arachnid and hemipteran predators patrolling for caterpillars. Hemipteran predators included a wheel bug (Reduviidae) and a soldier bug (Pentatomidae), both of which hatched from eggs laid on *Croton* and appeared to focus on feeding on *Anaea* caterpillars during each bug's larval development. Additional insects patrolling *Croton* and/or attacking *Anaea* caterpillars include green lacewings (Chrysopidae) and a small parasitoid wasp (Hymenoptera). Large polistine wasps (Polistinae) were seen searching *Croton*, and I observed several yellow jackets (Vespinae) attacking a large caterpillar of another species, but I did not observe predation events of *Anaea* caterpillars with these potential predators. Not observing predation by large predators such as wasps and birds does not mean that these predators did not have an effect. These predators tend to remove caterpillars from where they are found, leaving little if any trace (pers. obser.). There was a single instance where a rolled leaf shelter appeared chewed by a beak (triangular damage), leaving triangular markings and black hemolymph as the only remains. Predation rates by wasps and birds could not be quantified in this study. Disappearance of fifth instar larvae could mean either predation or pupation, but I controlled for this in the modeling by using an informative prior distribution for the probability of pupating.

The probability of growth (advancing either one or two stages) within 3 days decreased for progressively higher stages (Figure 2.3C). Concomitantly, the probability of remaining in the same stage for 3 days increases for later instars. Over the month of April, the probabilities of growth generally increased, mostly for early instars (Figure

2.5B). This increase in growth rates represents more rapid development and was linked to increasing temperature (Figure 2.5). Temperatures showed a warming trend during this survey, increasing on average about 20°F, but there was also significant temperature variation over time (Figure 2.5A). Warmer temperatures resulted in increased growth (Figure 2.5C) for all stages except for fifth instars. Despite a clear relationship between temperature and growth rates, model selection results suggested that neither temperature nor the progression of time in April were significant predictors compared with models that included only stage-dependent growth rates.

Discussion

In an effort to describe the recruitment process, which is not well quantified in most butterfly demographic models(Nowicki et al. 2009, McElderry et al. 2015), I applied relatively new techniques in mark-recapture statistics to estimate demographic rates among juvenile stages from mark-recapture data generated from field surveys of caterpillars. While my results established links between survival and caterpillar density and also between growth rates and temperature, model selection using AIC suggested that the recruitment process for *Anaea aidea* was best represented using constant survival for early instars, decreasing survival for late instars over the month of April, and growth rates that varied with stage but were not affected by the progression of time in spring. This result does not suggest that density and temperature had no effect on survival and growth rates of caterpillars, but does suggest that a simpler model was more efficient.

DETECTION – Overall high observation probabilities for first to fifth instars lended credibility to estimates of demographic rates for all caterpillar stages. Eggs and pupae were rarely observed in the field, so I did not expect model estimates for these stages to be meaningful. Eggs are tiny, often laid on the undersides of leaves, and time was not allocated to searching for eggs during this study. Fifth instar caterpillars left the host plant in search of a pupation site, so pupae were rarely found since I only searched host plants. The lack of observed fifth instar to pupa transitions did not confound fifth instar survival, because I set this transition probability to match what the transition probability estimated for caterpillars raised in the laboratory.

High observation probabilities estimated for caterpillar stages reflect the high fidelity of caterpillars to a particular area on a host plant. This high fidelity was likely due to the time caterpillars invested in building perches. First to third instar larvae constructed frass chain perches extending from the leaf tip, and fourth to fifth instars constructed rolled leaf shelters. The shift from frass chain to rolled leaf occurred during either the third or fourth instar, which may explain why third and fourth instar larvae were less likely to be observed. Often third instars molted on the frass chain and then moved to a new leaf to construct a leaf roll as a fourth instar, but some third instar larvae left the frass chain before molting. First and second instars generally remained on a single leaf for each stage; a single leaf providing sufficient food for the duration of these stages. As individuals grow progressively larger with each stage, they require many more leaves to complete development in each stage. In the laboratory, third instars required on average 2 leaves, fourth instars required 5 leaves and fifth instars required 20 or so leaves depending on leaf size (unpublished data from captive breeding). In the field, third and fourth instars were progressively more mobile, and fifth instars definitely occupied a larger area of the host plant, but were large and left behind obvious signs of herbivory making them easier to spot.

Motivated by greater nutritional requirements, fifth instar larvae had increased mobility and a larger foraging area relative to early stages. Fifth instars tended to defoliate all or a portion of their host and then moved to search for more food. Toward the end of the survey, host plants were increasingly defoliated and hungry caterpillars moved to adjacent host plants or were not observed again. All of the host plants in a patch were tagged and searched, so unobserved individuals most likely perished rather than crawling long distances to neighboring patches. This is one possible reason explaining why dead fifth instars were observed significantly less often than other instars.

SURVIVAL – High fifth instar survival early compared with late in the brood suggested a benefit to being born earlier. This brood was surveyed from the end of March to the end of April, a time during which spring progresses in Central Texas and several changes occur. I included an unspecified temporal effect in the model to include the myriad of changes occurring as spring progresses. Warming temperatures is one aspect of spring, and is a main driver of several other changes at this time, e.g., bud break, flowering, and the emergence of insects. It is likely that some subset of factors that increased with spring warming during my survey, like increasing insect abundance (Lowman 1982) and/or nesting and breeding in birds (Schwartz and Reiter 2000), was the driver of decreased survival of caterpillars during the month of April in this study. Leafwing caterpillar density certainly increased during this transition decreasing the amount of food available and attracting a number of predators, both directly linked to survival rates. Oviposition rate decreased and predation rate increased as the brood progressed, resulting in the observed initial increase then decrease in the number of individuals. There are two ways density could have affected survival in this system. Increasing caterpillar numbers and/or size decreased the amount of leaf tissue available for consumption. Similarly predator numbers and sizes seemed to increase over the survey period, resulting in the observed increase in per capita predation rate.

Although, models that included density as a factor received significantly less support than the best two models, the estimated effect of density on survival was interesting in that it implied that caterpillars were safer in high density. In classic models of predator-prey dynamics, predator numbers lag behind prey numbers and predation rate increases with the rise in predator density (Roughgarden 1998). Increasing numbers of predators coupled with decreasing prey numbers yields an increasing per capita death rate for the prey. Rather than caterpillars simply enjoying safety in numbers, these results were better interpreted in terms of predator-prey dynamics. As stated previously, the environment progressively changes during spring, characterized by stereotypical population and community dynamics. The leafwing recruitment pulse (prey) was followed by a predator recruitment pulse, meaning that *A. aidea* caterpillars were threatened by fewer predators early in the brood, and predation pressure increased as the predator density increased over time. The time lag meant that high prey numbers occurred with low predator numbers, giving the result that prey survival was higher at high prey densities.

The focus on predator-prey dynamics and positive density dependence ignores resource competition among prey, and the density-dependent survival estimates argue against the influence of limiting resources. However, the lack of support for negative dependence does not refute that it may have had an effect. In fact, the lasting effect of previous caterpillars was not included in the metric of density employed here, but it would provide an interesting representation of resource competition. While caterpillar density increased then decreased during the survey, none of the leaves consumed by caterpillars were replenished, meaning the abundance of edible leaves simply decreased over time. The abundance of leaves was not measured, but this resource certainly decreased in the month of April, possibly contributing to the observed decline in survival over time. Finding starved caterpillars would have supported this argument, but starving caterpillars would have wandered in search of food, making them almost impossible to find. Fifth instars were the most likely to starve, given their much higher nutritional needs relative to early instars, and were also the least likely to be observed dead. Overall decreasing survival over time was supported in the best model, which supports both the hypothesis that predators are having a progressively larger impact over time and that resource competition may increase over time.

The preeminence of predation as a factor decreasing survival of *A. aidea* juveniles is interesting not only for *A. aidea*, but it has implications for *A. t. floridalis*, for which a relatively large number of predators and parasitoids have been described (Salvato and Hennessey 2003, Salvato et al. 2008, Salvato et al. 2009). What is interesting is that *A. t. floridalis* is the only leafwing that does not construct rolled leaf shelters as fourth and fifth instars. All other *Anaea* (and many closely related genera) construct shelters either by rolling single leaves or tying leaves together with silk, presumably to evade parasitism and predation (Scott 1992, Smith et al. 1994). *Anaea t. floridalis* larvae have been observed tying leaves together, but this behavior is observed very rarely (Cech and Tudor 2005, Salvato et al. 2015), and may be due in part to the host plant architecture. The leaves of *Croton cascarilla* are generally too slender to roll and too sparsely arranged to tie multiple leaves together into a shelter, which is not the case for the leaves of *Croton* used by other *Anaea*. Without the protection of a leaf shelter, late instar *A. t. floridalis* are more exposed to predators, and the predation rates measured for *A. aidea* in this study likely underestimate those for *A. t. floridalis*. Indeed, predation/parasitism was listed as the most common cause of *A. t. floridalis* larval mortality in the field (Salvato and Salvato 2012).

GROWTH – Growth rates were stage dependent and time-invariant, suggesting development time was not extremely variable over the month of April. As caterpillars develop, they grow larger and increase rates of feeding rates to meet increasing nutritional requirements for each successive molting (Chown and Nicolson 2004). With this fundamental development plan, the time required in each stage increases in later instars, represented here by increased probabilities of remaining in the same stage for each 3-day survey. The probability of growing two stages within 3 days was non zero for early instars and was somewhat common only for eggs transitioning to first then second instars. Temperature did appear to affect development time, a phenomenon well described in ectothermic insects overall (Chown and Nicolson 2004), but temperature-dependent growth rates were not supported over constant growth rates for each stage. Increasing growth rates for first to fourth instars with warming spring temperatures indicated more rapid development later in the brood relative to early in the brood. Rapid development during a period of increased predation risk would be favorable, but of course this was not likely the cause.

The lack of variation for fifth instar growth rate was likely due to the stage duration relative to the sampling time step. Changing stage durations that are around the 3 or 6 day interval would be more likely to result in a change in the corresponding 3-day transition probabilities. In the laboratory, stage durations for fourth and fifth instar larvae were 4.9 and 11.8 days, respectively, under temperatures set to match those in the field at the end of April (R. McElderry, unpublished data). Increased development rates of fourth instars in the field likely decreased the stage duration from above 6 to below 6 days, resulting in an increased probability of individuals transitioning within two sample dates. Changes in stage duration for fifth instars likely had little effect on the probability of transitioning given that fifth instar caterpillars were most likely to remain in this stage for at least three sample dates.

This work describes the demographics of the butterfly recruitment pulse in a matrix model estimated using Bayesian procedures and a multi-state mark-recapture framework. Building from the current state of mark-recapture analyses (Fujiwara and Caswell 2002, Gimenez et al. 2012), and in line with similar innovations in mark-recapture history (e.g., Burnham 1993), I added dead recovery data to this multi-state model to improve survival and growth probability estimates. The state-space formulation of the multiple states scenario, simplifies the analysis of multi-state mark-recapture data, and in fact lends itself to adding information from dead recoveries. The requirement that the Markov model ($\boldsymbol{P_X}$) should be column stochastic, means that transitions from live to dead stages are included in the model. The frequent fortuitous encounters with dead caterpillars during surveys, carefully recorded, fed directly into this framework and improved the precision of demographic estimates.

CONCLUSION – Applying this level of mark-recapture field methods and statistical procedures to caterpillars is unprecedented in butterfly research, and has proven effective in illuminating the progression of individuals from egg to pupa, which comprises the recruitment process. The time-dependent estimates for matrix ***J***, the demographic matrix describing juvenile stage transitions, will be included in a periodic matrix model to describe the life cycle of *A. aidea* through multiple breeding events within a yearly cycle. The impact of each juvenile demographic rate on the population growth rate can then be quantified through perturbation analysis of the periodic matrix. In doing so, I may be able to identify particularly sensitive or influential life stages and/or seasons, which may have implications for the endangered relative, *A. t. floridalis*.

Table 2.1 Model selection table showing the information criteria (AICc and ΔAICc), model likelihood, and relative likelihood (Weight) for each model. Models were manipulated such that either survival (ϕ) or transition (ψ) probabilities were functions of stage, time, density, or temperature. For survival only, stage was grouped into early and late instar, represented as, st(E,L). All models used stage-dependent probability of observation .

model		**# Par**	**AICc**	**ΔAICc**	**Weight**
ϕ(st(E,L),time)	ψ(stage)	28	1588.86	0	0.5
ϕ(stage)	ψ(stage)	29	1588.87	0.01	0.5
ϕ(st(E,L))	ψ(stage)	26	1603.58	14.72	0
ϕ(stage,density)	ψ(stage,temp)	38	1605	16.14	0
ϕ(st(E,L),density)	ψ(stage,temp)	32	1606.37	17.51	0
ϕ(st(E,L))	ψ(stage,temp)	30	1607.96	19.1	0
ϕ(stage)	ψ(stage,time)	33	1608.09	19.23	0
ϕ(st(E,L),time)	ψ(stage,temp)	32	1612.62	23.76	0
ϕ(st(E,L),density)	ψ(stage,time)	32	1617.25	28.39	0
ϕ(stage,time)	ψ(stage,time)	38	1620.53	31.67	0
ϕ(stage)	ψ(stage,temp)	33	1621.13	32.27	0
ϕ(stage,density)	ψ(stage,time)	34	1629.24	40.38	0
ϕ(st(E,L),time)	ψ(stage,time)	32	1630.5	41.64	0
ϕ(st(E,L),density)	ψ(stage)	28	1633	44.14	0
ϕ(stage,time)	ψ(stage,temp)	38	1641.69	52.83	0
ϕ(stage,density)	ψ(stage)	38	1647.73	58.87	0
ϕ(st(E,L))	ψ(stage,time)	30	1663.16	74.3	0
ϕ(stage,time)	ψ(stage)	34	1665.02	76.16	0

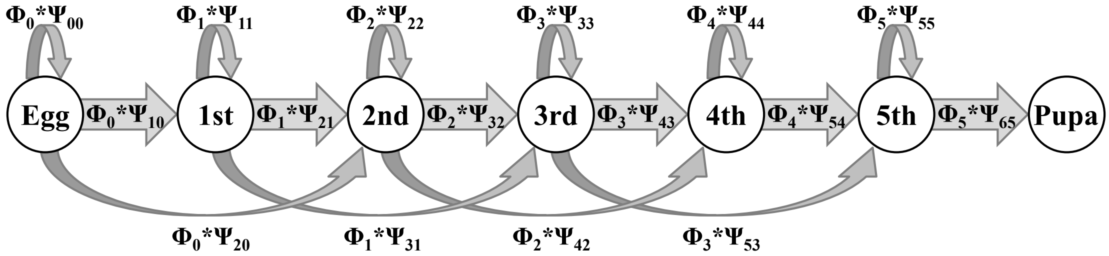

Figure 2.1 Diagram of the juvenile portion of the leafwing life cycle with circles indicating each developmental stage, and arrows indicating the one time step probabilities of transitioning (Ψ_{ji}) from stage *i* at time *t* to stage *j* at time *t+1* conditional on survival, (Φ_i).

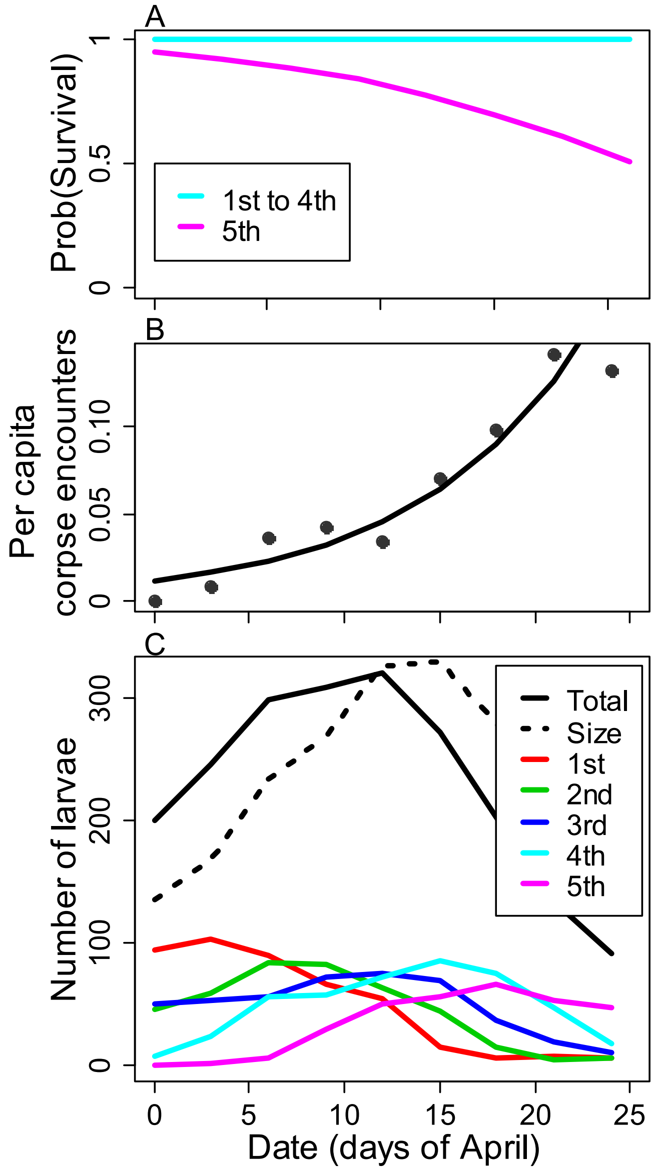

Figure 2.2 Line plots indicating the change over the survey period in (A) the mean probability of survival, (B) the per capita corpse encounter rate, and (C) the number of larvae in each stage and total caterpillar biomass. Points (B) represent the number of caterpillar corpses encountered per capita in each survey over the survey period, and the line represents the encounter rate; fit using Poisson regression. Size represents the size-scaled density measure estimated by summing the length of all live caterpillars at each survey.

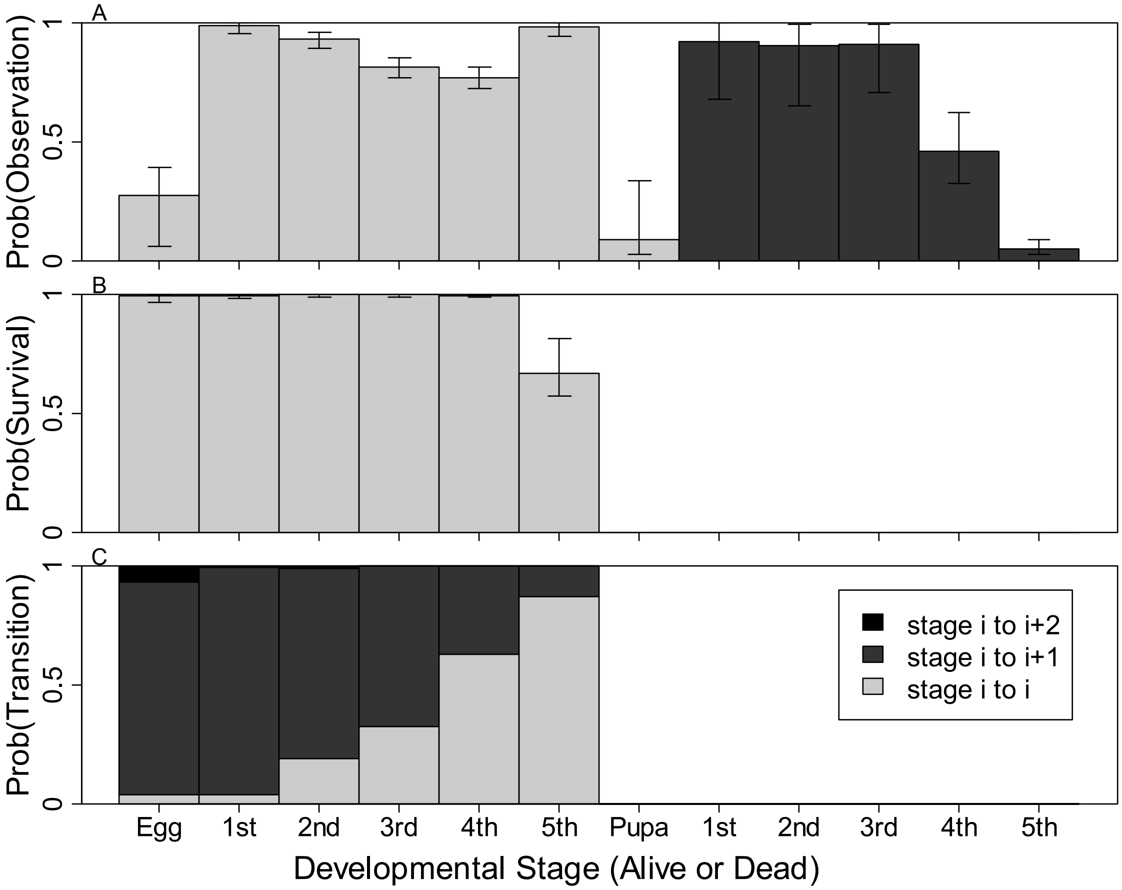

Figure 2.3 Bar plots indicating the stage-specific mean probabilities of (A) detection for live (light bars) and dead (dark) individuals, (B) survival, and (C) transitioning from each stage *i* to either *i*, *i+1*, or *i+2* during a 3-day interval, independent of survival. Detection probabilities (p_i) fill the detection matrix (P_0), and survival probabilities (ϕ_i) are combined with transition probabilities (ψ_{ji}) to fill the demography matrix (P_X). Error bars indicate the 95% credible interval.

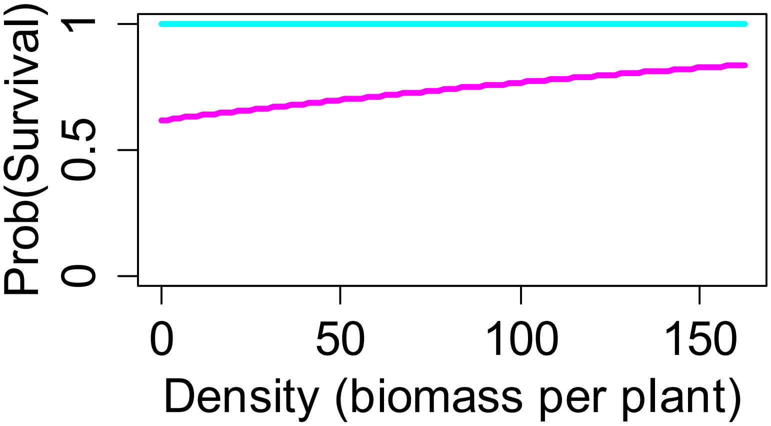

Figure 2.4 Line plot indicating the change in mean survival in response to size-scaled density. Size-scaled density was estimated by the sum of the lengths of all caterpillars on a plant at each survey.

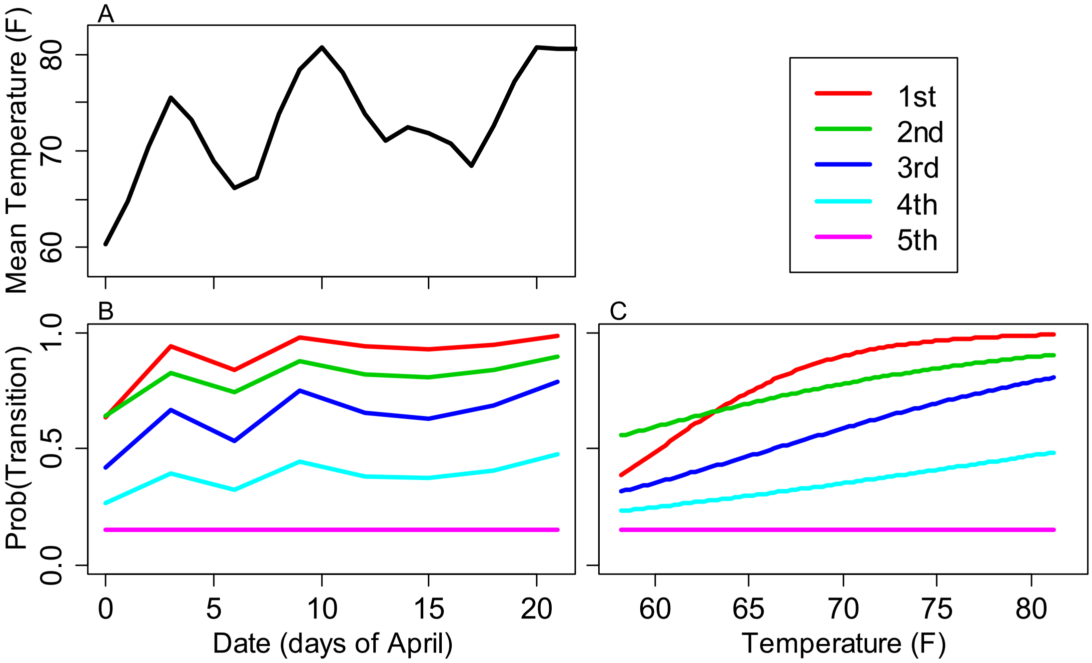

Figure 2.5 Line plots displaying the change over the survey period in (A) mean air temperature and (B) the probability of growing (defined as advancing either one or two stages) during a 3-day interval. The change in growth probabilities over time (B), is due to the relationship between transition probabilities and temperature (C). Temperature represents the hourly regional air temperature averaged over each 3-day time step.

Chapter III

Plant-mediated demographic performance in co-occurring yet widespread butterflies, one tropical and one temperate

Overview

Insect herbivores commonly feed on multiple plants throughout their geographic range, but feed locally on only one or a few plants. I asked how specialized were the diets of two related butterflies, *Anaea aidea* (tropical) and *Anaea andria* (temperate). Rearing almost 300 caterpillars in the lab on controlled diets of one of five *Croton* host plants, I evaluated the performance of caterpillars in terms of survival to pupation, development time, pupa mass, and adult size. Both leafwings showed a moderate ability to feed on multiple *Croton*, but tended to perform best on their *local host*. Even though each leafwing was capable of surviving to pupation on *non-local hosts* and *local non-hosts*, either growth or development time was less than ideal compared with the *local host*. The tropical leafwing performed well on all but one *Croton* in this experiment and performed better on its *local host* compared with the temperate leafwing on its *local host*. The temperate leafwing's *local host*, *C. argyranthemus* was a poor host for both leafwings, and *C. monanthogynus* (*non-local host* for the temperate leafwing and *local non-host* for the tropical leafwing) was a suitable host for both species. The results of this experiment and my experience with this system indicated to me that the endangered Florida leafwing might be restricted to a single host only because there are no suitable *Croton* nearby. I argue that the Florida leafwing could potentially feed successfully on other species of *Croton*, particularly those known as hosts of other *Anaea* in the Caribbean. Further experimental work would be needed to test this hypothesis and to determine if there would be management implications of finding alternative hosts.

Background

The coevolution of plants and insect herbivores has driven species diversity (Ehrlich and Raven 1964). Whether coevolved (in the narrow sense) or not, the close relationship between the life cycles of interacting plants and herbivores has an impact on the life history, fitness, population dynamics, and geographic distributions of each organism (Holland and Fleming 1999, Thompson and Cunningham 2002, Thompson and Fernandez 2006). For insect herbivores in particular, host plants supply not only all required nutrition, but also the microhabitat in which an individual completes all or at least the juvenile period of its lifetime (Jaenike 1990, Mira and Bernays 2002). Specialization leads to increased efficiency and is advantageous for many insects (Bernays 2001). It is therefore not surprising that many insects are highly specialized for life on single plant species (Thompson 1994), but numerous examples exist of highly generalized insects capable of surviving on any one of a long list of plant species (Bernays 1998). It is within this spectrum that I ask why some species are geographically widespread while others are narrowly distributed.

Many insects that use multiple host species over their geographic range specialize locally on one or very few hosts (Fox and Morrow 1981). This pattern generally involves phylogenetic conservatism, where diversification in host use tends to be restricted within a clade of plants sharing similar chemistry (Ehrlich and Raven 1964, Novotny and Basset 2005). In a review of insect diet breadth and geographic distributions, the distribution of plant genera determined the distribution of specialist insects, and specialist insects would feed on multiple species of the same genus when co-occurring locally (Novotny and Basset 2005). While specialization has advantages over generalization (Bernays 2001), it appears that some plasticity is beneficial to allow maximum utilization of all hosts occurring locally, and also to allow for a wider geographic range (Novotny and Basset 2005), which in butterflies is a key determinant of metapopulation viability (Hanski et al. 1996).

The link between host specialization and geographic range is important to understand if we aim to conserve endemic specialist herbivores threatened with extinction. I explored host plant specificity in leafwing butterflies, genus *Anaea* (Nymphalidae), which feed exclusively on host plants in the genus *Croton* (Euphorbiaceae)(Smith et al. 1994). The Florida leafwing, *A. troglodyta floridalis*, is endemic to southern Florida where its larvae feed on a single plant, *C. cascarilla*, and is threatened with extinction (McElderry et al. 2015). In this paper, I focused on two leafwing species with broad geographic distributions, one from mostly tropical latitudes, and the other from mostly temperate latitudes, both occurring in Texas. The *Croton* hosts for these *Anaea* differ in life history resulting in differences in the phenology and quality of available food for *Anaea*. Tropical leafwing (*A. aidea*), like the Florida leafwing, feed on perennial woody shrubs, while temperate leafwing (*A. andria*) feed on perennial and annual herbs(Scott 1992, Cech and Tudor 2005). Co-occurrence of these *Anaea* in Texas provided the opportunity to compare a tropical and temperate butterfly.

I measured the performance of the tropical and temperate leafwings on a variety of *Croton* diets, to evaluate how specialized each leafwing is to its local hosts relative to potential hosts in other parts of its range or in a relative's range. I distinguished among four categories of host plant with regard to each plants regional trophic connections to each leafwing species. I defined *local host* as a plant species known to host the target leafwing that was present in the region where I collected the leafwing. I defined *non-local host* as a plant species known to host the target leafwing somewhere in its range, but not present in the region where I collected the leafwing. Similarly, I defined *local non-host* as a plant species present in the region where I collected the leafwing that is known to host a different leafwing, but not the target leafwing. Finally, I defined *non-local non-host* to refer to plant species not present in the region where I collected the leafwing, but known to host a different leafwing, but not the target leafwing. In short, all of the plant species assayed were known to be hosts for *Anaea* somewhere in the range of the genus, but not necessarily in the region(s) where I collected the target species and not necessarily for the target species of *Anaea* in my experiment. Performance of caterpillars was measured by a composite of demographic parameters, survival to pupation, development time, and adult size (Thompson et al. 1990, Thompson 1996). I hypothesized that each leafwing would perform the best on its *local host*, indicating local adaptation, and better on *non-local hosts* than either *non-host* category. In a similar diet experiment with the *Papilio machaon* species complex, individuals performed the best on hosts from their local population of origin compared with performance on hosts from other parts of the species' range (Thompson 1988). This study utilized the following juvenile performance metrics: survival to pupation, development time from egg to pupa, and resulting adult size. These metrics affect the fitness of individuals, and when averaged over all individuals, the average fitness of a population.

Methods

CROTON-LEAFWING SYSTEM – Narrowly defined, *Anaea* includes nine species distributed throughout North and Central America and the Caribbean islands, and all *Anaea* larvae feed exclusively on *Croton* hosts (Comstock 1961, Smith et al. 1994). The *Anaea* selected for this study represent species from differing climates, temperate versus tropical. Each species feeds on more than one *Croton* host and includes hosts with different forms and/or life histories. *Anaea aidea* (tropical leafwing) is distributed throughout Central America and is reported to feed on the perennial shrubs, *C. cortesianus*, *C. fruticulosus*, *C. leucophyllus*, and *C. cascarilla*, (Scott 1992). *Anaea andria* (goatweed leafwing) is distributed throughout the southeastern United States and is reported to feed on annual and perennial herbs, *C. argyranthemus*, *C. capitatus, C. lindheimerianus, C. monanthogynus,* and *C. texensis* (Opler and Krizek 1984, Cech and Tudor 2005). For contrast with the tropical leafwing, I refer to *A. andria* as the temperate leafwing. All other *Anaea* are tropical, occurring in the Caribbean islands, and are each reported to feed on a few *Croton* shrubs (Smith et al. 1994).

CAPTIVE LEAFWING POPULATIONS – I collected both *Anaea* and *Croton* from natural populations in Gainesville, Florida and Austin, Texas, and transported all caterpillars and plants to Miami, FL where I established a captive *Anaea* population and a *Croton* crop. I obtained temperate leafwing both from Austin and Gainesville, tropical leafwing from Austin, and five *Croton* species for my experiments. I transplanted and/or propagated cuttings of three herbaceous hosts for the temperate leafwing, annuals *C. lindheimerianus* and *C. monanthogynus* from Austin and perennial *C. argyranthemus* from Gainesville, and two shrubs, the tropical leafwing host *C. fruticulosus* from Austin, and the Florida leafwing host *C. cascarilla* from Miami. In Austin, I made all collections from the Brackenridge Field Laboratory (30°17’00”N, 97°46’44”W) where the temperate leafwing feeds on *C. lindheimerianus* and *C. monanthogynus*, and the tropical leafwing feeds on *C. fruticulosus*. In Gainesville, I collected caterpillars from San Felasco Hammock Preserve (29°42’38”N, 82°27’04”W) where the temperate leafwing feeds on *C. argyranthemus*, and I collected the host *C. argyranthemus* from Ocala National Forest on a sand hill called Riverside Island, 35 miles southeast of Gainesville (29°27’12”N, 81°47’53”W). Eggs, caterpillars, and plants from both Gainesville and Austin were collected in April 2011 and then again from Austin in October 2012, and April 2013. The temperate leafwing was collected only from Gainesville in 2011 and in subsequent years only from Austin.

Once in Miami, I cultivated all crotons in a shade house at Fairchild Tropical Botanic Garden in Miami, FL. Transplants were repotted upon arrival and a mix of native soil and standard potting soil was added for each plant. The soil from Gainesville was primarily sand, which was quite different from the heavy clay soils collected with plants from Austin. Following advice from the nursery manager (Marlon Rumble pers. comm.), I mixed a standard potting soil with the native soil to provide some standardization, and added organic fertilizer as necessary to maintain vibrant plants. Stem cuttings from shrubs were encouraged to develop roots with a rooting hormone, potted in perlite, and cultivated in a propagation green house with a frequent misting schedule. After sufficient root formation, I potted these cuttings in the native-standard potting soil mix and placed them in the shade house. Plants grew under ambient conditions in the South Florida climate and received water from an automated system during the dry season.

Caterpillars grew in a growth chamber (Percival Scientific I-35), under lighting and temperature conditions programmed to mimic natural conditions during the month of April. The temperature schedule in the growth chamber started each day with a slow increase from 20°C to 30°C over 7 hours after the lights turned on. After remaining at this warm temperature for three hours, the temperature then gradually dropped back down to 20°C in a series of steps over the next 10 hours. The temperature then remained at the minimum of 20°C, and then the diurnal cycle repeated. Average daily temperature was 24.85°C. The lighting schedule was constant for each cohort of caterpillars.

In the field, I collected eggs and caterpillars along with the leaf or a sprig of the host where they were attached and placed them in petri dishes with moistened filter paper. Each wild-collected caterpillar, after hatching, was segregated into a separate petri dish lined with moistened filter paper and provided leaf material from the *Croton* on which it was found. Each day, I checked all individuals and recorded the developmental stage, cleaned the petri dish, and replenished leaf material. I moved all new fifth instar larvae from 9-cm diameter petri dishes to 15-cm dishes. Toward the end of the larval stage, caterpillars turned bright green, would cease feeding and start spinning a web of silk on the petri lid for attachment of the cremaster. After the pupa completed chrysalis formation, I weighed the lid with attached pupa and inserted a cylindrical mesh screen between the bottom and lid of the petri dish to create a small cage (15-cm diameter by 15-cm tall). The weight of lid was later subtracted to calculate pupa mass.

Adult butterflies emerged in these small cages still inside of the growth chamber and were provided with a small puddle of water and fermenting banana mash for food (about 2mL of each). For breeding, a single male-female pair was isolated in a flight cage with four host plants. Adults were free to fly within the 1-m^3 area, for courtship, copulation, and oviposition. I checked each plant daily for eggs and removed males from the cage when females began ovipositing. I removed all leaves with eggs attached, counted the eggs, and moved each leaf to a petri dish, which was placed in the growth chamber.

PLANT-MEDIATED CATERPILLAR PERFORMANCE – Originally, I designed this experiment to test for differences between the temperate leafwing in two regions. I hypothesized that temperate leafwing would perform better on its *local host* compared with the *non-local hosts*, indicating local adaptation. However, after discovering midway through the experiment that I had two different leafwing species as opposed to one species from two regions, I reformulated the question to match the realized experimental design. Nevertheless, my design still compares butterfly performance on plants that are known to be hosts locally versus non-locally, but it also compares butterfly performance on plants that are not known to be hosts that are local and non-local. Although my current design lacks reciprocity, some insights can still be gained.

To test for differences in offspring performance on different host plants, hatchlings from eggs laid in captivity were systematically assigned a diet of one of the five possible host plants, *C. argyranthemus*, *C. cascarilla*, *C. fruticulosus*, *C. lindheimerianus*, or *C. monanthogynus*. Once assigned a diet, this was the only *Croton* supplied to the individual. Developmental stage was recorded for each individual every day until death. For the temperate leafwing from Gainesville (n=85), *C. argyranthemus* was the *local host*, and *C. lindheimerianus* and *C. monanthogynus* represented *non-local* *hosts*, because they did not occur in Gainesville, but they are hosts for the temperate leafwing in Austin (Figure 3.1). There was no *local non-host* for the temperate leafwing, but both *C. fruticulosus* and *C. cascarilla* represented *non-local non-hosts*. For the tropical leafwing from Austin (n=185), *C. fruticulosus* was the *local host* and *C. cascarilla* was a *non-local host* not, because it did not occur in Austin, but it is a host for the tropical leafwing in Mexico, and several other *Anaea* (Smith et al. 1994). Both *C. lindheimerianus* and *C. monanthogynus* represented *local non-hosts* for the tropical leafwing, because the occurred in Austin, but are hosts for the temperate leafwing, and *C. argyranthemus* represented a *non-local non-host* (Figure 3.1).

To evaluate offspring performance I recorded survival to pupation, time to pupation, pupa mass, and forewing length of each individual. I used logistic regression to estimate the proportion of offspring hatching that survived to the pupa stage followed by analysis of deviance to evaluate the effect of two categorical factors, leafwing and *Croton*, and the leafwing × *Croton* interaction. Some leafwing-*Croton* combinations were simply not viable, and only 147 of the 344 hatchlings fed experimental diets survived to pupation (Figure 3.2). With low sample sizes at or near zero for certain leafwing-*Croton* combinations, I was unable to perform a full factorial analysis on performance metrics conditional on surviving to pupation. For each analysis, I evaluated the sample size in each factor combination category, and limited each analysis where appropriate. In general the temperate leafwing from Gainesville only survived on the *local host* (*C. argyranthemu*s) and one *non-local host* (*C. monanthogynus*). The tropical leafwing survived on all hosts except for the *non-local non-host* (*C. argyranthemus*) (Figure 3.2).

Offspring performance metrics conditional on surviving to pupation were restricted to these groups only.

A multivariate analysis of variance (MANOVA) tested the differences between centroids (multivariate means) of the pair of response variables, log-transformed number of days from oviposition to pupation and pupa mass, that were due to leafwing and *Croton*. Using a factorial design would be inappropriate because of the lack of sample size (for reasons described above) for many factor levels. Instead, I combined the factors for this model into one predictor variable that identified leafwing species, city of origin, and diet. Data did not deviate significantly from the assumptions of multivariate normality and homogeneity of variances. Pairwise comparisons between group means were made using Tukey post-hoc tests for each response variable. I considered all p-values less than or equal to 0.05 as significant in all analyses.

Results

I collected a total of 210 caterpillars and eggs in the field and reared them in the laboratory. From these individuals, I obtained 593 eggs from only 10 females, of which 295 successfully hatched and were reared in the lab. Of the total number of caterpillars reared for this study, 279 survived to pupation, and 244 survived to adulthood.

The proportion of individuals surviving from hatching to pupation differed significantly among each controlled diet of *Croton* (Figure 3.2), and the effect of each *Croton* was dependent on leafwing species, which is indicated by the significant *Croton* × leafwing interaction (Table 3.1). The tropical leafwing had higher survival on a wider range of hosts than the temperate leafwing, and was unable to survive to pupation only on the *non-local non-host* (Figure 3.2). The temperate leafwing actually survived better on one of its *non-local hosts*, *C. monanthogynus*, had relatively low survival on its *local host*, and barely survived to pupation on any other host. The annual, *Croton monanthogynus*, was the only host both leafwings were capable of surviving with (Figure 3.2).

For those surviving to pupation, development time (time from oviposition to pupation) and pupa mass were both significantly affected by *Croton* diet (Figure 3.2), indicated by a significant difference among group centroid means (Pillai's trace = 0.634, $F_{12,226} = 8.74$, $p < 0.001$). Univariate ANOVAs support this result indicating a difference among groups in development time ($F_{6,133} = 13.09$, $p < 0.001$) and pupa mass ($F_{6,133} = 5.42$, $p < 0.001$). Post-hoc pairwise comparisons indicated that temperate caterpillars pupated sooner on their *non-local host* than on their *local host*, but the increased time spent feeding on the *local host* resulted in much larger pupae (Figure 3.2). Tropical leafwing developed more quickly on *local hosts* compared with *local non-hosts*, and more quickly on *local non-hosts* compared with the non-local host (Figure 3.2). Despite pupating sooner on *local non-hosts*, tropical leafwing pupated at smaller sizes compared with either the *local host* or *non-local host* (Figure 3.2).

As an additional test of development time differences among leafwings, I analyzed time to pupation separately using accelerated failure-time regression. This analysis compared only tropical and temperate leafwings that were fed their *local host* (n=93) in order to represent development times that I may expect to measure in the field. I found that the log-logistic probability distribution provided the best fit to the data (AIC: exponential = 815, Weibull = 526, log-logistic = 488, and lognormal = 493), which indicates that the probability of pupating increased to a maximum at some time, then decreased (Figure 3.3). Leafwing species explained a significant amount of the total deviance, indicating a significant fit of the model to the data (df = 1, $\chi^2 = 69.9$ $p < 0.001$). This model predicted that a minimum of approximately 20 days were required for an individual to pupate, after which the probability of pupation increased to nearly 0.5 at 30 days and 49 days for tropical and temperate leafwings, respectively (Figure 3.3). The tropical leafwing developed on average 7 days more quickly than the temperate leafwing (Figure 3.3).

Discussion

By comparing two widespread leafwing butterflies, I showed that both *Anaea aidea* (tropical) and *A. andria* (temperate) have the ability to feed on multiple *Croton* host species that occur within their geographic range, and there is moderate evidence of specialization to feed on *local hosts*. This general result is not new (e.g., Fox and Morrow 1981), but does provide perspective when considering why *A. t. floridalis* is narrowly distributed, while *A. aidea* and *A. andria* are widespread. The ability to feed on multiple hosts likely enables a broad geographic range (Novotny and Basset 2005), but it is arguable whether an expanded diet precedes geographic range expansion, or if an expanded diet results from intermixing of locally specialized populations. Texas is rich in *Croton* (51 species) relative to Florida (11) (USDA NRCS 2015), which suggests that there are more opportunities for diet expansion in Texas. In my experience, *Croton* in Texas also appeared more regularly in the landscape, and often occurred in mixed species patches or in patches adjacent to congeners (pers. obser.). *Croton* in Florida appeared to be rare, and were not found co-occurring with congeners. It was also noteworthy that all of the *Croton* that grow in Florida also occur in Texas (USDA NRCS 2015), but I did not observe *Anaea* in Texas using these *Croton* species. From this information, it seems reasonable to infer that *A. aidea* and *A. andria* are widespread, and have a moderate ability to feed on multiple *Croton*, because they occur in a *Croton*-rich region. On the other hand, *A. t. floridalis* is narrowly distributed and restricted to a single host, because that is essentially the only host available in South Florida.

Smith et al. (1994) described South Florida as an ecological island for Caribbean butterflies. *Anaea t. floridalis* is nested within the Caribbean species *A. troglodyta*, which reportedly feeds on a number of *Croton* shrubs and occupies a region where *Croton cascarilla* and other *Croton* shrubs are common (Smith et al. 1994, Askew and van B. Stafford 2008) and diverse (Van Ee and Berry 2009). My research supported the argument that *A. t. floridalis* is a Caribbean butterfly at its northern extreme, and range expansion northward appears restricted by a lack of suitable hosts.

Overall, caterpillars did not survive better on *local hosts* compared with other hosts. However, both leafwings survived poorly on their *non-local non-hosts*, *Croton* on which another leafwing species feeds and that occurs in some other region. Time to pupation was best considered along with pupa mass. In general, rapid development should be considered high performance, but I observed that caterpillars that pupated early did not necessarily grow more quickly on some hosts. Specifically, the temperate leafwing on the *non-local host* pupated sooner, but at a smaller size, which did not represent quicker growth. The same was observed for the tropical leafwing on *local non-hosts*; pupation was achieved quickly, but pupa mass was less than caterpillars fed either the *local* or *non-local host*. In fact, the tropical leafwing on its *non-local host* required more time to develop, but grew to a size equal to the average size achieved on the *local* *host*. These results did not provide strong evidence for local specialization, although they did provide stronger support for specialization for the tropical leafwing than they did for the temperate leafwing.

In general, *Croton argyranthemus* proved to be a poor host. The tropical leafwing did not complete development on this host, and temperate caterpillars also performed relatively poorly on this host. All of these *Croton* when cut produce resinous, typically amber-colored sap (Salatino et al. 2007), but *C. argyranthemus* seemed to produce more and deeper colored sap than the other *Croton* when cut. Even if the chemistry of this sap is not toxic to leafwing caterpillars, it is quite sticky and may prevent feeding by reducing movement in a caterpillar's mandibles. The sap of several *Croton* are used in traditional medicine, and sap toxicology has been well studied in several *Croton*, including *C. cascarilla*, which has been reported to contain compounds that are lethal to insects (Salatino et al. 2007).

Moderate performance of the tropical leafwing on *non-local host*, *C. cascarilla*, was not surprising considering the broad distribution of *C. cascarilla* and that it has been reported as a host for at least three leafwings in the Caribbean (Smith et al. 1994, Askew and van B. Stafford 2008) and also for the tropical leafwing (Scott 1992). The temperate leafwing, after being isolated in northern latitudes, likely has not come into contact with *C. cascarilla* (*non-local non-host*) or other *Croton* shrubs in the tropics for a long time. It is possible *C. cascarilla* was unpalatable or toxic to the temperate leafwing, but the structure of the leaves also seemed to pose a problem for all leafwings in my experiment. *Croton cascarilla* leaves were thicker and tougher than all other *Croton* in this study and may have been difficult for these caterpillars to chew and digest. Temperate leafwing were simply unable to feed as early instars and generally starved. Tropical leafwing took much more time to develop, perhaps from difficulty digesting the tough leaf fragments.

Croton monanthogynus appeared generally suitable considering that all leafwings performed equally well on this host. This finding may also be due to the widespread geographic range of *C. monanthogynus*, which includes the Great Plains, the Midwest, and the Southeast United States (USDA NRCS 2015), and may explain its broad palatability. Both *C. lindheimerianus* and *C. monanthogynus* have pliable, thin leaves with few trichomes, and produce very little sap when cut. However, *C. lindheimerianus* is absent from the Southeast U.S.(USDA NRCS 2015), which may explain the poor performance of temperate caterpillars on this host. *Croton fruticulosus* leaves were intermediate in thickness and toughness in comparison to the annuals and *C. cascarilla*, but it had a thick layer of trichomes that caterpillars generally did not consume.

Temperate leafwing required more development time than tropical leafwing when each was fed its *local host*. This may be due to the size and phenology of each *local host*. *Croton* shrubs can grow up to two meters tall, and can produce enough leaves to support the complete development of numerous caterpillars, whereas annual *Croton* grow less than half a meter tall and produce many fewer leaves, perhaps not enough to support a single caterpillar's development. When leafwings begin breeding in the spring, *Croton* shrubs are full of leaves, while annual and perennial *Croton* herbs are just sprouting and much smaller than they will be by the end of the *Anaea* breeding. For these reasons, temperate leafwing may not evolved to have rapid development, because caterpillars may be required to spend more time foraging for neighboring hosts to complete development. Scriber and Feeny (1979) showed that caterpillars developed more rapidly on herbaceous plants relative to trees and shrubs, and suggested leaf toughness and water content were the main factors. Leaves of *C. fruticulosus* were thicker, but not much tougher than annual *Croton* leaves, which may help explain why tropical leafwing developed faster than temperate leafwing. Rapid development is advantageous considering the significant impact of predation during the juvenile stage (Benrey and Denno 1997; Chapter 2), but for the annual *Croton* this meant smaller pupae as well.

CONCLUSION – In this paper, I described a controlled diet experiment to test a tropical and temperate leafwing butterfly in terms of their level of feeding specialization as caterpillars. Evaluating survival to pupation, development time, and pupa mass, I documented the ability of each leafwing to feed on multiple hosts. Neither leafwing survived well on *non-local non-hosts*, and considering all performance together, they each performed better on their *local host*, but this was more pronounced for the tropical leafwing.

Table 3.1 Analysis of deviance table for the generalized linear model with binomial error of caterpillar survival from hatching to pupation on experimental *Croton* diets. The amount of deviance explained by each factor follows a χ^2 distribution so I used a χ^2 test to test the significance of each factor. The fit of the model to the data, i.e., the total deviance explained, was significant (df = 9, $\chi^2 = 85.85$, $p < 0.001$).

Factor	**df**	**Deviance**	**Residual df**	**Residual Deviance**	**p-value**
Leafwing	1	22.1	292	371.4	<0.001
Croton	4	31.4	288	340.0	<0.001
Leafwing × *Croton*	4	32.3	284	307.7	<0.001
Intercept only			293	393.5	

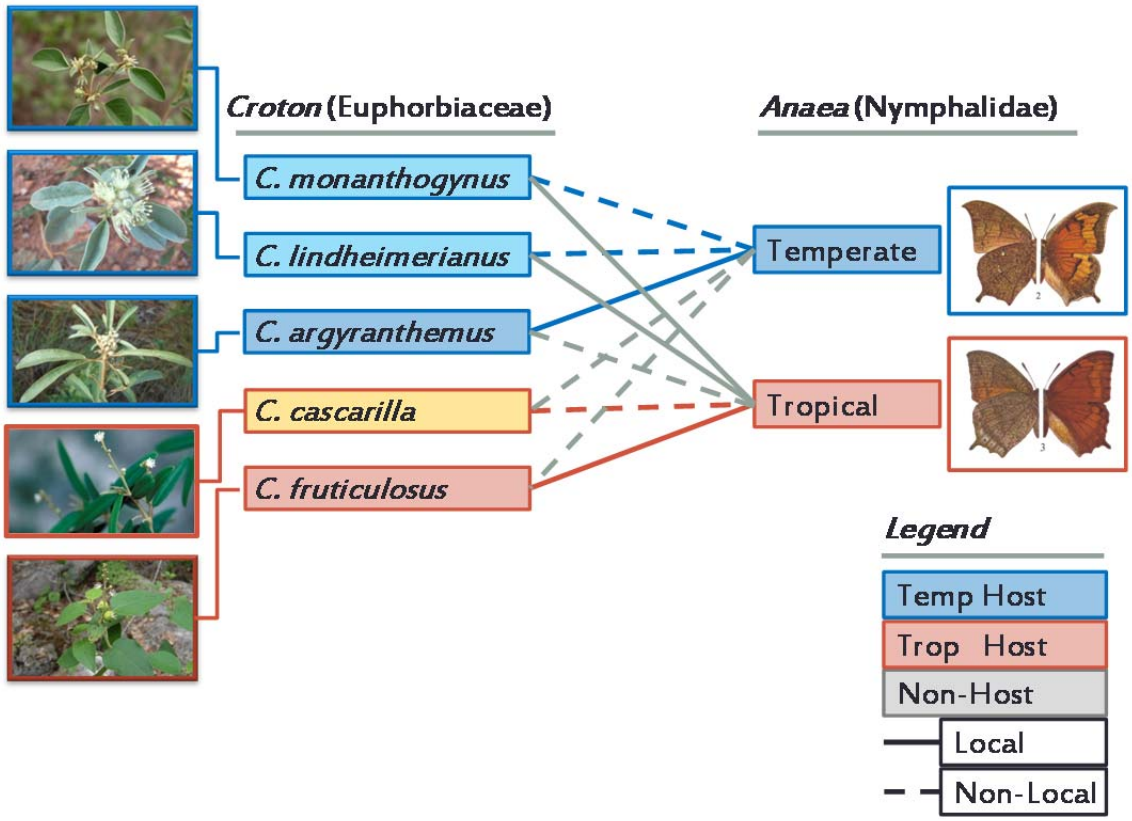

Figure 3.1. Diagram indicating the trophic connection between each *Croton* and *Anaea*. Blue lines indicate known hosts for the temperate leafwing, red lines indicate known hosts for the tropical leafwing, and gray lines indicate non-hosts, which are known hosts for other leafwings, but not the target leafwing. Solid lines indicate hosts that were found where the target leafwing was collected, and dashed lines indicate hosts that were collected in another region, not from where the target leafwing was collected.

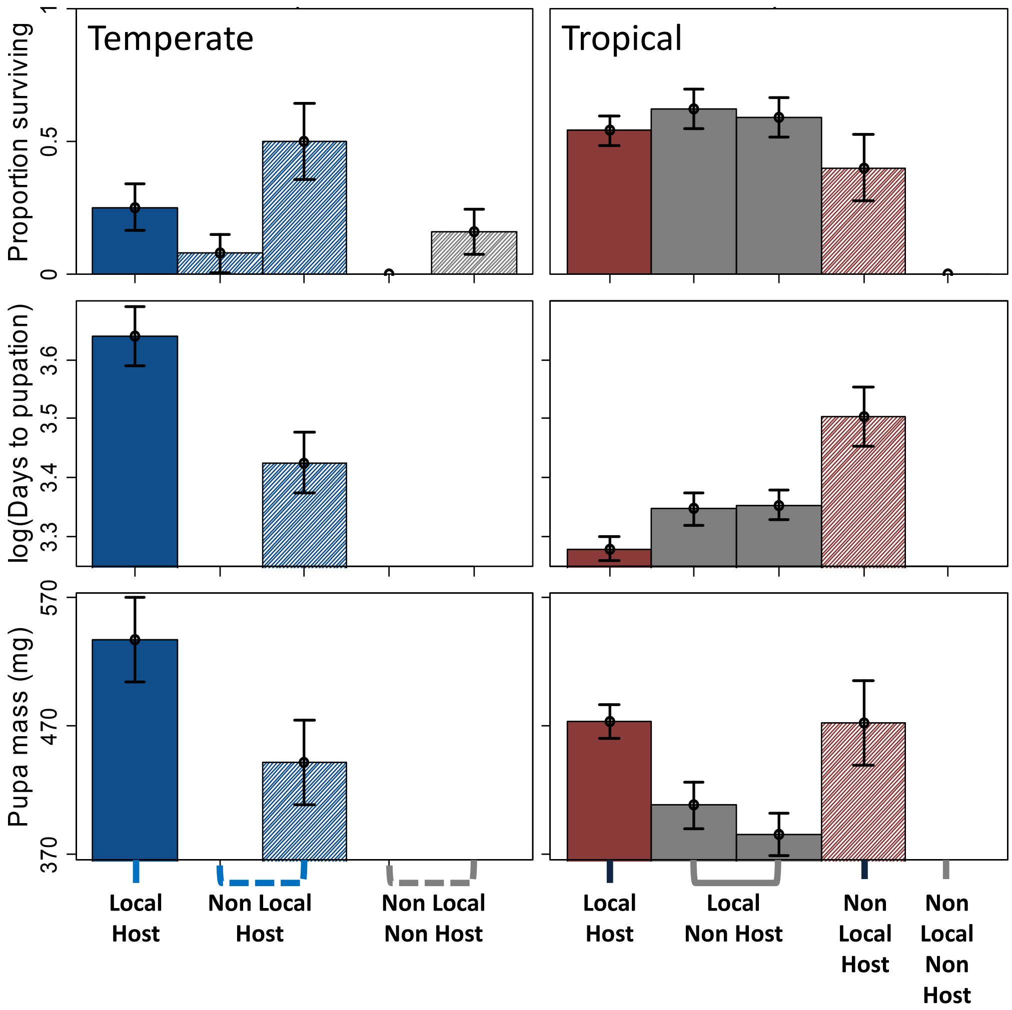

Figure 3.2. Effect of diet on proportion of individuals surviving from hatching to pupation (top), time from oviposition to pupation conditional on surviving to pupation (middle), and pupa mass (bottom) for temperate leafwing (*A. andria*) from Gainesville, FL and tropical leafwing (*A. aidea*) from Austin, TX. Each individual was assigned to one of five possible *Croton* species. The temperate leafwing rom Gainesville was assayed on its *local host* (*C. argyranthemus*), 2 *non-local hosts* (*C. monanthogynus* and *C. lindheimerianus*), and 2 *non-local non-hosts* (*C. fruticulosus* and *C. cascarilla*). The tropical leafwing was assayed on its *local host* (*C. fruticulosus*), 2 *local non-hosts* (*C. monanthogynus* and *C. lindheimerianus*), 1 *non-local host* (*C. cascarilla*), and 1 *non-local non-host* (*C. argyranthemus*),. Height of bars and error bars represent group means and standard errors estimated either from Analysis of Deviance with binomial errors (A) or from MANOVA including time to pupation, pupa mass, and all independent variables (B and C).

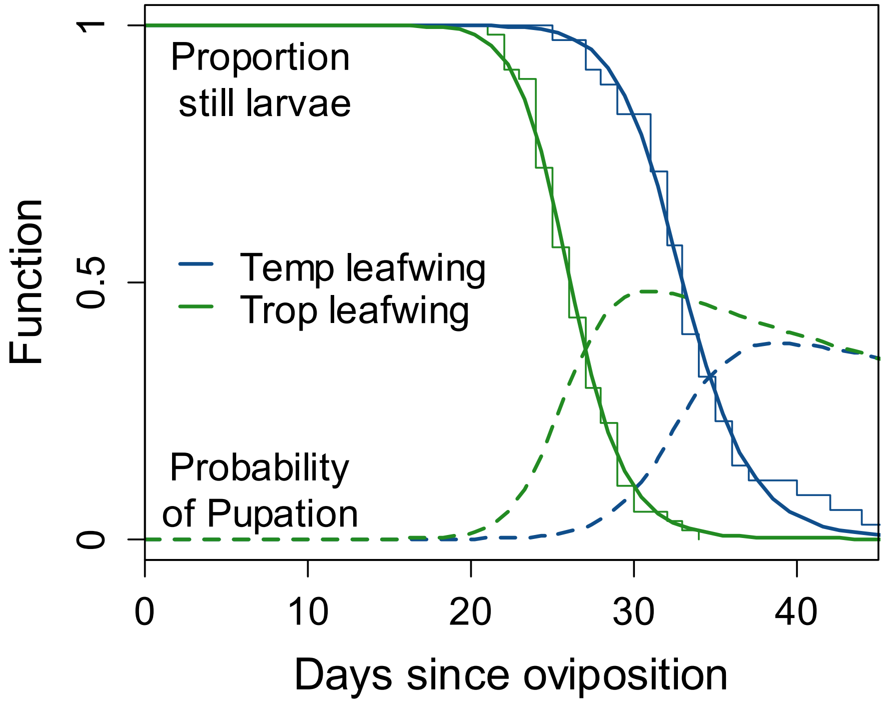

Figure 3.3 Analysis of age at pupation conditional on survival to pupation for the temperate (*A. andria*) and tropical (*A. aidea*) leafwings. The thinner, stepped lines represent the proportion of individuals that have not yet pupated by a given age, i.e., they are still larvae. Solid, smooth lines represent the fit of the accelerated failure time model (or survival function), estimated with the log-logistic distribution. Dotted lines represent the instantaneous rate of pupation (transition from larval to pupal stage) at each age, given by the hazard function.

Chapter IV

Life expectancy differences between seasonal forms of two leafwing butterflies

Overview

Surviving inhospitable periods or seasons may greatly affect fitness. Evidence of this exists in the prevalence of dormant stages in the life cycles of most insects. Here I focused on butterflies with distinct seasonal morphological types in which one morphological type, or form, delays reproduction until favorable conditions return, while the other form develops in an environment that favors direct reproduction. For two butterflies, *Anaea aidea* and *A. andria*, I tested the hypothesis that the development of each seasonal form involves a differential allocation of resources to survival at eclosion. I assayed differences in adult longevity among summer and winter forms in either a warm, active environment or a cool, calm environment. Winter form adults lived 40 times longer than summer form but only in calm, cool conditions. The magnitude of this difference provided compelling evidence that the winter form body plan and metabolic strategy favor long term survival. Neither adult feeding nor reserve size appeared to be the main cause, leaving resource conservatism as the primary explanation. This research suggests that winter form adults maintain lowered metabolic rate, a common feature of diapause, to conserve resources and delay senescence while overwintering.

Background

In variable environments, survival of harsh conditions has the greatest impact on fitness, especially for short-lived organisms (Morris et al. 2008). The ability of organisms to store nutrients and energy can provide an important buffer against the difficulties presented by environments that change state on a temporal scale that is shorter than the lifetime of organisms. One important example is seasonality, a pattern in which the environment changes state on a regular basis each year. In response to seasonality, many insects are capable of entering into diapause or a similar dormant state in which they can endure periods of otherwise unsuitable conditions (Hahn and Denlinger 2011). Diapause serves not only to allow insects to endure unsuitable conditions, but also synchronizes the life cycle with seasonal resource dynamics (Hahn and Denlinger 2011). In addition to storing resources, diapausing insects can reduce their metabolism to conserve energy reserves (Hahn and Denlinger 2011). Although diapause has typically received attention in temperate regions, where unsuitable conditions occur during the winter, diapause has also gained attention for its prevalence during summer (Masaki 1980) and also among tropical species where dry seasons may restrict suitable conditions (Denlinger 1986).

In this paper, I explored adult longevity in leafwing butterflies, genus *Anaea* (Nymphalidae). I focused on two leafwing species with broad geographic distributions, one from tropical latitudes, and the other from temperate latitudes. Co-occurrence of these *Anaea* in Texas provides the opportunity to compare seasonality between a tropical and temperate butterfly in a a subtropical climate. There are two distinct seasonal forms of adult *Anaea* (Riley 1980) that diverge in life history. Winter form adults develop in the fall and survive the winter to reproduce in the spring, while summer form adults develop in the spring and reproduce in the summer and fall (Riley 1980). The development of each seasonal form is day length dependent (Riley 1988a), so they do not represent genetic-based polymorphisms. These seasonal forms are morphologically (Riley 1980) and physiologically distinct (Riley 1988b), suggesting the differential allocation of resources to survival and reproduction. Summer form *Anaea* are reproductively viable as early as two days following adult emergence, while winter form individuals are reproductively non-viable for at least one month after emerging (Riley 1988b, pers. obser.).

Many butterflies depend on the resources acquired as caterpillars (Boggs 1997), meaning the energy budget is set before eclosing as adults. Thus resource allocation to survival and reproduction has typically been evaluated simply by the pattern of resource distribution throughout the bodies of adults at eclosion. In general larger female butterflies allocate an increased proportion of both biomass and nitrogen to the abdomen for reproduction (Wickman and Karlsson 1989), suggesting that when butterflies obtain sufficient resources to survive, any remaining resources are allocated to reproduction. However, in butterfly seasonal forms the allocation patterns differ in the proportion of resources invested in either survival or reproduction. An experiment comparing winter and summer forms in *Polygonia c-album*, a butterfly with similar life history to *Anaea*, found that winter form adults invested a higher proportion of resources to the thorax compared with the abdomen (soma versus reproduction) (Karlsson and Wickman 1989). In other words, winter form adults allocate resources preferentially to survival. Summer form females invested a higher proportion of body mass and total nitrogen to the abdomen relative to the thorax (Karlsson and Wickman 1989), representing preferential investment in reproduction. Within these seasonal forms there is a clear switch in investment strategies, but to evaluate whether or not this switch constitutes a tradeoff, we need to evaluate the fitness cost.

A trade-off, by definition, is an observed negative association between life history traits that implies differential allocation of limited resources to one function vs another at particular times in the life cycle (Zera and Harshman 2001). The observed allocation is expected to be the one that has the highest fitness, such that alternative allocations would have lower fitness (Stearns 1989). For example if a species allocates all its energy to growth and survival during the early years, but allocates a substantial portion of energy to reproduction later in life, it is expected that altering this allocation pattern, for example reproducing early, would result in lower fitness. The link between the preferential investment in the abdomen and fecundity has been well established across insects, with increased abdomen size resulting in increased fecundity (Wickman and Karlsson 1989, Karlsson and Wickman 1990, García-Barros 2000). However, increased life expectancy resulting from preferential investment in the somatic tissues has not been experimentally verified, although winter form butterflies do seem to live longer in nature. I asked if summer form adults are capable of the achieving the same life expectancy if provided the same living conditions as winter form adults. Important to this question is the finding that preferential allocation of larval-obtained resources to the abdomen decreases in species that forage for nitrogen-rich food as adults (Boggs 1981). Several butterflies, including *Anaea* and *Polygonia* feed on potentially nitrogen rich foods, e.g., rotting fruit, fermenting sap, or dung (Scott 1992). If resources are not limiting, then how important is the preferential investment in reproduction over survival at eclosion if adult feeding can balance resource needs?

I tested the hypothesis that the development of each seasonal form involves differential allocation of resources to survival at eclosion, which will result in differences in life expectancy. Using the fact that the development of each seasonal form is photoperiod dependent (Riley 1988a), I created each seasonal form in the laboratory. I recorded adult size at eclosion and measured the time to death for each adult under two experimental living conditions. I subjected adults of each seasonal form to either a warm, sunny environment that encouraged an active lifestyle similar to foraging adults in the summer, or to a cool, calm environment that encouraged inactivity of individuals similar to quiescence in winter. To remove resource limitation, I continuously supplied adults with fermenting bananas and water. I presumed that adult size at eclosion corresponds the size of resource reserves and included size as a covariate in the analysis to control for differences in adult life expectancy due to adult size alone. I predicted summer form adults will survive better in the active conditions, and winter form adults will survive better when inactive. Further, I expected larger adults to live longer based on resource allocation patterns documented for *Polygonia* (Karlsson and Wickman 1989). Although adult feeding and lack of reproductive activity in summer form adults might offset resource needs in favor of surviving, I expected that winter form adults would exhibit physiological differences in addition to the altered allocation strategy that would allow longer life expectancy.

Methods

I studied adult longevity for two leafwing butterflies *Anaea andria* and *A. aidea* (Nymphalidae), each from a different climate, temperate and tropical, respectively. *Anaea andria* (temperate leafwing) is distributed throughout the southeastern United States and *Anaea aidea* (tropical leafwing) is distributed throughout Central America (Scott 1992). *Anaea* are relatively unique among temperate butterflies in that they endure the winter as adults, as opposed to a majority of temperate butterflies that overwinter in a juvenile stage (WallisDeVries et al. 2011). *Anaea aidea* and *A. andria* exhibit two seasonal morphological types of adults called either winter and summer forms or dry and wet forms (Comstock 1961). While some morphological features have been described for *Anaea* of the Caribbean suggesting seasonal forms in these tropical species, the distinction between broods seems less distinct (Comstock 1961). Day length regulates whether pupae will develop into summer versus winter form adults with photoperiods of at least 14 hours and winter form adults with photoperiods no longer than 12 hours (Riley 1988a).

I collected *Anaea* caterpillars from natural populations in Gainesville, Florida and Austin, Texas, and transported caterpillars along with local host plants to Miami, FL where I established a captive *Anaea* population. In Austin, I collected caterpillars from the Brackenridge Field Laboratory (30°17’00”N, 97°46’44”W), and in Gainesville, I collected caterpillars from San Felasco Hammock Preserve (29°42’38”N, 82°27’04”W). I collected temperate leafwing from Gainesville and Austin, and tropical leafwing from Austin only on three separate occasions, April 2011, October 2012, and April 2013. The temperate leafwing was collected only from Gainesville in 2011 and in subsequent years only from Austin.

Caterpillars grew in a growth chamber (Percival Scientific I-35), under lighting and temperature conditions programmed to mimic natural conditions during the month of April (see Chapter 3 for details on rearing procedures). After each caterpillar pupated, I inserted a cylindrical mesh screen between the bottom and lid of the petri dish to create a small cage (15-cm diameter by 15-cm tall). Adult butterflies emerged in these small cages still inside of the growth chamber and I provided each individual with a small puddle of water and fermenting banana mash for food (about 2mL of each). The lighting schedule was constant for each cohort of caterpillars, but differed among cohorts to produce each seasonal form. I set the photoperiod to 14L:10D to produce summer form adults and 12L:12D to produce winter form adults (Riley 1988a).

To test for differences in life expectancy I focused on the adult stage only. Adults of each seasonal form were subjected to one of two experimental living conditions meant to mimic either the conditions endured by summer form adults that do not enter a quiescent state or the conditions endured by winter form adults that are quiescent through the winter and become active in the spring. To encourage an active life style, I kept butterflies outside in a shade house to represent a warm, sunny (20% sunlight) environment where metabolism would be increased with warmth and activity, and individuals were free to fly in 1-m^3 cages. Average summer temperatures in South Florida remain around 33°C , and about 200 mm of rain falls on average each month from May to October (Chen and Gerber 1990). To encourage an inactive life style, I kept butterflies inside the cool, dim laboratory where metabolism would be decreased, and individuals were essentially unable to fly in small 15-cm × 15-cm cylindrical cages. Conditions in the laboratory remained fairly consistent with an average temperature of 22°C, and I provided a diurnal lighting schedule with a small full spectrum fluorescent light set to a 12L:12D photoperiod. I checked each individual every day, and supplied fresh water and fermenting banana mash as needed until all butterflies died.

I used accelerated failure-time regression to evaluate adult life expectancy, i.e., the number of days from eclosion to death, in response to age, leafwing species, seasonal form, sex, forewing length, and experimental living conditions. Accelerated failure-time models are generalized linear models for which a number probability distributions can be tested, each with a direct link to how the probability of mortality and the instantaneous risk of mortality changes with age (Fox 2001). I evaluated the appropriateness of four probability distributions, and in doing so I tested three alternative hypotheses; mortality was constant over all ages (exponential), mortality increased or decreased monotonically (Weibull), or mortality increased to maximum at some age then decreased (log-logistic and lognormal). To select among these probability distributions, I evaluated statistical significance of the scale parameter, which estimates the age effect on mortality (Fox 2001), and also compared the values of each model's Akaike's information criteria (AIC), which estimates the fit of the model relative to the number of parameters used (Burnham and Anderson 2002).

I used a backward stepwise regression approach to reduce the full model, and consulted AIC to evaluate relative support of each step. The full factorial model had 33 parameters, which with 226 adults in this study means less than 7 data points contributed to each parameter estimate. In this process, I sequentially removed statistically non-significant parameters in steps, starting with higher level interaction terms, and repeated the process until no non-significant parameters could be removed. Lower level interaction parameters and main effects were not removed if higher level parameters that included these lower level parameters were significant. To cross-validate the results of step-wise model reduction, I evaluated the AIC values corrected for small sample size (AICc) of the model at each step.

Leafwing, seasonal form, sex, and experimental living condition were each categorical factors, and forewing length was a covariate. Forewing length, a measure of adult size, represented the quality of the adult at eclosion. To standardize for differences in starting quality, I standardized forewing length and used analysis of deviance of the best failure-time model to evaluate the effect of each factor at the mean forewing length. The fact that there were no significant interactions with forewing length made this approach possible, and enabled me to evaluate the effect of each factor and their interactions while holding constant the influence of adult size on life expectancy.

Results

The log-logistic probability distribution provided the best fit to the data (ΔAIC: exponential = 88, Weibull = 35, log-logistic = 0, lognormal = 41), indicating that mortality increases to a maximum at some age, then decreases. Using backward stepwise regression for model reduction, I excluded 22 non-significant interaction terms, and arrived at a model that included all factors and only four interactions. AIC model selection metrics supported this model with 65% likelihood relative to the other models in my stepwise approach (Table 4.1). Although forewing length explained a fraction of the deviance that seasonal form and living conditions explained, it did explain a statistically significant amount of deviance (Table 4.2). Larger adults lived longer on average, and this result was not dependent on any other factor, i.e., all interactions were non-significant; so I evaluated the effect of all other factors at the mean forewing length to control for size effects. The significant interactions terms: treatment × season, treatment × sex, and treatment × season × sex, indicated that differences in life expectancy between winter and summer seasonal forms and also between the sexes depended on the experimental living conditions (treatment in Table 4.2). The interaction between season and sex indicates that seasonal differences in life expectancy are sex dependent, and the lack of significant interactions including leafwing species indicates that the effects of these factors and their interactions do not differ among leafwings (Table 4.2).

Winter form adults had the longest life expectancy, outliving summer form adults by a factor of almost 40 (Figure 4.1). However, this only occurred in the cool, dim environment, where individuals were encouraged to be inactive. In the warm, sunny environment, where individuals were encouraged to be active, winter form adults had much lower life expectancy than summer form adults (Figure 4.1). There was a much smaller difference in life expectancy for summer adults between active and inactive living conditions than there was for winter form adults, but similar to winter adults, summer adults did live longer when encouraged to be inactive (Figure 4.1). This pattern was more pronounced for males than it was for females (Figure 4.1). Females lived longer on average, but had lower life expectancy than males when inactive and in the winter form. Tropical leafwing lived longer than temperate leafwing regardless of sex, seasonal form, or activity level (Figure 4.1), but this result was marginally significant.

Discussion

For butterflies with seasonal forms, winter (dry) season forms delay reproductive maturity to survive until breeding season conditions return in spring (Karlsson and Wickman 1989, Brakefield and Reitsma 1991, Torres et al. 2009). I experimentally verified for both *Anaea aidea* (tropical) and *A. andria* (temperate) that winter form adults do have much greater live expectancy than summer form adults. The maximum life span achieved was 466 days (oviposition to death). Fruit feeding butterflies have been shown to have relatively long lives in nature with one individual living at least 293 days (Molleman et al. 2007). The great disparity in life expectancy between seasonal forms provides compelling evidence that the winter form body plan and metabolic strategy favors long term survival. Resources used for reproduction are not available for maintenance (Stearns 1989, Zera and Harshman 2001), and the fact that winter form leafwing delay reproductive maturity (Riley 1988b) and increase life expectancy in doing so suggested that these resources were used for maintenance to survive.

My research also showed that adult feeding had little impact on the differences between seasonal forms. Although some studies have documented a positive effect of feeding on survival (Karlsson 1994), many studies did not find evidence to support this (Boggs and Ross 1993, Molleman et al. 2008a, Molleman et al. 2008b). My results demonstrated that adult feeding did not offset the cost of reproductive development paid by summer form adults. Winter form adults delayed this cost, and achieved greater life expectancy. The finding that adult size in my study was positively correlated with life expectancy may suggest that leafwings rely on stored resources obtained as larvae. However, life expectancy only changed a small amount relative to changes in adult size. A millimeter difference in forewing length predicted little more than a day of extra life on average, and the maximum difference in forewing length among adults was only 12 mm.

The energy budget of diapausing, aestivating, or quiescent adults responds to resource reserves, income, and conservation. The results reported here discount large effects of either reserves or income, but strongly support a difference in resource conservation. Differing morphology between seasonal forms already suggested different physiology, and the extreme difference between seasonal forms was striking. Summer form adults were simply unprepared to survive as long winter form adults. The fact that this result was only true in a cool calm environment added support, given that warmer temperatures increase metabolism. Lower temperatures have been linked to decreased resting metabolic rates (Pijpe et al. 2007) and for adults also greatly increases life expectancy (Bauerfeind et al. 2009, Wikström et al. 2009, Hahn and Denlinger 2011). Lowered metabolic rate decreases resource demands reducing the need to be active, which also conserves energy. Insects have multiple physiological pathways for sensing resource reserves (Hahn and Denlinger 2011), and starvation as adults actually increases life expectancy of fruit-feeding butterflies (Pijpe et al. 2007, 2008). The level of feeding activity varied among individuals in our study, but most butterflies appeared at rest most of the time, and fed infrequently. The inability to survive in the warm, active environment shows that the longevity of winter form adults required quiescence to achieve high life expectancy, which is important when considering the effects of warming climates on overwintering butterflies (see WallisDeVries et al. 2011).

Summer form adults lived short lives whether active or inactive, which indicates that these butterflies would only have limited aestivation abilities to survive during inhospitable periods. The age of the oldest summer form adult was 126 days which is very similar to the time between spring and fall breeding seasons, about 120 days from May to September. Austin has a subtropical climate with two inhospitable seasons (NOAA NWS 2015), which differs from either temperate climates to the north where *A. andria* occurs or tropical climates to the south where *A. aidea* occurs. The wet-dry seasons in Mexico are comparable in timing and duration to the summer-winter seasons in United States. Summers may be especially hot and dry in Texas, making recruitment difficult for butterflies when fresh foliage is hard to find and creating a situation where survival strategies are optimal. Similar to other Texas butterflies in this environment

(Gilbert 1985), leafwing likely retreat to wetter, shaded habitats along rivers and streams, where conditions are more favorable. This behavior has also been documented for tropical butterflies (Denlinger 1986, Brakefield and Reitsma 1991).

CONCLUSION – I found a striking difference in life expectancy between seasonal morphological types of adults for a tropical and temperate leafwing, *A. aidea* and *A. andria*, in a subtropical environment. Although the tropical leafwing outlived the temperate leafwing, the pattern of differences in response to sex, season, and living conditions was unchanged between species. Winter adults outlived summer adults, but only in a calm, cool environment. My results suggest that winter adults achieved high life expectancy not just through resource storage, but also resource conservatism.

Table 4.1. Model selection criteria among competing models predicting time to death of adult butterflies with the factors, leafwing species, seasonal form, sex, and living conditions and the covariate forewing length. The lowest Akaike Information Criteria corrected for small sample size (AICc) was subtracted from each model AICc to obtain the ΔAICc, which was then used to calculate the relative likelihood (Weight) of each model. The column "Step" indicates the step in the backward stepwise regression model reduction process. The global was step 0, and the last model, which was also the best model according to AICc, was step 6. The best model had the following structure: log(time to death) = treatment + season + leafwing + sex + forewing length + treatment×season + treatment×sex + season×sex + treatment×season×sex, where treatment refers to the experimental living conditions and season refers to the seasonal form.

Model	**Step**	**# Par.**	**AICc**	**ΔAICc**	**Weight**
1	6	11	1684	0.00	0.65
2	5	12	1685	1.67	0.28
4	3	20	1689	5.71	0.04
5	4	17	1691	7.29	0.02
6	2	21	1692	8.10	0.01
7	1	26	1703	19.53	0.00
8	0	29	1708	24.84	0.00

Table 4.2. Analysis of deviance table for the accelerated failure-time model predicting time to death of adult butterflies with the factors, leafwing species, seasonal form, sex, and living conditions and the covariate forewing length. Results were for the reduced model, which scored the lowest AICc (Table 3.4). The amount of deviance explained by each factor followed a χ^2 distribution so I used a χ^2 test of the significance for each factor. The fit of the model to the data, i.e., the total deviance explained, was significant (df = 9, $\chi^2 = 363.78$, $p < 0.001$). The factor treatment refers to the experimental living conditions and season refers to the seasonal form.

Factor	**df**	**Deviance**	**Residual df**	**Residual Deviance**	**p-value**
Treatment	1	145.3	223	1879	<0.001
Season	1	32.8	222	1846	<0.001
Leafwing	1	3.9	221	1842	0.048
Sex	1	13.8	220	1828	<0.001
Forewing Length	1	14.8	219	1813	<0.001
Treatment × Season	1	137.6	218	1676	<0.001
Treatment × Sex	1	6.9	217	1669	0.009
Season × Sex	1	2.4	216	1667	0.120
Treatment × Season × Sex	1	6.2	215	1660	0.013
Intercept only			224	2024	

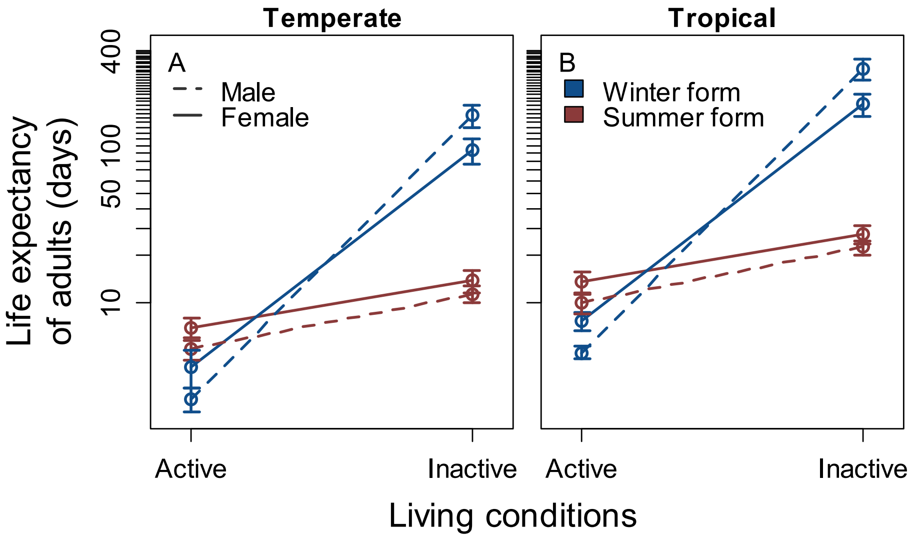

Figure 4.1. Interaction plots comparing the mean life expectancy between males (dashed) and females (solid) of each seasonal form of the temperate, *A. andria* (A), and tropical, *A. aidea* (B), leafwings. Adults were maintained under two experimental conditions and provided with water and food continuously. Living conditions refers to one of two treatments. Active conditions were maintained outside in a shade house and represent a warm, sunny environment where butterflies were able to fly. Inactive conditions were maintained inside a laboratory and represent a cool, low light environment where butterflies were unable to fly.

Chapter V

Seasonal sensitivity in the life history of a tropical leafwing in a temperate climate[2]

Overview

Seasonal climatic fluctuations affect the life history of most organisms. The tropical leafwing butterfly, *Anaea aidea* (Nymphalidae) has a complex life cycle that is characterized by there being multiple generations within a year, distinct seasonal forms of adults, and relatively long-lived adults who feed on nitrogen-rich sources. During a single year, the stage structure of the population changes seasonally as do the probabilities of survival and growth and the amount of reproduction. This paper addresses the question: when the fates of individuals depend on both life stage and season, how do life stage transitions within each season contribute to population dynamics evaluated over the entire seasonal cycle?

We combined demographic rates estimated for the tropical leafwing in the field and in the laboratory to construct a periodic stage-structured matrix model that encapsulates the progression of the population through each seasonal phase along with the demography within each phase. We used a megamatrix form of the periodic matrix model. This approach is distinct from compiling a series of annual matrices as the particular products of the single phase matrices, which is the more commonly utilized approach. We show that these two approaches to the problem are equivalent and that the analytical properties of the megamatrix provide the seasonal population dynamic properties of interest.

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Our results show that stable stage structure, reproductive value, and elasticity all changed seasonally, highlighting the importance to annual dynamics of different life stages in different seasons. Using elasticity of the annual growth rate to demographic rates for each stage and season, we found that overwinter survival of adults has the largest overall effect on average annual dynamics. During the breeding season, from spring to fall, reproduction and growth have the largest effect.

This research is the first application of a periodic megamatrix to model seasonal butterfly population dynamics. With this approach we demonstrated that the importance of each life stage depends on season, and our results suggest which selection pressures maintain two distinct seasonal forms and life histories in leafwing butterflies, *Anaea*.

Background

Timing and the amount of reproduction during a lifetime depends upon inherent tradeoffs in allocation of limited resources, resources used for reproduction are not available for maintenance (Stearns 1989, Zera and Harshman 2001). In a variable world, organisms must 'decide' whether to reproduce now or later – a decision made by the allocation of resources to either survival or reproduction (Boggs 1981, Karlsson and Wickman 1989). However, the energetic costs of producing offspring are not the same as the cost of reproduction, which refers to the effect on future survival and reproductive potential (Clutton-Brock 1984). The cost of a tradeoff, when one trait benefits at the expense of another, is paid in the currency of fitness (Stearns 1989) and fitness can be described as an individual's success in either surviving or producing offspring. In seasonal environments, resources vary in abundance across the year and organisms must allocate effort to growth, survival, and reproduction differently across the year to minimize costs and/or maximize benefits in general. Nonetheless, the relative importance of different activities throughout the year to overall success is poorly understood.

The ability of organisms to store resources (nutrients and energy) can provide an important buffer that allows organisms to endure challenging conditions that occur within their lifetimes. In temperate regions, butterflies must reproduce during the growing seasons and also survive the winter. Tropical regions may also have seasonal rain patterns that similarly affect the phenology of larval resources and butterfly activity (Torres et al. 2009). Given their relatively short lifespan, butterflies are capable of producing one or more generations in a growing season so that offspring of individuals that live through the winter (overwinter), or dry season, typically do not have to live through the following winter (Scott 1992). For butterfly species that overwinter as adults, individuals must survive to reproduce in the spring, requiring preferential investment in survival over reproduction (Hahn and Denlinger 2011). By contrast, adult butterflies developing in spring are able to invest directly in reproduction, but their offspring will again have to overwinter. This seasonal investment decision point favors reproduction in early season broods and survival in late season broods.

Leafwing butterflies (genus *Anaea*; Nymphalidae) are an ideal study system for investigating a seasonal tradeoff switch. Two distinct morphological types of adults occur in different seasons (Riley 1980, Torres et al. 2009) and differ in the timing of life events and investments (life history strategies) (Riley 1988b; Chapter 4). *Anaea* adults eclose as winter form in the fall and survive winter to reproduce in spring. Summer form adults eclose during spring and summer, and reproduce in summer through fall (Riley 1980). These seasonal forms are identifiable by differences in wing shape (Riley 1980), but more importantly, differ in their ability to reproduce following eclosion (Riley 1988b) and in life expectancy (Chapter 4). Summer form adults are reproductively viable as early as two days following adult emergence, while winter form individuals are reproductively non-viable for at least one month after emerging (Riley 1988b). The benefit gained from delayed reproductive maturity is increased survival. Winter form adults are capable of outliving summer adults by almost 40 times (Chapter 4), thus demonstrating a clear life history tradeoff. In another butterfly with similar life history, *Polygonia c-album*, summer form compared with winter form females invested a higher proportion of body mass and total nitrogen to the abdomen relative to the thorax, demonstrating a tradeoff in resource allocation favoring reproductive development in the abdomen over storage for survival in the thorax (Karlsson and Wickman 1989).

We developed a periodic stage-based matrix projection model that projects the population through two or more generations in a single year and includes an inherent tradeoff in survival and reproduction. Using life table data from complete lifetimes of both seasonal forms reared in captivity (Chapters 3 and 4) and also from mark-recapture surveys of caterpillars (Chapter 2) and adults in the field, our cyclical model projects individuals through each juvenile stage, pupation, and adulthood, tracking the fate and reproductive output of an individual in each season for an entire year. Using the model, one could project the demographic paths and performances for a collection of individuals and calculate the average fitness of the population of individuals. As in any matrix model of population dynamics, the annual per capita population growth rate given by analysis of the matrix is a metric of the average fitness of individuals that share the common set of structured demographic rates contained in the matrix (Hamilton 1966, Caswell 2001). A key feature of our model is that adult butterflies transition between two states. In the active state adults can reproduce have low survival, and in the quiescent state adults can exhibit high survival but defer reproduction; i.e., cost of reproduction is decreased survival while cost of high survival is delayed reproduction. The mechanism is that leafwings can endure inhospitable periods in a quiescent state, a term used here to refer to both avoidance of adverse conditions and true adult diapause. Increased survival in quiescent adults compared with active adults (Chapter 4) demonstrated the benefit gained by inactivity, but inactivity has a cost since reproduction requires activity. The body plan of winter form adults favors survival over reproduction, which suggests a deferment of the energetic costs of breeding to supplement a survival strategy, typical of adults that enter diapause (Denlinger 1986, Hahn and Denlinger 2011).

Adult food intake may be crucial to supplement nutritional reserves and offset the energetic cost of breeding, especially following long periods of quiescence during winter or dry season. Juvenile and adult butterfly stages differ dramatically in resource type and requirements, and the phenology and quality of larva and adult resources affect the differential investment in either survival or reproduction. Butterfly larvae depend on the availability of specific species and growth stages of host plants, and are generally responsible for the accumulation of a large proportion of an adult's nitrogen budget (Boggs 1981). Nectar-feeding butterflies rely heavily on nutritional reserves stored during the larval stage (Boggs 1997), but butterflies feeding on non-nectar sources, e.g., pollen, rotting fruit, or fermenting sap, may be less dependent on nitrogen obtained as larvae when food sources are rich in nitrogen (Gilbert 1972, Karlsson 1994, Boggs 1997). Like many of their relatives (Charaxinae), adult leafwing feed on fermenting sap, rotting fruit, and dung (Scott 1986) food sources with potentially high nitrogen content. The life history of leafwings, namely the importance of adult survival through inhospitable periods, suggests the need for high quality adult food to sustain long-lived adults.

We asked several questions concerning the emergent population dynamics that arise from the seasonally-dependent demographic rates. To do this, we constructed a megamatrix form of a periodic model, which is a Leslie-style block matrix that holds all the phase-specific matrices in chronological order, where the phase-specific matrices have a time step that is much shorter than annual. Each of the phase-specific matrices has all the life cycle stages, so that one entry in the megamatrix represents the probability of a stage in a certain phase of the year to contribute to or become another stage during the subsequent phase of the year. The properties of this matrix provide the annual population growth rate (dominant eigenvalue), stable stage-within-phase distribution (right eigenvector), reproductive values for each stage in each phase (left eigenvector), and the proportional effect of perturbing a matrix parameter on the population growth rate (elasticity). For a population at equilibrium, we asked which stages are most abundant during different phases and what is their contribution to future population size? Which life history transitions, e.g., survival and stasis of overwintering adults or growth and survival of juveniles, when perturbed would contribute the most to changes in the population growth rate? How does elasticity change over time in one year and in which seasons were these transitions most important? Given the existence of seasonal forms in *Anaea* and the hypothesized tradeoff in survival and reproduction, we predicted that population growth would be most sensitive to fecundity in spring and summer, and survival during the winter, rather than the reverse.

Methods

Anaea aidea is found in tropical dry forests throughout Central America and Mexico (Scott 1992), and at the northern limit a population has persisted many years in Austin, U.S.A., (Larry Gilbert, personal communication). The climate of Austin is classified as humid subtropical with hot summers, mild winters, and on average 830 mm of rainfall per year (NOAA 2015). Summer temperatures typically exceed 90°F (32°C), and temperatures exceeding 100°F are common during hot summers (NOAA 2015). Rainfall occurs throughout the year, but on average peaks in May and September (although few years are "average") and generally occurs as thunderstorms with brief periods of abundant rain (NOAA 2015). However, hot dry conditions have been more common than usual in Texas since 2000 due to global ocean temperature patterns (Nielsen-Gammon 2011). In fact, the year in which a majority of the data were collected for this study was the hottest and driest year (October 2010 – September 2011) on record (Nielsen-Gammon 2011). Herbaceous and shrubby vegetation desiccate and senesce during hot dry periods, but these plants can be quick to sprout or flush new leaves in response to brief and episodic periods of rain. All species of the genus *Anaea* occur in seasonally dry habitats so we expect that *A. aidea* and its relatives are adapted to respond to intermittent drought and rain. Caldas (1995) showed oviposition increased in response to increases in humidity for *Memphis ryphea* a close relative native to subtropical forests of Brazil.

Leafwing populations (*A. andria*) in the northern range are reported to have two generations per year, with three generations becoming theoretically possible further south (Scott 1992) and overlapping, non-discrete generations reported for *A. troglodyta* in tropical regions (Smith et al. 1994). Seasonality in Texas differs from that of the tropical dry forests of Mexico at the center of *A. aidea* distribution. In tropical dry forests, Torres et al. (2009) reported that *A. aidea* were active and abundant during the wet season, from April to November, but very few adults were observed during the dry season, November to April. In Austin, *A. aidea* is abundant in the spring (March to May) and fall (September to November), and inactive during the winter, (pers. obser.). These peaks in *A. aidea* abundance in Austin correspond to peak rainfall during these months, but their activity level and abundance are not known during summer months. Periods of rain in spring and fall rejuvenate local vegetation, which allows two pronounced breeding seasons per year in the spring and fall. The possibility of a third generation or overlapping generations between the spring and fall broods depends on summer heat and lack of rain. Droughts occur periodically during Texas summers and may inhibit a third generation in some years if host plants senesce. Leafless host plants during drought would force summer form adults to delay reproduction until the rains return.

We conducted the majority of our field work at the University of Texas Brackenridge Field Laboratory (BFL), which comprises 82 acres along the Colorado River in Austin (30°17’00”N, 97°46’44”W). Additional brief surveys were conducted at Barton Creek Wilderness Park (30°14’43”N, 97°48’40”W) and west of Austin at Pedernales Falls State Park (30°18’34”N 98°14’47”W) to place our study site in the larger context. The habitat where *A. aidea* is found with its local host, *Croton fruticulosus*, was similar at each of these sites and characterizes suitable habitat in the region west of Austin. This region, called the Texas Hill Country, is at the eastern edge of the Edwards Plateau and above the Gulf Coastal Plain. Low limestone capped hills are dissected by a network of rivers and creeks forming rugged canyons and creating a mosaic of open grasslands, oak-cedar woodland, and riparian forest (Wrede 2005). At each study site, oak-cedar woodland occurred in a narrow band along low limestone outcrop above alluvial terraces and riparian habitats. *Croton fruticulosus* occurred primarily in oak-cedar woodlands, typically on rocky slopes beneath small limestone bluffs trailing down to riparian forest (Wrede 2005; pers. obser.). We observed *Anaea aidea* adults in these woodlands and also occasionally in riparian forest where conditions may be favorable for adults during hot, dry periods. Open meadows adjacent to oak-cedar woodlands and along streams on alluvial terraces are rich in annual *Croton* species, which may provide ephemeral hosts for the offspring of opportunistic *A. aidea* females (see Chapter 3).

MODEL DESCRIPTION – To represent the leafwing life cycle, we constructed a model of leafwing population dynamics within and across years that incorporates all development stages including summer and winter seasonal forms both when active and quiescent. To represent time in a meaningful way with respect to leafwing development, we divided the year into $M = 122$ ordered phases or time steps, each 3-days in duration. In each phase during the year, individuals may occur in one to several of ten discrete stages: egg, first to fifth instar, pupa, summer adult, winter adult, and quiescent adult (Figure 5.1). Although not all stages are present in all seasons, we used well-known techniques of periodic matrix analysis to create a stage based-projection matrix for each phase, and multiplied these matrices in sequence to obtain the full dynamics across the full life cycle from one year to the next (Caswell and Trevisan 1994, Caswell 2001).

The number of individuals (*n*) in each stage at time *t* was represented by the vector *n*(*t*), with dimensions 1 × 10. The state variable, *n*(*t*), was always non-negative and we used a set of real nonnegative matrices, $\boldsymbol{B}(m)$ to project the population from time *t* to time $t + 1$ (Caswell and Trevisan 1994, Caswell 2001).

$$n(t+1) = \boldsymbol{B}(m)n(t) \tag{1}$$

The cycle was composed of $M = 122$ phases, so there were 122 matrices, $\boldsymbol{B}(1)$, $\boldsymbol{B}(2)$, . . . , $\boldsymbol{B}(M)$, and the cycle was periodic such that $\boldsymbol{B}(m + M) = \boldsymbol{B}(m)$. The matrix product of all $\boldsymbol{B}(m)$ yielded an annual projection matrix, $\boldsymbol{A}$, that projects the population through the entire cycle (Caswell 2001).

$$n(t+M) = (\boldsymbol{B}(M)\cdots\boldsymbol{B}(2)\boldsymbol{B}(1))n(t) \tag{2}$$

$$n(t+M) = \boldsymbol{A}n(t) \tag{3}$$

We noted that for each of the 122 phases of the year it was possible to construct an annual matrix, each one starting in a different phase of the year and each one projecting from one year to the next year at that phase(Caswell 2001). Because order matters in matrix multiplication, each of these annual matrices was different.

Projecting a population with each of these annual matrices for many years will lead to an asymptotic equilibrium stage structure for a particular phase of the year and an asymptotic population growth rate. While these matrices all shared the same asymptotic growth rate given by their dominant eigenvalues λ_A, they each had a different equilibrium stage structure given by the right eigenvector associated with the dominant eigenvalue of each matrix. The very nature of the seasonal environment dictates that stage structure changes within the year, however when we fixed on one phase of the year as a reference point to track annually, the stage structure had an equilibrium distribution. A similar point can be made about the left eigenvectors of each annual matrix and their correspondence to reproductive values of each stage for a given phase of the year.

We found that a convenient way to represent this process and study these equilibrium structures was to construct a large square matrix that combines the phase-specific matrices into a single block-structured matrix. This approach is equivalent, but less well-known, and encapsulates the progression from one phase to the next as well as the demography within each phase (Le Corff and Horvitz 2005, Caswell and Shyu 2012). The megamatrix, $\mathbb{B}$, arranged each demographic matrix along the sub-diagonal in chronological order, with the matrix for the final phase in the upper right corner to return to the beginning (Bru et al. 1997, Coll et al. 2012).

$$\mathbb{B} = \begin{pmatrix} 0 & \boldsymbol{B}(M) \\ diag\big(\boldsymbol{B}(1),\ldots,\boldsymbol{B}(M-1)\big) & 0 \end{pmatrix} \tag{4}$$

The dimension of $\mathbb{B}$ was the product of the number of phases with the number of stages, in our case 122×10 = 1220. The state variable, $\mathcal{N}(t)$, was similarly a structured version of $n(t)$ with dimensions 1×1220 and contained the number of individuals in each stage in each phase. In this arrangement, the dynamics of the system were described in the following equation, where the time step remained in 3 day intervals.

$$\mathcal{N}(t+1) = \mathbb{B}\mathcal{N}(t) \tag{5}$$

This approach is useful if all the phases of the individual matrices are of the same duration. Then, the block matrix represents a kind of dynamic averaging of all the phases of the life cycle and can readily provide insights into the relative importance of transitions during different phases to the overall annual dynamics.

Each projection matrix, $\boldsymbol{B}(m)$, contained the stage-specific survival and growth probabilities and the amount of reproduction for a particular phase of the year. In each phase, there was stage-specific probability of surviving, ϕ_{i}, and a stage-specific probability of growing from stage i to stage j, ψ_{ji}, conditional on survival. Individuals could either remain in the same stage, $j=i$ (called stasis), grow to the next stage, $j=i+1$, or early instars could grow two stages, $j=i+2$. Adults could not grow any further, but could contribute to recruitment via egg laying, F_i. Each column represented a particular stage at phase m, each row represented the stage at phase $m+1$, and each element a_{ji} represented the probability of survival and growth or the amount of reproduction from or by stage i to stage j.

$$\boldsymbol{B}(m) = \begin{bmatrix} P_1 & 0 & 0 & 0 & 0 & 0 & 0 & F_8 & F_9 & 0 \\ G_1^1 & P_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ G_1^2 & G_2^1 & P_3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & G_2^2 & G_3^1 & P_4 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & G_3^2 & G_4^1 & P_5 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & G_5^1 & P_6 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & G_6^1 & P_7 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & G_7^1 & P_8 & 0 & R_{10}^2 \\ 0 & 0 & 0 & 0 & 0 & 0 & G_7^2 & 0 & P_9 & R_{10}^1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & G_8^2 & G_9^1 & P_{10} \end{bmatrix}$$

Each matrix in $\boldsymbol{B}(m)$ was a ten by ten, diagonal matrix with nonzero elements along the first, $a_{i-1i}(m) = R_i^1(m)$, and second, $a_{i-2i}(m) = R_i^2(m)$, super-diagonals, the main diagonal $a_{ii}(m) = P_i(m)$, the first , $a_{i+1i}(m) = G_i^1(m)$, and second, $a_{i+2i}(m) = G_i^2(m)$, sub-diagonals and in the upper right corner, $a_{18}(m) = F_8(m)$ and $a_{19}(m) = F_9(m)$. Active summer and winter form adults were the only reproductive stages. The probability of survival in stage i and stasis in stage i at phase m was represent by $P_i(m) = \phi_i(m)\psi_{ii}(m)$. The probability of survival in stage i and growth from i to i+1 at phase m was represented by $G_i^1(m) = \phi_i(m)\psi_{i+1i}(m)$, and growth from i to i+2 was represented by $G_i^2(m) = \phi_i(m)\psi_{i+2i}(m)$. The probability of survival in stage i and regression from i to i-1 at phase m was $R_i^1(m) = \phi_i(m)\psi_{i-1i}(m)$, and regression from i to i-2 was $R_i^2(m) = \phi_i(m)\psi_{i-2i}(m)$. Regression in this model described quiescent adults becoming active.

The 122 phases of the year belong to different seasons, where phase 1 begins January 1 in one year and phase 122 ends January 1 of the following year. Enumerating the phases in this way, the active season for *A. aidea* spans the time between the last frost date in spring and the first frost date in winter (NOAA NWS 2015); March 1 to the end of November, phases m = 20-109. Juvenile growth and survival, $P_{1-6}(m)$, $G_{1-6}^1(m)$, and $G_{1-3}^2(m)$, were nonzero and constant over this time period, except for the last four phases (Figure 5.2). For these phases we decreased survival by 10% each phase to represent dropping temperatures. Pupa survival and stasis, $P_7(m)$ was constant over this period, but transition to the summer form adult, $G_7^1(m)$, was non zero from late March to late September (m = 29-88), and transition to the winter form adult, $G_7^2(m)$, was non zero from late September to late November (m = 89-109) (Figure 5.2).

Transitions between active and quiescent adult stages were more dynamic, but in general winter adults were absent from the summer and summer adults were absent from the winter. Both summer and winter adults had the option to enter the quiescent state to achieve higher survival, as opposed to remaining active in which state reproduction was possible. Inactivity of both seasonal forms in the laboratory greatly increased adult survival and life expectancy (Chapter 4). During the winter, all adults were winter form and quiescent, $P_{8-9}(m) = G_9^1(m) = G_8^2(m) = R_{10}^1(m) = R_{10}^2(m) = 0$, and $P_{10}(m) = \phi_{10}$ for m = 1-17, 117-122. Starting in late February (m = 18-27), we used a gradient in transition probabilities ($\psi_{9,10}(m)$ = 0.1, 0.2, . . . , 1.0) to represent the population of quiescent adults gradually becoming active as winter transitions to spring (Figure 5.3). These active winter form adults laid eggs and remained in this stage for the remaining life, $P_9(m) = \phi_9$ for m = 18-50. The offspring of the winter form adults in spring gave rise to summer form adults, which were present from the end of March to mid-November, $P_8(m) = \phi_8\psi_{8,8}$, for m = 30-107. Active adults went quiescent with a steady low probability during this time period, $\psi_{10,8}(m)$ = 0.2 for m = 37-60, then gradually became more likely to go quiescent as the summer grew hotter and drier in July and August, $\psi_{10,8}(m)$ = 0.25, 0.3, 0.35, . . . , 0.8 for m = 61-72, and $\psi_{10,8}(m)$ = 0.8 for m = 73-78 (Figure 5.3). During this time, quiescent adults were less likely to become active, $\psi_{8,10}(m)$ = 0.1 for m = 38-78, which represented some commitment in seeking out a place to be quiescent. As rains returned at the end of August, the probability of becoming quiescent decreased, $\psi_{10,8}(m)$ = 0.7, 0.6, . . . , 0 for m = 79-83, and the probability of becoming active increased, $\psi_{8,10}(m)$ = 0.2, 0.3, . . . , 1 for m = 79-84, so that all summer form adults were active and laying eggs for the fall breeding season. The offspring of these adults will became winter adults due to shortening day length in fall. Winter form adults began emerging in late September, $\psi_{9,9}(m)$ = 1 for m = 90-103, and then active winter form adults became quiescent as fall transitioned to winter, $\psi_{10,9}(m)$ = 0.05, 0.1, . . . , 0.5, 0.6, 0.7, 0.9 for m = 104-116.

ADULT ABUNDANCE – To estimate the abundance of adults in the field, we used mark-recapture methods. Leafwing butterflies are elusive, and we used Van Someren-Rydon traps baited with fermenting banana mash to lure and capture adults (DeVries 1987, Torres et al. 2009). Traps consisted of a vertical cylinder, 30-cm in diameter and 65-cm tall, constructed of two wire hoops and nylon chiffon, and open on both ends. A square platform, 35-cm x 35-cm, made from 0.6-cm plywood covered the top and bottom of the cylinder, with the lower platform suspended 1-cm below the cylinder to allow butterflies to enter the trap. Each morning we refreshed the banana mash bait in a petri dish placed in the middle of the lower platform and revisited each trap in the evening to check for *A. aidea* and to release all other insect captives. Captured individuals were marked with a unique number on the ventral surface of the hind wing using a Sharpie fine point permanent marker then released.

We set out eight traps in fall 2010 for a total of 8 survey days (4-11 November) and 20 traps in spring 2011 for a total of 24 survey days (28 March – 19 April). In 2010, traps were roughly 75 meters apart along the long axis of the croton bearing habitat. In 2011, we randomly selected trap locations throughout BFL, with ten traps outside of croton bearing habitat. However, only one of these outer trapping locations successfully attracted leafwings, so we removed the other nine traps on April 6th.

We arranged the adult capture data for all survey dates in groups of three, and used a closed population mark-recapture model to estimate adult abundance in each three day time period. Closed population models assume that no individuals enter or leave a population between samples (Williams et al. 2002). While we expected that butterflies may in fact have died, left, or entered the study area during our surveys, we felt that reducing each survey period to three days reduced the chances of this occurring. The encounter history for each individual was a binary vector with a 1 indicating capture, and 0 indicating not captured, e.g., 1 0 1 indicated that an individual was captured, not captured, then captured again. We estimated adult abundance for three sampling occasions using a method that expanded the 2 sample Lincoln-Petersen method (Williams et al. 2002). This method treated the frequency of each of 2^3 possible capture histories for three sampling occasions as a multinomial probability (Williams et al. 2002), and we allowed for a different probability of being captured, p_i, at each sample date i. The probability of each capture history was the product of the probabilities of being captured or not at each occasion, e.g., Pr(capture history = 1 0 1) = $p_1(1-p_2)p_3$ (Williams et al. 2002). The probability of being captured at least once, $p^* = (1 - \prod_{i=1}^{3}(1-p_i))$, was proportional to adult population size, $n_A = c/p^*$, where c was the number of unique butterflies captured (Huggins 1991). We used Bayesian procedures to estimate capture probabilities and population size. In this framework, n_A was treated as a random variable with a Poisson distribution, and capture probabilities for each sample date were random variables, uniformly distributed between zero and one. Using Markov chain Monte Carlo methods, we discarded the first 120,000 iterations, then used an additional 10,000 iterations to estimate the posterior distributions of each parameter in each model. All procedures were performed using R (version 3.1.0 (2014-04-10), R Core Team 2014) and JAGS (Plummer 2003).

JUVENILE ABUNDANCE AND STAGE DYNAMICS – For the dynamics and abundance of juvenile stages we incorporated data from mark-recapture of larvae (see Chapter 2) and life table data for laboratory-raised larvae (Chapter 3). In 2010, we marked and searched 354 plants for larvae and signs of herbivory by leafwing caterpillars on October 8, 11, 27 and November 1. In 2011, McElderry (chapter 2) marked 578 plants and surveyed all larvae every three days from March 28 to April 23. We marked the leaf where each caterpillar was found at each survey and recorded recaptures for each individual.

Analyzing these mark-recapture data in a state-space Markov model, McElderry (Chapter 2) estimated the survival and growth of juvenile stages. The constant model with stage-specific survival and growth rates constant in time was one of the two best models according to AIC model selection (Chapter 2), and we used the time constant model here, because of its parsimony compared with the model where survival varied in time. Field data allowed for robust estimates of all transitions from first to fifth instars, but the lack of consistent observations prevented robust estimates of survival and growth for eggs, pupae, and adults. For these stages, we used daily observations of individuals raised in the laboratory where the stage and survival of each individual was known for all individuals from birth to death (Chapter 3). Data from *A. aidea* raised in the laboratory on its local host, *C. fruticulosus*, were divided into three day phases and analyzed using the same procedures that were described in Chapter 2. These analyses differed only in that the transition matrix $\boldsymbol{P_X}$ included the adult stage with all juvenile stages.

SEASONAL FECUNDITY – Fecundity was estimated using a combination of the field surveys and the periodic matrix model, $\boldsymbol{B}(m)$, described above. We modified $\boldsymbol{B}(m)$ to estimate the birth date of each caterpillar observed in either the fall 2010 or spring 2011 field surveys. We created a matrix, $\boldsymbol{J}$, by reducing a spring time transition matrix, $\boldsymbol{B}(20)$, to only juvenile stages and used $\boldsymbol{J}$ to project retrospectively from each observed caterpillar to birth. In matrix $\boldsymbol{J}$, we removed survival and transposed $\boldsymbol{J}$ so that each column represented a vector of the probabilities that an individual in stage i at time $t + 1$

was in stage j at time t. Each column needed rescaling to sum to one, so we divided each element by the column sum. Matrix $\boldsymbol{J}$ in this form represents the retrospective transition projection matrix for living individuals observed during the breeding season. We stochastically projected live individuals from their initial observation into the past using the multinomial probability distribution to randomly assign each individual a sample path to birth according to the transition probabilities in the appropriate column of $\boldsymbol{J}$. Averaging over 1,000 independent sample paths to birth for each individual, we estimated each individual's mean date of birth. To include the number of caterpillars that died before the opportunity to be counted in our surveys, we estimated the initial cohort size at each birth date in spring and fall that would have resulted in the observed stage distribution for each date, $n(t)$. In the spring cohort we added 41 individuals, and in the fall we added 117, because our survey occurred later in the fall brood. Total reproductive output of the population was then the numbers of births at each date $b(t)$. From these procedures, we estimated the reproductive output for the spring ($b_{spring} = b(20\text{-}35)$) and fall ($b_{fall} = b(78\text{-}97)$) reproductive phases, but these estimates needed to be converted to per capita birth rates (fecundity) to match other per capita demographic rates in $\boldsymbol{B}(m)$.

To convert total reproduction to per capita fecundity, we predicted the number of adults during each reproductive phase, and modeled the number of eggs laid per adult in each phase. We treated each time series of the number of eggs, $b(t)$ in spring ($t = 20\text{-}35$) and fall ($t = 78\text{-}97$) as a random Poisson variable described by a single parameter, the product of per capita birth rate, $f(t)$, and number of adults, $n_A(t)$. To match the rise and fall in the number of eggs laid in each reproductive pulse, we used a quadratic function and Bayesian procedures to fit the following statistical model for each season separately.

$$b(t) \sim Poisson\big(f(t) n_A(t)\big)$$

$$\log\big(f(t)\big) = -a(t-h)^2 + v - \log(n_A(t))$$

The parameters a, h, and v estimated the speed of increase and the horizontal location and height of the peak, respectively. Both a and v were given uninformative lognormal prior distributions, and h was given a normal prior, restricted to the range of birth dates. Using the Bayesian statistical procedures and programs described above, we estimated per capita birth rate for each brood.

Accurate estimation of per capita fecundity relied on accurate measures of adult abundance during each reproductive phase, but the estimates of adult abundance from our mark-recapture analyses were confounded with local weather. Rather than using these estimates, we projected each individual forward from birth to death using $\boldsymbol{B}(m)$ to predict the number of adults alive during each brood. Similar to before, we took the mean over 100 independent sample paths, in which each individual was projected forward through a random sample path according to the multinomial probability distribution.

The schedule of fecundity for winter form adults was non zero only during spring, $F_9(20\text{-}35) = f(20\text{-}35)$, and fecundity for summer adults is non zero for summer, $F_8(35\text{-}77) = f(35\text{-}77)$, and fall, $F_8(78\text{-}98) = f(78\text{-}98)$. We were unable to measure fecundity in the field during summer months, due to extreme drought, so we modified the best fit curve for the spring brood, $f(20\text{-}35)$, by stretching the curve to fit the summer time interval and decreased peak birth rate by 75%. These modifications created a low summer birth rate peaking in early June just after the peak rainfall period in May, and also represented high competition among females for oviposition sites, given high adult densities and scarcity of suitable host plants due to recent caterpillar feeding and hot and dry conditions.

MODEL ANALYSIS – The periodic nature of the process we modeled with the megamatrix, $\mathbb{B}$, means that $\mathbb{B}$ did not have a dominant eigenvalue, but it did have a certain kind of "asymptotic" dynamics. It had a set of co-dominant eigenvalues all of equal absolute magnitudes, including real and complex ones, and each associated with its own left and right eigenvectors. There was inherent cyclicity in stage structure within a year. Focusing on the positive real eigenvalue in the set of co-dominants, we found that it had a real right eigenvector in which all the elements had the same sign. Taking the absolute magnitudes of the right eigenvectors for each of the co-dominants yielded equivalent shapes for all. Similar results were found for the left eigenvectors. For convenience, we focused on the positive real eigenvalue and its real eigenvectors, connecting their interpretation to the properties described above for the phase-specific annual matrices.

The positive real root that was a co-dominant eigenvalue, $\lambda_{\mathbb{B}}$ provided a geometric mean measure of population growth rate for each phase of the year. To turn it into an annual rate, we raised it to a power equal to the number of phases in the year. In fact, ${\lambda_{\mathbb{B}}}^{122} = \lambda_A$. The right and left eigenvectors associated with this eigenvalue were comprised of 1220 components corresponding to the ten stages in each of the 122 phases of the environment. In fact each set of ten components in these long vectors were equivalent to the eigenvectors of the phase-specific annual matrices. The eigenvectors associated with $\lambda_{\mathbb{B}}$ can be used to directly calculate sensitivity and elasticity of the geometric mean of the overall annual population growth to changes in demography during each phase of the life cycle. Our calculations using the megamatrix approach gave results that are equivalent to the sensitivity calculations described in earlier treatments of periodic matrix models (Caswell and Trevisan 1994, Caswell 2001).

We calculated elasticity values, which measure the proportional change in population growth rate to proportional changes in each element in $\mathbb{B}$ (de Kroon et al. 1986). Elasticity is useful because it quantifies the relative contributions to the population growth rate of alternate life history transitions, e.g., survival and stasis versus survival and growth (de Kroon et al. 1986). The fact that elasticity values sum to one, when summed over all demographic rates throughout the year (Horvitz et al. 2005), allowed us to evaluate the relative contributions to the annual population growth rate from each demographic rate in each phase, in each season, and within one year. In each phase, we summed elasticity values for five types of life history transitions, quiescent adults going active, active adults going quiescent, remaining in the same stage (stasis), fecundity, and growing to the next one or two stages. In each season, we summed elasticity values for each stage-specific demographic rate, and we also summed elasticity values for each stage-specific demographic rate over one year.

Results

We captured 34 unique adults with 6 recaptures in fall 2010, and 43 unique adults with 25 recaptures in spring 2011. Capture probabilities and population size estimates were similar between seasons, except during the early spring sample dates, Mar 28 to April 7 (Figure 5.4). Only 9 of the 68 captures occurred during this first half of the spring survey. Population size estimates were very low (mean = 2.5) in these early days compared with later spring days (mean = 16), which were comparable with the number of adults in the fall (mean = 15). Temperature and sunlight are two environmental factors that affect butterfly activity(Wikström et al. 2009). A majority of early spring trapping days were cloudy (7/12), and average daytime temperatures were cooler (72°F) compared with warm (82°F) days with clear skies (10/12) later in the spring.

Fitting the Markov model, $\boldsymbol{P_X}$ (Chapter 2), to laboratory data we estimated the survival rates for eggs (0.959) and pupae (0.974), and we also estimated growth rates from egg to first (0.690) or second instar (0.068), fifth instar to pupa (0.301), and pupa to adult (0.326). Only eggs that hatched in the lab were included in the analysis, which means we overestimated true egg survival. However, estimating egg survival in this way is congruent with our data and facilitated estimating fecundity with retrospective projections from individuals that already hatched from eggs. Fecundity in our model represented the per capita rate of laying eggs that hatched. For this reason, including the number of eggs that died before hatching would not contribute our model so we left this out. Adult survival was lower for quiescent summer form adults (90.4%) compared with quiescent winter form adults (98.9%), and survival of active adults was much lower than quiescent adults. For our model, we increased our estimates for active adults, equal between seasonal forms, from 74.7% to 82.6% (halfway between active and quiescent summer adults) to provide a more realistic survivorship in projected populations.

Stochastically projecting each juvenile we observed in the field into the past until birth resulted in a reasonable frequency distribution of birth dates (Figure 5.5). Fall oviposition appears to have started before September 3, peaked in late September, and then ended October 18. Spring oviposition started on March 1, peaked near the end of March, and then ended on April 15, according to retrospective projections. According to the quadratic fit to these dates and assuming a smooth rise in fecundity, fall oviposition started August 22. To account for the fewer plants searched in fall compared with spring, the quadratic fit shown in Figure 5.5A was scaled up to match the spring sampling effort. The quadratic model predicted a lower and wider frequency distribution of births in the fall (height = 54 eggs, after scaling, width = 63 days) compared with spring births (height = 81 eggs, width = 48 days).

The curve in figure 5.5 represents the number of eggs over time, which is the product of the per capita birth rate and the number of adults. A feature of our demographic model is that a pulse of adults becomes active at the beginning of each brood. This feature causes the peak number of active adults to precede the peak number of eggs laid, which means that per capita birth rates in spring and fall, increase then decrease over time (Figure 5.6). At maximum fecundity the model predicted that females on average laid about 10 eggs per day, which was congruent with reproductive output in the laboratory.

Assembling all components of $\boldsymbol{B}(m)$ and projecting forward, the population started in January with all quiescent winter form adults (Figure 5.7). In March, quiescent adults became active (dashed to solid blue lines; Figure 5.7) and began laying eggs. Eggs developed through all juvenile stages and emerged as active summer form adults (red line; Figure 5.7) that began laying eggs with a low per capita fecundity. Despite the low fecundity, adult density was high resulting in a substantial number of eggs laid in May and June. Peak summer egg production coincided with the transition of active to quiescence summer adults (solid to dashed red lines; Figure 5.7). The number of adults and eggs laid declined in July and August, during which time adults were mostly quiescent. At the end of August, quiescent summer form adults became active and per capita fecundity increased. The offspring developed into winter form adults that went quiescent at the end of November then overwintered (Figure 5.7).

Analyzing the properties of the periodic megamatrix $\mathbb{B}$, we calculated the co-dominant eigenvalue, $\lambda_{\mathbb{B}} = 1.00045$ which represented a positive low annual population growth rate, ${\lambda_{\mathbb{B}}}^{122} = 1.056$. The stable stage distribution summed over the entire year indicated that the population on average was composed primarily of third to fifth instars, quiescent adults, and eggs (Figure 5.8A). The reproductive value of each stage summed over the year indicated that quiescent adults were the most valuable in terms of contributing to future population size, and pupae, summer form adults, and winter form adults were intermediate between larvae and quiescent adults (Figure 5.8B). This pattern was due to the timing of suitable hosts for oviposition; individuals developing in or surviving to the right seasons had higher contributions to future population size. Throughout the year, both the stable stage distribution and reproductive value changed over time (Figure 5.9A-B). In winter months, only quiescent adults were present and reproductive value of these adults increased closer to spring time. Quiescent adults also had relatively high reproductive value in September. Overwintered adults becoming active in the spring had the largest value for active adults, and reproductive value of active adults increased again during the fall brood (Figure 5.9B). Juvenile and pupa reproductive value increased from March to May, with a slight drop in midsummer, then peaked at the end of August, when developing caterpillars could eclose as adults and contribute to the fall brood (Figure 5.9B). There was an additional peak toward the end of October for developing caterpillars that will become valuable overwintering adults.

The life history transition, survival and stasis, would have the largest proportional effect on the annual population growth rate, but the importance of this transition changed over time within a year (Figure 5.9C). The total elasticity for each phase was equal to 1/122, and this phase-specific total elasticity was divided among life history transitions. In March, quiescent adults becoming active claimed about 20% of the elasticity, after which fecundity and growth became important transitions (Figure 5.9C). Growth accounted for over half of the elasticity in April, when caterpillars of the spring brood were developing, and about 40% in early to midsummer when recruitment was necessary to maintain the population before the hot, dry conditions of late summer (Figure 5.9C). Active adults transitioning to the quiescent state accounted for about 10% of the elasticity during late summer, and quiescent adults transitioning back to the active state in September accounted for almost 40% of the elasticity. Growth and fecundity again became important during the fall brood, accounting for about half of the elasticity. Active adults transitioning to the quiescent state in late fall accounted for almost 20% of the elasticity, then quiescent adults remaining quiescent, i.e., survival and stasis, accounted for all elasticity, because all individuals must overwinter in the quiescent state (Figure 5.9C).

Summing over each season, survival and stasis of quiescent adults was most important only in summer and especially in winter (Figure 5.10). In spring, summer, and fall, several other transitions had proportionally large effects on population growth. In spring and fall, survival and reproduction of active adults were most important. Early instars had little effect relative to other stages, but survival of fourth instars and pupae stood out in their contributes to annual population growth (Figure 5.10).

Discussion

When the fates of individuals depend on life stage and season, then changes in the importance of each life stage in each season will affect population dynamics, especially for organisms with lifespans shorter than seasonal cycles. Our periodic stage-structured matrix model of butterfly population dynamics represented multiple generations developing within a single year and contrasted two opposing life history strategies that occur in different seasons. Incorporating field and laboratory studies of *Anaea aidea* into our periodic model, projections provided a realistic population trajectory *A. aidea*, with low positive population growth. Our model induced a tradeoff in survival and reproduction, and results indicated that a survival strategy is optimal in winter while reproduction is the optimal strategy in summer. However, our results also suggested a substantial benefit to the ability of summer adults to switch behaviors and improve survival at the cost of leaving breeding habitat. What is interesting is that these properties emerge from the timing of multiple coincident processes. Seasonal temperature and rainfall patterns drive the phenology of host plant foliage abundance and leafwing activity patterns, i.e., breeding or waiting. By controlling demographic rates to match these seasonal patterns, our model has provided insight into the seasonal constraints on the alternating life cycles of summer and winter form cohorts.

LIFE HISTORY TRADEOFFS – Explicitly modeling seasonal variation in life history revealed a seasonal switch in the direction of the tradeoff between survival and reproduction. The average lifetime fitness of the population, represented by the annual population growth rate, was most sensitive to changes in survival of overwintering adults, than it was to a proportional change in other demographic rates. However, this sensitivity was seasonally restricted, and demographic rates such as fourth instar and pupa survival, and adult survival and fecundity were more important in non-winter months. In terms of strategies, winter form butterflies invest in survival rather in than in reproductive development (Riley 1988b, Karlsson and Wickman 1989), and our results suggested that overwintered adults that survive to reproduce had the highest reproductive value, which means they had the largest potential contribution to future population size. This result highlights the crucial connection between growing seasons allowed by surviving the winter. We do not know how the preferential investment in survival might reduce future reproductive potential in terms of energetics, but adult feeding may offset this cost of long-term survival (Gilbert 1972, Boggs 1997). We observed adults feeding on fermenting sap of injured live oaks and dung during the study, both renewable nitrogen resources that could enable overwintered adults to replenish nutrient stores in order to realize an undiminished reproductive potential. For *A. aidea* in a Mexican tropical dry forest, (Torres et al. 2009) showed that peak fruit abundance coincided with the end of dry season when quiescent adults became active and began foraging and breeding. Although we did not model energetics, we did consider energetic constraints and decided not to penalize the fecundity of overwintered adults despite the potentially large energetic investment required to survive the winter.

A feature of our model was that the cost of reproduction was reduced survival only. Adults were either active and reproduced at the cost of lower survival, or they were quiescent and had higher survival with the cost of no reproduction. A subtlety in this feature was that the cost-benefit ratio varied seasonally, which resulted from a constant cost of survival to reproduce, while reproductive potential varied seasonally. In nature, caterpillars in the spring recruitment pulse consume host plants and reduce the number of suitable plants for ovipositing females. The seasonal synchrony of life cycles in the spring cohort should result in high leafwing densities, which coincide with this decrease in oviposition sites reducing per capita fecundity. As spring transitions to summer, hot and dry weather becomes more frequent and host plants again are likely reduced if their leaves senesce. Early autumn rain replenishes host plant suitability and low adult densities allow increased per capita fecundity. We represented this phenomenon, with our fertility schedule, which imitated two peaks in fecundity in spring and fall, and one low peak in summer. We restricted adults from achieving their potential fecundity because adults are likely capable of higher fecundity than the habitat will allow in most seasons. However, those adults ready to lay eggs at the right time had increased fecundity relative to those living during other time periods.

Clutton-Brock (1984) pointed out that natural selection will operate through reproductive cost. Indeed our results indicated that adults in spring and late summer had more reproductive value despite similar reproductive costs in other seasons. Sensitivity and elasticity (proportional sensitivity) both represent selection pressures, in that they represent the change in average lifetime fitness (population growth) in response to a change in demographic rates, e.g., survival, growth, and reproduction (Benton and Grant 1996, Caswell 2000, Horvitz et al. 2010). According to the seasonal elasticities, selection should favor winter form individuals that can survive until spring and reproduce. Long-term survival was not favored during the growing season due to the high reproductive value of juvenile stages. Population growth was sensitive to development and growth at this time, and more sensitive to the survival of active, reproducing adults than to quiescent adults. Summer form adults do require some ability to become quiescent and prolong their life, as was indicated with high sensitivity of population growth to survival of quiescent adults in summer, but this need likely varies year to year in the severity of high temperatures and drought, and is less certain and shorter in duration than winter.

It was interesting that fecundity itself did not stand out as having a large effect on annual population growth. There are at least two possible explanations; offspring were relatively low in reproductive value and/or reproductive potential took time to be realized. Supporting the first explanation, our results indicated that juvenile stages had low reproductive value, and stages preceding fourth instar had a negligible effect on population growth. The chances of a single egg becoming an adult were slim, but individuals surviving to adulthood, especially those surviving the winter had high value. Overwintering in the adult stage was therefore costly because, adults were more expensive than eggs or early instars, a finding that may explain why temperate butterflies tend to overwinter in demographically cheaper stages (WallisDeVries et al. 2011).

The second explanation rests on theoretical demographic models of ageing, in which the net reproductive rate is simply the product of average reproductive rate and life expectancy (Baudisch and Vaupel 2010). Per capita reproductive rate in our model was seasonally limited, and survival of active adults was always relatively low. Baudisch and Vaupel (2010) showed that net reproductive rate can be increased via raising either reproductive rate or life expectancy. Our results suggested that increasing life expectancy of active adults during the spring and fall broods would have a proportionally large effect on annual population growth. Due to seasonal per capita fecundity fluctuations, extended survival would not only increase time available for reproduction, but would also increase the chances of being alive, when reproductive rates are higher.

QUIESCENCE – Quiescence in our model represented the strategy of waiting until the best time to forage and reproduce. Winter form adults have this strategy imprinted in their body plan, and enter diapause during winter. Similarly the life cycles of many other insects include a diapause stage that is synchronized with predictable periods of harsh conditions (Denlinger 1986). A variety of environmental factors, e.g., temperature, rainfall, humidity, and day length, may provide cues that indicate when to breed (Topp 1990, Caldas 1995) and when to diapause (Riley 1980, Denlinger 1986). However, unpredictable environmental extremes may clash with optimal schedules. Contrary to an "average world", seasonal extremes may dominate for the majority of a butterfly's lifetime, and natural selection should favor the ability to 'decide' when to reproduce and when be quiescent when faced with uncertain environmental conditions.

We enforced quiescence during winter to represent true diapause, strongly encouraged it in late summer to escape extreme heat and drought, and mildly encouraged it in late spring to summer. Summer adults were not expected to enter into true diapause (sensu Masaki 1980), but may behave similarly to adults in diapause. In order to achieve higher survival, active adults transitioning to quiescence were hypothesized to leave the breeding habitat and seek out some refuge. The ability to 'choose' quiescence as opposed to entering a true diapause state may be better for summer form *A. aidea*, because it would allow a quick response to infrequent rainfall and local leaf flush during otherwise hot and dry summers.

Refuge for *A. aidea* may be found along stream corridors, and quiescent adults may select from a variety of roosting sites including crevices in tree bark, a hollowed tree or perhaps a cave, similar to other nymphalid butterflies that diapause as adults (Dvořák et al. 2002). We observed most adult activity within woodland croton patches and at the woodland edge in close proximity to croton patches. These habitats were largely associated with streams, occurring upslope in dry upland as opposed to riparian habitat. Riparian habitats in the Hill Country support a diversity of large hardwood tress that may provide holes and hollowed caverns for inactivity in a sheltered environment (Wrede 2005). In the tropics, diapause of adult insects including butterflies is well documented, and adults in diapause tend to aggregate in moist areas during the dry season (Denlinger 1986). Gilbert (1985) described the summer diapause behavior of adult *Kricogonia* during dry summers in south Texas. Females in particular selected sites in shaded stream corridors which were cooler on average than surrounding habitat, and entered reproductive diapause until local conditions improved (Gilbert 1985). This butterfly's host was leafless during this period, so behavior focused on improving survival until a favorable time for oviposition should be adaptive. Whether or not summer form *A. aidea* enters true diapause, they may select similar sites and behave similar to adults in diapause to survive harsh periods when reproduction is not optimal.

Implicit evidence for this behavior is common in butterfly research, in that it is well known that butterflies are active only under certain weather conditions (Pollard 1977, Wikström et al. 2009). Not surprisingly, *A. aidea* adults essentially ceased activity during cool cloudy days. However, butterfly activity seemed to remain very low on warm days among predominantly cool weather days. We captured very few adults during a period of 12 days of mostly overcast cool weather, despite 5 warmer days with clear skies. This slow response to improved weather suggests that there was a time commitment to avoiding activity, which may have involved moving between active and quiescent habitats. We enforced a time commitment in seeking refuge and becoming quiescent by either not allowing winter form adults to leave quiescence until early spring, or enforcing only a low probability of summer form adults leaving quiescence until early autumn.

CONCLUSION – We constructed a periodic megamatrix for the butterfly, *Anaea aidea*, to evaluate how life stage transitions in each season contribute to overall population dynamics. With this approach, which has not previously been applied to butterflies, we demonstrated seasonal constraints on fitness and the maintenance of distinct seasonal life histories. Our results show that natural selection should favor reproduction in summer adults, juvenile growth during the breeding season, and survival of winter adults. In general the benefit gained through the increase in fitness for butterflies reproducing after surviving inhospitable conditions far outweighed the cost of delayed reproduction.

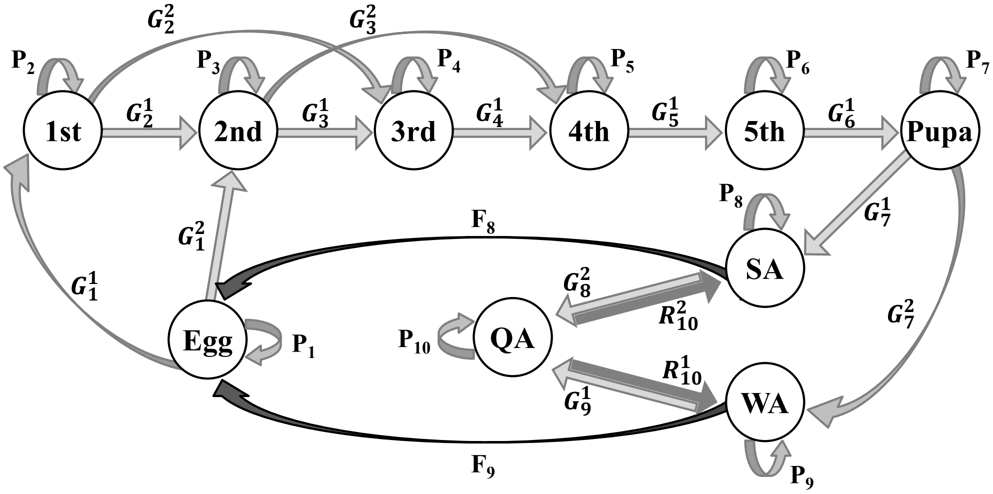

Figure 5.1 Life cycle graph for the population dynamics of *A. aidea* showing all possible stages and transitions. Adult stages are QA = quiescent adult, SA = summer form, and WA = winter form. The parameters correspond to the diagonals of the matrix $\boldsymbol{B}(m)$, and represent the stage-specific probabilities of survival and stasis, $\boldsymbol{P_i}$, survival and growth one, $\boldsymbol{G_i^1}$, or two stages, $\boldsymbol{G_i^2}$, and survival and regression one, $\boldsymbol{R_i^1}$, or two stages, $\boldsymbol{R_i^2}$.

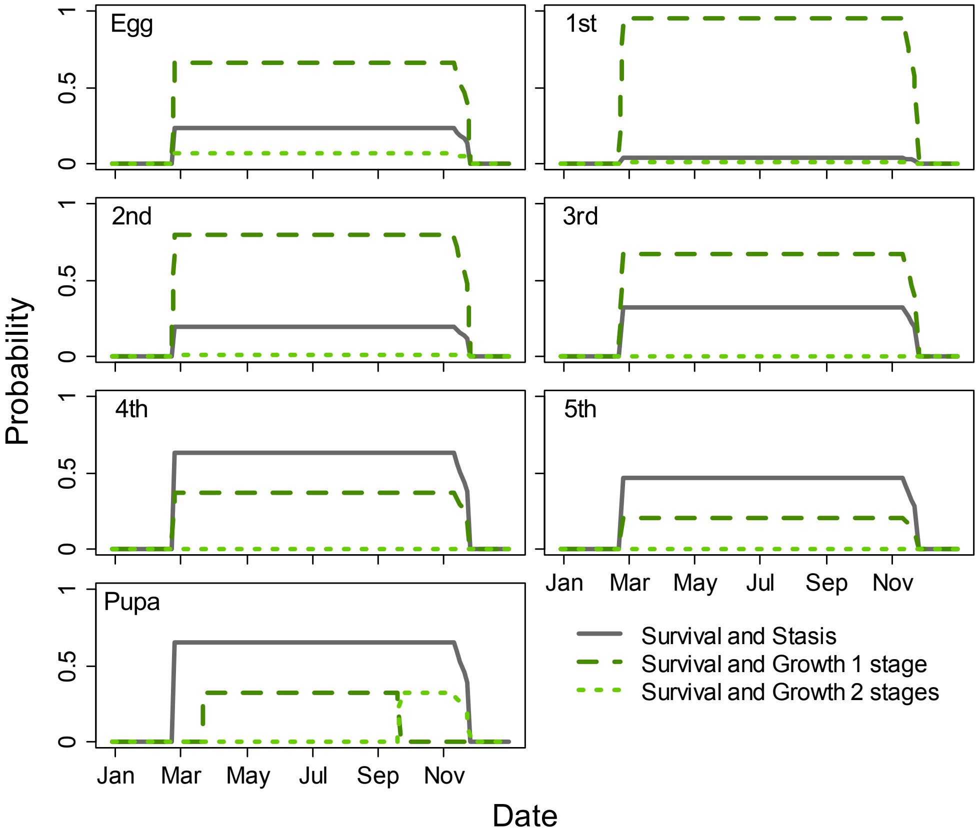

Figure 5.2 Line graphs of life history transition probabilities for *A. aidea* juvenile stages. Each line represents an element of $\boldsymbol{B}(m)$ over time. $\boldsymbol{P}_{\mathbf{1-7}}$ are the probabilities of surviving and remaining in the same stage. $\boldsymbol{G}^{\mathbf{1}}_{\mathbf{1-7}}$ and $\boldsymbol{G}^{\mathbf{2}}_{\mathbf{1-7}}$ are the probabilities of surviving and growing either one or two stages, respectively. For the pupa stage $\boldsymbol{G}^{\mathbf{1}}_{\mathbf{7}}$ and $\boldsymbol{G}^{\mathbf{2}}_{\mathbf{7}}$ are the probabilities of surviving and eclosing as either a summer adult or winter adult, respectively.

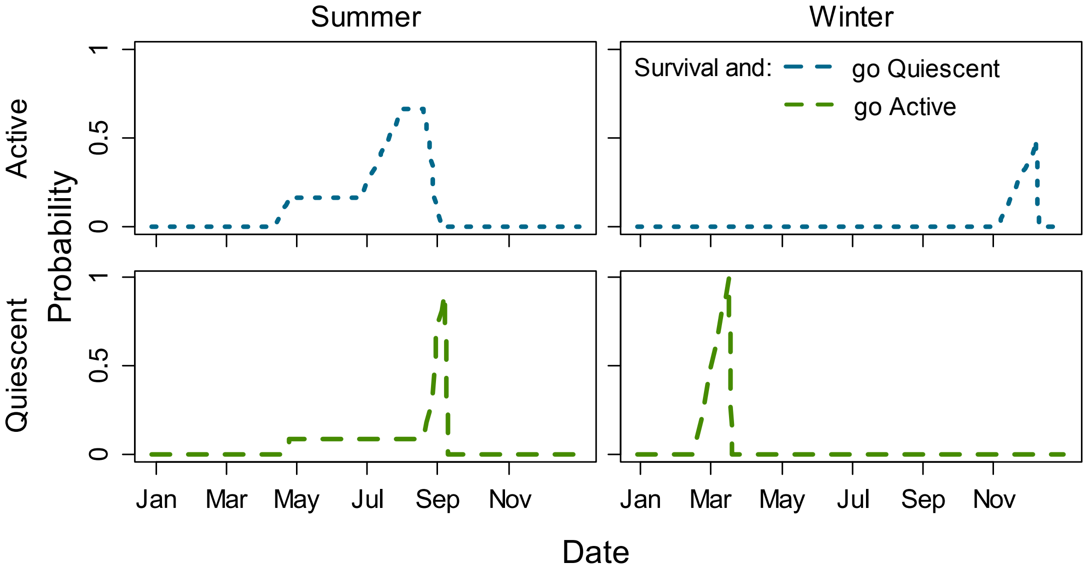

Figure 5.3 Line graph of life history transition probabilities for *A. aidea* summer and winter form adults that are either quiescent or active. Each line represents an element of $\boldsymbol{B}(m)$ over time. $\boldsymbol{G}_8^2$ and $\boldsymbol{G}_9^1$ are the probabilities of active summer and active winter adults going quiescent (top panels). $\boldsymbol{R}_{10}^2$ and $\boldsymbol{R}_{10}^1$ are the probabilities of quiescent summer and quiescent winter adults going active (bottom panels).

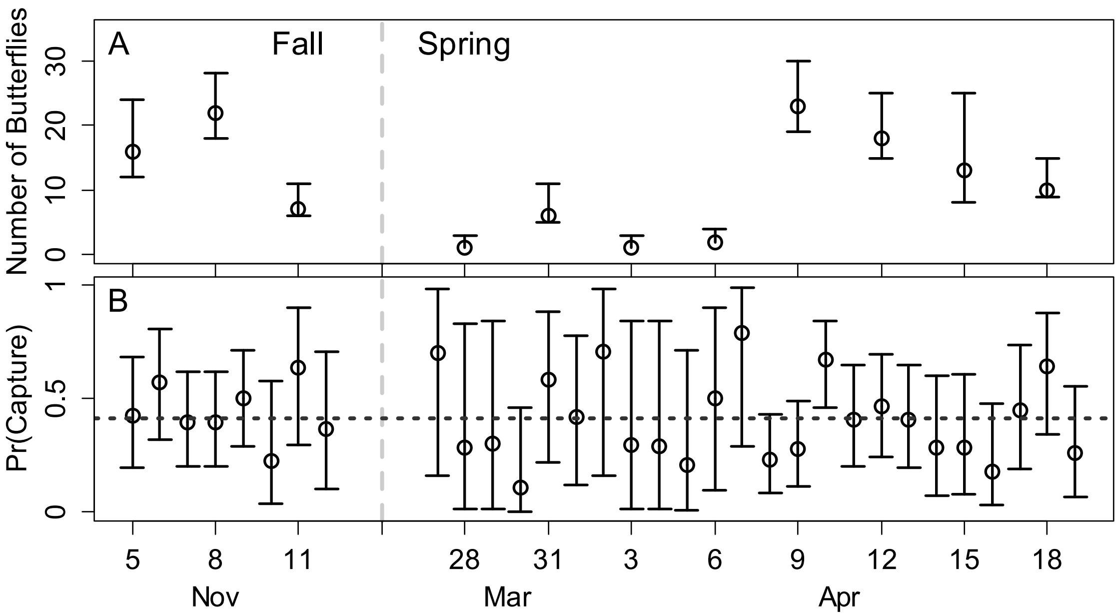

Figure 5.4 Estimates of the number of *A. aidea* butterflies for each 3-day sample (A) and capture probabilities for each sample day from capture-recapture data in fall 2010 and spring 2011. Circles indicate means and error bars indicate the 95% credible limits. The dotted horizontal line indicates the mean capture probability over all samples.

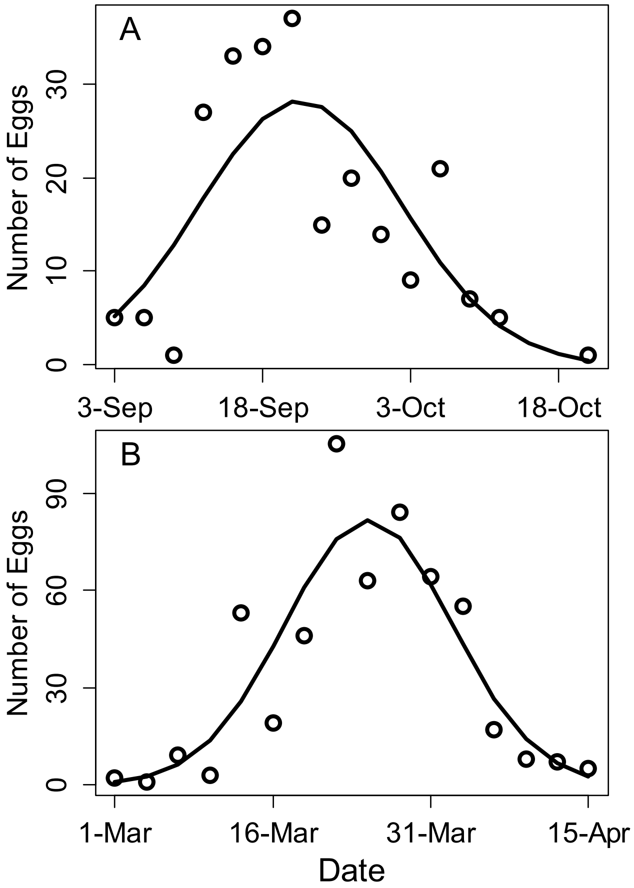

Figure 5.5 Scatterplot of the number of *A. aidea* eggs laid at each date in either fall 2010(A) or spring 2011(B). Data points represent the sum of all individuals born on each date. For each juvenile observed in the field and also for individuals predicted to have died before our surveys, we projected each individual 100 times from their stage at first observation retrospectively to birth individuals to estimate the mean date of birth. The quadratic curve was fit using Poisson regression.

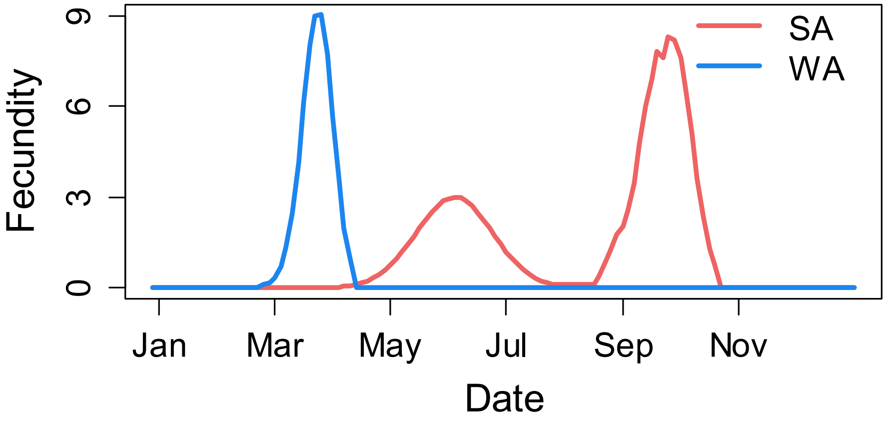

Figure 5.6 Line graph of average number of eggs laid per female per 3-days for *A. aidea* summer ($\boldsymbol{F_8}$) and winter ($\boldsymbol{F_9}$) form adults. Each line represents per capita fecundity in the upper right hand corner of $\boldsymbol{B}(m)$ over time.

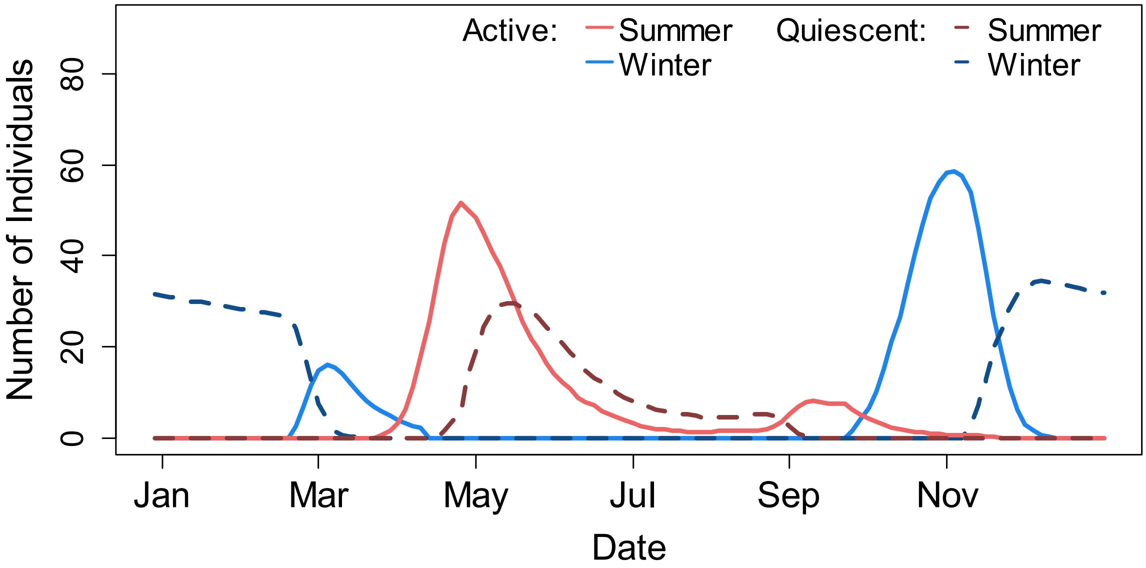

Figure 5.7 Line graph displaying the number of adults predicted over one year by the periodic model ***B***(*m*), projecting from the number and birth date of eggs estimated over the 2010-2011 field season. Each individual starts as an egg on its estimated birth date, and is projected forward stochastically until death. Each individual was projected through 100 independent sample paths and the number of adults was summed over the population at each step and averaged over all 100 iterations.

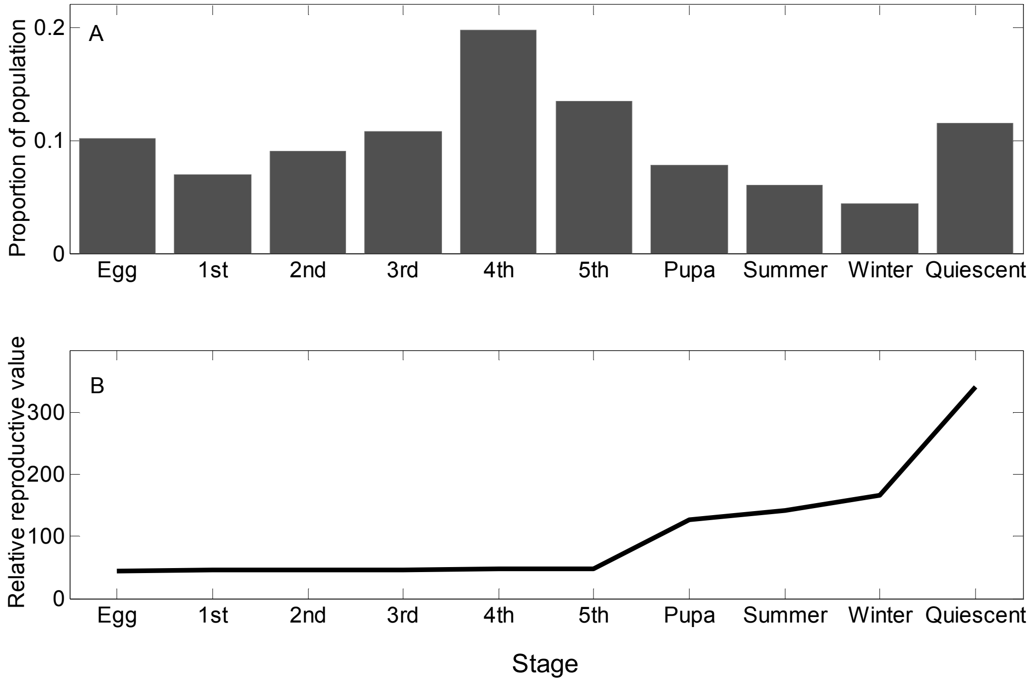

Figure 5.8 Plots of the stable stage distribution (A), and reproductive value (B) for each stage summed over one year. The stable stage distribution represents the proportion of the population in each stage when the population is at equilibrium. The relative reproductive value represents the contribution of each stage as a seed of future population size.

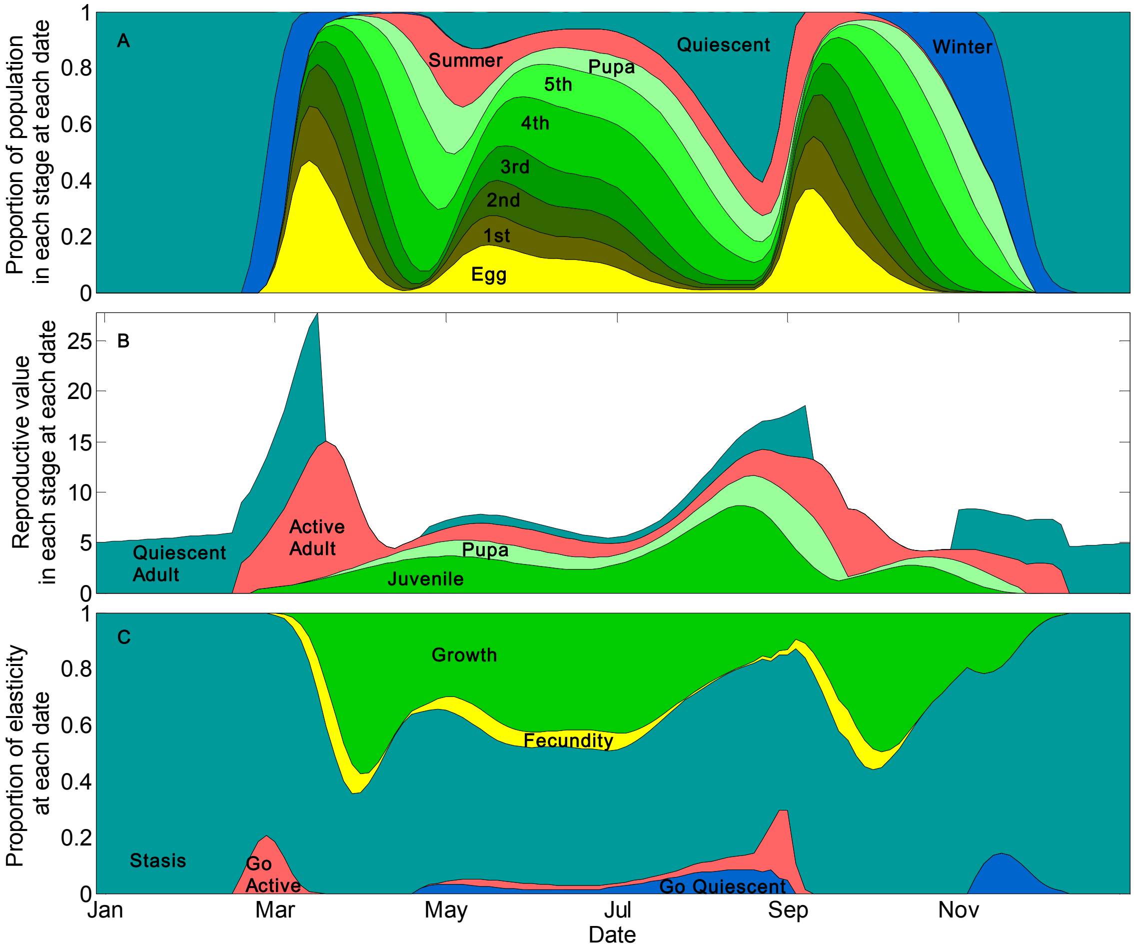

Figure 5.9 Area plots of the stable stage distribution (A), reproductive value (B), and elasticity (C) over the period of one year. The area of each colored region in A indicates the proportion of the population that is in each stage at each date. Area of colored regions in panel B indicates the contribution of each group of stages at that date to future population size. Total height of all areas in panel B represents the reproductive value at each date. In panes B and C, quiescent and active adults include both winter and summer adults, and juvenile includes egg to fifth instar in panel B. Area of colored regions in panel C indicates the proportional change in the annual population growth rate for a unit change in each life history transition at that date. We summed elasticity over all stages for each life history transition (quiescent to active, active to quiescent, remaining in the same stage, fecundity, and growth). The proportion of elasticity at each date to the total elasticity in one year is constant over time such that $e_{ij}(m) = 1/122$.

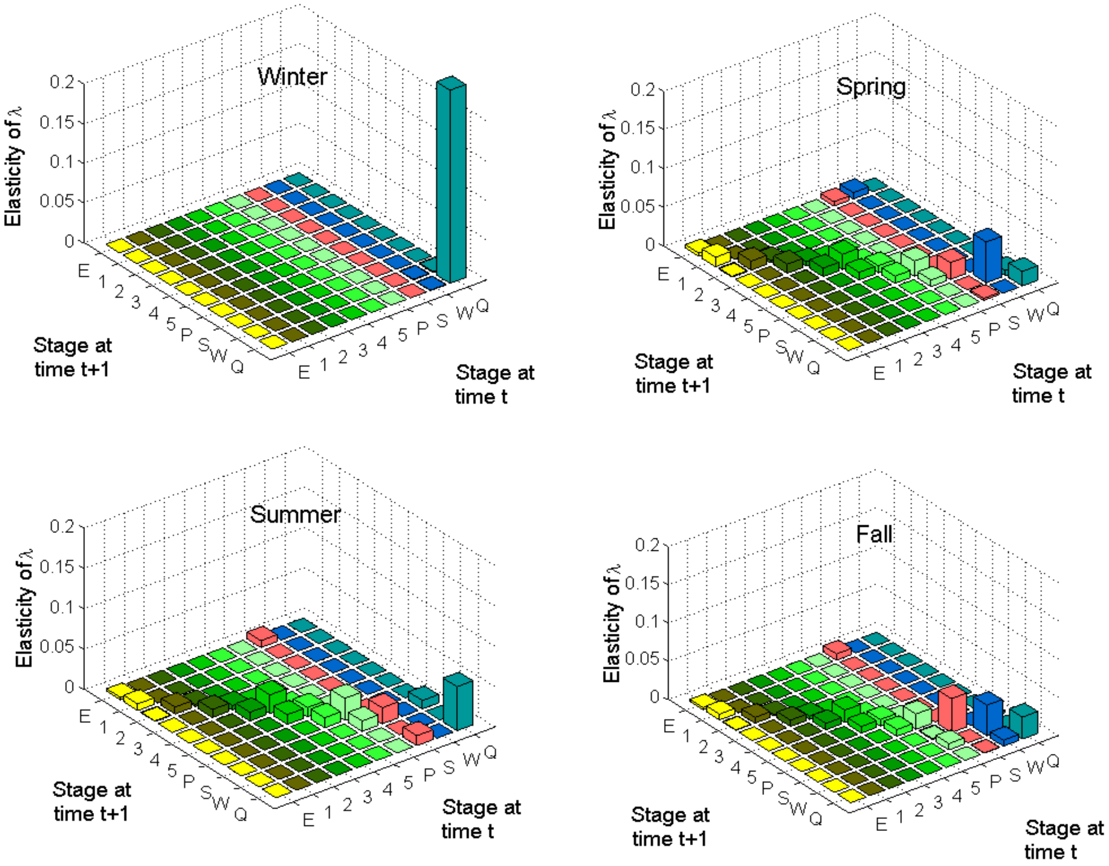

Figure 5.10 Elasticity for all stage transitions summed by season. The height of each bar indicates the proportional change in population growth for a proportional change in the vital rate in each season represented by that matrix cell.

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