

ABSTRACT

HENRY, ERICA. Disturbance and Conservation of At-risk Butterflies. (Under the direction of Dr. Nicholas M. Haddad).

Across the globe, insect populations are declining. For many species, loss of historic disturbance is a main driver of habitat loss. This threat, however, has the potential to be reversed through responsible land management. I answered the following two questions: 1) how best can we restore historic disturbances in the presence of rare species? 2) how will climate change affect future population dynamics? To answer these questions, I focused on three endangered butterfly species, Bartram's scrub-hairstreak, St. Francis' satyr, and Miami blue.

I studied the hostplant of Bartram's scrub-hairstreak, *Croton linearis*, to understand how hostplant populations respond to different types of disturbance-based management. I compared survival, recruitment, and population growth rates for *C. linearis* following prescribed fires, mechanical understory removal, and in control plots. In the first year post-management, survival rates did not differ across the treatments. In the second year, survival was significantly higher in fire plots than mechanical and control plots. Both disturbance treatments stimulated a flush of recruitment in the first year post-treatment, but there was almost no recruitment in control plots. Despite this recruitment, two years after treatments population growth rates in mechanically cleared plots were <1 and no different from controls; only prescribed fire led to increases in croton population.

For St. Francis' satyr, I evaluated the utility of using another butterfly as a substitute species to determine the success of disturbance-based restoration actions. This strategy assumes the response of the substitute mirrors that of the endangered species. I tested this assumption, comparing population size, behavior, survival, and habitat selection of a common butterfly, Appalachian brown, to St. Francis' satyr, a very similar endangered butterfly, in wetland

restoration plots and no-treatment controls. I found the two butterflies exhibited opposite patterns in almost all response variables I measured. Endangered caterpillars foraged under more cover than common ones, endangered caterpillar survival was highest in restoration sites and common caterpillar survival was highest in controls. These survival differences translate to differences in adult population sizes, with endangered butterfly populations highest in restoration sites and common butterfly populations highest in controls. These differences demonstrate that Appalachian brown is not a good substitute for St. Francis' satyr, and that restoration strategies designed based on Appalachian brown may perform poorly for St. Francis' satyrs.

Miami blue butterflies are vulnerable to climate change. To understand how climate change will affect Miami blue butterfly population dynamics I developed estimates of population density, determined how climate variables control phenology, and built a population model to explore how our uncertainty about the butterfly's biology and future climate change interact to affect future Miami blue persistence or extinction. I found peak densities of Miami blues were in the 100's of butterflies per hectare and that butterfly phenology was controlled by precipitation patterns. My population model used daily precipitation projected by downscaled climate models to drive butterfly population dynamics. I compared extinction dates predicted by 20 climate models across various combinations of diapause survival values (life stage about which we know nothing) and initial population sizes. I found that Miami blue population was more sensitive to variation in diapause survival than future changes in precipitation.

Results from these three case studies highlight the importance of understanding the natural history of the endangered species we manage to design effective conservation strategies and reliably predict their future fates. By focusing on demography and population dynamics I was able to both identify key life history traits important for maintaining populations as well as

compare outcomes of management strategies for different species. This is a powerful approach for predicting how species might respond to future changes in both landscapes and climate.

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Disturbance and Conservation of At-risk Butterflies

by
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DEDICATION

To all the land managers working hard to implement science-based habitat restoration and management of endangered species populations.

BIOGRAPHY

I grew up on the shores of Puget Sound in Washington State, digging for clams and geoducks, and backpacking in the Cascade mountains. This instilled in me a love of the outdoors and motivation to protect natural resources and open spaces. After graduating from Willamette University in Salem, Oregon, I traveled widely across the United States, working on a variety of research projects centered on endangered species habitat restoration and management. Eventually this winding path led me to a Master's degree at Washington State University. Through my Master's research I learned that conservation programs could be very successful when based on scientific findings that are rooted in a deep understanding of species natural history. After finishing my Master's, about a year into working with rare butterflies in south Florida, I realized I had opinions about what research should be done to move the conservation work forward and wanted to have a hand in directing those efforts, for that, I needed a PhD.

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CHAPTER 1

Two surrogates for wildfire, prescribed burning and mechanical clearing, differentially affect demography of a rare, endemic plant

ABSTRACT

Alteration of historic disturbance regimes is one of the biggest threats to species' persistence. This is particularly true for species adapted to frequent, low-intensity fires. When restoration via prescribed fire is not an option, surrogate methods of clearing vegetation are needed. We use an observational study of fire effects and an experimental study of mechanical clearing to test how restoration techniques affect individual vital rates and population growth rate of a rare, endemic plant *Croton linearis*. We found that survival rates one-year post-disturbance were similar across mechanical clearing, prescribed fire, and control plots. Two-years post treatment, however, survival in burned plots was much higher than that in either mechanically cleared plots or controls. Both fire and mechanical treatments stimulated a flush of recruitment in the first-year post disturbance but not in the second year, and we saw very little recruitment in control plots in either year. These vital rates combined to produce stable populations two years post treatment in the fire plots and shrinking populations in both mechanically cleared and control plots. Our results suggest that when possible, prescribed fire should be used to manage *C. linearis* populations. Because mechanical clearing increases recruitment, it is potentially a better alternative than no action. By focusing on vital rates instead of community level responses, we were able to calculate population growth rates and therefore predict how management will affect the trajectories of species of interest in the future. This mechanistic approach is especially important for maintaining and restoring rare species populations in an era of rapid change.

INTRODUCTION

Fire adapted ecosystems are among the world's most endangered (Noss et al. 1995). Loss of fire-dependent ecosystems is highest near human development, as people restrict or eliminate fire near their homes, cities, and farms. Loss of natural fire regimes remains a threat even within protected areas. In many cases, this is a legacy of historical management, in which fire was viewed as a neutral or even negative process in maintaining habitat. Without fire, however, succession fundamentally alters the competitive landscape for plants and animals, changing habitat structure and species composition, ultimately leading to extirpation of species adapted to historic fire regimes (Leach & Givnish 1996; Bond & Keeley 2005; Ratajczak et al. 2011). The preferred management strategy in these systems is prescribed fire. However, lighting fires can be difficult due to high costs, narrow prescription windows, and the threat of escape (Miller & Wade 2003). This results in the need for habitat management that replicates the effects of fire such as mechanical understory removal. Mechanical methods mimic the effects of fire on vegetation structure but have different effects on below ground processes and litter dynamics (Moghaddas & Stephens 2007; Youngblood et al. 2008; Stephens et al. 2012); differences that could result in altered population dynamics for fire-adapted species. We test how prescribed fire and a surrogate, mechanical understory removal, affect survival, recruitment, and population growth rates of a rare plant.

The success of fire management and its surrogates, in terms of rare species restoration, will be determined by the effects a management strategy has on population size and growth rates of target species. Efforts to understand how fires and their surrogate management strategies affect populations should, therefore, focus on estimating responses of vital rates. The differential effects of restoration strategies have been examined in many fire prone systems, however, these

comparisons focus on community responses or changes in species cover rather than individual vital rates (Perchemlides et al. 2008; Steen et al. 2013a; 2013b). Studies that do focus on vital rates generally only examine the effects of fire and do not compare fire to other management strategies (Liu et al. 2005; Menges 2007, but see Gross et al. 1998). The advantage of focusing on vital rates is that it allows for estimation of population growth rates, and therefore, of the future trajectory of the population. This information is especially important for land managers tasked with the job of ensuring the survival of threatened and endangered plants and animals. Understanding the likely trajectory of a population following a specific management action feeds into the decision-making process. With this information, land managers can consider the full range of costs, benefits, and risks when deciding between different management strategies (van Wilgen 2013).

By focusing on how vital rates are affected by different management strategies, we can begin to tease apart the mechanisms that underlie species responses to habitat management. As an example that is particularly relevant to our study, plants that are adapted to frequent fires generally fall into one of two categories, seeders and sprouters (Keeley 1977). This distinction between seeders and sprouters is generally based on the vital rate that contributes the most to a plant's population growth rate following a fire. Seeders are killed by fires, but have extensive seed banks, the germination of which is stimulated by heat or chemical cues, such as smoke or charcoal (Keeley et al. 2011). For these species, conditions that promote germination from the seedbank are most important for population recovery post-fire. Sprouters lose their above-ground biomass as a result of fires but survive and re-sprout following the fire. Re-sprouting often depends on resources stored underground in carbohydrate rich lignotubers or other structures which are unaffected by the fire (Bond & Midgley 2001). Unlike germination, re-sprouting post-

fire does not rely on specific environmental cues but is instead a response to the loss of above ground biomass. The goal of mechanical fire surrogates is to replicate the vegetation structure of frequently burned systems by removing standing above ground biomass and reducing canopy cover, thereby increasing the amount of sunlight reaching the ground (Stephens et al. 2009). These methods differ from fires in that they leave a layer of mulch and debris behind, plant material that would be consumed and recycled in a fire (Youngblood et al. 2008). This key difference between mechanical treatments and fires has the potential to interact with species life history, potentially promoting a specific set of adaptations over others. For example, we might expect mechanical treatments to favor sprouters that respond to biomass loss over seeders that rely on heat or chemical cues for germination. If this is the case, we predict mechanical surrogates and prescribed fires to have similar effects on sprouter vital rates, but very different effects on seeder vital rates. We might also expect these two restoration treatments to differentially effect animal species through differential effects on key resources such as nesting sites or food. This kind of detailed information about vital rates will allow us to better design fire surrogates to promote target species and potentially predict how different species may respond to a given treatment based on life history traits.

We test the effects of fire and mechanical restoration treatments on vital rates of pineland croton (*Croton linearis* [Euphorbiaceae], hereafter referred to as croton), a rare plant endemic to fire-adapted pine rocklands in South Florida. Although not listed as endangered itself, croton is the only host for two endangered butterflies, Bartram's scrub-hairstreak (*Strymon acis bartrami*) and Florida leafwing (*Anaea troglodyta*). After habitat loss, loss of disturbance regime is the primary reason for declines in these species. Most of the remaining pine rockland fragments are surrounded by people in Miami-Dade county and on Big Pine Key, making fire difficult to

implement and mechanical treatments an attractive alternative. Working in an experimental study of mechanical clearing and an observational study of fire effects, we ask: do fire and mechanical clearing result in similar survival and recruitment responses and population growth rates? This research is essential for understanding population level responses of this rare plant to habitat restoration and has implications for restoration of other species, specifically the butterflies that rely on croton.

METHODS

Study system

Pine rocklands are found only in south Florida, The Bahamas, and Turks and Caicos where they grow directly out of the limestone substrate. These forests are characterized by open stands of South Florida slash pines (*Pinus ellioti* var. *densa*) and an open shrub/palm subcanopy, below which a rich herbaceous community thrives. In south Florida, pine rocklands once extended along the Miami rock ridge, from North Miami Beach to Everglades National Park, and a few islands in the Florida Keys. Ninety percent of the pine rockland on mainland south Florida and much of the habitat in the Keys has been cleared for development in the last 100 years. The largest remaining pine rocklands are protected within Everglades National Park, Navy Wells – a Miami-Dade County Natural Area, and the National Key Deer Refuge on Big Pine Key. Given this large decline of pine rockland habitat, croton populations have declined and become increasingly fragmented. Pine rockland habitat loss affects not only croton, but the butterflies that rely on it, Bartram’s scrub-hairstreak and Florida leafwing. Declines in these butterfly populations are closely linked with the fragmentation and loss of croton populations in the landscape.

Remaining pine rocklands are vulnerable to lack of disturbance and subsequent forest succession, which excludes croton, this is true even within protected areas. Historically, pine rocklands burned frequently, maintaining the forest in an early successional state. Croton is well adapted to fire; based on anecdotal data, it appears to both seed and re-sprout following fires (C. Anderson unpublished data). Without fire, pine rocklands convert to hardwood hammocks, and croton eventually disappears from the ecosystem. Since 1951, croton has gone from being one of the 10 most common plants in pine rocklands on Big Pine Key (Alexander 1972) to covering less than 0.01% of pine rocklands in 2014 (Bradley 2009); this decline has occurred simultaneous with decline in fire across the island.

Fire is the preferred management strategy for maintaining croton and habitat for Bartram's scrub-hairstreaks and Florida leafwings; however, there exist multiple obstacles to implementing prescribed fire, especially at the wildland-urban interface and in instances where fuels have accumulated to levels that make it unsafe to burn. In these areas, mechanical clearing through mowing, brush hogging, etc. has been proposed as a possible habitat maintenance strategy. This is motivated by anecdotal observations of croton plants sprouting and thriving in newly cleared fire breaks. While these observations are encouraging, evidence-based data are necessary for making informed management decisions.

Mechanical clearing experiment

To test the effects of mechanical clearing on croton demography, we implemented a clearing experiment on Big Pine Key. In July 2015, we established four, half-hectare replicates each of two treatments, mechanical clearing and a no treatment control. US Fish and Wildlife Service fire staff implemented clearing treatments by haphazardly driving a skid steer with a

masticating head through the treatment unit. The prescription for the treatment was to clear and mulch the understory vegetation in a mosaic pattern covering 50% of the plot with the goal of mimicking the mosaic burn patterns that might result from a prescribed fire.

Prior to treatments, we tagged all croton plants in 15 subplots in each replicate. To ensure that we tagged croton plants across the replicate, we identified croton sampling plots by establishing a grid of parallel 50-meter transects spaced 10 meters apart in all treatment units (total of 10 transects). In each unit, we randomly selected five transects and established three, permanent 2.5m-radius subplots on each one, in the middle and each end of each transect. We marked subplots by hammering a 25cm galvanized spike at the subplot center and attached a numbered, metal plant tag to identify the subplot number. In each subplot, we tagged all croton plants by wiring metal plant tags around the base of each plant. If we did not reach our goal of 30 plants tagged in the first five transects, we randomly sampled additional transects until we reached at least 30 plants. We tagged plants in 5-10 subplots in each unit, depending on croton density.

We first tagged plants in July 2015 and implemented mechanical treatments in August of the same year. We re-sampled the croton plots in July 2016 and July 2017. Each time we re-sampled we located all previously tagged plants and recorded if they were alive or dead. We also tagged new seedlings as well as plants that we missed in the previous round of sampling. New seedlings were easily distinguished from missed plants by their orange-colored stems that were not noticeably woody. From these data, we estimated annual survival and recruitment in the two years following mechanical treatments. To quantify recruitment, we divided the number of seedlings tagged after the disturbance by the number of tagged plants in the year prior to disturbance.

Prescribed fire monitoring

To test the effects of fire on croton demography, we used long-term croton monitoring data that has been collected over the last decade in Everglades National Park. There are four different fires represented in this data set, in 2006, 2007, 2013, and 2016. The 2006, 2007, and 2013 fires occurred in three different fire units within the park, and the 2016 fire burned the same unit as the 2006 fire. In each of these fire units, Everglades National Park monitors fire effects on croton in one 20m X 50m plot. Every croton plant within the monitoring plot was tagged with a metal plant tag and its location within the plot was mapped prior to burning, the number of croton plants in each fire unit ranged from 7-84. Following each fire, plants were re-surveyed monthly and, in three of the four fires, new seedlings were mapped and tagged as they were discovered. Only two of these fire units were monitored two years post-fire, one of which had new recruits that were tagged. To estimate annual survival we compared the croton surveys from the month before each fire to the same month in subsequent years in which data were available. We standardized recruitment as detailed above.

Statistical analyses

All statistical analyses were done in R version 3.3.1 (R Core Team, 2016).

Survival: To test for differences in overall survival, we used a generalized linear model with a binomial response variable and fixed effects of treatment (mechanical, fire, control) and year (one year and two years post treatment) using the glm function and Anova function from the car package (Fox & Weisberg 2011).

Recruitment: We tested for differences in recruitment between the three treatments with a linear model, using our standardized recruitment estimates as the response variable, and post-hoc tests with the TukeyHSD function.

Recruit vs. established plant survival: We compared survival of new recruits to survival of previously established plants in the second year post-treatment in our mechanical experiment only (we did not have these data in our prescribed fire dataset). To do this we used a two-way ANOVA with main effects of age (recruit vs. established) and treatment (mechanical vs. control).

Population growth rate: We calculated count-based population growth rates for the two years following treatments as $N(t+1)/N(t)$. Because we did not have recruitment data for year two post-fire, we assumed there was no additional recruitment in that second year. This was based on our observations of very little (almost no) recruitment following mechanical treatments, and results in the most conservative estimate possible. We then used a linear model and post-hoc Tukey test to test for differences between population growth rates across treatments and years.

RESULTS

We tagged a total of 588 croton plants, 161 in control plots, 292 in fire plots, and 135 in mechanically cleared plots.

Overall survival by treatment depended on year (interaction: Wald's chi-square=24.74, $df=2$, $p<0.0001$). In the first year post-treatment, survival was similar across all treatment types. In the second year post-treatment, survival in both disturbance treatments increased, while survival in control plots showed the opposite trend (Figure 1a).

Recruitment was highest in mechanically cleared plots and was significantly higher in mechanical plots than controls ($F_{2,8}=5.85$, $p=0.027$). Recruitment in fire plots was intermediate between control and mechanical treatments, and not statistically different from either, although it was marginally significantly greater than controls ($p=0.075$; Figure 1b). Survival of new recruits was not different from survival of already established plants (Wald's chi-square=0.059, $df=1$, $p=0.81$). However, there was a trend in mechanically cleared plots of survival being higher for new recruits (0.85 ± 0.084) than previously established plants (0.77 ± 0.059), and a trend suggesting that recruit survival was higher in mechanically cleared plots (0.85 ± 0.084) than controls (0.64 ± 0.17). The interaction between plant age and treatment was not significant (Wald's chi-square=1.12, $df=1$, $p=0.29$; Figure 2).

Differences in survival and recruitment across treatments combined to produce differences in population growth rates. There was a significant treatment effect ($F_{2,,18}=4.33$, $p=0.033$), with population growth rate in the fire treatment significantly higher than controls, mechanical treatment fell between the two and was not statistically different from either (Figure 1c). Growth rates were higher in the first year following disturbance than the second ($F_{1,19}=8.04$, $p=0.013$). Mean growth rates were above 1 in both disturbance treatments one year post treatment, but fell below 1 in mechanically cleared plots two years post-clearing. Fire treatment was the only one in which population growth did not fall below one, and thus was the only treatment that resulted in a stable population.

DISCUSSION

We demonstrated that mechanical surrogates for fire are a useful restoration strategy, but are unable to replace fire completely. Mechanical understory removal results in similar patterns

of vital rate responses as found after prescribed fire, however, the magnitude of the response differed between treatments. These differences do not yield growing populations in mechanical clearing treatments as are found after prescribed fire. In mechanically cleared plots survival declines quickly to a rate that is similar to controls. It is this vital rate that appears to have the greatest influence on population growth rate. Recruitment is also important to increase population growth, and thus conditions should be maintained in which croton seeds can readily sprout post disturbance. Because mechanical clearing leads to more recruitment than no action, it is better restoration strategy than doing nothing, but efforts should be made to use fire as a restoration tool when possible.

Like many shrubs in fire-adapted landscapes, croton relies heavily on re-sprouting post disturbance to maintain extant populations. Eighty and seventy percent of croton plants survived and re-sprouted following fires and mechanical clearing, respectively. There are tradeoffs associated with sprouting and seeding, such that plants that invest heavily in below-ground carbohydrate stores for re-sprouting invest less in reproduction (Bond & Keeley 2005). Because of this, sprouters are expected to be good competitors, but poor dispersers (Higgins et al. 2008). Although we documented post-treatment recruitment, it only occurred in plots where croton was present prior to treatment, suggesting that croton seeds do not disperse far from natal patches (as far as we know, croton dispersal is limited to dehiscence, although animals may disperse seeds further). These results highlight the importance of promoting the survival of existing croton patches. Once patches are extirpated, they are unlikely to be re-colonized rapidly.

A key question can be answered by focusing on demographic responses to disturbance: which vital rate contributes most to the overall population growth rate, or has the highest elasticity? For many organisms, the vital rate with the highest elasticity is the one that is least

variable (Pfister 1998). Of the two vital rates we measured, survival was half as variable as recruitment (Figure 1), suggesting that survival has higher elasticity. This is yet another line of evidence that highlights the importance of using management strategies that promote survival of existing plants. If this is the goal of management and restoration efforts in these habitats, fire is the best method to accomplish that goal; mechanical clearing did not increase survival above controls in either year post-disturbance. We acknowledge that there are additional vital rates that could have even higher elasticities than recruitment and survival, such as fecundity and seed survival. However, for other plants that resprout at high rates following disturbance, survival and growth of existing plants generally have the highest elasticities (Menges & Quintana-Ascencio 2004; Liu et al. 2005; Altwegg et al. 2014).

Even vital rates with low elasticity contribute to overall population growth rates. Without restoration, croton populations on Big Pine Key are shrinking at a rate of 20-30% per year. Mechanical clearing abated this trend as it was followed by a flush of recruitment (Figure 1b). These new recruits had slightly higher survival than established plants in both cleared and control plots (Figure 2). If this trend continues, these new recruits could have a positive impact on long term population persistence (Pascarella & Horvitz 1998). Since there was no recruitment and low overall survival in control plots in both years, mechanical clearing, while not as effective a restoration tool as prescribed fire, is better than no restoration at all.

Regardless of life-history strategy, too frequent and too long of a disturbance return interval leads to declining populations of disturbance-adapted species (Altwegg et al. 2014). To understand the optimal disturbance return interval, we would ideally estimate all vital rates in populations at different years post-disturbance and then simulate the effects of varying disturbance return intervals on long-term population growth rates (Gross et al. 1998; Menges &

Quintana-Ascencio 2004; Liu et al. 2005; Menges et al. 2006). For croton, this will require more information than we currently have. One of the mechanisms through which altered disturbance return intervals affect populations is by altering the variability of vital rates between disturbance phases (Morris et al. 2006). For example, for many disturbance-adapted species, including croton, the variability of recruitment between disturbance phases is high, with high recruitment immediately following disturbance and almost no recruitment between disturbances (Figure 1B). If disturbance frequency was to increase, it is possible that post-disturbance recruitment would decrease, due to insufficient time for mature plants to replenish the seedbank. This would in effect decrease the between-phase variability of this vital rate, and negatively affect the long-term population growth rate. On the other end of the disturbance frequency spectrum, long return intervals allow succession to reduce the survival of fire adapted plants by increasing vegetation structure and therefore competition for light. Based on the population declines we measured in our control plots on Big Pine Key that have not burned in 15 years, we can infer that a 15 year disturbance return interval is too long to maintain growing croton populations. In addition to increasing competition, long fire return intervals increase the threat of catastrophic wildfires. Unlike the frequent, low intensity fires that native species are adapted to, these catastrophic fires are generally much hotter and may result in population declines or even extinction. Hot fires can kill seeds in the seedbank and consume below ground resource stores thus limiting the probability that fire adapted plants survive (Moreno & Oechel 1993; Odion & Davis 2000; Pausas & Keeley 2014). The catastrophic fires that result from fire suppression and long fire return intervals also have the ironic consequence of threatening homes and other valuable infrastructure. Understanding optimal fire return intervals is therefore important for both maintaining rare species populations and reducing threats to human development.

We measured a plant's response to two different types of disturbance-based management, but the question remains: what happens when disturbances interact? Mechanical clearing has been proposed as a pre-fire treatment in this system to reduce vertical fuels and therefore the risk of prescribed fire escape. What we do not yet understand is how this would affect the responses that we measured. Given that we saw very little recruitment in the second year following both disturbance treatments, we might hypothesize that the two disturbances in sequence act antagonistically, with the first disturbance, mechanical clearing, stimulating germination and thus depleting the seed bank such that there are few seeds left to germinate following the second disturbance, prescribed fire. Additionally, mechanical clearing is likely to alter fire behavior since it concentrates fuels on the ground, thus increasing ground fire temperature and duration. This could have the unintended consequence of killing seeds present in the seedbank (Busse et al. 2005) or burning up the underground resource stores necessary for re-sprouting post-fire. In this case, the two responses again act antagonistically by reducing recruitment and/or survival. Conversely, if the prescribed fires occurred before seeds germinate, they could act synergistically with the mechanical clearing, further enhancing germination rates by increasing the amount of bare ground and providing additional chemical cues that may be necessary to stimulate germination.

Understanding how interacting disturbances affect vital rates is necessary to predict how communities will be shaped in the future. This is true both in the management context described above, and in the context of climate change. Systems like the pine savannahs in the SE US are subject to fire disturbances as well as hurricanes, both of which are projected to shift in frequency with a changing climate. This is especially the case in south Florida that is exposed to near annual hurricanes, and to hurricanes that are increasing in intensity due to climate change.

Beckage et al. (2006) used simple Lotka-Volterra competition models to demonstrate that the return intervals of both fires and hurricanes interact to maintain pine savannas, and alteration of either return interval has the ability to shift the system to an alternative state; more frequent disturbance of either kind leads to grassland, and less frequent disturbance to closed canopy forest. This model predicts that these disturbances act synergistically to accelerate habitat shifts over time, but this has yet to be tested empirically. As global change accelerates, it is more likely that disturbance events simultaneously shift in their intensity and frequency, increasing the probability of interactions between multiple disturbances.

We have shown that mechanical disturbance is not a perfect surrogate for fire, but is a better alternative than no action. Our experimental test of the demographic effects of both prescribed fire and its mechanical surrogate on a rare plant provides a framework for understanding the mechanisms underlying population level responses to these restoration strategies. These mechanisms are important to include into predictions of species responses to global change (Gilman et al. 2010; Buckley et al. 2010). In the face of changing climate, it may become even more difficult to meet the environmental conditions necessary for prescribed burning (Terando et al. in prep), therefore other habitat maintenance and restoration strategies may become increasingly important in fire adapted systems. By focusing on vital rates instead of community level responses, we acquire the data needed to predict how disturbance will affect the trajectories of species of interest, and thus establish ecologically-based management strategies to recover rare species.

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FIGURES

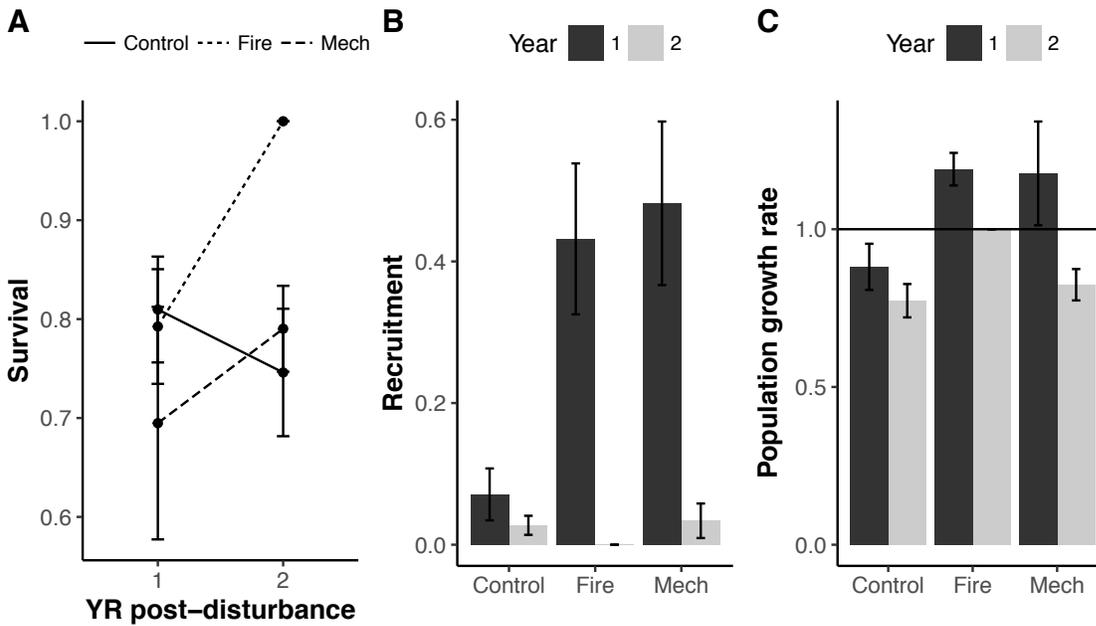


Figure 1.1. Survival, recruitment, and population growth rates across treatments. A) Proportional survival following fire (dotted line), mechanical clearing (dashed line), and no treatment control (solid line). B) Standardized recruitment and C) population growth rates in year one (black bars) and year two (grey bars) post-treatment. Error bars are standard error in all panels. Population growth rates >1 indicate growing population.

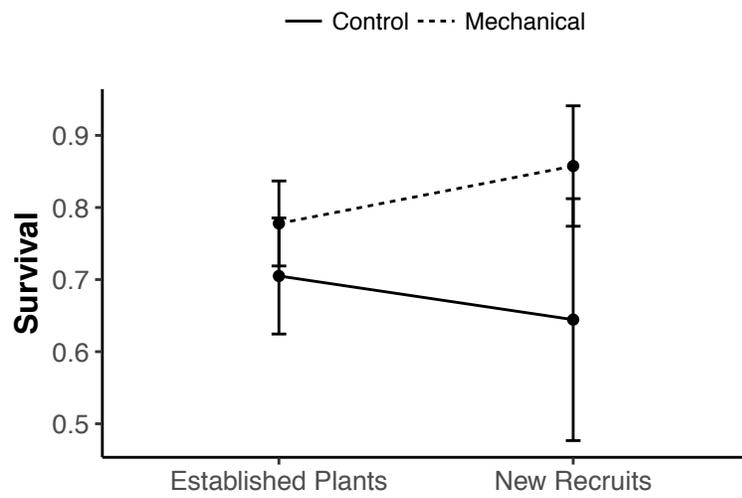


Figure 1.2. Survival of previously established plants and new recruits in mechanically cleared plots (dotted line) and controls (solid line), error bars are standard error.

CHAPTER 2

Are substitute species the white whale of endangered species conservation?

ABSTRACT

The use of substitute species is widespread and continues in the face of broad criticism. We addressed many of the criticisms of substitute species by selecting a substitute that is closely related to, overlaps in distribution, habitat, and host use with, and exhibits similar life history to the target endangered species. We used this endangered-substitute pair to test the validity of the substitute species concept in a habitat restoration experiment. In our test, we integrated fine-scale measures of behavior, habitat preference, and demography and found that the common species was not a good substitute for answering questions related to local scale movement and demography. Despite nearly identical habitat preferences, differences in caterpillar host use and behavior translated to differences in caterpillar survival, adult behavior, and population size across restoration treatments. Our small-scale manipulations of the endangered species led to important natural history discoveries that improve our ability to design conservation strategies. We have demonstrated that fine-scale differences between two species cause the substitute relationship to fail, highlighting that management decisions based on invalidated substitute species run the risk of doing more harm than good for endangered species conservation.

INTRODUCTION

Despite widespread criticism (Landres et al. 1988; Lindenmayer et al. 2002; Cushman et al. 2010; Lindenmayer & Likens 2011), surrogate species (species used as a proxy for another species, ecological community, or environmental condition) continue to be used in endangered

species conservation (Caro 2007). Because of restrictions on manipulation of endangered species, scientists and practitioners are often forced to evaluate the effectiveness of conservation strategies with a common, non-target species instead of with the endangered species itself. This type of surrogate is commonly referred to as a substitute (Caro et al. 2005). The great risk associated with this strategy is that if substitute species do not respond to conservation actions in the same way the target endangered species responds, they provide false insight into the effects of conservation actions (eg. Murphy et al. 2011). In this paper, we empirically test the suitability of using substitute species in a conservation context.

Reviews on surrogate species generally conclude that ecological processes should determine selection of a surrogate, not simply species co-occurrence (Henle et al. 2004; Roberge & Angelstam 2004; Caro 2007; Seddon & Leech 2008). For substitute species selection, the ecological processes most important to consider are behavior (Caro 2007) and demography (Caro et al. 2005). These two ecological processes allow assessment of how conservation actions affect both population growth rates and landscape connectivity.

Individual behaviors are useful for understanding how organisms move across the landscape and scale up to dispersal parameters that can predict whether or not a habitat patch is likely to be occupied by a target species (Brown & Crone 2016). This is especially true for species that exist in metapopulations. Dispersal distances and patch occupancy have been found to be relatively consistent across closely related species that occupy the same landscape of habitat patches and matrix (Wahlberg et al. 1996; Fric et al. 2010). However, when dispersal and occupancy are measured in different landscapes, there can be as much variation among different populations of the same species as there is between species (Fric et al. 2010; Stevens et al. 2010). In addition to occupying the same breeding habitat, a good dispersal substitute species will also

use the same habitat type for dispersal (Breckheimer et al. 2014). It follows that to be a good behavioral substitute species, a species must be closely related to, occupy the same landscape as, and overlap in dispersal habitat with the target species.

Understanding the demographic response of a target organism to disturbances or management actions is fundamental to sensitivity analyses, population viability analyses, and other common tools used to develop and compare the effects of different conservation strategies (Morris & Doak 2002; Mills 2013). When using a substitute species to assess disturbance effects on rare species, or to parameterize population models, demographic responses of the two species must be similar. Studies on the effects of environmental contaminants on anadromous fish and parasitic wasps have found that substitute species can be useful to predict mortality rates, but sub-lethal demographic responses are more nuanced (Stark et al. 2004; Banks et al. 2010). Demographic responses of organisms to disturbance are affected both by the effect of the disturbance on particular life histories and by the effects of vital rates on population level response (Hartway & Mills 2012). Therefore, to exhibit similar demographic responses, substitute and target species must have similar life histories and also similar vulnerabilities to disturbance (Stark et al. 2004; Murphy et al. 2011; Banks et al. 2014). Demography, like movement, is context dependent. Reproduction and survival are sensitive to local environmental conditions such as microclimate, resource availability, and habitat condition. Because of this, vital rates estimated in one population rarely predict population dynamics for different populations of the same species (Johnson et al. 2010; Hernández-Camacho et al. 2015). Therefore, an effective demographic substitute species must share similar life history with and occupy the same habitat patches as the target endangered species.

We address what has been a key limitation in use of substitute species, the subtle differences in movement and demography that are often undetectable or unknown without detailed investigation. Correlational approaches that identify surrogate species based on co-occurrence may miss important mechanisms that drive co-occurrence patterns. Experiments provide the most direct approach to identify causal factors that make two species more or less similar. Experimental restoration action provides the “acid test” (Bradshaw 1987) of assessment of the substitute species concept. In this paper, focus on the mechanisms of movement and demographic responses of target and substitute species in response to conservation action (Schultz & Crone 1998; Haddad & Baum 1999).

The question remains: how broadly can one species substitute for another, if at all? We use a habitat restoration experiment to test the validity of the substitute species concept using an endangered-substitute pair of butterflies. In our test, we focus on integrating fine-scale measures of behavior, habitat preference, and demography to assess whether a common species can, in fact, be a good substitute for a rare species. We ask whether using a species that is closely related to, overlaps in distribution and host use with, and exhibits similar life history to the target species, is sufficient for identifying a valid substitute species.

METHODS

Study Species and Site

St. Francis' satyr (*Neonympha mitchellii francisci*) is an endangered butterfly found in sedge-dominated wetlands. The global distribution of the species is confined to Fort Bragg Army Installation in North Carolina. Historically, these open, sedge-dominated wetlands were maintained by frequent fires and beaver activity. In the last 150 years, both natural fire regimes

and beaver populations were nearly extirpated from the southeast, and with them the habitat on which St. Francis' satyrs depend. Today, the largest St. Francis' satyr populations are found in the artillery impact areas at Ft. Bragg. In the impact areas, live fire military training ignites fires that burn across the landscape and beaver activity is not curtailed by humans. The resulting riparian areas are open and free of thick, shrubby vegetation.

Appalachian brown butterflies (*Satyroides appalachia*) are a member of the same subfamily Satyrinae, as St. Francis' satyrs. They range across eastern North America from north Florida to southern Canada, and as far west as Minnesota. In eastern North Carolina, however, Appalachian browns are largely restricted to Ft. Bragg. At Ft. Bragg, they inhabit wetlands and have strong overlap in their habitat use with St. Francis' satyr. Both butterfly species feed on sedges as caterpillars, and overlap in use of *Carex mitchelliana* (a plant that is located at all habitat patches occupied by both species). Additional hosts are possible but as yet unknown. Both species have two to three generations per year (likely dependent on spring temperatures (Wepprich 2017)), overwintering as early instar caterpillars.

We previously considered Appalachian brown butterfly as a substitute species for St. Francis' satyr in research focused on the movement of butterflies across the landscape (Hudgens et al. 2012; Breckheimer et al. 2014). In this context, Hudgens et al. (2012) tested the substitute approach by comparing boundary crossing behaviors and movement (step-lengths and turning angles) between the two species. Although they found differences in behavior between the two species within their wetland habitat, these differences were smaller than the differences in behavior among habitat types. Hudgens et al. (2012) concluded that Appalachian brown is a strong substitute for assessing landscape connectivity for St. Francis' satyrs. It remains unknown

whether Appalachian brown is a good surrogate for demography, especially in response to habitat restoration, which is what we examined here.

To test the effects of habitat restoration on the demography of St. Francis' satyr, we took advantage of an existing habitat restoration experiment (Aschehoug et al. 2015). In 2011, we delineated four blocks of four 30m X 30m plots, placing each block within 250m of a historic St. Francis' satyr population. To mimic the disturbance created by beavers, each plot was randomly assigned one of the following four treatments: tree removal, installation of temporary dams, both tree removal and dam installation, and a no treatment control (see Aschehoug et al. 2015 for additional details). Damming treatments did not affect total sedge cover, but tree removal increased total sedge cover four-fold (Aschehoug et al. 2015). For the purposes of the current study, we pooled data from all tree removal plots and refer to them as restoration plots, and we pooled data from plots with no tree removal which we refer to as controls. Succession happens quickly in these environments. Therefore, each spring we remove encroaching hardwoods from restoration plots by hand in an attempt to maintain the open habitat structure required by the butterflies. From 2012 to 2015 we released captive-reared St. Francis' satyr adults into one experimental block. This combination of restoration and re-introduction has resulted in the largest St. Francis' satyr population outside of the artillery impact area (617 butterflies in 2015).

Caterpillar host use and survival

We measured St. Francis' satyr caterpillar survival in the field by releasing first instar caterpillars into experimental arenas, allowing them to develop, and counting adult butterflies that emerged from arenas. We used experimental arenas composed of a 57cm tall X 37cm diameter ring of a polyethylene food grade drum buried approximately 10 cm into the ground

(Aschehoug et al. 2015). Arenas were installed in 2012 around naturally occurring *C. mitchelliana*. When preparing arenas in 2016, we chose those that still contained *C. mitchelliana* so we would not need to supplement larval diets. Because our federal permit limited us to a small number of caterpillars, we only measured caterpillar survival in the control and tree removal + dam treatments in two blocks of the original restoration experiment, releasing caterpillars into two arenas per plot for a total of 8 arenas. We released 6-7 first instar caterpillars into each arena over two days. One arena in each treatment was invaded by ants after the first day, so no additional caterpillars were released into them and they were dropped from the experiment. We left arenas open to predation and counted caterpillars daily. Once we observed pupae, we enclosed the arenas with mosquito netting. We continued to visit arenas daily, recording and releasing all adults that emerged. By releasing St. Francis' satyr caterpillars into arenas, we observed larval feeding and host choice in the wild for the first time. In addition to counting caterpillars daily, we recorded the species of sedge on which each caterpillar was feeding.

We tested whether larval survival differed among treatments between St. Francis' satyr and Appalachian Brown (data from control and tree removal + dam treatments in Aschehoug et al. 2015). To do this, we used a generalized linear model with a binomial response variable, including a species*treatment interaction main effect. We conducted this analysis using the glm function of the lme4 package, and Anova function of the car package in R.

Caterpillar behavior

We measured Appalachian brown and St. Francis' satyr caterpillar behavior using captive individuals. These originated from wild-caught females brought into the greenhouse to lay eggs. When the eggs hatched, we allowed caterpillars to crawl onto potted *C. mitchelliana* plants in

screened captive rearing enclosures. We measured the height above soil level for 20 caterpillars of each species twice a week for four weeks. These experiments were done in a greenhouse at Ft. Bragg which is programmed to replicate outside environmental conditions. To test for differences in foraging height, we used a two-way ANOVA with a species*date interaction term to account for differences in foraging as caterpillars develop. Foraging heights were square-root transformed to meet ANOVA assumptions.

Caterpillar microhabitat

We measured sedge canopy above captive St. Francis' satyr and Appalachian brown caterpillars as a measure of how likely a caterpillar is to be shaded by sedges. We did this by dropping a pin through the sedges to the location of each caterpillar and counting the number of sedge blades that contacted the pin. As with height data, we collected sedge canopy on 20 caterpillars of each species twice a week for four weeks. To compare sedge canopy above the two species, we totaled the number of individuals counted with zero, one, two, three, and four hits above them on the pin for each species across all eight surveys. We used a chi-square test to assess differences between sedge canopy cover above St. Francis' satyrs and Appalachian browns.

Adult locations

To test whether adult butterflies select habitat within wetlands based on host resources, microclimate, or some combination of the two, we marked with a pin flag the location where we first detected each adult butterfly during our surveys. At each location we estimated canopy cover, total sedge cover, *C. mitchelliana* cover and distance to structural boundary. We estimated

canopy cover by attaching a GoPro camera to a 2m long pole and taking a photo of the canopy from 1m above each flag. We analyzed photos by converting each image to a two-tone black and white image. In converted images, vegetation becomes black pixels and sky becomes white. We calculated canopy cover as the percentage of pixels in each image that were black. All photo analyses were done using ImageJ. We visually estimated total sedge cover and *C. mitchelliana* cover in the following bins: 0-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-100%. We also visually estimated distance to the structural boundary of each treatment to the nearest meter. For tree removal plots, we measured the distance between the observation and the edge of the tree removal area. In control plots, we measured the distance between the observation and the edge of riparian vegetation. We used two-way ANOVAs to compare each environmental variable between the two species and the two treatments using the `lm` function in R. We interpreted a non-significant species*treatment interaction to indicate that butterflies select similar habitat regardless of plot-type. Prior to analysis, proportion canopy cover was squared and the proportions of *C. mitchelliana* and total sedge cover were arcsine square-root transformed to meet the assumptions of ANOVA.

Adult behavior

Concurrent with population monitoring in 2016, we collected data on habitat use and movement within the plots. To understand how vegetation structure affected movement of both butterfly species, we focused on butterflies that we first detected flying. We recorded vegetation type at the initial detection location for each butterfly and observed it until it changed vegetation type once or until it was lost or landed and recorded the last vegetation type within which it was observed. We defined three structural vegetation types as follows: 1) Sedge: open, meadow-like,

and sedge-dominated with little to no canopy; 2) Shrub: shrubs and vines; and 3) Forest: dominated by closed canopy of trees with little undergrowth. To evaluate differences in habitat use and movement between St. Francis' satyrs and Appalachian browns we conducted chi-square tests using the `chisq.test` function in R. For habitat use, we compared the habitat types at initial locations of each butterfly species. For movement, we compared the final habitat in which butterflies were observed given they were initially detected in the same habitat. Because very few butterflies were observed moving into forest habitat we simulated p-values with the `simulate.p.value` option using 1000 iterations.

Index of Population Size

Each demographic and behavioral response to disturbance we measured has the potential to result in differences in population size between treatments. To estimate population size in each 30m X 30m plot, we established an identical, fixed transect composed of 5cm x 15cm boards that allowed us to access plots without trampling vegetation. We conducted modified Pollard walk surveys daily (Pollard & Yates 1993). These involved counting each St. Francis' satyr and Appalachian brown butterfly observed during a 15-minute survey on each transect. All surveys occurred between 13:00 and 17:00, the time when St. Francis' satyrs are most active (Kuefler et al. 2008). To estimate population size in each restoration plot, we calculated the Pollard-Yates index of population size (Pollard & Yates 1993). This is done by calculating the average number of butterflies seen each day for each week surveyed. Weekly averages are then summed across the survey period, yielding an index for population size that is highly correlated with more rigorous capture-recapture estimates (Haddad et al. 2008). We compared Pollard-Yates indices for both species across plot types. We analyzed data from the second flight period in 2015

because the total Pollard-Yates index at our reintroduction site was nearly identical for the two species. To compare population sizes across plot-type we conducted a chi-square test using `chisq.test` function in R.

RESULTS

Caterpillar host use and survival

We released a total of 37 St. Francis' satyr caterpillars into field arenas, 10 of which successfully survived to become adult butterflies (21% survival). This is considerably higher than the 4.2% survival we expected based on our estimates of egg laying and survival rates in the greenhouse.

Small, green caterpillars are difficult to find within field arenas. However, we were able to find some caterpillars each day. In the four arenas that contained multiple sedges, $67 \pm 27\%$ of the St. Francis' satyr caterpillars fed on *C. atlantica*, until they were fourth instars, at which point 100% of caterpillars fed on *C. mitchelliana*. The remaining 33% of St. Francis' satyr caterpillars fed on *C. mitchelliana* as early instars. We did not release Appalachian brown caterpillars into arenas as part of this project so we do not have data on their host preferences. However, in prior experiments in these same arenas (Aschehoug et al. 2015), we never observed Appalachian browns feeding on *C. atlantica*. Occasionally they will feed on grass species when grass is abundant within arenas, but *C. mitchelliana* is by far the preferred host of Appalachian browns.

Caterpillar survival did not differ between species (Wald's chi-square=0.65, df=1, p=0.42) or treatments (Wald's chi-square=0.30, df=1, p=0.58). The interaction term is marginally significant (Wald's chi-square=2.66, df=1, p=0.10) and in the context of small

sample sizes for St. Francis' satyr indicates a trend toward differential survival between Appalachian browns and St. Francis' satyrs across treatments (Figure 1).

Caterpillar behavior

Appalachian brown caterpillars foraged at heights twice as great as St. Francis' satyrs ($F_{1,353}=149.3$, $p<0.0001$); Appalachian browns foraged at 12.6 ± 5.2 cm off the soil and St. Francis' satyrs at 6.5 ± 3.7 cm. This difference was consistent across caterpillar development, the species*date interaction was not significant ($F_{8,346}=0.25$, $p=0.98$).

Caterpillar microhabitat

Appalachian brown and St. Francis' satyr caterpillars forage under different levels of canopy cover ($\chi^2=134.87$, $df=4$, $p <0.0001$). Fifty-eight percent of Appalachian brown caterpillars are found with no sedge cover above them, only 4% and 1% are found under 3 and 4 sedge blades, respectively. Just over half of St. Francis' satyrs, were found under the shelter of 3 or 4 sedge blades, and only 10% forage in the open with no cover.

Adult locations

We marked the location of a total of 148 St. Francis' satyr and 135 Appalachian brown adults. Canopy cover was the only variable that differed between the two species ($F_{1,301}=114.3$, $p<0.0001$; Table 2). Appalachian brown butterflies were found under an average of $70 \pm 12\%$ canopy cover, whereas St. Francis' satyrs were found under only $53 \pm 16\%$ canopy cover. *C. mitchelliana* cover, total sedge cover, and distance to structural boundary were not significantly

different between the two species and none of the species*treatment interaction terms were significant.

Adult behavior

We recorded the flight behavior of 230 St. Francis' satyrs and 370 Appalachian browns. The two species differed in initial habitat preference ($\chi^2=93.94$, $df=2$, $p<0.0001$; Figure 2a), and probability of changing habitat, regardless of initial habitat (starting in sedge: $\chi^2=8.88$, $p=0.005$; starting in shrub: $\chi^2=7.01$, $p=0.03$; Figure 2b, c). Eighty-four percent of St. Francis' satyrs were first observed in sedge; 58% of these stayed in sedge and 26% flew into shrubs. Very few St. Francis' satyrs were observed in forest habitat type. Appalachian browns were almost equally likely to be found in sedge and shrub and 15% of the butterflies were found in forest habitat type. Appalachian brown butterflies stayed within the habitat in which they were initially observed; only 17% changed habitat.

Index of population size

We counted a total of 215 St. Francis' satyrs and 305 Appalachian browns in the second flight period of 2015. These numbers translate to total population indices of 34 and 54. The St. Francis' satyr population was two times larger than the Appalachian brown population in restoration plots and Appalachian brown population size was five times higher than St. Francis' satyrs in control plots ($\chi^2=23.6$, $df=3$, $p<0.0001$, Figure 3).

DISCUSSION

By focusing on measures of habitat preference, behavior, and demography, we have demonstrated that even two species that are nearly identical in broad and specific ways (e.g. same subfamily, overlapping habitat, overlapping host use, same life history, same phenology) are not good surrogates for answering most local-scale questions in ecology and restoration. Fine scale differences in resource use translated into differences in habitat selection, behavior, and survival, the combination of which resulted in differences in population size following restoration. Because of this, it may be necessary to conduct limited experiments with the target species directly. In cases where a surrogate must be used, it is important to select a surrogate based on fine-scale similarities that relate directly to the action at hand.

We chose our substitute species based on the essential requirement that substitute and target species overlap in habitat requirements. We found that although this was true at broad scales, it was not true at local scales which are of greatest importance in restoration. We have regularly found Appalachian brown caterpillars feeding on *C. mitchelliana*, but have found only two St. Francis' satyr caterpillars in the wild. By releasing St. Francis' satyr caterpillars into arenas in the field, we learned that, unlike Appalachian browns that forage on *C. mitchelliana* throughout development, early instar St. Francis' satyrs prefer to feed on *C. atlantica* before switching to *C. mitchelliana*. This difference in host use likely drives other local-scale differences we measured between the two butterfly species, such as adult habitat selection and differences in population size across treatments. *C. atlantica* cover is four times greater in restoration plots (23%) than controls (6%, unpublished data). Within restoration plots, *C. atlantica* is unlikely to occur under canopy cover (EH personal observation). The preference for restoration plots and open canopy cover that we observed for adult St. Francis' satyrs is likely

driven by a preference for staying close to host plants, further reinforcing the importance of understanding the fine-scale resource needs of both substitute and target species.

The substitute species we tested had previously been shown to be a good substitute for landscape connectivity questions (Hudgens et al. 2012); however, we found that habitat selection and movement patterns differed between the two species at the local, within-patch scale. St. Francis' satyrs were more likely to initially be found in open sedge than Appalachian browns (Figure 2). Appalachian browns were more likely to stay in their initial habitat, suggesting that they view both sedge and shrub as "habitat". St. Francis' satyrs, on the other hand, were biased to stay in sedge but did cross structural habitat boundaries from open sedge to shrub at a high rate (0.44). Like St. Francis' satyr's preference for open canopy, this movement preference is likely related to the distribution of *C. atlantica* within the wetlands. Butterflies are known to exhibit biases toward host plants and avoid crossing structural boundaries (Schultz 1998; Kuefler & Haddad 2006; Schultz et al. 2012). Biases ultimately affect the rate at which butterflies move through the landscape, or their diffusion rates, measured as net displacement over time. These fine-scale differences in movement are important to understand in the context of restoration planning (eg. Schultz 1998). If the goal is to restore habitat patches that are likely to be naturally colonized by St. Francis' satyrs, a sedge dominated corridor may facilitate the movement of these butterflies between sites (Haddad 1999a; 1999b).

A fundamental problem with the substitute species concept is that in the same habitat, different species are *expected* to occupy different niches (MacArthur 1958). We found that two closely related species that overlap in resource needs differed in feeding behavior and thus their vulnerability to altered microclimates. St. Francis' satyr caterpillars forage closer to the ground and under more cover than Appalachian brown caterpillars. These differences in foraging

behavior expose them to different microclimates and different levels of direct solar radiation (Turlure et al. 2010; 2011), especially in restoration plots where the tree canopy has been removed and air temperatures are 3-5 degrees C warmer than control plots (unpublished data). By foraging at the end of the sedge blades, Appalachian browns are exposed to direct solar radiation which increases caterpillar body temperature above air temperature (Casey 1976; Turlure et al. 2011). Higher air temperatures and increased exposure to direct solar radiation in restoration plots leads to a higher risk of desiccation and death for Appalachian brown caterpillars than St. Francis' satyrs (Turlure et al. 2010). In fact, we found multiple first instar Appalachian brown caterpillars desiccated at the end of sedge blades in our caterpillar arenas in restoration sites, but never in control sites (EH personal observation). These subtle behavioral differences provide a possible mechanism to explain the differences in caterpillar survival that we measured between species across treatment type.

By studying a species with similar life history as a demographic substitute, we assumed that the two species would respond to restoration in the same direction (Caro et al. 2005). However, fine-scale differences in behavior and demography following restoration add up to differences in population size across treatments. St. Francis' satyr adults are more likely to be found in open locations under little canopy cover but high sedge cover, and to stay in open sedge habitat when in flight. Since caterpillars then develop in sunny areas, they forage closer to the ground and under cover where they are protected from direct solar radiation. Because both host plant cover and larval survival are highest in restoration sites, the St. Francis' satyr population size is highest in restoration sites as opposed to controls. Appalachian brown butterflies, on the other hand, prefer locations with higher canopy cover. If oviposition follows adult habitat preference, Appalachian brown caterpillars forage under a tree canopy where they are protected

from solar radiation even though they forage at the exposed ends of sedge blades. Because caterpillar survival is slightly higher in control than in restoration plots and adult butterflies prefer shaded locations, population size is highest in control as opposed to restoration treatments. Although these two butterfly species are very similar, both adult and larval life stages are adapted to different microclimatic conditions within the wetlands in which they overlap. Therefore, restoration actions that promote the conditions necessary for one species do not necessarily result in growing populations of both species.

The “hands off” approach of avoiding collecting data on the endangered species directly is prevalent in endangered species conservation, especially for species that are very rare and poorly understood. These are the exact species for which well planned, targeted experiments within the context of ongoing conservation efforts have the ability to provide insights necessary to develop effective conservation strategies. Even small-scale manipulations of the target species, like those we have reported, can lead to important natural history discoveries that improve the ability to design effective conservation strategies. For example, by conducting targeted experiments with St. Francis’ satyrs, we learned what caterpillars are actually eating in the wild and how caterpillar behavior may promote survival. Had we relied only on data collected on Appalachian browns (Aschehoug et al. 2015), we would have concluded that tree removal is unnecessary as long as there is enough *C. mitchelliana* to sustain populations, and our next steps might be to determine how much *C. mitchelliana* is enough. Instead, based on our results with St. Francis’ satyrs, we concluded that tree removal is necessary to promote both host species and is not detrimental to survival. Our next steps involve understanding the successional dynamics within the sedge community and how often we must “re-restore” restoration sites to maintain viable populations of St. Francis’ satyrs. By thinking creatively about how to conduct

conservation actions in an experimental context, we, as a conservation community, could improve and better target our efforts in the future.

We have demonstrated that fine-scale differences between two species cause the substitute relationship to fail. Management decisions based on invalidated substitute species run the risk of doing more harm than good for endangered species conservation. Working with the endangered species directly is especially important in the case of demographic questions, since demographic rates are highly dependent on local conditions and species-specific behaviors. Substitute species are never a complete alternative for an endangered species. There are limited circumstances where a species may substitute for very specific behaviors or demographic rates. In those rare instances, focusing on the key ecosystem process(es) or ecological theory(ies) underlying the question at hand can serve as a guide to which life history components are important in substitute selection. Thinking carefully about substitute selection in this way will improve the ability of a substitute species to inform conservation actions.

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TABLES

Table 2.1:

Trait	St. Francis' Satyr	Appalachian brown
<i>Caterpillar</i>		
Host plant	<i>C. atlantica/C. mitchelliana</i>	<i>C. mitchelliana</i>
Microhabitat	Under cover	Exposed
Height on plant	6cm	12cm
Survival	Highest in tree removal	Highest in control
 <i>Adult</i>		
Microhabitat	Sun	Shade
Movement	Prefers open sedge	Equally likely in sedge and shrub
Population Size	Highest in restoration	Highest in control

Table 2.2. ANOVA results. SFS refers to St. Francis' satyr; ABB refers to Appalachian brown. Bolded values are statistically significant at $p < 0.05$

Response variable	Mean (SD)		Species		Treatment		Sp X Treat	
	SFS	ABB	f-value	p-value	f-value	p-value	f-value	p-value
Canopy	0.30(0.17)	0.49(0.15)	114.3	<0.0001	54.3	<0.0001	3.1	0.8
Sedge	0.36(0.31)	0.30(0.23)	3.43	0.065	0.21	0.651	2.44	0.12
Mitch	0.27(0.28)	0.23(0.27)	1.72	0.19	6.3	0.013	0.4	0.53
Dist. To edge	5.09(2.73)	4.77(3.35)	0.75	0.39	3.45	0.065	0.84	0.36

FIGURES

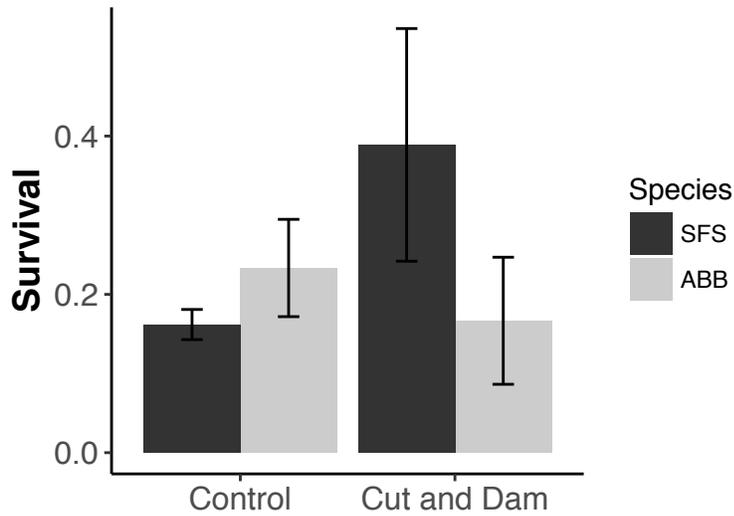


Figure 2.1. Means and standard error of St. Francis satyr (SFS) and Appalachian brown (ABB) caterpillar survival.

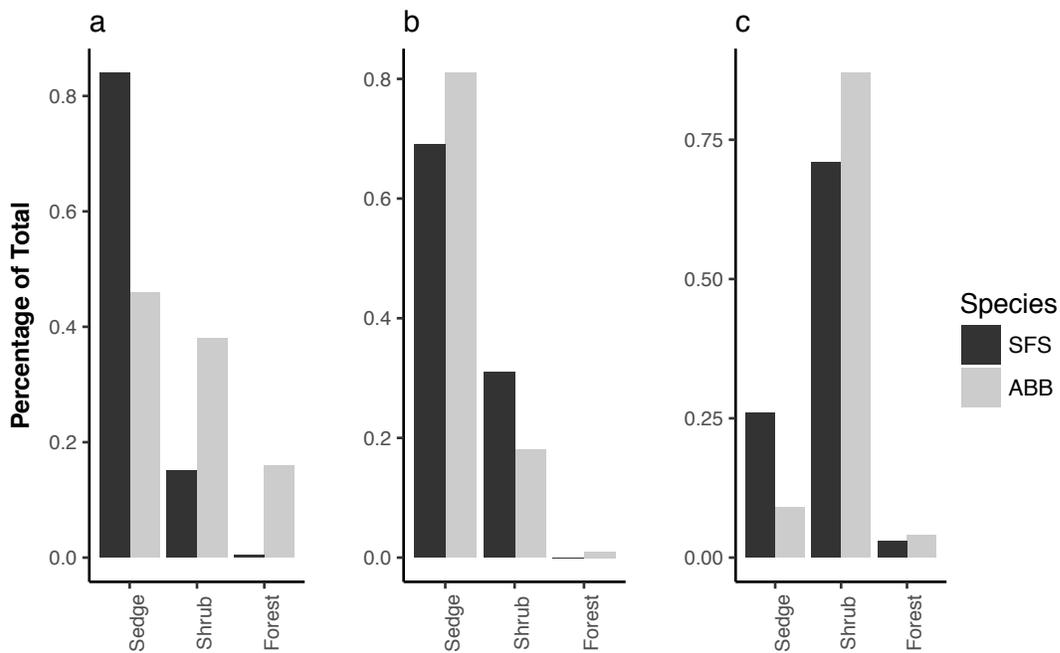


Figure 2.2. Initial habitat (a) and behavior of butterflies that were first observed in sedge (b) and those first observed in shrubs (c). X-axis labels indicate habitat in which butterflies were first observed (a) or habitat into which they moved (b and c). SFS=St. Francis' satyrs, ABB=Appalachian brown.

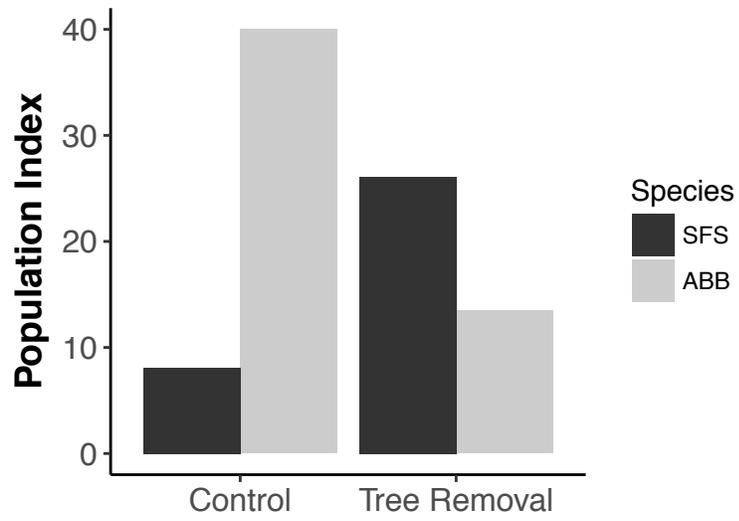


Figure 2.3. Population indices measured for St. Francis' satyr (SFS) and Appalachian browns (ABB).

CHAPTER 3

Point count methods to monitor butterfly populations when traditional methods fail: a case study with Miami blue butterfly

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ABSTRACT

Established butterfly monitoring methods are designed for open habitats such as grasslands. Not all rare species occupy habitats that are easy to see across and navigate, in which cases a new approach to monitoring is necessary. We present a novel use of point transect distance sampling to monitor the Miami blue, a highly endangered butterfly that occupies dense shrub habitat. To monitor Miami blue density, we developed surveys consisting of butterfly counts in semi-circular plots. We examined the rate at which an observer detects new butterflies to determine the survey duration that meets the key assumption that butterflies are detected at their initial location. As a related secondary goal, we identified the determinants of adult flight phenology to target monitoring efforts during periods of high adult abundance. We observed peak Miami blue densities in April and July/August 2012, and July/August 2013. We estimated density using detections from a 10-second survey, our most defensible and conservative estimate. Peak daily density estimates ranged from 592 – 680 butterflies per hectare. Adult density was related to precipitation patterns, with high densities occurring 4-6 weeks after particularly wet 4-week intervals. For butterfly species that exist in high enough densities, we recommend using

point transect distance sampling in habitats where traditional methods are impossible to implement.

INTRODUCTION

Rigorous monitoring and abundance estimates for rare insects are essential to conserve and recover their populations. However, defined monitoring protocols and rigorous abundance estimates are lacking for many endangered species, including a number of rare butterflies. The most rigorous approaches to population estimation: mark-recapture and transect counts (Brown and Boyce 1998; Haddad et al. 2008), are not always possible to implement. We implement a new approach for butterfly population estimation, based on distance sampling from point transects (Buckland et al. 2001), that can be applied when assumptions of traditional methods fail.

The most common methods used for monitoring butterfly populations are mark-recapture and transect counts. Mark-recapture methods are the most rigorous because they allow for estimation of daily and total population sizes, recruitment, survival, and detection probabilities (Haddad et al. 2008). However, these methods are resource intensive and have the potential to harm fragile butterflies in the marking process (Murphy 1987). As an alternative, transect counts are commonly used to monitor butterfly populations because they are non-invasive, have a long history in population and community monitoring, and allow for estimation of detection probabilities by way of distance sampling (Pollard 1977; Thomas 1983; Brown and Boyce 1998; Isaac et al. 2011). Distance sampling from line transects is increasingly used to estimate butterfly density and abundance of rare species (Brown and Boyce 1998; Hamm 2013) as well as butterfly metapopulation structure, habitat associations, and response to habitat restoration treatments

(Boughton 2000; Powell et al. 2006; Pocewicz et al. 2009; Moranz et al. 2014). Transect counts are easily deployed in open habitats such as grasslands, but can be problematic in dense vegetation where it is difficult to simultaneously count butterflies and navigate. Adding distance sampling to transect counts is also complicated by dense vegetation because of the need for transect locations to be random (Buckland et al. 2001). Another method used to incorporate detection probability into abundance estimates is occupancy modeling (Bried and Pellet 2011; van Strien et al. 2013). One of the fundamental assumptions of this method, however, is that a population is closed between survey visits (Mackenzie et al. 2006), an assumption that may be hard to satisfy for butterfly species that are logistically difficult to access, have overlapping broods, and are surveyed sporadically. We overcome the limitations imposed by other, more traditional butterfly monitoring approaches and develop methods for point transects for insects, adapting methods originally developed for estimating bird abundance (Rosenstock et al. 2002; Buckland 2006).

We develop our new approach using a case study of an extremely rare and threatened butterfly, Miami blue (*Cyclargus thomasi bethunebakeri*). Miami blue is a US Federally Endangered species (USFWS 2012) for which rigorous monitoring protocols and abundance estimates have yet to be developed. Traditional monitoring approaches are nearly useless for these butterflies; Miami blues are small (3/4 inch wingspan) and occupy remote, uninhabited islands at the western end of the Florida Keys (Figure 1). Access to these islands is often impossible because they can only be approached by small boats dependent on favorable weather and tides. Additionally, Miami blue habitat is characterized by dense, shrub-dominated, coastal thickets. These thickets are bordered on the inland side by impenetrable mangroves and on the seaward side by coastal prairie composed of vines, sand-spurs, waist high sea oats, and old

hurricane debris. All three habitat types are particularly difficult to traverse. Although trails could be created, these would harm butterfly habitat, and would provide access points for illegal visitors to restricted wilderness islands. These size and habitat restrictions make Miami blue an ideal candidate for testing new abundance estimation methods.

A second goal of our study is to make the monitoring protocol we develop more efficient by pinpointing periods of high adult flight activity within years. Nearly all established butterfly monitoring protocols are developed for species in temperate regions where butterflies exhibit predictable phenology patterns. Generally, temperate butterflies fly as adults in spring and summer, and diapause during winter months when cold temperatures limit insect development. This makes it relatively easy to target monitoring efforts during periods of high adult abundance. In the sub-tropics and tropics, however, temperatures vary little and remain warm throughout the year. Instead, it is precipitation that varies annually with distinct dry and rainy seasons. Tropical butterfly responses to seasonality are varied, with some species flying at high abundances at the start of the rainy season, others at the end of the rainy season, and still others flying during the dry season (Wolda 1988). This presents a considerable challenge for monitoring sub-tropical butterflies, especially if the phenology of a species is poorly understood and occupied habitat is difficult to access. The limited data that exist for the extant populations of Miami blue butterflies suggest a high amount of intra- and inter-annual variation in flight phenology (Daniels 2010; Cannon et al. 2010; Henry et al. 2012). We investigate the relationship between rainfall and Miami blue abundance, developing a predictive model of adult flight to streamline future monitoring efforts

METHODS

Study species and sites

Miami blues were once abundant in south Florida, especially in the area in and around Miami and throughout the Florida Keys (Saarinen and Daniels 2012). Miami blue habitat was destroyed as Miami, in the first half the 20th century, and then the Keys, in the second half, developed. By 1992, there were so few Miami blues that the butterfly was presumed extinct when Hurricane Andrew destroyed what was then the last known population on Key Biscayne in the Florida Keys. The butterfly was re-discovered at Bahia Honda State Park (hereafter, Bahia Honda) in 1999 (Calhoun et al. 2002), and in the Key West National Wildlife Refuge (hereafter, Key West Refuge) in the winter of 2006 (Cannon et al. 2010). Because of its accessibility, the Bahia Honda population was the subject of the majority of Miami blue research efforts until the population went extinct in 2010 (Saarinen and Daniels 2006; Saarinen et al. 2009; Trager and Daniels 2010). Very little is known about the butterflies in Key West Refuge because the islands occupied by Miami blues are 16 – 25 miles west of Key West and only accessible by small boat. Winter winds and summer thunderstorms greatly limit the number of days that it is possible to access the islands to study the butterflies. Existing, limited, data reveal notable differences between butterflies at the Key West Refuge and Bahia Honda. At Bahia Honda, Miami blues used nickerbean (*Caesalpinia bonduc*) as the larval host, and peak adult counts were observed during the summer (Emmel and Daniels 2008). In contrast, populations in the Key West Refuge use blackbead (*Pithecellobium keyense*) as their host plant, and preliminary data (pre 2012) suggested that adults were most abundant during the winter/spring (Henry et al. 2012).

In the Key West Refuge, Miami blues occupy seven beaches on five islands including Boca Grande Key and the Marquesas Keys (Cannon et al. 2010). These beaches range in length

from 250 meters to three kilometers (Table 1) and contain most of the undeveloped, upland coastal berm habitat in the Florida Keys. The coastal berm is dominated by blackbead, the butterfly's host, which grows in dense thickets (FNAI 2010). This impenetrable, linear strip of blackbead ranges in width from one shrub to 60 meters, and individual shrubs can grow up to four meters tall. Female butterflies lay eggs singly on newly emerging leaves and flower buds of blackbead. The distribution of the butterflies is closely tied to the distribution of blackbead on the islands.

Counts of adult butterflies

We conducted Miami blue surveys as often as weather conditions allowed boat access to the Key West refuge. Our goal was to survey each occupied beach a minimum of once per month from March 2012 through December 2013. On each visit, we performed point transects along the length of each beach bordered by blackbead-dominated coastal berm. On four beaches, this encompasses the entire length of the blackbead. On the other three beaches the blackbead extends behind the mangroves, beyond the reach of the beach. To maximize our time spent surveying on these islands, we limited our survey to the blackbead accessible from the beach.

We defined a survey "point" as the centroid of the location where we conducted our butterfly counts. To select our survey points, we used ArcGIS 10.0 (ESRI, Redlands CA 2010) to determine a line parallel to the beach extending the length of blackbead habitat. We then marked a location every 30m along that line. For each visit, we chose a new, random starting point along the line and generated new point locations. Upon arriving at a location along the beach, we walked inland until we intersected the blackbead edge. This point at the edge of the blackbead was our survey point. On all beaches except the longest (Long Beach) in the Marquesas, we

surveyed all points on each visit, identifying the location of the point with a handheld GPS. Because all points on Long Beach could not be surveyed in one day, we randomly selected 30 of the 100 points each visit and surveyed as many of those as time/tides/weather allowed.

Most of the butterflies we counted were first detected in flight, therefore, we restricted our survey area to a semi-circle. We oriented the flat edge of the semi-circle perpendicular to the shrub edge to incorporate both dune grass and blackbead habitat. Restricting our survey to a semi-circle allowed the observer to keep track of individual butterflies, thus reducing the likelihood of double counting individuals during the survey. At each survey point, we recorded all Miami blues, Cassius blues (*Leptotes cassius*) and unknown blues (butterflies we could not confidently identify as Miami or Cassius blue) that we detected within the semi-circle during a 1-minute period. Cassius blues and Miami blues look so similar that the common species, Cassius blue, is listed as Threatened under the US Endangered Species Act within the range of Miami blue (USFWS 2012). By recording Cassius blue detections we were able to include Cassius density as a covariate that might influence our ability to accurately detect Miami blue butterflies.

Distance sampling involves three key assumptions (Thomas et al. 2010) that we were able to address through our methods. First, butterflies at the point must be detected with certainty. To reduce the risk of flushing butterflies from the point, we approached survey points by walking toward the flat edge of the semi-circle, avoiding the area to be surveyed. We also started our survey the moment we arrived at the survey point and recorded any butterflies flushed from the point upon approach as detected at the start of the survey period.

A second assumption of distance sampling is that butterflies are detected at their initial location (Thomas et al. 2010). For point transects it is important to select an appropriate time interval to assure that butterflies at the point and close to the observer are detected without

allowing much movement of butterflies towards the observer. The longer the duration of a point count, the more likely that butterflies move from their initial location towards the observer where they are more likely to be detected, resulting in overestimation of density (Buckland 2006). Because point transects have not been previously used to monitor butterfly populations, we recorded the time that each butterfly was detected and examined the relationship between survey duration and density.

A third assumption of distance sampling is that distance measurements are exact (Thomas et al. 2010). When we detected a butterfly, we visually estimated distance from observer in 1-meter intervals. This was the smallest distance interval we were confident we could accurately estimate visually. We did not restrict the radius within which we included butterfly observations; with fixed radius counts, observers often overestimate the number of butterflies present by pulling detections into the specified radius (Simons et al. 2007).

Detection probability can be influenced by a number of covariates related to climatic and habitat conditions (Dover et al. 1997; Wikström et al. 2008). In our case, we expected that blackbead cover, total shrub cover, and wind speed were all likely to influence our ability to detect Miami blue butterflies. Therefore, at each point, we estimated blackbead and shrub cover using modified Daubenmire cover classes (Daubenmire 1959), and estimated wind speed using the Beaufort scale (NOAA). Temperatures below 17°C and hours outside of peak sun can also result in reduced detectability in temperate zones (Wikström et al. 2008). In our case, all surveys occurred at temperatures above 23°C and within a relatively narrow time window (10:00 – 15:30) so we did not expect these factors to dramatically influence detectability and therefore did not include them as covariates in our models.

Estimating density

To determine the survey duration that best represents the butterflies initially present at a survey point, we investigated the rate at which detections accumulated during one-minute surveys. Over time, we expect the number of new butterflies observed in a given time period to stabilize, representing the constant addition of butterflies moving into the observer's field of view. To estimate this time, we broke our one-minute survey into five-second intervals and determined how many new butterflies were detected in each interval. We then identified the time at which the number of new butterflies per five-second interval leveled off, and set our survey duration at the next time lower than that time interval.

To obtain estimates of detection probabilities (p – the probability that a butterfly in the survey area is detected) from point transects, we fit a function to our detection data that describes the observed decline in the number of detections with distance from observer. This detection function can be used to estimate $p(0)$ and the distance at which we miss as many butterflies as we detect (the effective detection radius) (Buckland et al. 2001). Using these parameters, we can estimate the density of Miami blues. Buckland et al. (2001) recommend a sample size of at least 40-60 detections to accurately fit a detection function to the data. Because we never detect 40-60 butterflies on any individual survey, we pooled data from all points surveyed across all 7 beaches during time periods when we observed peak densities. We binned our data at the following cut-points – 2.5m, 3.5m, 4.5m, 5.5m, and 6.5m – and truncated the data at 6.5m. By having a large interval close to the observer (0 – 2.5m) we can account for movement of butterflies prior to detection at close distances where movement is more likely to affect estimation of detection probability (Buckland et al. 2001). We tested the following key function and adjustment combinations for our detection function: half-normal model with cosine adjustment, half-normal

model with hermite polynomial adjustment, and hazard rate model with simple polynomial adjustment (Thomas et al. 2010). We used Akaike's information criterion (AIC) values, a chi square goodness-of-fit test, and coefficients of variation to select the best model (Thomas et al. 2010).

By pooling data across multiple beaches, we assume that the detection function does not vary between beaches. This is biologically reasonable because the habitat on each beach is similar and survey methods are constant. Still, we assessed whether this was a valid assumption by fitting data from individual beaches to the same model we used for the pooled data set. We then compared the AIC value from the pooled model to the sum of the seven individual AIC values (Buckland et al. 2001).

Once we established our final detection function, we included the following covariates in our analysis: blackbead cover, total shrub cover, wind speed, and Cassius blue butterfly detections. We added each covariate to our detection function individually and compared AIC values to determine the best model. We then applied this model to the discreet time periods when we observed the highest Miami blue densities to compare densities across peak periods.

We fit all models in Program DISTANCE 6.0 ((Thomas et al. 2010)). DISTANCE calculates density by dividing the number of detections by the total area surveyed (scaled by the detection function). For a point survey with a full circle (such as for birds), the area surveyed for point transects is $k\pi w^2$ where k =number of survey points and w is the effective detection radius. Because we conducted surveys over half-circles, we multiplied the density estimated in DISTANCE by two. Confidence intervals were estimated analytically and are the sum of the variation in encounter rate (n/k) and variation in detection probability.

Estimating area of suitable habitat

Daily estimates of Miami blue population size require estimates of population density, as well as estimates of the area of suitable Miami blue habitat. Because we know very little about the habitat requirements for Miami blues, we broadly defined potentially suitable habitat as the area that is occupied by its hostplant, blackbead, within each key in the currently known range. On all beaches except for Long beach, blackbead is mostly contiguous and contained. On these beaches, we physically mapped blackbead with a handheld GPS. On Long beach, blackbead is less contained and it is impossible to physically map its entirety. Instead, we used aerial imagery to delineate the portion of the coastal berm that is mostly dominated by blackbead based on known blackbead locations.

Predicting adult flight

Early Miami blue surveys in the Key West Refuge conducted by United States Fish and Wildlife Service, Florida Fish and Wildlife Conservation Commission, and University of Florida personnel revealed high variability in the timing of adult flight periods. For example, on February 3, 2007 Cannon et al. (2010) counted 431 Miami blue butterflies on Boca Grande; the following February, Daniels (2010) counted only 3 butterflies on the same beach. We observed a similar phenomenon in 2012 and 2013 with peak abundances occurring at different times each year.

Two environmental factors that varied substantially between 2012 and 2013 were precipitation amount and seasonality. The spring months of 2012 were much wetter than in 2013. By the end of April 2012, Key West had received 31.3 cm of rain, whereas by the end of April in 2013 Key West had received only 16 cm. To investigate the relationship between precipitation

and Miami blue abundance, we acquired daily precipitation data from the Key West Airport weather station – the weather station closest to Miami blue habitat – through the National Climate Data Center (www.ncdc.noaa.gov). We examined the correlation between observed Miami blue densities and cumulative precipitation. We varied the interval over which precipitation was accumulated by 1) summing precipitation in week-long intervals up to 10 weeks prior to a survey and 2) moving the starting date of the summed interval back in one week time-steps prior to the survey date, up to 10 weeks. Each combination of these two strategies resulted in a sum of precipitation over a given length of time starting a given number of days prior to a survey. We correlated that sum of precipitation to the number of Miami blues observed per point on each survey date.

RESULTS

We made a total of 79 trips to the Key West National Wildlife Refuge between March 2012 and November 2013, during which we surveyed a total of 1991 points. We surveyed at least one beach each month, all beaches were surveyed at least 15 times – the most accessible was surveyed 26 times. We observed three distinct periods of peak abundance: late March – early April 2012, late July – early August 2012, and late July – early August 2013 (Figure 2).

Estimating density

Longer observation periods resulted in higher densities. Thirty percent of Miami blue butterflies observed during one-minute point counts were detected during the first 5 seconds of the survey. At the end of 10 seconds, we had detected 40 percent of butterflies. After that, butterfly detections accumulated at a fairly constant rate of 6-8 percent of the total detections

every five seconds (Figure 3). Based on this analysis, we chose 10 seconds as our survey duration as it resulted in the most conservative, minimum estimates of population size.

Using pooled data from 10 second surveys, the detection function with the lowest AIC_C was a half-normal model with a cosine adjustment. When we fit the data from individual beaches to this model, the combined AIC_C was greater than the AIC_C of the pooled model ($\Delta AIC_C=7.4$). This favors our approach of pooling data from multiple beaches to estimate density. Adding covariates to the model did not have strong effects on the AIC_C values or densities; all models, except for the model that included Cassius blue density, had ΔAIC_C values <3 (Table 2) and estimated butterfly densities only varied by one Miami blue per hectare. The high ΔAIC_C of the Cassius blue model indicated that Miami and Cassius blue butterfly densities did not co-vary. Additionally there was no relationship between daily Miami blue and Cassius blue counts per point ($r^2=0.020$) indicating that the presence of the two butterfly species did not affect the observer's ability to correctly identify each species.

Density estimates from all three peak periods were similar. In spring and summer 2012, we estimated the density to be 592 (95% CI: 500 – 702) and 680 (95% CI: 574 – 806) Miami blue butterflies/hectare, respectively. In early 2013, we observed very low Miami blue activity, until we again detected high densities in the mid-late summer. For this period, we estimated density to be 554 (95% CI: 468 – 656) butterflies/hectare. The coefficient of variation for these peak estimates is 0.086. DISTANCE estimated detection probability to be 0.12 (95% CI: 0.099 – 0.14) and the effective detection radius to be 2.22 meters (95% CI: 2.04 – 2.42).

Estimating area of suitable habitat

We estimated a total of 18 hectares of upland coastal berm in the Key West National Wildlife Refuge (Table 1).

Predicting adult flight

The best predictor of adult abundance (Miami blues/point) was the sum of precipitation over the 49 day period ending 28 days prior to the survey date ($r^2=0.41$, $p<0.001$; Figures 4 & 5). Once cumulative precipitation over a 49 day period exceeds 13 cm, we expect to observe Miami blue densities greater than one butterfly per point 28 days later.

DISCUSSION

Point transect distance sampling provides an efficient method to obtain reliable and statistically rigorous estimates of butterfly densities. Whereas point counts have been used previously to estimate butterfly species richness in tropical forests (Sparrow et al. 1994), we demonstrated that by adding distance sampling we are able to effectively estimate density. This method is especially useful in butterfly habitats like those dominated by shrubs, wetland, or dense forest, where navigating transects is difficult. For example, the US Endangered St. Francis' satyr (*Neonympha mitchellii francisci*) occupies early successional wetlands dominated by sedges and small hardwood saplings (Kuefler et al. 2008). Traversing this habitat involves wading in sometimes knee-deep muck, and tramples sensitive wetland vegetation, including the butterfly's host. Using point transect distance sampling to estimate density in these habitats would allow for the estimation of detection probability and subsequent abundance with minimal damage to the habitat. These methods are not only useful for butterflies, but could be applied to

insects more broadly, especially for other species that can be injured during marking and for which transect counts are logistically difficult, such as damselflies (Cordero-Rivera et al. 2002). Mark-recapture remains an important method for estimating demographic parameters and total population size. However, point transect distance sampling is a good alternative for long-term monitoring because it is relatively cheap and easy to implement.

We made one key adjustment to traditional point transect distance sampling methods to ensure we did not violate the assumption that butterflies are detected at their initial location: we further developed methods to determine the proper survey duration. Our analysis of the rate at which we accumulated detections validated our use of data from a 10 second survey for our density estimates, the optimal duration for Miami blue butterflies. This is much shorter than the 5-minute survey recommended for birds (Rosenstock 2002), although surveys as short as 2 or 3 minutes have been recommended for particularly active bird species (Cimprich 2009; Peak 2011). Short survey duration is necessary for Miami blues because they are most often detected on the wing and move toward and away from the observer rapidly. Additional methods could be used to validate survey duration. With knowledge of movement rates, one could simulate the rate at which butterflies move into and out of the estimated detection radius for a particular species. Repeat sampling at sites could also help determine survey duration by allowing for estimation of butterfly availability. Future attempts to apply point transects to insects that behave differently than Miami blues will need to evaluate the survey duration that best capture realistic densities of the target species (Lee and Marsden 2008).

In an ideal survey scenario, point locations would either be randomly distributed throughout the butterfly's habitat, or stratified in a pre-defined grid (Buckland et al. 2001). This would allow for unbiased estimation of butterfly density. In the impenetrable blackbead thicket

characteristic of Miami blue habitat, navigating to random or stratified point locations is not feasible. Because of this limitation, we located survey points randomly on the edge of blackbead habitat. This decision has the potential to bias our density estimates either high or low if butterflies are attracted to, or avoid the blackbead edge.

A number of studies have examined butterfly behavior and density with respect to habitat edges (Haddad 1999; Haddad and Baum 1999; Ries and Debinski 2001; Schultz and Crone 2001; Ross et al. 2005; Schultz et al. 2012). In these cases, butterfly densities are greatest in the center of a habitat patch (Haddad and Baum 1999) and butterflies avoid patch edges from as far as 20 meters away (Ries and Debinski 2001; Schultz and Crone 2001; Ross et al. 2005; Schultz et al. 2012). If Miami blues follow this established pattern, then we consistently underestimate Miami blue density. Previously studied landscapes, however, are very different from Miami blue habitat. Butterfly habitat in previous edge studies is generally open compared with the surrounding matrix (except Schultz and Crone 2001, where habitat and matrix are both open grassland), nectar and host plant resources co-occur, and resources are unavailable in adjacent habitat. Under these conditions, butterflies are attracted to openings, and avoid forests (Haddad and Baum 1999; Ries and Debinski 2001; Ross et al. 2005; Schultz et al. 2012). The structure of Miami blue habitat, however, is not open and grassy, but dense and shrubby, and nectar resources are not always available within host plant patches. Instead, many flowering plants occur in the adjacent, open, coastal prairie community. When important resources are located within adjacent habitat types, butterflies are less likely to respond to structural habitat boundaries (Schultz et al. 2012) and may even be attracted to the edge (Ries et al. 2004). Under this scenario, we would consistently overestimate Miami blue densities by only surveying along the blackbead edge. We surveyed a limited number of points within blackbead, and recorded

individual Miami blues that we stumbled upon while mapping blackbead. We encountered a number of butterflies within the center and back edge (adjacent to mangrove) of the blackbead, even during periods of low abundance. These observations confirm that butterflies are at least present throughout blackbead, and suggest it is unlikely that we are grossly over or underestimating Miami blue density.

Another aspect of our design that has the possibility of biasing density estimates is our decision to orient our survey semi-circles perpendicular to the blackbead edge. If Miami blues are mainly detected on the blackbead side of the semi-circle, we are consistently underestimating butterfly density. We did not collect data on the habitat type in which we detected butterflies, but future monitoring efforts could. These data would allow us to determine the proportion of butterflies detected in blackbead vs. beach dune and refine our density estimates further.

The density and abundance estimates we present are an index of population size, the number of adult individuals estimated to be flying on a given day; they are not total population size. This number will be sufficient to track the size and trends of the Miami blue population over time. The true population size, however, would account for all adults that emerge over a flight period within one generation. Total butterfly population size is generally estimated by combining daily counts across a flight season (Mattoni et al. 2001). This approach works in temperate regions for butterflies with distinct flight periods, each representing a single generation. For Miami blues, we cannot simply sum counts because of the possibility of overlapping generations. This fact makes it nearly impossible to estimate total population size without estimating demographic parameters such as daily survival and generation time (Williams et al. 2002). Some estimates of both daily survival rates and generation time exist for Miami blue

in captivity (J. Daniels, personal communication), but these estimates do not represent the seasonal or temporal variability that is expected for the Key West Refuge population. Given the variability we observed with respect to inter- and intra-annual phenology, estimating these demographic parameters in the field would be necessary to calculate accurate estimates of total population size.

Implications for an imperiled butterfly

Our estimates of peak Miami blue daily density are the first for the Key West Refuge. Peak daily densities of 500-600 butterflies per hectare are much higher than previously expected for Miami blues. In the winter of 2006/2007, when the butterflies were initially discovered in Key West refuge, Cannon et al. (2010) observed peak daily counts of 441 and 521 Miami blues on Boca Grande and Main Beach, respectively. Since those initial reports of abundances in the hundreds, surveys have been sporadic and failed to produce a daily count higher than 14 Miami blue butterflies (Daniels 2010). Rather than indicating a dramatic loss of butterflies, these surveys more likely reflect the difficulty of targeting surveys around periods of high adult abundance. By surveying continuously throughout the year, we captured time periods of both low and high Miami blue densities.

Peak Miami blue densities in 2012 and 2013 were similar, but intra-annual fluctuations were large (Figure 2). It is unknown if low adult density translates to low overall population size or simply low *adult* abundance, therefore, low adult densities are concerning for management of Miami blues. The observed swings from low to high densities could result from erratic boom and bust population cycles not uncommon among insects. Our surveys, however, detected rapid increases from low adult density to high in just a week, instead of the gradual increase across

multiple generations expected in the boom and bust scenario. A better explanation for the observed fluctuation in adult population size is that periods of low (or no) adult abundance represent times when immature Miami blue development rate is dramatically reduced, likely due to environmental conditions. This scenario fits with the precipitation model we developed (Figure 5). Likely, early or mid-instar Miami blue larvae diapause during droughts as their blackbead host desiccates and becomes less palatable. Precipitation then initiates new growth of blackbead and larvae emerge from diapause, complete development, and fly as adult butterflies 4-6 weeks later. This period of larval dormancy explains the lag we observed between increased precipitation and peak butterfly abundance. Because we almost always saw at least one Miami blue, there is likely a high amount of variation in when, and for how long, an individual diapauses as well as how long an individual butterfly lives.

Despite high densities, the global distribution of Miami blues remains small and limited to low-lying islands where sea level rise will undoubtedly accelerate habitat loss over the next few decades. Another, perhaps more imminent, climate change related threat is the potential for changes in precipitation patterns. Current climate projections for south Florida (although highly uncertain) predict decreases of at least 5% in mean annual precipitation and up to 10-15% reduction in June-July-August precipitation (IPCC 2007). If Miami blue larvae diapause during dry periods, prolonged drought could have negative effects on the population through reduction in adult size (Hahn and Denlinger 2007) which could result in reduced fecundity (Trager and Daniels 2007). Extended diapause also has the possibility of increasing the butterfly's vulnerability to predation and parasitism. In cold climates, parasitoids and invertebrate predators often diapause simultaneously with their prey (Hahn and Denlinger 2007). This is unlikely to be

the case in the warm Florida Keys, where diapausing larvae are likely susceptible to a number of unknown invertebrate assassins.

Using point transect distance sampling to estimate insect density is a reasonable option for species whose habitat or life history traits make traditional methods impossible to implement. Still, careful attention must be paid to assure that assumptions of the method can be met. The need for 40-60 detections to accurately fit a detection function to the data may still not make point transects a feasible option for rare species that exist at extremely low densities. For these species, pooling data across space and/or time could allow for the development of a “global” detection function, as long as it is unlikely that detection probability varies in space and/or time. Double observer approach for estimating detection probability from points (Nichols et al. 2000) could also be applied to low-density butterflies. For some very rare species that are logistically challenging to monitor, land managers may have to rely on count index methods, or shift focus to tracking occupancy instead of abundance over time. Although Miami blue butterflies are rare globally, difficult to access, and occupy challenging habitat, they are still locally abundant and exist at densities high enough to allow for quantitative estimation of their population density.

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TABLES

Table 3.1. Name, length, number of survey points, and estimated habitat area for each occupied beach.

	Boca Grande	East	Snook	Short	Third	Main	Long	Total
Beach length (m)	1100	221	529	503	638	1023	2800	6814
Number of survey points	24	8	7	9	15	22	100	185
Habitat area (ha)	1.8	2.9	0.35	1.7	3.6	2.8	5*	18.15

*Habitat area was physically mapped on all beaches except for Long where it was estimated using known blackbead locations and aerial imagery

Table 3.2. Delta AIC values for detection functions with covariates. # par = number of parameters in the model, X^2 p-value = result of X^2 Goodness-of-fit test.

Model	# par	X^2 p-value	ΔAIC_c	AIC_w
Null	2	0.454	1.66	0.201
Blackbead	3	0.210	2.55	0.129
Total shrub	3	0.212	0.00	0.461
Wind speed	3	0.210	1.60	0.207
Cassius density	2	0.001	13.12	0.001

FIGURES

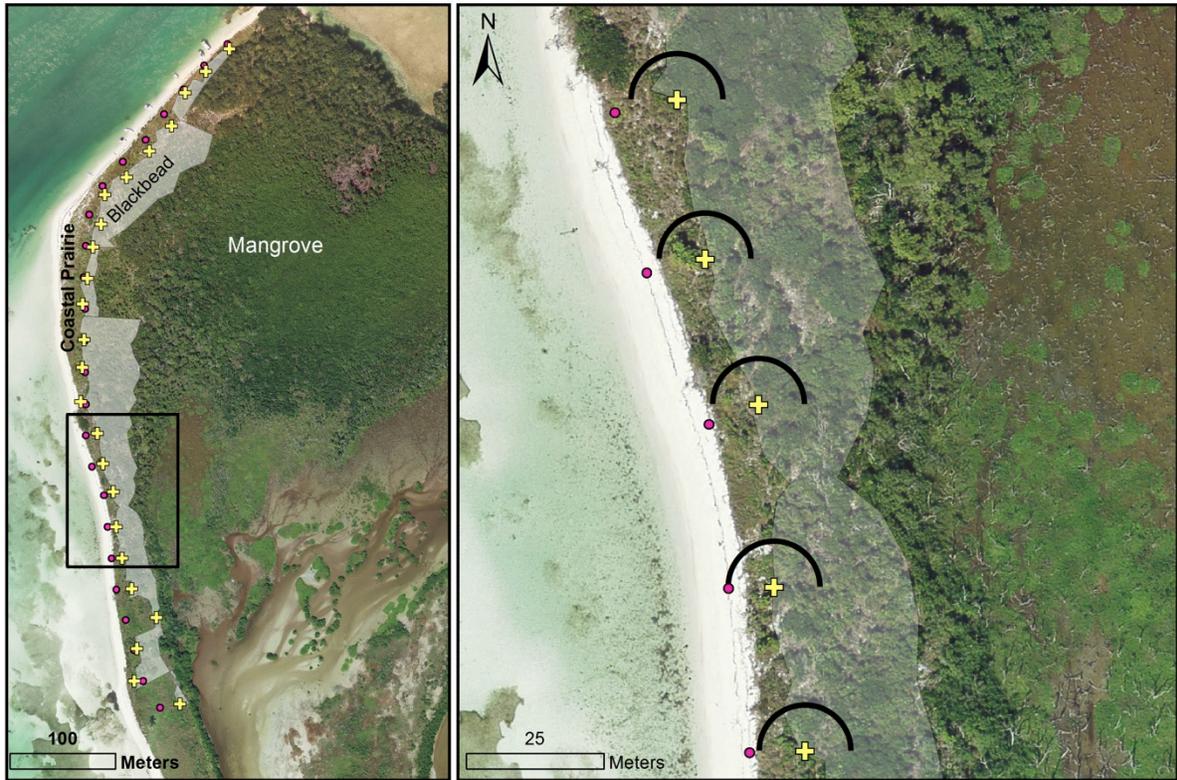


Figure 3.1. Example Miami blue butterfly habitat and survey locations. Coastal prairie, blackbead and mangrove habitats are labeled. Dots represent locations mapped every 30 meters along the beach, crosses represent actual survey locations, and semi-circles have a radius of 8m, the farthest detection distance in our data set.

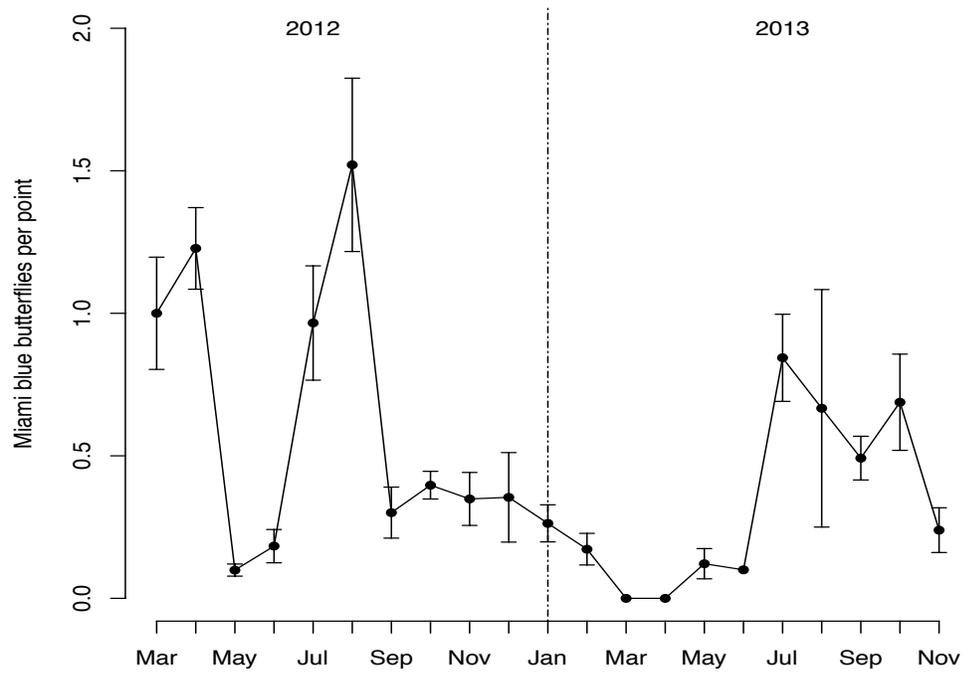


Figure 3.2. Phenology of Miami blue butterfly adults in 2012 and 2013. Values are mean butterfly count per point \pm SE.

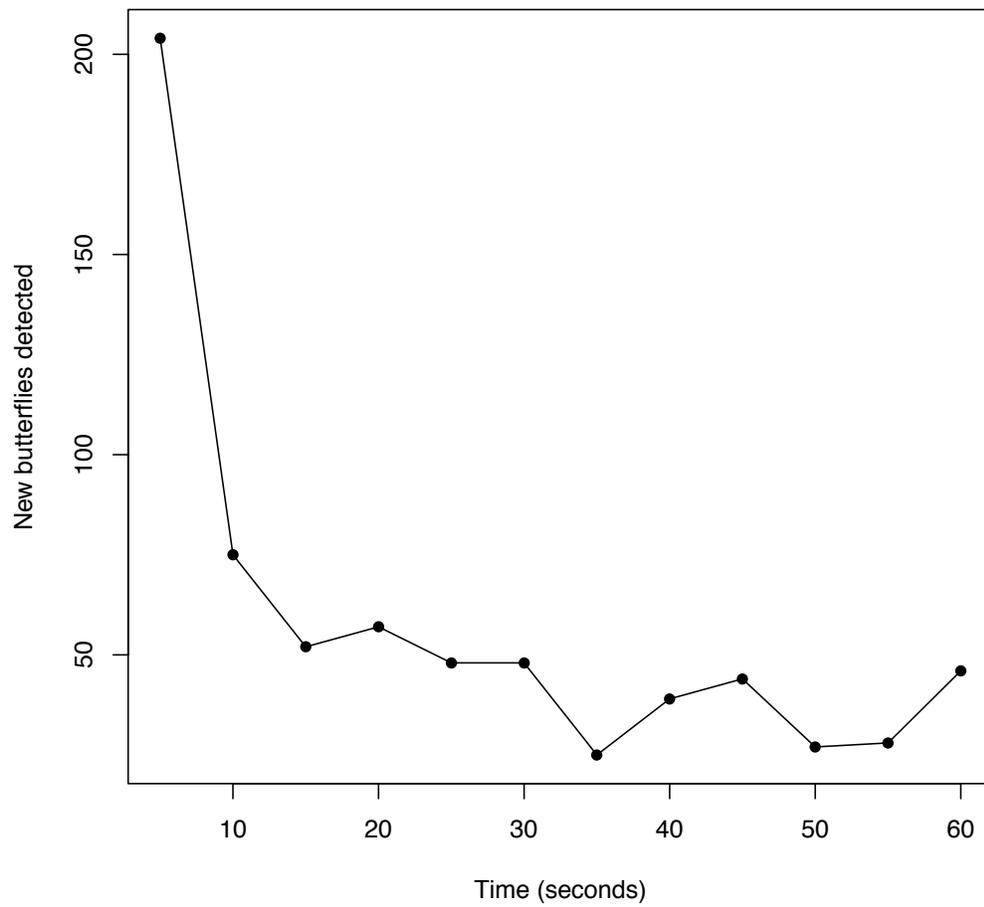


Figure 3.3. The number of new butterflies detected in each five-second interval during a one-minute survey. The total number of detections is equal to all butterflies detected during surveys in 2012 and 2013.

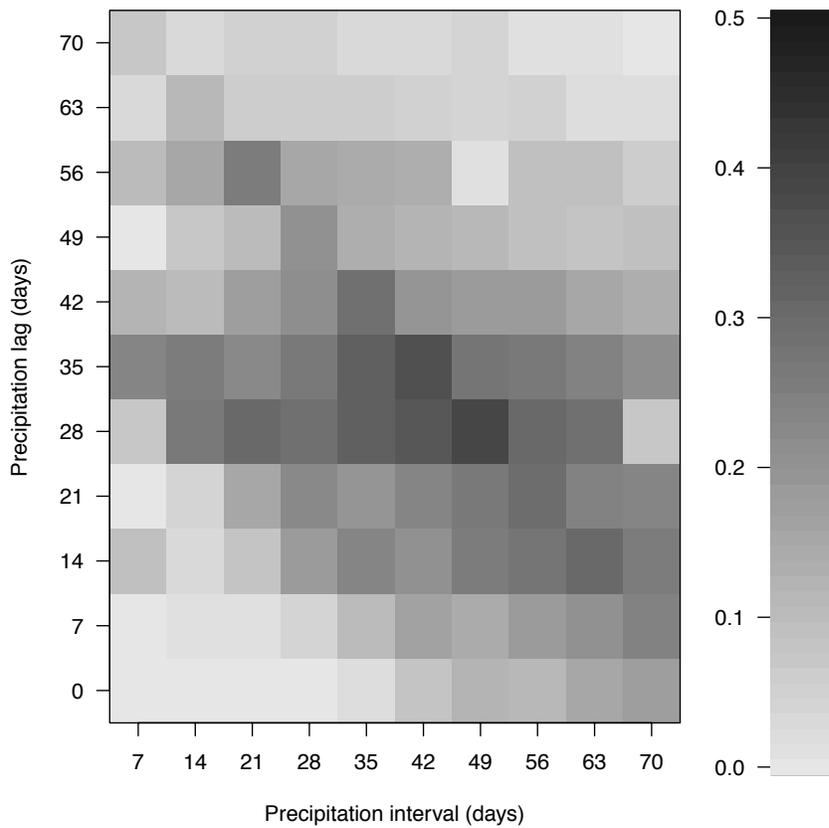


Figure 3.4. Matrix of r^2 values for correlations between precipitation and Miami blue butterfly density. Each correlation uses a different number of days over which precipitation is summed (interval) ending a different number of days prior to the survey date (lag). Darker squares equal higher r^2 value.

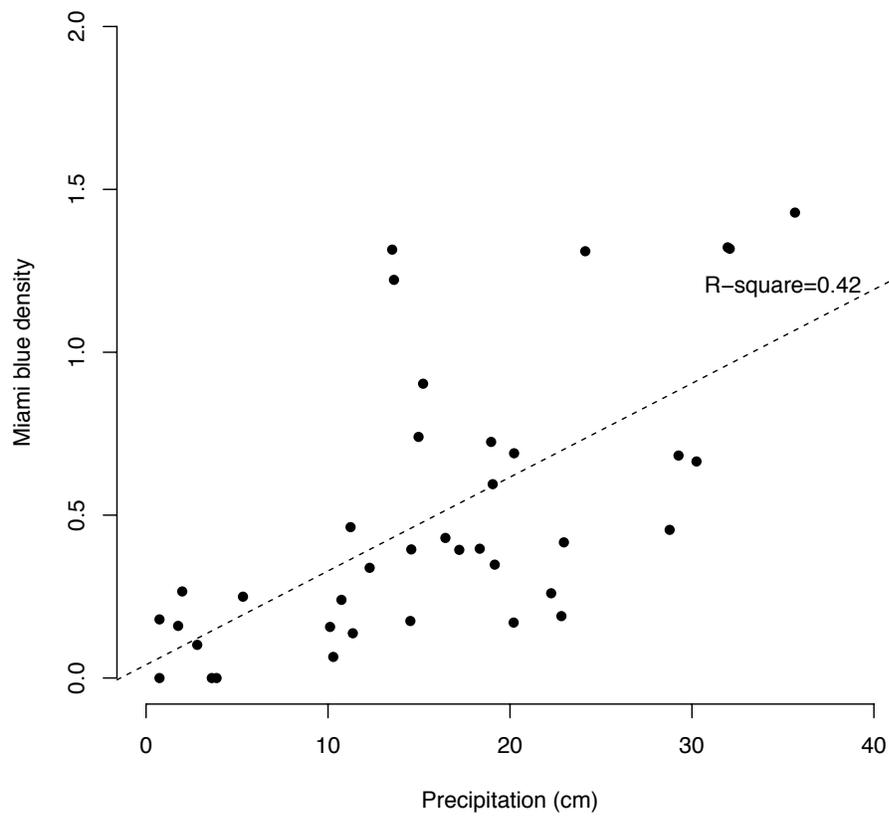


Figure 3.5. Relationship between precipitation and butterfly density. X-axis represents the cumulative precipitation over 42-day period ending 6 weeks before survey date.

CHAPTER 4

Demographic uncertainty, not climate, drives extinction patterns for a rare butterfly, Miami blue

ABSTRACT

Predicting how species respond to changes in climate is a critical part of conserving biodiversity into the future. Until now, much of this effort has focused on predicting the effects of warming temperatures and relied on statistical approaches. However, precipitation changes may be more important in driving future population dynamics, and mechanistic approaches are better suited to tease apart the effects of individual climate drivers and intrinsic variability within populations. Building upon previous research on the phenology of Miami blue butterfly, we constructed a mechanistic population model that explicitly tied butterfly phenology to climate variables. We used projections from downscaled climate models to project butterfly population size into the future and evaluated whether uncertainty about butterfly biology or changes in climate had larger impacts on extinction risk. We found the butterfly population to be highly sensitive to daily survival during diapause; changes in this parameter of <1% determined long-term persistence or extinction. Changes in precipitation patterns had significant effects on population growth rates, but at a very narrow range of values of diapause survival. A mechanistic approach will often be needed to evaluate population responses of rare species to climate change.

INTRODUCTION

As the Earth's climate warms, so do the ranges and population dynamics of a diverse array of species (Parmesan & Yohe, 2003). Understanding and predicting how species will

respond to environmental change is paramount to effectively conserving biodiversity. Most research on range shifts and population change focuses on the effects of temperature, largely excluding other climate drivers such as precipitation (Chen *et al.*, 2011; Valladares *et al.*, 2014; Aguilée *et al.*, 2016). These efforts to predict future population dynamics often rely on simple correlative approaches (Urban *et al.*, 2016). Recently there has been a call to incorporate mechanisms into these models as a powerful way to explore population sensitivities and potential impacts of management actions (Pacifci *et al.*, 2015). This is especially important for threatened and endangered species, the species which are some of the most vulnerable to climate change due to restricted ranges and small population sizes. In this paper, we use a mechanistic approach to evaluate extinction risk due to precipitation change for one of the world's rarest butterflies.

Models of species responses to temperature are common likely because there are simple linear predictions to test, such as latitudinal and elevational range shifts. Climate model predictions of temperature change are less uncertain than those of precipitation, and organism response to temperature change is relatively easy to measure. Including changes in precipitation patterns in these predictions, however, can produce counter-intuitive results relative to the expected response to warming temperatures, such as down slope migrations (Crimmins *et al.*, 2011), especially in regions where water is a highly limiting resource. A recent review (Siepielski *et al.*, 2017) found that precipitation patterns drive natural selection on plants and animals more strongly than temperatures. This suggests that changes in precipitation patterns may present challenges for species that are unable to easily track shifts in climate, especially for populations of rare species with reduced genetic variation and limited capacity for evolution.

Most models that assess extinction risk as climate changes rely on correlational approaches. Predictions from correlational models such as bioclimatic envelopes can be useful

for revealing the relationship between climate variables and broad landscape patterns. However, including the mechanisms that relate population changes or range shifts to climate variables is a more powerful model to aid in identifying strategies to improve species' resilience to a changing climate (Pacifiçi et al., 2015). Population viability and sensitivity analyses are well established methods that use demographic information to model the probability of persistence into the future and are common tools used by researchers and land managers to evaluate potential effects of management strategies (Morris & Doak, 2002; Mills, 2013). Population models that incorporate demographic rates as functions of climate variables and then use climate projections to drive the future population dynamics (eg. Jenouvrier *et al.*, 2012) can tease apart a population's sensitivity to both demographic stochasticity and climate uncertainty. This is one way to include mechanisms into predictions of extinction risk which results in improved understanding of species dynamics in the face of climate change.

Butterflies exhibit population dynamics that are particularly suited to these types of mechanistic models. Because butterflies are ectothermic, they are especially vulnerable to temperature changes. Many papers have documented temperature driven range shifts, both latitudinal and elevational (Parmesan, 1996; Parmesan *et al.*, 1999; Crozier, 2004; Chen *et al.*, 2011). Butterfly phenology is also affected by temperature. Cayton et al. (2015) demonstrated that growing degree days (accumulation of heat over time in the spring) accurately predict spring emergence date and peak abundance across butterfly species, and suggest tracking degree days as a simple way to predict species response to climate. In addition to temperature effects, temporary declines in butterfly abundances have been correlated with drought (Oliver *et al.*, 2015), and the extinctions of individual bay checkerspot populations have been attributed to increasing variability in precipitation (Ehrlich *et al.*, 1980; McLaughlin *et al.*, 2002b). Almost all of these

findings result from correlational models. Precipitation patterns likely have their strongest effects on populations through their effects on resources. This is particularly important for specialist butterflies that rely on one species of host plant. For example, in years of low precipitation, Bay checkerspot butterfly caterpillars race to reach 3rd instar, the stage in which they overwinter, before their hostplants senesce (McLaughlin *et al.*, 2002a). This type of system lends itself nicely to a mechanistic approach for predicting future climate-driven population changes. Despite a call to build these kinds of models for butterfly populations in 1990 (Murphy *et al.*, 1990) there are still only a few examples in the literature, and none that use climate model output to drive future butterfly dynamics.

We modeled the effects of changes in precipitation patterns on population dynamics for a very rare butterfly, Miami blue (*Cyclargus thomasi bethunebakeri*) in the face of climate change. Building upon previous research that shows how precipitation controls phenology (Henry, *et al.* 2015), we used available data sources to construct a mechanistic population model that explicitly tied butterfly phenology to climate variables. With this model we asked: 1) How sensitive is population size (or growth rate) to future changes in climate? 2) How sensitive is the population to vital rate variation? 3) Is the population more vulnerable to extinction based on climate or demographic uncertainty? We demonstrate the value of this approach in improving future endangered species management.

METHODS

Miami blue butterflies

Miami blue butterflies were once common along the east and west coasts of peninsular Florida. Historical collection records document their existence as far north as Cape Canaveral on

the east coast and Tampa area on the west. As the human footprint in Florida expanded in the 20th century, the range of Miami blues contracted. They are one of the rarest known butterflies in the world and are confined to a few low-lying islands in the lower Florida Keys. These islands are 25-40 km west of Key West and protected within the Key West National Wildlife Refuge. This remote location means that monitoring activities are opportunistic, and dependent on favorable weather; winter winds and summer thunderstorms can make conditions unsafe for boating to the islands. Because of this, the island closest to Key West, Boca Grande Key, is the most accessible and the one for which we have the most monitoring data.

Miami blues have been known to feed on at least three different host plants, including balloon vine (*Cardiospermum halicacabum*), nickerbean (*Caesalpinia bonduc*), and blackbead (*Pithecellobium keyense*). In their current locations, black bead is their primary host. Female butterflies lay eggs singly on newly emerged plant growth or tucked into flower buds. Caterpillars hatch and feed on this new plant tissue. Like many Lycaenids, Miami blues are myrmecophilic, meaning that their caterpillars are protected from parasitoids and predators by ants in exchange for a honeydew that is secreted by caterpillars. This mutualistic relationship increases larval survival in the wild. In fact, of all Miami blue caterpillars that have been collected from the wild, not one has been parasitized (J. Daniels personal communication).

In captivity, Miami blues develop continuously from one generation to the next, regardless of season or time of year. In the wild, however, periods of high adult abundance are followed by periods of low, or no, adult butterflies (Henry *et al.*, 2015). Henry *et al.* (2015) found that the phenology of Miami blue butterflies is conditioned on precipitation patterns rather than temperature and hypothesized that between periods of peak adult abundance, immature butterflies enter diapause. This relationship between abundance and precipitation reveals a clear

mechanism through which future changes in precipitation patterns could impact Miami blue butterfly populations. If the periods between flight periods becomes longer, butterflies will spend more time in diapause. Extending the diapause period could lead to decreased survival due to exposure to predation and parasitism in addition to exhausting resource stores (Hahn & Denlinger, 2007).

Butterfly Model

We constructed a model of Miami blue butterfly population dynamics based on the relationship between butterfly abundance and rainfall detailed by Henry et al. (2015). The authors determined that the amount of precipitation that falls in the 48-day period that ends 28 days prior to a survey date is the best predictor of butterfly abundance on the survey date. To tie butterfly dynamics to precipitation patterns, we determined a threshold amount of precipitation that results in high Miami blue densities. To identify the appropriate precipitation threshold, we tested thresholds from 150 – 300mm in 25mm increments and visually inspected how the modeled population compared to our observed counts from Boca Grande in 2012 and 2013 (the most accessible island in Miami blue's range, and therefore the island for which we have the most continuous data). Our primary concern was that the interval between peak butterfly densities predicted by the model matched the timing of our observed peak densities.

For every day in our simulation, we calculated the amount of precipitation that fell in the previous 48 days and identified the dates on which that value was equal to or exceeded the precipitation threshold. To each of these dates, we added a number of days drawn from a uniform distribution between 18 and 38 to account for the 28-day lag in Henry et al.'s precipitation model. We add a random number to account for variability in emergence across a flight period

and to spread emergence out over a number of days. If we added the constant 28 to each period, all adult butterflies would emerge on the same day. The resulting dates are the dates on which butterflies will emerge as adults in our population model. And the number of days between any given date and the next emergence date is the parameter x in Equation 1, which is roughly the number of days that butterflies will spend in diapause.

Equation 1:
$$N_{(t)} = N_{(t-1)}S_a + \frac{N_{(t-x)}}{2}ES_l^{D_l}S_d^{(x-D_l)}$$

To model butterfly population dynamics, we used a model with a daily time-step (Equation 1). In our model, the number of adult butterflies present on any given day ($N_{(t)}$) is equal to the number of butterflies that were present the day prior ($N_{(t-1)}$) that survived at rate S_a , plus those that emerge on day t . The number of butterflies that emerge on day t is the number of butterflies that were flying on day $t-x$ divided by two (assuming half are female), that laid eggs at rate E . Eggs hatch, then larvae survive at a daily survival rate S_l for D_l days. For the remaining days until the precipitation threshold is met ($x-D_l$), immature butterflies are in diapause surviving at daily survival rate S_d . We parameterized the model with survival and development data from captive individuals in Gainesville at the Florida Museum of Natural History (Table 1).

Table 4.1. Parameter definitions and data sources.

Parameter	Estimate	Data source
Egg laying rate (E)	14 eggs per day	Saarinen (unpublished dissertation data 2009)
Daily adult survival (S_a)	0.5	Population model fit to daily adult counts in field enclosure (Zonneveld, 1991)
Development time (D_l)	22 days	Captive individuals raised on blackbead (Daniels et al. unpublished data)
Daily larval survival (S_l)	0.96	Captive individuals raised on blackbead (Daniels et al. unpublished data)
Daily diapause survival (S_d)	Variable	This is the parameter we vary because we have no information on daily diapause survival in captivity or the wild

Climate data

We used a suite of climate models to evaluate how future changes in daily precipitation patterns affect the persistence of Miami blue butterflies. Global climate models (GCMs) are the models the IPCC uses in their predictions of global patterns of climate change. The resolution of these GCMs is two-degrees, an area much larger than the entire range of Miami blue butterflies. Precipitation patterns in south Florida are largely driven by local climate forcings of convective thunderstorms and island cloudlines, phenomena that occur on a scale much smaller than 2 degrees and similar to the range of Miami Blue. For this reason, we used downscaled climate projections in our model. Specifically, we used data from the MACAv2-METDATA dataset. Climate forcings in this dataset were drawn from a statistical downscaling of GCM data from the Coupled Model Intercomparison Project 5 (CMIP5, Taylor et al. 2012) using the Multivariate Adaptive Constructed Analogs (MACA) method (Abatzoglou & Brown, 2012). This method statistically downscales the two-degree resolution of the GCM data to four-kilometer resolution. Like all statistical downscaling techniques, this method relies on a training dataset that is based on historical meteorological observations from weather stations. The training dataset used to produce MACAv2-METDATA projections is METDATA (Abatzoglou, 2013). The southern extent of METDATA, and therefore MACAv2-METDATA projections, is 25°N, which includes the southernmost tip of mainland Florida and part of Key Largo, but excludes the lower Florida Keys and the range of Miami blue butterflies. Since we could not select model output directly from the lower keys for our projections, we instead chose the location in the data set that was closest to Key West while still in the Florida Keys, which was in Key Largo (-80.4939E, 25.2764N).

The relationship between precipitation and Miami blue abundance in Henry et al. (2015), and the resulting precipitation threshold used in our population model was based on daily precipitation data from the Key West Airport. To correct for potential bias in the climate projections that could arise from relying on a grid cell located 160km from the location used to estimate the relationship between weather and abundance, we used historical Key West Airport data from 1979-2006. For every day in this dataset we calculated the number of days between it and the next threshold event. We did the same calculation with METDATA from the Key Largo location across a range of thresholds from 100mm to 300mm in one millimeter increments. To find the threshold for the Key Largo location that most closely matched the chosen precipitation threshold from the Key West Airport, we summed the squared differences between these dates in the historical data set and METDATA and identified the threshold for which this value was smallest.

We examined trends in the time between threshold events over time by fitting a linear model with the date precipitation thresholds are reached as the predictor and the number of days between precipitation thresholds as the response variable. We excluded the intervals that were less than our measured development time of 22 days, since an interval of less than 22 days would result in direct development from egg to adult without the need for diapause. We did this for all 20 climate models for two greenhouse gas emissions scenarios RCP 4.5 (stable emissions rate followed by a decline in emissions by mid-century) and RCP 8.5 (near status quo emissions). For each climate model, we noted if there was a significant trend toward increasing or decreasing intervals between thresholds over time. We interpreted a positive slope as a drying climate (more time between threshold events over time) and a negative slope as a wetter climate (threshold events get closer together over time).

Future Miami blue population projections

We used downscaled climate data to evaluate how future precipitation patterns will affect Miami blue populations. We were especially interested in how projected changes in precipitation interact with daily diapause survival to affect population persistence, since we know almost nothing about this life stage. To do this, we varied daily diapause survival from 0.98 (the value that produced realistic dynamics in the model validation process) to 0.976 in increments of 0.001, and simulated population dynamics under our two climate change scenarios for the period 2018-2099. In addition to varying daily diapause survival, we also varied initial population size over three orders of magnitude, 100, 1000, and 10,000. For each combination of diapause survival (n=5), initial population size (n=3), downscaled climate model (n=20), and emissions scenario (n=2), we extracted the last date on which at least one adult butterfly was predicted to occur in the simulation. This resulted in a total of 300 simulations per emissions scenario that we used to infer potential climate change effects on population persistence of the Miami blue.

RESULTS

The precipitation threshold that resulted in the best fit between the population model and the historical adult butterfly count data was 200mm. Thresholds below this amount resulted in butterflies emerging between the peak abundances we measured, and thresholds above 200mm did not predict adult butterflies beyond summer 2012. We found a similar pattern with diapause survival in which 0.97 resulted in the population going extinct immediately, 0.98 resulted in dynamics that represented our observations, and 0.99 predicted butterfly populations that grew by an order of magnitude between summer 2012 and summer 2013, which we did not observe in the field (Figure 1).

The precipitation threshold for the Key Largo grid cell that best matched (lowest sum of squares) the 200mm threshold in the historical Key West data was 226mm. This is the threshold we used in our projections of future butterfly population dynamics. None of the climate models in the RCP 4.5 ensemble revealed a trend in interval length change over time, there was no significant relationship between period between threshold events and date in the climate projection (Figure 2a). Under the RCP 8.5 scenario, however, 7 of the 20 models had significant positive correlations between intervals between threshold events and date, indicating increasing dry periods over time (Figure 2b).

Miami blue butterfly population predictions were much more sensitive to varying the daily diapause survival rate than they were to projected future changes in climate. For both emissions scenarios, whether or not the population persisted to the end of the century depended strongly on diapause survival. For a starting population of 10,000 butterflies, daily diapause survival >0.978 resulted in the population persisting until 2100, and daily diapause survival of 0.976 resulted in extinction within in the next 20 years regardless of climate model (Figure 3). For this very narrow range 0.976-0.978, there was large variation in population extinction date among the 20 climate models with some climate models predicting immediate extinction, and others persistence to 2100. Decreasing the initial population size by an order of magnitude, increased the values of daily diapause survival under which the butterfly population persisted by 0.001. When the initial population size was 100, the model predicted rapid extinction for all values of daily diapause survival.

DISCUSSION

We found Miami blue population dynamics to be more sensitive to poorly understood demographic parameters and initial population size than to projected changes in precipitation patterns. These results highlight the importance of understanding basic natural history to predict how populations will respond to climate change. In fact, a better understanding of how all vital rates are affected by climate variables could reveal new population level sensitivities to future change. There is a high level of uncertainty in what the future precipitation will be in the Florida Keys, and for specific values of survival this uncertainty was important. This interaction between demography and climate demonstrates the importance of including precipitation dynamics into models of future climate-driven population dynamics. Our case study emphasizes the power of mechanistically linking precipitation to population dynamics to inform management actions and conservation strategies even for species with highly uncertain vital rates.

Our results highlight two strategies for increasing the probability of Miami blue butterfly persistence through 2100: 1) ensuring that populations are large and 2) maximizing diapause survival rate. There are a number of reasons that small populations are susceptible to extinction, mainly demographic stochasticity, and genetic issues such as inbreeding depression. In our Miami blue model, the whole population of butterflies has the same daily diapause survival rate, so a small population declines rapidly, and a large population can persist in diapause for a very long time. Current estimates of the total Miami blue population size are around 10,000 butterflies, but populations on individual islands number in the thousands (Henry et al. 2015). The diapause survival value at which the model best represented historical counts was 0.98. For a population of 1000 butterflies, this value is perched on the edge of what could lead to precipitous population declines in the future (Figure 3); any decrease in population size *or* survival rate

could be disastrous for the butterflies, regardless of the climate scenario. In the case of rapid population decline, perhaps the result of a hurricane or unusual cold snap, the best action to improve the population's probability of persistence, if available, may be population augmentation.

Another strategy for ensuring persistence would be to enact conservation measures that increase an important demographic rate, daily diapause survival. Diapause is a crucial life stage because of its duration; for most temperate butterflies, a majority of the life cycle is spent in diapause. Each butterfly species has a specific life stage in which diapause occurs. For Lycaenids, this is generally either at the larval or pupal stage. Most temperate Lycaenids diapause through the winter as mid-instar larvae at the base of their host plants, emerging in the spring to feed on newly emerged plant tissues before pupating and flying as adults (Warchola *et al.*, 2015). Much less is known about the phenology of sub-tropical butterflies like Miami blues. A number of myrmecophilic species, such as Miami blues, are adopted into the nest of their ant hosts where they overwinter (Thomas *et al.*, 2009). Refining our understanding of what happens to Miami blues when they are not readily observable as adults, and what contributes to growth and survival of difficult-to-observe larval Miami blue butterflies should be the highest research priority going forward. This information will allow managers to prioritize conservation efforts that increase survival probability in this important life stage, thereby increasing Miami blue's resilience to climate change.

Rare butterflies are not the only group of organisms with cryptic life stages that influence population persistence but about which we know very little. This problem extends to other taxa of conservation concern as well as those of economic importance. Some examples include overwinter survival for migratory birds (Norris *et al.*, 2004), larval survival of bonefish and

tarpon (Adams & Cooke, 2015), and survival of migratory stages of many pest insects (Hobson *et al.*, 2018). Understanding how sensitive (or not) a population is to variation in an understudied life stage, and how that life stage may or may not be influenced by climate will improve our understanding and management of these important species. This knowledge will also aid in deciding where to allocate research and monitoring resources in the future.

The lack of sensitivity of Miami blue population dynamics to climatic changes is in part a result of the simplicity of our modeling strategy. We assumed that all demographic rates remained constant regardless of climate, but this is unlikely to be true. Both field and lab studies have shown that all butterfly life stages are affected to some degree by changes in temperature and humidity (Thomas *et al.*, 2009; Klockmann & Fischer, 2017; McDermott Long *et al.*, 2017). Species at low latitudes are more likely to currently exist at temperatures precariously close to their thermal maximum (lethal temperature) than species at higher latitudes (Deutsch *et al.*, 2008; Sunday *et al.*, 2011). This signifies that slight increases in temperature may result in profound negative fitness and survival consequences for low latitude species, such as Miami blues (Deutsch *et al.*, 2008; Sunday *et al.*, 2011). For many species, the diapause stage is particularly sensitive to drought (McDermott Long *et al.*, 2017); Thomas *et al.* (2009) found a 56% reduction in large blue butterfly (*Phengaris arion*) diapause survival in drought years. These studies report results of survival rates across the entire diapause period, but to our knowledge, the effect of drought on daily diapause survival is unknown. In 7 of the 20 climate models at the high emissions scenario, there is an increase in the interval between precipitation threshold events over time. If daily diapause survival decreases even slightly due to drying conditions, the future scenario for Miami blue becomes quite bleak. Another potential reason we did not detect strong climate effects is that we do not have an upper limit to the number of days

an individual butterfly can survive in diapause, and how precipitation might affect this trait. In reality, butterflies are unlikely to survive in diapause indefinitely. There are some examples of butterflies that are capable of persisting in diapause for up to two years (eg. Taylor's checkerspot (*Euphydryas editha taylori*), Schaus' swallowtails (*Heraclides aristodemus ponceanus*) and at least one Lycaenid larva documented to survive for over a year in its host ant's nest (Elmes *et al.*, 1991). Identifying the maximum number of days Miami blues can spend in diapause would reveal the maximum number of days between threshold events that Miami blues can survive. In 7 of the climate models, the time between threshold events increases over time, up to three years in some models. If butterflies cannot survive these increasingly long periods of diapause, the future is likely much bleaker for Miami blues than predicted by the population model.

Our model focuses purely on environmental drivers of population regulation. Population ecology has long focused on how both environmental conditions (Birch, 1948), and intrinsic mechanisms (Pearl, 1927) interact to regulate populations. By focusing entirely on the former, we neglected the latter, assuming instead that vital rates would remain constant regardless of the population size. Because of this assumption, combinations of high diapause survival and high initial population size, predicted time periods in which butterfly populations grew nearly without bound. This is likely responsible for the ability of populations to persist in the face of long intervals between threshold events and is quite unrealistic. Nowicki *et al.* (2009) found that density dependence was much more important in regulating butterfly populations than were environmental conditions. Density-dependent effects are most likely to occur at the larval stage as a result of competition for food resources (Ehrlich *et al.*, 1980) or at the egg laying stage if females are unable to find suitable oviposition locations (Himes Boor *et al.*, 2017). Observations from the captive population of Miami blues suggest this as well, as at high densities caterpillars

begin to cannibalize each other when food resources run low (J. Daniels unpublished data). Therefore, to improve the ability of our population model to realistically predict butterfly dynamics at high densities, we would include a function for larval survival that decreases with density.

For larger population sizes of 1,000 and 10,000 butterflies, there was still narrow range of diapause survival values (± 0.002) for which the uncertainty between individual climate models was the difference between population persistence and extinction. For this narrow range of survival values, the population is very sensitive to different precipitation patterns and the uncertainty in the climate models makes it impossible to predict the most likely fate of Miami blues by the end of the century. If this were true at all levels of diapause survival, reducing uncertainty in the climate models would be necessary in order to predict future dynamics and develop conservation strategies. Our approach of evaluating the relative threats of uncertainty in demographic parameters to climate change related threats highlighted that learning more about natural history is a critical need. Management to address aspects of demography is possible in a way that refining climate projections may not be. If we refine our understanding of life history and, in the case of Miami blues, identify strategies that result in increased diapause survival, we have the ability to increase the populations' resilience to climate change, regardless of the amount of uncertainty about the future.

Our mechanistic approach is an improvement over a purely correlational approach in terms of being able to identify research and management priorities that will enhance resilience to climate change. However, because of the large data requirements and detailed natural history knowledge required for mechanistic models, it may not be possible in many research settings. Despite the limitations of our data and knowledge of species biology, the results of our

population model provide useful recommendations for land managers and identify potential demographic tipping points at which the butterfly population may become extra vulnerable to extirpation. Our results point to opportunities to use model predictions to design lab and reintroduction experiments that will answer key questions and refine important parameter estimates. A logical first experiment would be to test the effect of rainfall on diapause duration and survival. This is the first step in the adaptive modeling framework proposed by Urban et al. (2016) and provides an example of how thinking mechanistically about species responses to climate change can help to elucidate specific vulnerabilities and identify potential management actions.

To manage species to increase their resilience in the face of a changing climate, we must know how climate interacts with species biology and leaves species vulnerable (or not) to climate change. There are many rare species that are understudied and likely highly vulnerable to climate change because of small population sizes and/or limited distributions. It is for these species that this adaptive mechanistic modeling approach could be extremely useful for identifying research priorities and management strategies. Even with limited biological data, we have shown that structuring models and experiments in this way can be used to evaluate population sensitivities to environmental change and potential methods for building adaptive capacity for populations, be that enhancing survival of a specific life-stage or improving connectivity among habitat fragments. Our mechanistic modeling approach provides a tractable way to test whether climate change or species demography is more likely to influence future persistence. Answering this question is fundamental for both understanding threats and prioritizing actions that will improve the odds of survival for vulnerable species in a rapidly changing global environment.

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FIGURES

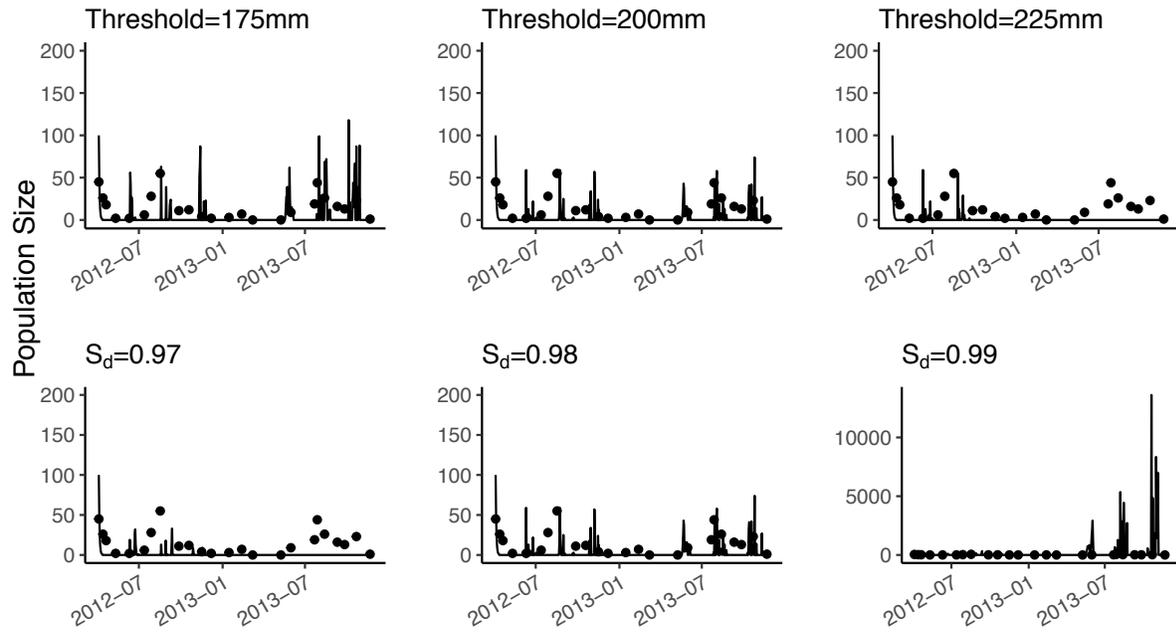
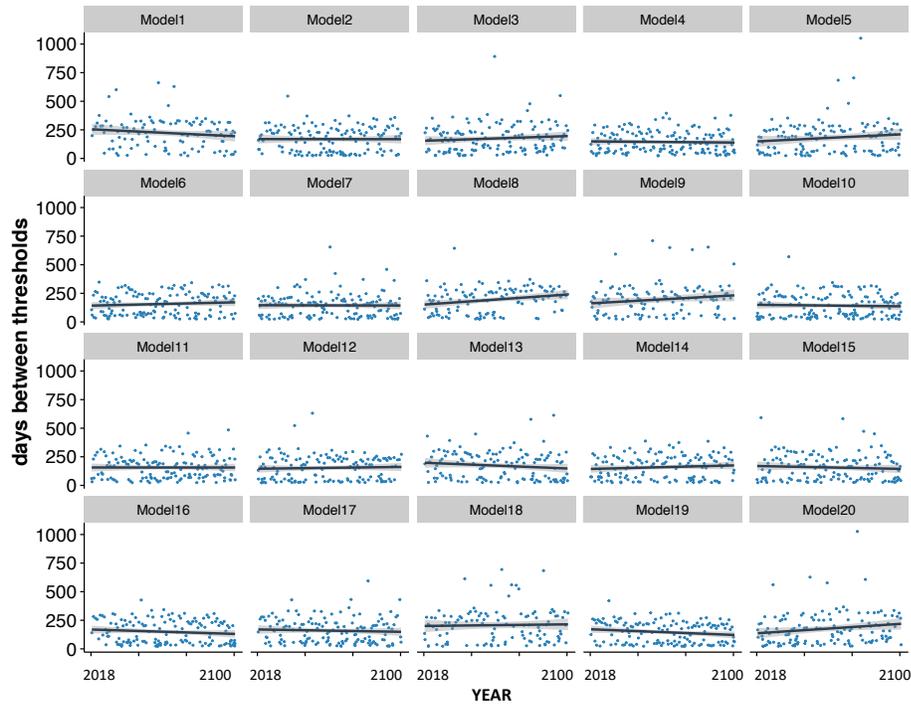


Figure 4.1. Model results of predicted butterfly abundance (solid line) compared to butterfly counts from Boca Grande in 2012 and 2013 (dots). Top panels depict effect of different precipitation thresholds, bottom panels depict different values of daily diapauses survival.

A



B

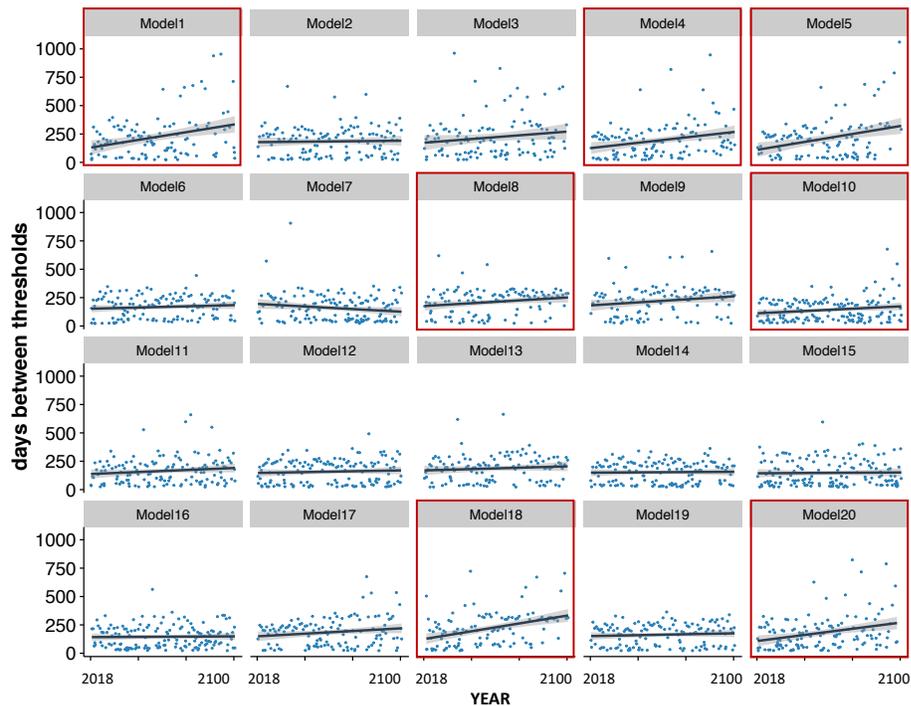


Figure 4.2. Correlations between days between threshold events and time for RCP 4.5 (A) and RCP 8.5 (B) for all 20 climate models in the ensemble (Model1 – Model20). Models outlined in red contain significant positive correlations ($\alpha < 0.05$) indicating drying trends through time.

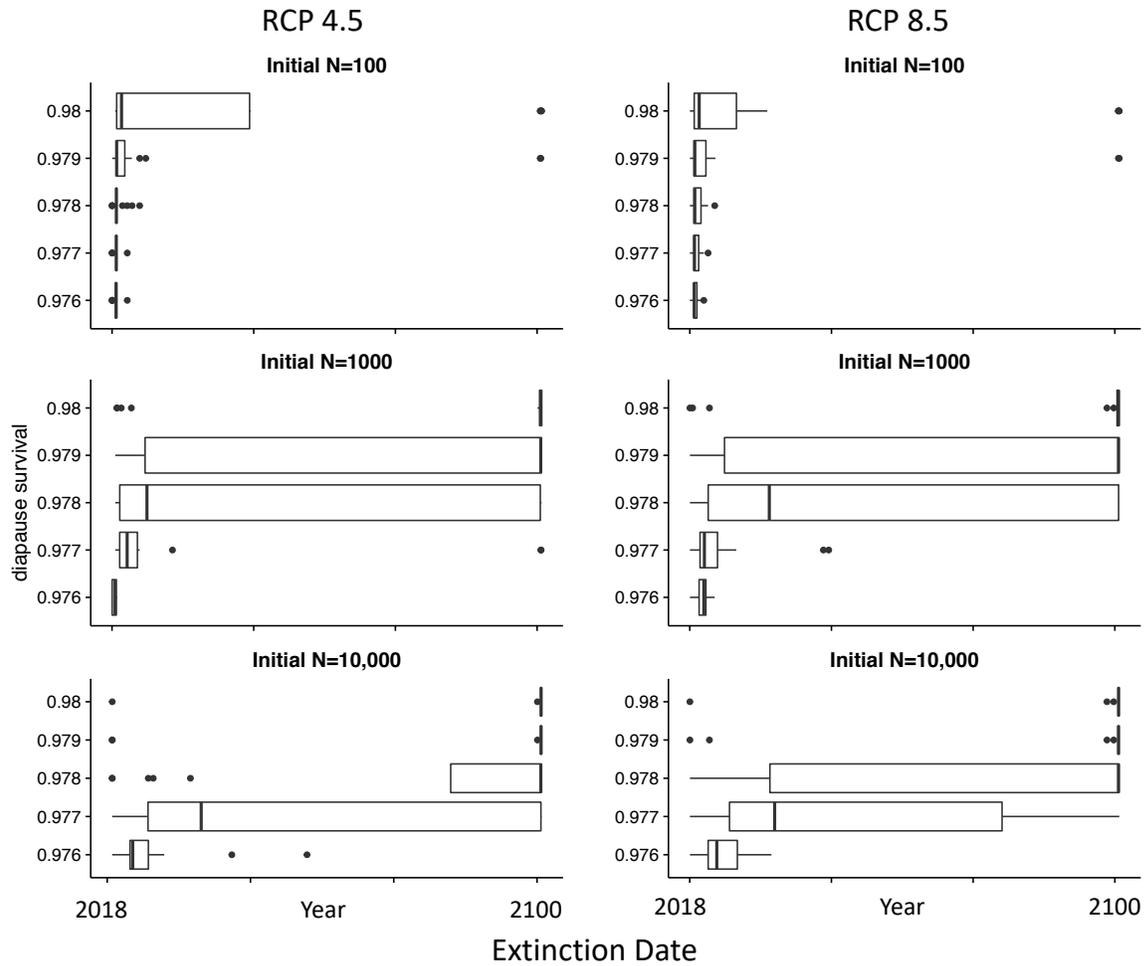


Figure 4.3. Extinction dates (day last butterfly is present in simulation) for all 20 models at each combination of daily diapause survival (along x-axis) and initial population size of 100 (top panels), 1000 (middle panels), and 10,000 (bottom panels). Models with “extinction dates” at 2100 predict population persistence to next century.