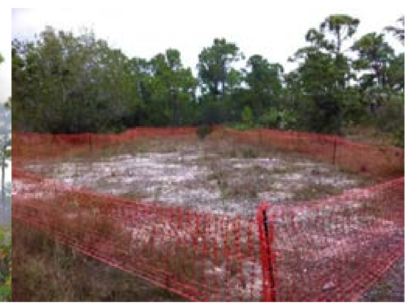
U.S. Fish & Wildlife Service

Synthesis of Research, Monitoring, Management of the Bartram's Hairstreak in the National Key Deer Refuge 2009-2014



Submitted by:

Chad Anderson, Biologist
U.S. Fish and Wildlife Serice
Florida Keys National Wildlife Refuges Complex

Erica Henry
North Carolina State University

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Introduction

The Bartram's scrub-hairstreak (*Strymon acis bartramii*) is a small (1" wingspan), grey, butterfly dependent on pineland croton (*Croton linearis*), its only host. Females lay eggs singly on the racemes of croton plants and the butterfly's distribution is therefore closely tied to the distribution of their host. Pineland croton populations are confined to pine rocklands in south Florida. These forests once extended from north of Miami to Everglades National Park (ENP), 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. Given this dramatic decline of pine rockland habitat, both Bartram's hairstreak and pineland croton populations have accordingly declined and become increasingly fragmented. There are likely 3 metapopulations of Bartram's scrub-hairstreak remaining in extreme southern Florida: Long Pine Key in Everglades National Park, pineland fragments in Miami-Dade County, Big Pine Key in the National Key Deer Refuge (NKDR) in the Florida Keys (USFWS 2014). Habitat loss is further compounded by degradation of remaining habitat due to loss of disturbance. Bartram's hairstreak habitat protected within ENP and NKDR is vulnerable to lack of fire and subsequent forest succession. Historically, pine rocklands burned frequently, maintaining gradual succession and a robust herbaceous and shrub layer (Albritton 2012, Harley 2013). These factors, and others, have contributed to the Bartram's hairstreak recently being listed as federally endangered (USFWS 2014).

Because of the growing number of threats to the species and its apparent decline, the Bartram's hairstreak has increasingly become a conservation priority. Previous efforts to determine trends generally focused on counts of adult butterflies from established reference transects or informal surveys. While these surveys where instrumental in learning some basic some life history information and provided relative abundance trends, many questions remain unanswered. Data gaps regarding habitat needs, threats, and even some basic life history information still exist. The most comprehensive survey to date, conducted by Mark Salvato, (Salvato 1999, Salvato and Hennessy 2004, Salvato and Salvato 2010, ect.) has reported seemingly drastic declines in counts of Bartram's hairstreaks since 1999. Recently, these surveys have been unable to detect trends due to low counts on reference transects. While this likely represents a long term decline, it is unclear whether these trends accurately represent the population or are a result of loss habitat quality within reference transects. To better inform management actions, a survey methodology is needed which is highly adaptable (i.e. can incorporate all known croton patches spatially and temporally), can accurately estimate abundance in order to track population trends over long time frames, and is practical to implement. In addition, detailed studies on preferred habitat are needed in order to set management goals. Several studies (Hennessey and Halbeck 1992, Salvato 1999, Pierce 2009, Bargar 2013) have focused on other potential causes of decline, such as mosquito control chemicals. However, no previous studies have defined the island-wide distribution, estimated population numbers, or quantified key habitat characteristics in a way that can help to inform management actions. For these reasons, the following objectives were identified based on the goal of collecting information to better inform management.

1. Design and implement a monitoring methodology to determine seasonality, track trends, estimate abundance, and provide spatially referenced data to assist in species management and recovery efforts.

2. Quantify vegetative characteristics to describe current habitat characteristics and analyze historic data
3. Collect data on the effects of fire and mechanical clearing on croton.
4. Explore methods to augment existing *Croton linearis* population such as planting and seeding.

Objective 1: Design and implement a monitoring methodology to unbiasedly track trends, estimate abundance, and provide spatially referenced data to assist in species management and recovery efforts.

The first step to implementing an effective monitoring approach was to define the potential habitat that could be used by the Bartram's hairstreak. Croton count data from Bradley's (2007) pine rockland vegetation survey was used to create an interpolated surface with the natural neighbor calculation in Arc GIS 9.2 (ESRI Inc., Redlands, CA). Six, one-hectare reference plots were then established within the areas of highest *Croton linearis* (here forward croton or *C. linearis* or croton) density, 2 in the northern part of Big Pine Key (BPK), 2 in the central, and 2 in the south. These plots were surveyed for Bartram's hairstreaks beginning in July 2010. Each plot was surveyed for 30 minutes in north-south or east-west transects. These sites were surveyed bi-monthly throughout the flight season of 2010 until Bartram's hairstreak butterflies were no longer found in September, thereafter only one plot per area (north, central, south) was surveyed until August 2011with the assumption that peak flight would again occur in August as witnessed the previous year. However, the peak of activity in 2011 occurred earlier in the late spring/summer (Figure 1). The highest density recorded was 7 butterflies per/ha in the northern area of BPK in May of 2011. From these data, the "flight season" could roughly be defined as spring through summer, however the species was found at low numbers in nearly all months.

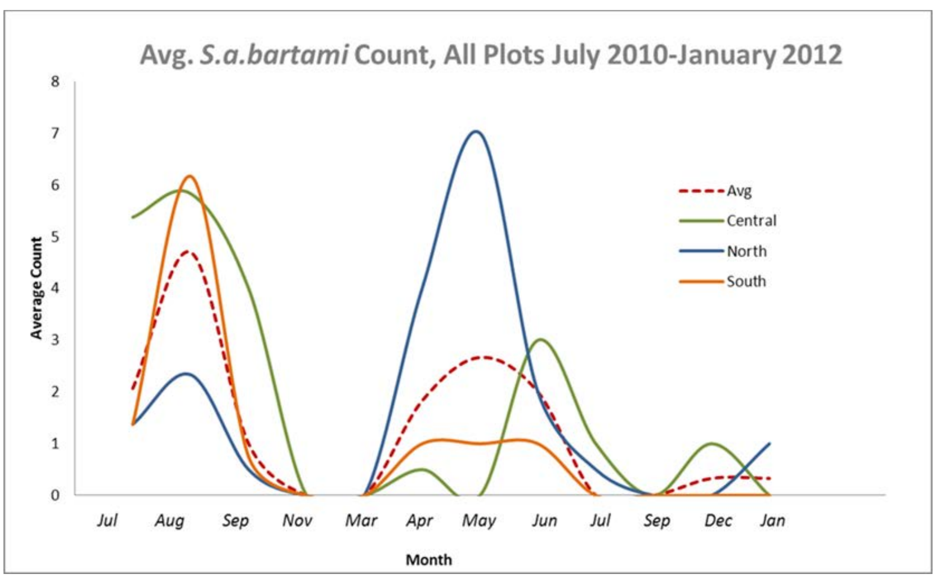


Figure 1: Average number of monthly observations of Bartram's Hairstreaks in reference transects on Big Pine Key July 2010 through January 2012.

One of the main purposes of this survey was to determine the optimum sampling period by searching the best areas possible on a regular basis to collect the maximum number of detections. While generalizations could be made, the irregular shape of the flight curves in an individual patch and the asynchronous nature of the peak densities prevent definitive answers regarding "optimum" sampling period.

To move beyond the standard monitoring and enact a survey method that could account for detection probabilities to more accurately estimate abundance, new techniques and survey methods were explored beginning in 2012. The incorporation of detection probability into estimates of animal abundance is increasingly common among rigorous wildlife research studies. Detection probabilities improve indices generated by survey counts by accounting for individuals that were available to be detected but were not observed. The two methods commonly used to estimate detection probabilities for butterflies are mark-recapture (capture-recapture) and distance sampling from line transects (Ehrlich and Davidson 1960, Burnham and Overton 1979, Gall 1984, Brown and Boyce 1998, Baguette and Schtickzelle 2003, Haddad et al. 2008). Both methods provide reliable estimates when assumptions are met, but also have limitations, particularly when the species of interest is rare. In addition to detection probabilities, the mark and recapture technique allows for estimation of multiple demographic parameters such as abundance, survival, and recruitment. However, this method requires the recapture of a large number of individuals over a long period of time. In addition, mark and recapture includes the potential risk of damaging individuals (Murphy 1987). For these reasons, use of mark and recapture is often impractical for federally threatened or endangered species. A small-scale mark-recapture study was previously attempted for Bartram's hairstreaks (Salvato pers. comm.). Due to the low densities of the butterflies, no marked butterflies were ever re-captured. Unlike mark-recapture, distance sampling is minimally invasive, but requires at least 40 detections to accurately model the decline in number of detections with distance. This number may be reasonable for common butterflies, but detecting a sufficient number of rare butterflies may be difficult, especially when the target population is small. Another method for estimating detection probabilities is the double-observer survey (Nichols 2000); this method is rarely used in butterfly research but has been suggested as an alternative method for estimating butterfly abundance (Haddad 2008, Nowicki et al. 2008). The limitation inherent in the double-observer method is the need for two observers, which increases the cost of surveys.

Double-observer and distance sampling methods could both prove useful to estimate the abundance of Bartram's scrub hairstreaks. Distance sampling is the most cost-effective method because a single observer can collect the data. The biggest challenge to surveying Bartram's scrub hairstreaks, however, is that emergence times and corresponding peak abundances are do not always occur in the same season and are not always synchronized across habitat patches. For this reason, no apparent sampling window can be pre-identified, potentially making it difficult to meet the minimum requirement of 40 detections for distance sampling (Buckland et al 2005). Double observer methods can be applied when only 10 individuals are detected (Nichols et al. 2002), making this an attractive method for monitoring a species that occurs at low densities. To estimate peak Bartram's scrub hairstreak abundance, we collected data using both survey methods simultaneously, and evaluated the utility of each approach. While distance sampling has been widely used, no studies have compared estimates to those produced by double-observer surveys. Here we present a comparison of methods that may have broad applications to rare species of many taxa and provide alternative methods to land managers with similar logistic, financial, and time constraints.

Data collection

To sample the entire potential habitat that could be used by the Bartram's hairstreak survey grid data from Bradley (2009) was used to create an interpolated surface using Arc GIS 10.0 (ESRI Inc., Redlands, CA). Based on the interpolated surface, high value croton areas and the adjacent raster cells were systematically ground-truthed on a 200x200 meter raster grid for the presence of croton. Only the highest value croton areas of the (>15 plants/ha) were retained. Areas which are routinely occupied and unoccupied from 2009-2013 where found to have an average of 103 and 15 plants per/ha, respectively (Anderson and Henry 2014a).On Big Pine Key there are three core patches that meet this criteria, the north (4.8 ha), central (6.6 ha), and south occupied patches (1.5 ha) Figure 2. In each patch we established a grid of parallel 50m transects spaced 10 meters apart.

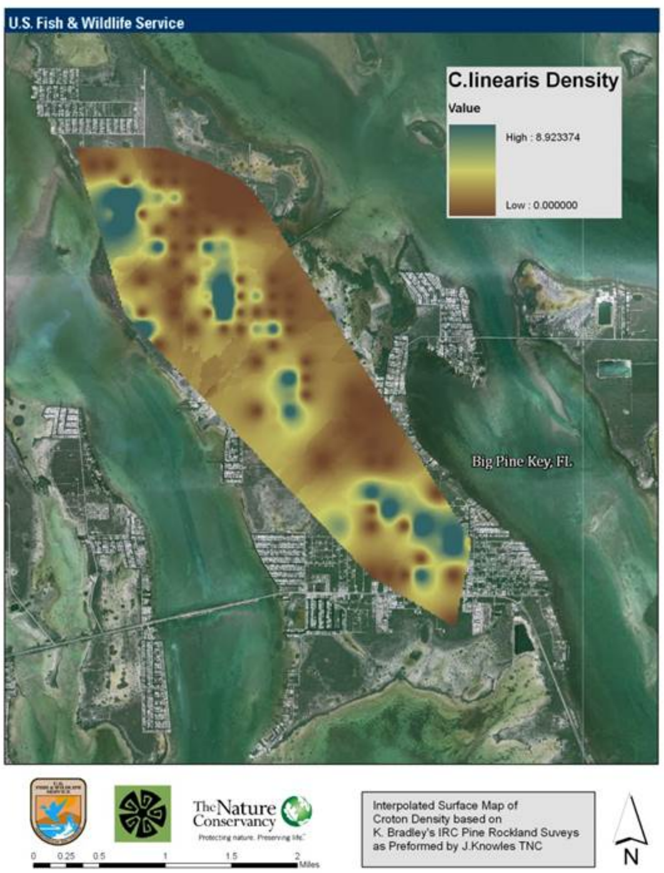


Figure 2: Map showing interpolated surface created from 2007 croton data (Bradley 2009).

We oriented transects either north-south or east-west according to the shape of the patch (Figure 3). We conducted surveys once per month from March 2013 – December 2013 with effort increased to weekly surveys when butterflies were present. All surveys were conducted between 0900 and 1530 h when temperatures ranged from 22-36° C and winds were light to moderate (<15kts).

On each survey date, we randomly selected one third of all potential transects in each croton patch (20 in the north, 30 in the center, and 10 in the south). Two observers walked each transect simultaneously and independently counted all Bartram's scrub hairstreaks she/he observed. For each butterfly detected, both observers estimated the perpendicular distance (to the nearest half meter) from the transect line to the location where he/she first detected the butterfly. At the end of each transect, the two observers reconciled which butterflies were detected by observer A only, by observer B only, or by both observers. For butterflies that were detected by both observers, they determined which observer was the first to detect the butterfly and recorded the detection distance for that observer, although, there was typically little variation in distance estimates between observers. Reconciling observations for an individual butterfly was easily achieved because the maximum number of butterflies observed on one transect was four.

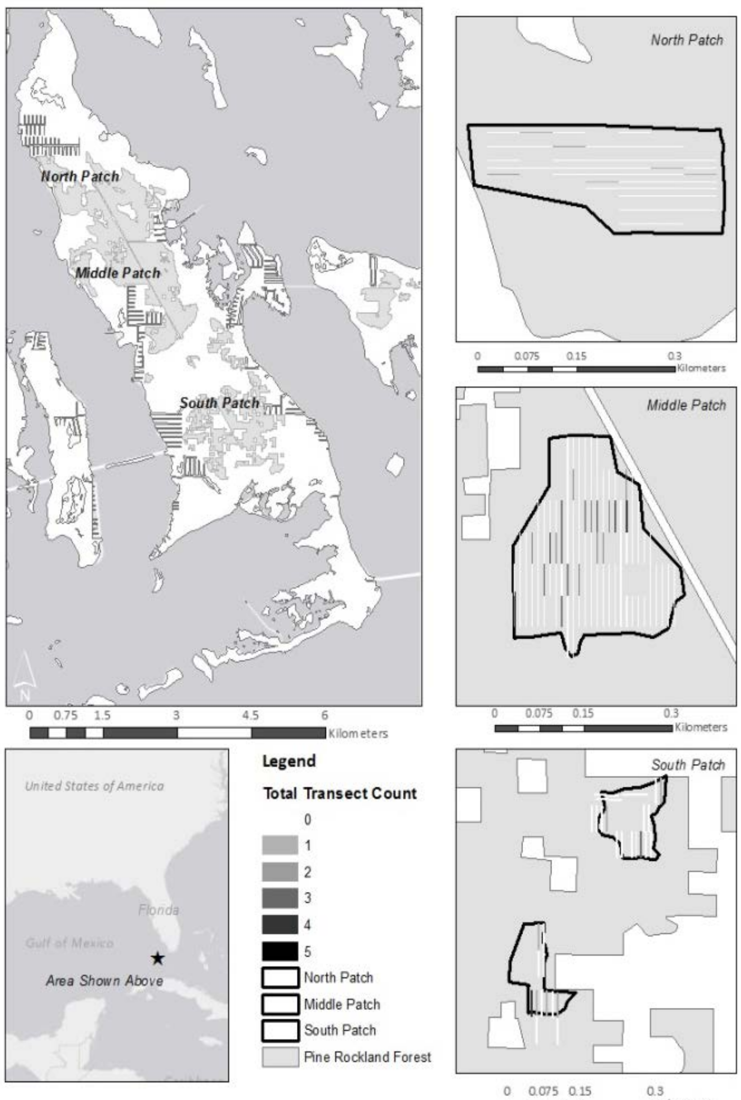


Figure 3: Survey areas and counts during 2013 peak flight period

Distance analysis

To accurately fit a detection function to distance data, Buckland et al. (2005) recommend a minimum of 40 detections. Bartram's scrub hairstreaks occur at relatively low densities, we did not detect 40 butterflies on any individual survey. Therefore, we pooled distance data from all butterflies detected during our 2013 surveys to create a global detection function that we could then apply to butterflies observed during distinct periods throughout the year. By pooling data, we are assuming that detection probability is uniform across both time and space.

Using the pooled data, we tested the following model/series expansion combinations in program DISTANCE (Thomas et al. 2010): half normal model with cosine adjustment, half-normal model with hermite polynomial adjustment, and hazard rate model with simply polynomial adjustment (Thomas et al. 2010) and used Akaike's information criterion (AIC) values and chi-square goodness-of-fit statistics to select the best model. These models approximate the shape of the decline in detections with distance from the observer and allow for the estimation of detection probability and the effective strip width – the distance at which the observer misses as many butterflies as she detects. We then applied this detection function to the detections from the survey period of the flight season during which we observed peak counts and estimated a peak daily density for Bartram's scrub-hairstreak.

Double-observer analysis

To estimate detection probability and a peak daily abundance using the double observer method, we used the multinomPois procedure in the unmarked package (Fiske and Chandler 2011) for R v3.0 (CRAN 2006). For this analysis we used observer data from the week during which we observed peak, island-wide counts. To evaluate model fit, we used the parametric bootstrap (parboot) function to generate a chi-square statistic for the model. The multinomPois analysis estimates abundance of butterflies per transect. Unlike distance sampling, double-observer method does not estimate transect width based on the data. Instead, it assumes that observers walked transects and counted all butterflies detected within a pre-determined distance. Because we are interested in comparing the estimates of detection and abundance derived from the two methods, we defined the width of the transects using the effective strip width estimated by Distance as a guideline. For the purposes of distance analyses, an "effective strip width" is the area in which an observer detects more individuals than they miss. Therefore, in our double-observer analysis we included only observations within that distance. To estimate total daily density, we multiplied the estimated abundance per transect by transect area and converted that number to butterflies per hectare.

Results

We conducted 16 complete, island-wide, surveys in 2013, detecting a total of 59 Bartram's scrub hairstreaks. Throughout the year, we picked up one major flight period between April 30 and May 23, 2013 (Figure 2). During this peak flight period, we conducted 5 complete surveys in which we counted a total of 43 butterflies. Thirteen of those butterflies were observed in one complete, island-wide survey on May 3, 2013. These 13 detections were used for estimating density with both distance and double observer methods. Bartram's scrub-hairstreak numbers began to decline shortly after our peak count and by the end of June we no longer detected the butterflies on our surveys.

Distance

The model with the lowest AIC value was the half normal model with cosine adjustment (X^2=3.27, p=0.35). The estimated detection probability was 0.3 (95% conf interval 0.26-0.41). Our estimated effective strip width was 1.83m. Estimated density during the peak of Bartram's flight in 2013 was 12 ± 3 butterflies per hectare. When applied to the total area of croton habitat we surveyed on Big Pine Key we estimate a peak daily abundance of 156 ± 31 Bartram's scrub-hairstreaks.

Double observer

The multinomPois procedure estimated a detection probability of 0.67 ± 0.12. This is the probability that a butterfly will be detected by at least one of the observers. The bootstrapped chi square goodness of fit test was non-significant (p=0.278). The estimated detection probability translated to an abundance estimate of 0.26 ± 0.08 Bartram's scrub hairstreaks per transect. Using the ESW of 1.83m estimated by DISTANCE as a guide, we defined the appropriate width of transects to be 4 meters. When we applied this abundance to our transects (50m x 4m) we estimate a density of 13 butterflies per hectare, which translates to a peak abundance estimate of 169 ± 51 Bartram's scrub hairstreaks on Big Pine Key.

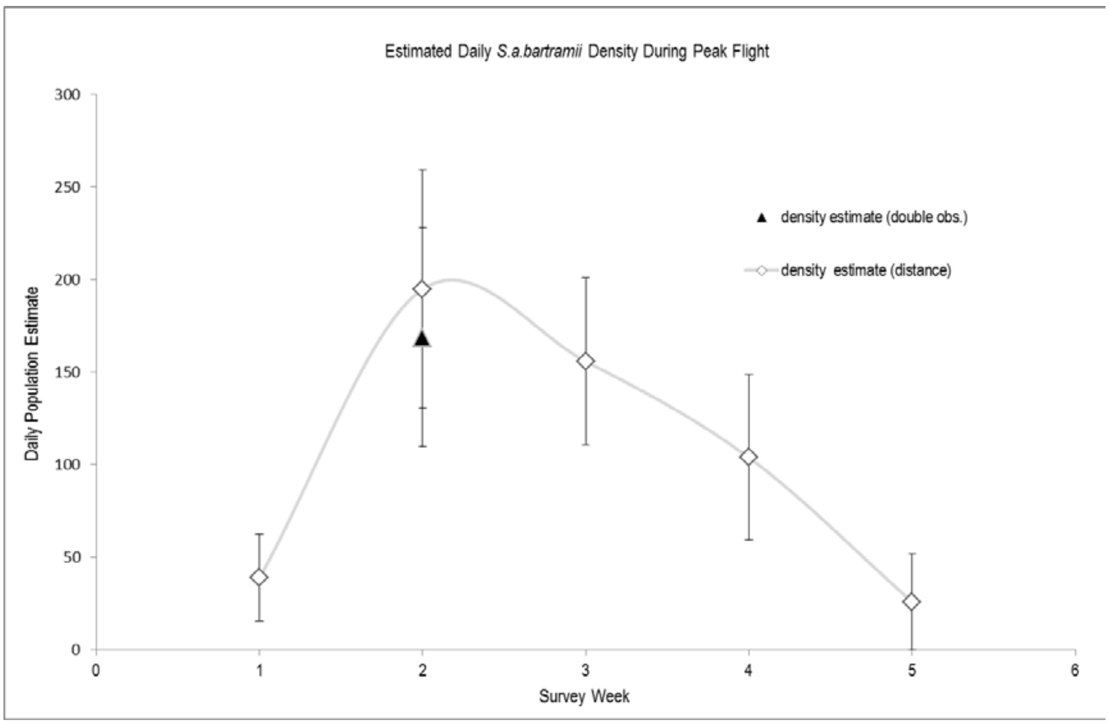


Figure 4: Weekly estimates for Bartram's hairstreak butterflies using distance and double-observer methods over the peak flight period of 2013.

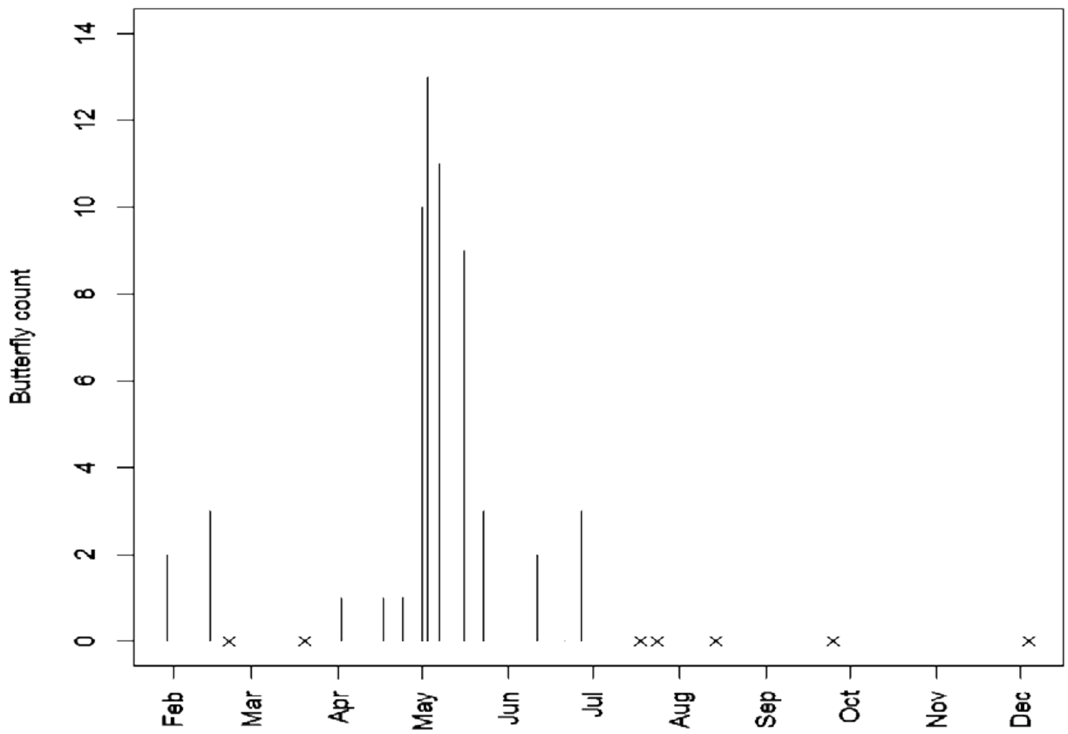


Figure 5. Adult butterfly counts from island-wide surveys in April 2012 and December 2013

Discussion

Double observer and distance sampling and methods are both feasible options for estimating detectability and abundance of a rare species. Because double observer methods can be used to estimate detection probability when as few as 10 individuals are detected (Nichols et al. 2000), this method is useful in the estimation of abundance for very rare insect species. With so few detections, the variability in detection probability estimates can be high, however, the method can still provide important information when species are so rare that other methods are impossible to implement. The drawback to double observer surveys is the cost associated with two observers. If the detection probability of a target species proves to be stable over time, a single estimate could be applied to data collected by one observer, thus reducing future survey costs.

The key to applying these methods to species that occur at low densities is to ensure that assumptions and necessary sample size requirements are met. The most crucial assumption for a double observer survey is that observers are independent and can easily reconcile observations at the end of each transect (Nichols et al. 2000). When species occur at high densities – likely more than 5 reconciling observations could be difficult. For these species, however, it would likely be easy to detect the minimum of 40 butterflies necessary to fit detection curves to distance data (Buckland et al. 2005). Conversely, when species occur at low densities, this target of 40 detections can be a difficult to obtain. Pooling data across multiple surveys and/or sites and developing a global detection function is one strategy that can be used to overcome the limitation of rare species (Henry et al. in review) This method assumes that detection probability of the target organism is constant across time and/or space. If this is unlikely to be true, pooling data can lead to biased abundance estimates. Bias, however, can be minimized by accounting for environmental variables likely to influence detection, such as temperature and wind, by only surveying under specific weather conditions. Additionally, covariates can be incorporated into the detection probability modeling process, as long as sufficient detections exist. To use distance sampling for Bartram's scrub-hairstreaks, we pooled data across time periods *and* occupied patches. Pooling across time periods was reasonable because the butterflies occur in sub-tropical habitat; there are not dramatic vegetation changes across the year that would significantly affect detectability. Pooling data from different habitat patches on Big Pine Key is also reasonable; all three habitat patches are structurally similar in terms of vegetation that could impede detection of butterflies.

The biggest remaining challenge associated with monitoring Bartram's scrub-hairstreak is our lack of understanding of the drivers of the butterfly's lifecycle. Individuals have been detected in every month of the year (Salvato 1999, USFWS 2013b, See Obj. 1). This inter-annual availability in adult flight makes it difficult to target surveys during periods of peak abundance, because we are attempting to optimize the amount of time spent surveying throughout the year with the number of butterflies we are likely to detect. With a solid understanding of the phenology of the species, we could target survey effort during time periods when we know we will detect butterflies and avoid year around sample efforts which make a comprehensive monitoring program burdensome to implement. This would likely increase the number of butterflies we detect, therefore, potentially decreasing the variability in our density and abundance estimates.

Management Implications

Our Bartram's scrub-hairstreak density and abundance estimates are the first to utilize a systematic survey of occupied habitat and incorporate detection probability; the methods that we used on Big Pine Key could be incorporated in other conservation areas within the range of the species, and elsewhere. The Bartram's hairstreak persists in areas with a wide variety of management needs and questions that remain largely unassisted by monitoring. The methods described here could be utilized effectively at any scale and could be used to move beyond informal survey and "hot spot" index site or transect sampling, especially given the considerable rarity of this species.

For the Bartram's scrub-hairstreak, both distance sampling and double observer methods produced similar estimates of abundance despite the different approaches used in the estimation process, and these numbers were low. Our surveys confirm that Bartram's scrub-hairstreak is rare and therefore further conservation efforts are needed. Efforts to expand and connect existing patches such as mechanical clearing and prescribed fire are likely necessary to provide the habitat needed to prevent declines or possible extirpation of this subpopulation given the limited area which remains in a condition suitable for occupancy. To the extent possible, efforts such as host plant augmentation to sites should be expanded to maximize total available habitat. The recent proposed listing of the Bartram's scrub hairstreak seems appropriate given our estimates and the butterflies relative rarity among all butterflies, globally.

Objective 2: ***Quantify vegetative characteristics to describe current habitat characteristics and analyze historic data***

Quantification of Vegetation characteristics

Quantification of habitat parameters is an important factor in predicting occurrence of a species, setting management targets, and identifying appropriate survey areas. Little is known about the preferred habitat characteristics of the Bartram's scrub-hairstreak. Previous efforts have roughly described their habitat, however explicit efforts to quantify habitat variables have not occurred. Defining these variables could be useful in management efforts to improve the quality of habitat for recovery of the Bartram's hairstreak. Large shifts in vegetation composition and structure have occurred over the last 60 years concurrent with the butterfly's decline (Dickson 1955, Bradley et al. 2014). While little is known about historic hairstreak population sizes and changes over time, some information exists about the habitat, which may be used to understand the relationship between the apparent loss and decline of taxa, including the Bartram's hairstreak. Surveys to understand and quantify the existing habitat and historic habitat are important improve understanding how recovery might be achieved.

To accomplish this, we sampled vegetation in 2.5-meter radius plots at both ends of each butterfly survey transect (n=132 plots). Vegetative cover classes were recorded using ocular estimates (similar to Daubenmire 1959) of broad groups, shrub, graminoid, palm, bare ground, and leaf litter. In addition, croton plants were counted in each plot. The values collected within survey areas were then compared to adjacent plots in unoccupied pine rockland areas (n=63).

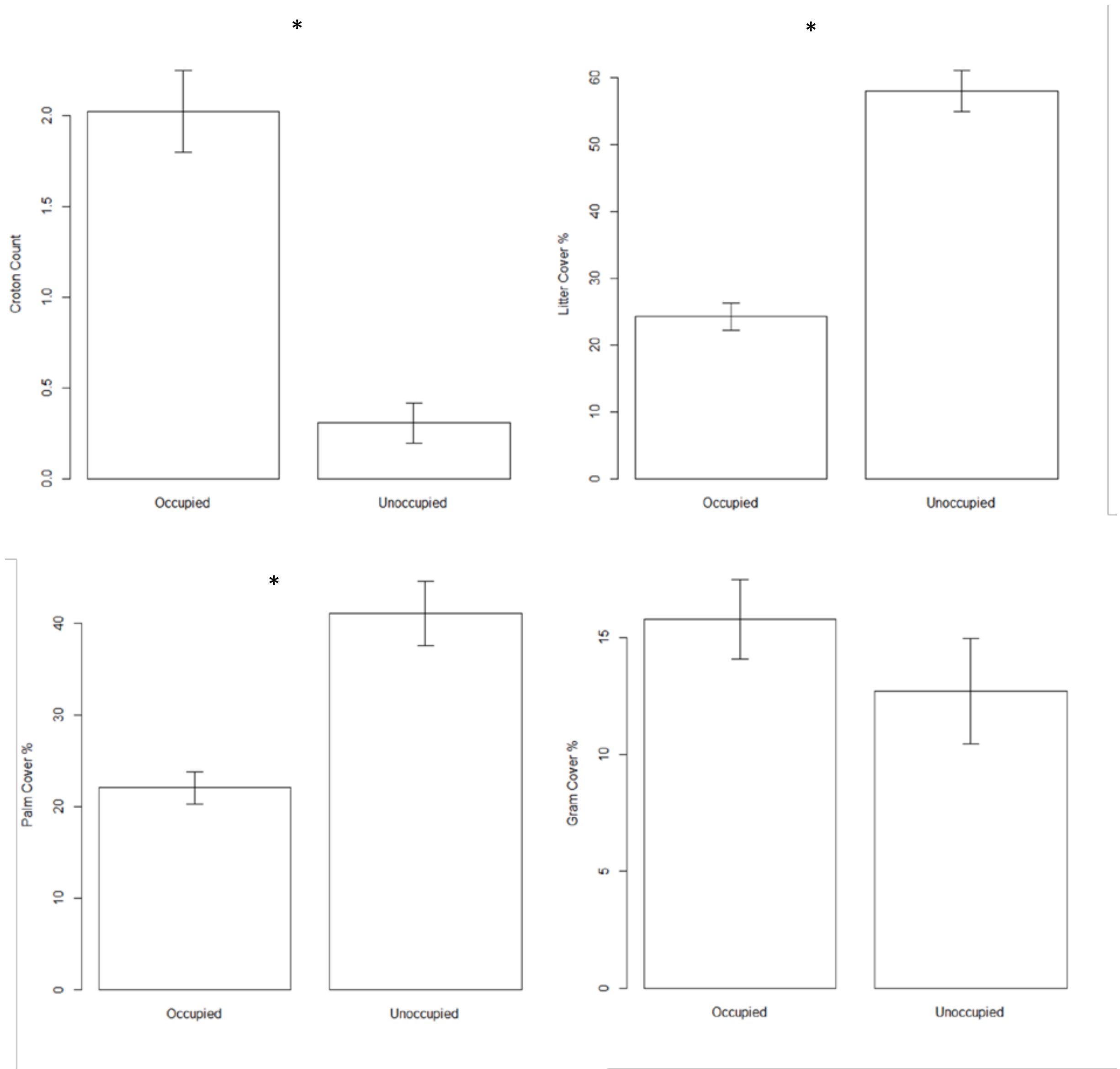
In addition to vegetation plots in currently occupied and unoccupied areas, historic data was used to track trends of the host plant, *C. linearis*, which is strongly associated with butterfly success. All known vegetation data sets for Big Pine Key where searched for croton counts, frequency, density, or percent cover.

Results

Vegetation in occupied and unoccupied areas

Areas that were generally occupied during peak flights had a significantly higher croton count (W=6130, $p < .0001$), less palm cover (W=2166, $p < 0.0001$), and less litter cover (W=1277, $p < 0.0001$) but not percent bare ground or graminiods (Fig. 4). Hairstreak occupancy occurred where croton count values were on average 2.02 (±1.57-2.50) per plot or 0.11 per m^2 (.09m^2-.13m^2). In contrast, mean croton count in unoccupied areas was significantly lower (W=6130, $p < .0001$) at .22 (.08-.53) per plot or 0.02 (.005-.03) m^2. As one would expect, occupied areas were significantly correlated with a greater proportion of croton (Kendall's tau= 0.399, p

<0.0001). Litter cover (needle cast, fallen leaves, ect.) was significantly lower in occupied (58.03% ±4) than unoccupied areas 24.24% ±6.28). Occupancy was negatively correlated to litter cover (Kendall's tau= -0.50, $p < 0.0001$). Palm cover was significantly higher in unoccupied (41 ±7.14%) than unoccupied areas (22 ± 3.54%). Occupancy was also negatively correlated to palm cover (Kendall's tau= -0.35, $p < 0.0001$), though not as strongly correlated as to litter cover. Hardwood shrub cover in occupied areas was 14.11% (±2.57). Comparisons of bare ground (rock, soil) and graminoid (grass-like plants) cover in occupied and unoccupied areas were non-significant (Fig. 4).



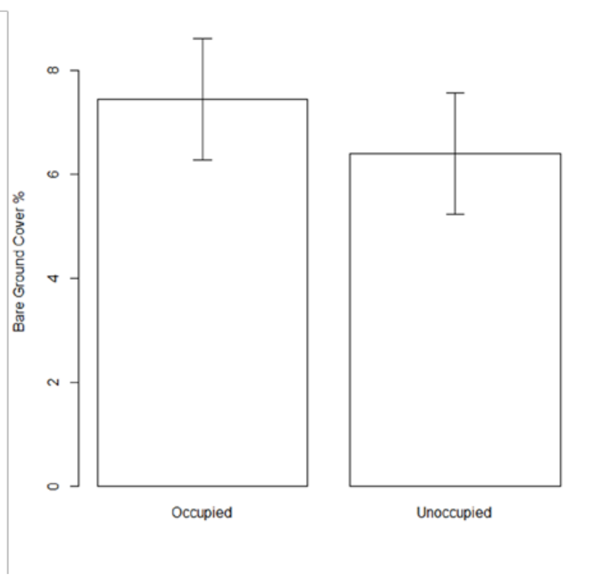


Figure 6: Comparison of habitat variables in occupied and unoccupied areas. Wilcoxon Rank Sum Test significance (shown as *= p <0.0001). Error Bars ± 1 standard error

In order to assess differences across occupied patches (north, central, south) Kruskall-Wallis rank sum tests for all habitat variables were conducted. Significant differences in locations were found in graminoid cover (χ^2=30.92, p <0.0001), Shrub Cover (χ^2= 10.35, p <0.001), pine cover (χ^2=29.49, p <0.0001), palm cover (χ^2= 15.32, p <0.001), and percent bare ground (χ^2= 11.88, p <0.01) but not found in croton count or litter cover.

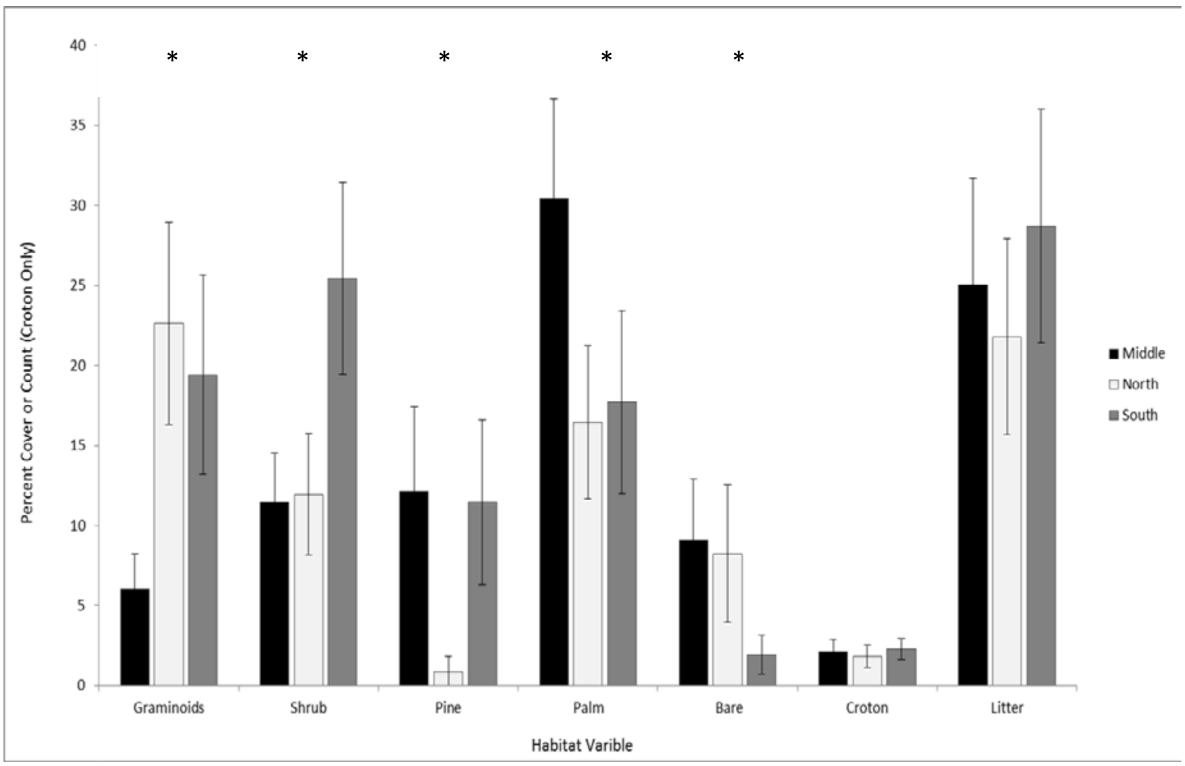


Figure 7: Mean percent cover per plot and count per plot (pineland croton) for all Bartram's Hairstreak Patches. Asterisks represent level of significance difference within patches using Kruskall-Wallis test (**= p <0.001, ***= p <0.0001). 95% confidence intervals are shown around the mean.

Though differences between occupied patches are present (Fig. 5), interestingly, little difference exists in the values that delineate occupied from unoccupied areas (Fig. 4) namely croton count, litter, and palm cover.

Discussion

These data indicate which habitat parameters are associated with Bartram's hairstreak occupancy. Patches with butterflies have significantly more croton, and lower litter and palm cover. These variables would be best used for target setting, especially during prescribed fire operations or mechanical habitat treatments. Based on these results, key habitat targets should be average palm cover no more than 25%, average litter cover of no more than 25%, and croton density should be at least 0.1 plants/m^2. Currently mean palm cover across BPK is 29.39%, mean shrub cover is 41.3%, and mean croton cover is 0.014 plants/m^2 (Bradley et al. 2014). Average shrub cover was 14.11% in occupied areas, which is substantially lower than the mean shrub cover across BPK (41.3%) – however, it should be noted that this percentage includes palm cover in total shrub. Regardless, shrub cover is likely a significant factor in Bartram's occupancy. The plots in occupied areas considered shrub cover independently of palm, so statistical comparisons of total shrub between occupied and unoccupied areas cover could not be drawn. Regardless, efforts to reduce palm cover and leaf litter would similarly reduce shrub cover. Croton density requirements derived from this study could be realistically used when planning host plant augmentations to improve habitat for the species, especially when paired with our finding that the smallest regularly occupied croton patch was 1ha. Combining these findings one could assume that 1000 plants within a 1 ha area could be minimally sufficient for an independent patch. While a good starting point, caution should be exercised as many other factors could influence distribution, fitness, and extinction/colonization rates of a Bartram's hairstreak patch. Restoration sites, and all management efforts, should be monitored intensely due to the large information gaps regarding the species biology and ecology.

Historic Vegetation Change

To understand why the Bartram's hairstreak has become endangered, it may be important to understand what long-term habitat changes have occurred which could have contributed, and continue to contribute to the species decline. Little information (i.e. adult counts) exists for the butterflies before the 1990's. Even so, changes in habitat and host plant abundance can be gleaned from historic studies of pine rockland habitat on BPK. Many studies have been done to assess pine rocklands in general and the habitat of the Key deer. Dickson (1955), Alexander (1972), Folk (1990), and Bradley (2005, 2007, 2014) have recorded *C. linearis* density, cover, and/or frequency which can be used to interpret trends over time. While not directly comparable to adult counts, host plant quantity and distribution is assumedly a major driver of potential population size. Because *C.linearis* in the sole host plant for the Bartram's hairstreak, understanding long-term changes (50+ years) to its abundance may be insightful for understanding the apparent decline of the species.

Dickson (1955) completed a fairly comprehensive vegetation survey in the early 1950's that lends a tremendous amount of information about vegetation on Big Pine Key, especially pine rockland plants. In Dickson's initial study, he noted that croton was one of the fifteen most common plants on the island, at 20% frequency, 0.3 plants per m^2, and 5-25% cover in plots. Alexander (1972) resampled Dickson's plots in 1972 because of the large changes, namely forest succession and hurricane damage, which had occurred during the 1960's. Alexander noted that croton was no longer one of the fifteen most common plants, and had declined to 13% frequency, 0.2 plants per m^2, and 2.5% cover in plots. Alexander implicated fire suppression as causal factor for plant community change at that time. Folk (1990) also recorded *C. linearis* and reported that average percent cover in 1990 was 2.97% and frequency was 20%. Interestingly, Folk's study reported a similar frequency to Dickson (1955), but a percent cover which was similar to the much reduced percent cover average presented by Alexander (1972). Keith Bradley et al. preformed three samples of all pine rocklands on BPK (2005, 2007, 2014) that provide information on croton cover and density. From 2005-2014 croton declined in percent frequency from 13.1% in 2005, to 9.6% in 2007, to 6.5% in 2013. Density was only recorded in 2007 and

2013, but also declined from 0.024 to 0.013 plants per m^2. Comparisons of these data may not be entirely appropriate as the sampling methods, intensities, and locations vary across studies. However trends implicating croton declines seems to be fairly consistent across all surveys, and all similarly repeated surveys show declines over time (Figs. 6, 7).

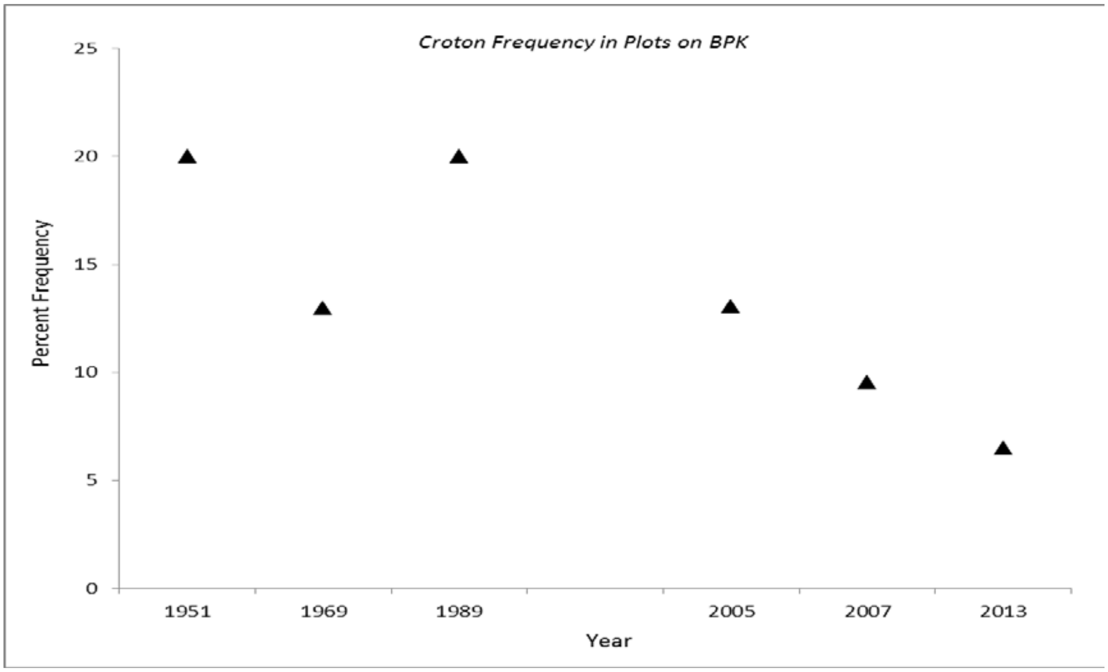


Figure 8: Frequency of C. linearis in plots on BPK 1951-2013

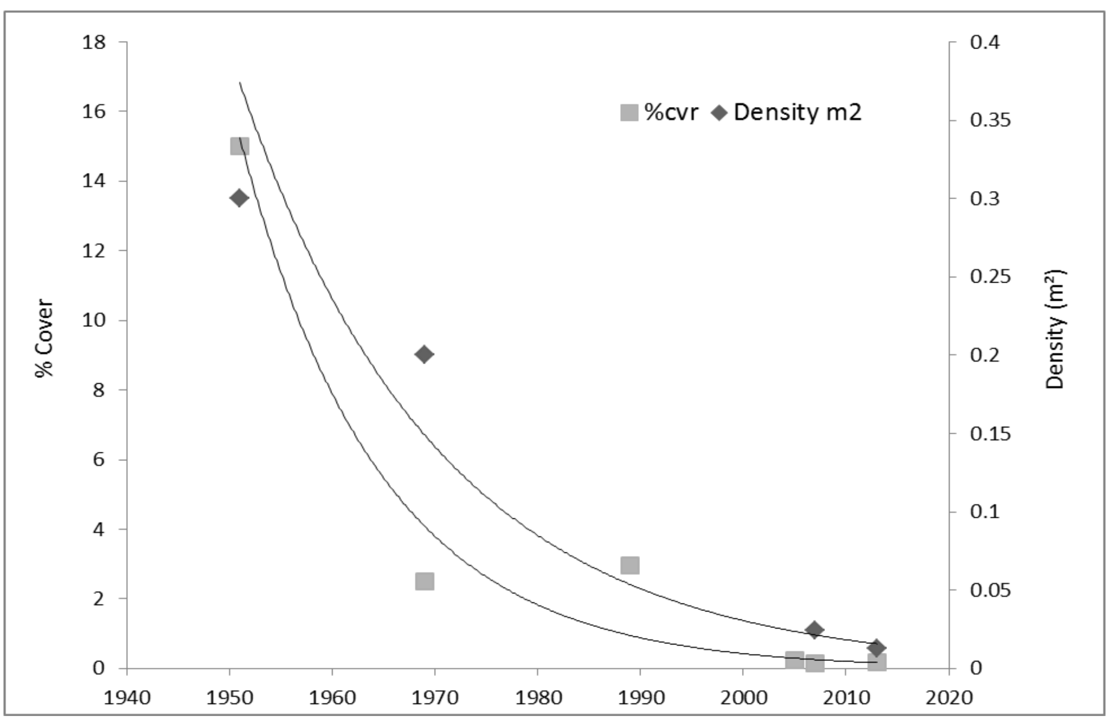


Figure 9: Percent Cover and Density (m^2) of C. linearis in plots on BPK 1951-2013

Objective 3: Collect data on the effects of fire on croton.

Introduction
C. linearis is an important nectar source for many pine rockland species. Most importantly it is the sole larval food source for two extremely rare candidate butterfly species, the Bartram's Hairstreak and the Florida Leafwing. Prescribed fire is commonly used as a management tool to restore pine rocklands, but little is known about the post fire response of many non-listed plants such as pineland croton.

Methods
Before the prescribed fire of 2009, 147 *C. linearis* plants were marked and then observed one year post burn. The original 147 plants, and 219 new seedlings, were revisited and measured for height, maximum crown size, presence of larvae and larval feeding damage one year post burn. In 2012 (3 years post burn) the plants were revisited. Due to the large number of new plants and seedlings which were present, plots were established to reduce sampling effort. In 2012, 33 10m x 10m plots were searched and observers counted and measured croton, and looked for signs of Bartram's hairstreak herbivory.

Results

1. Plants that exhibited signs of Bartram's larvae feeding damage were taller than plants without, averaging 42.37 (2 SE= ± 4.73 cm), and 28.53 (2 SE= ± 3.03m), respectively pre-burn.

2. There is a significant, positive relationship ($r= 0.30$, $t=1.51$, $p<0.001$) between plant height and herbivory.

3. One year post burn, 59 (41%) of the original plants resprouted and 219 additional seedlings germinated. Thirty-nine (27%) of the original plants were never burned due to mosaic pattern burning (Fig. 8).

4. One year post burn the average height of croton plants was 18.16 ± 1.1cm. Feeding damage was found on plants ranging from 16-83cm suggesting that the minimum height preferred by larvae was attainted on year post burn.

5. Three years post burn, there was an average of 3.37 croton plants per plot. Extrapolating this sample out to the entire burned area would result in an estimate of 348.41-890.52 plants (Fig.8). Mean height had decreased 3 years post burn from 33.4 cm to 16.23 cm, likely due to increased seedling recruitment.

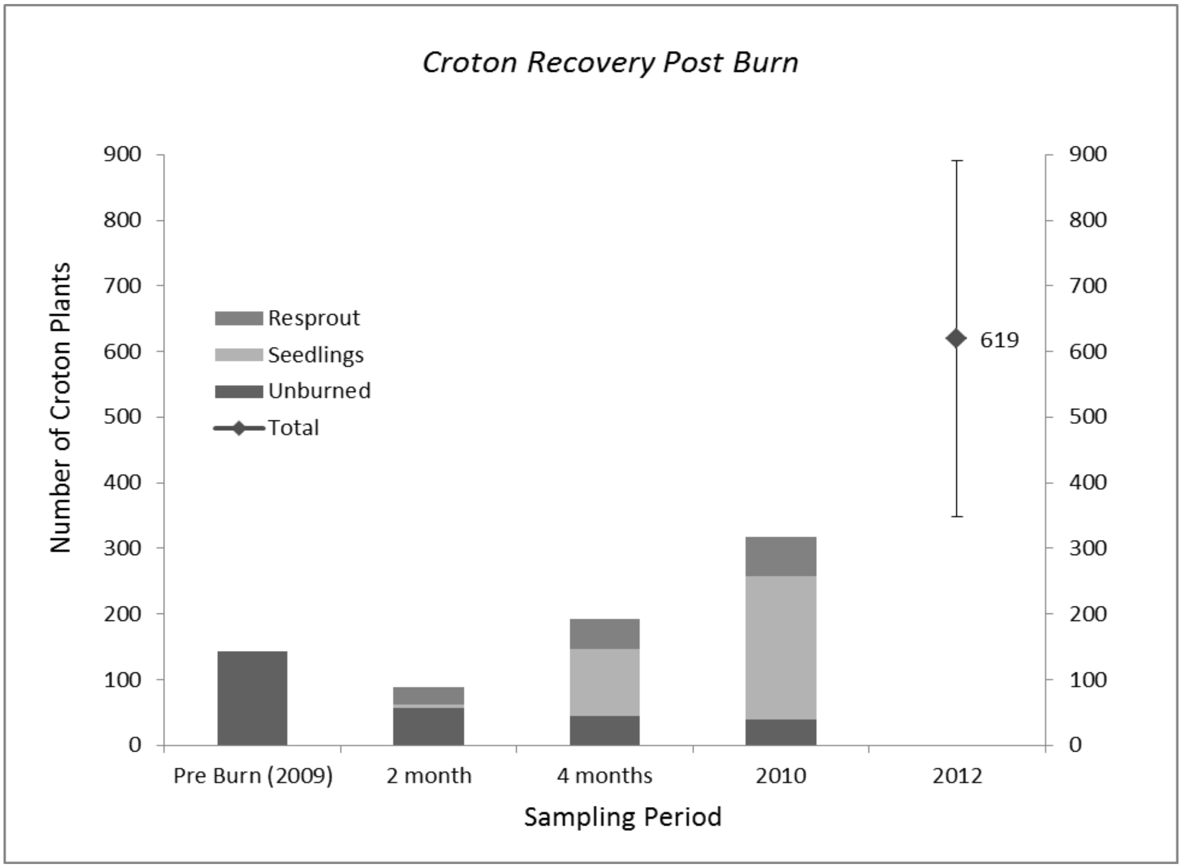


Figure 4: Croton counts, pre-fire through 3 years post fire; year 3 shown with ± 1 standard error bars

Management Implications

This limited study indicates that croton can positively respond to fire. However, more sampling should be conducted to explore if this trend continues. During this study period (2009-2014) limited opportunities were provided to expand upon this work due to the scarcity of both croton and burning opportunities. The burn unit from this study was directly adjacent to one of the largest, most dense, areas of croton which have may have influenced germination. However, there was a significant number of large croton in the unit pre-burn which remained to serve as a seed source post burn. Even so, seedling recruitment was found >100m from the nearest known source, indicating that some seed bank recruitment occurred. More information on fire effects, germination, phenology, seed bank longevity is needed to better anticipate the outcomes of either prescribed fire or wildfire to croton populations.

Objective 4: ***Explore methods to augment existing croton population such as planting and seeding.***

In addition to burning, host plant augmentation could be used to improve habitat. Two options explored during this time period were ex-situ cultivation and augmentation of wild populations, and in-situ direct seeding of croton.

Planting

To augment croton populations across Big Pine Key, we planted croton seedlings at three sites on the island, nature trail parking lot, Blue Hole, and The Nature Conservancy tract (TNC tract). Volunteers at Pennekamp State Park propagated seedlings from seeds we collected on BPK. In 2012-2014 we planted over 1000 seedlings and tagged 411 of them so we could monitor seedling survival and growth. Plants were installed during wet periods, typically early to late summer, to take advantage of rainfall. To monitor growth, we measured the size of each tagged plant by measuring the maximum length, width, and height of the plant (this roughly approximates the plant volume). Some plantings were fenced while others were not to test if key deer exclusion was necessary to increase croton success. While croton is not a primary food for deer, they are known to uproot seedlings and generally cause disturbance to plantings. Survival of the tagged croton seedlings within fenced enclosures was 60% and 66% while survival outside of fenced enclosures was 71.2%. Survival was not dramatically different between fenced and un-fenced planted areas. However, the unfenced seedlings were less vigorous; unfenced seedlings were, on average, 30% smaller (per plant volume approximation) than fenced seedling. Signs of browsing and trampling from deer were present at all sites where croton was unfenced. In fenced plantings, seedling volume was larger by 79.5% and 69.9% after one year and two years post planting. Overall, 3 areas were planted (Fig. 9) the two most significant ones (Blue Hole and TNC tract) were monitored.

About 50 plants were placed at the Nature Trail site initially, few if any of these plants were still alive 3 years after planting. After the Blue Hole fire the area was transformed from a mainly mulch ground cover to dense grass cover post fire. Between heavy deer use of the area (bedding, acacia browse, ect.), the fire, and the subsequent competition from fast growing grass, none of the initial plants survived. However, a small area on the northern portion of the parking lot (~25 plants) survive in a small fenced area, unfortunately, none of these initial plants were tagged. While none of these plants were tagged they are probably among the most robust and successful of all the plantings, likely due to nutrient and moisture supplementation from the mulch at the site.

Another site exists across from the Blue Hole parking lot which was planted in January of 2013. 82 plants were planted here, all of them tagged and unfenced. Within one year 28% of plants were lost and average plant size (length x width x height), decreased by 1568 cm^3, likely due to deer browsing and uprooting (Table 1).

Table 1: Summary of Blue Hole planting site. Original planting January 2013.

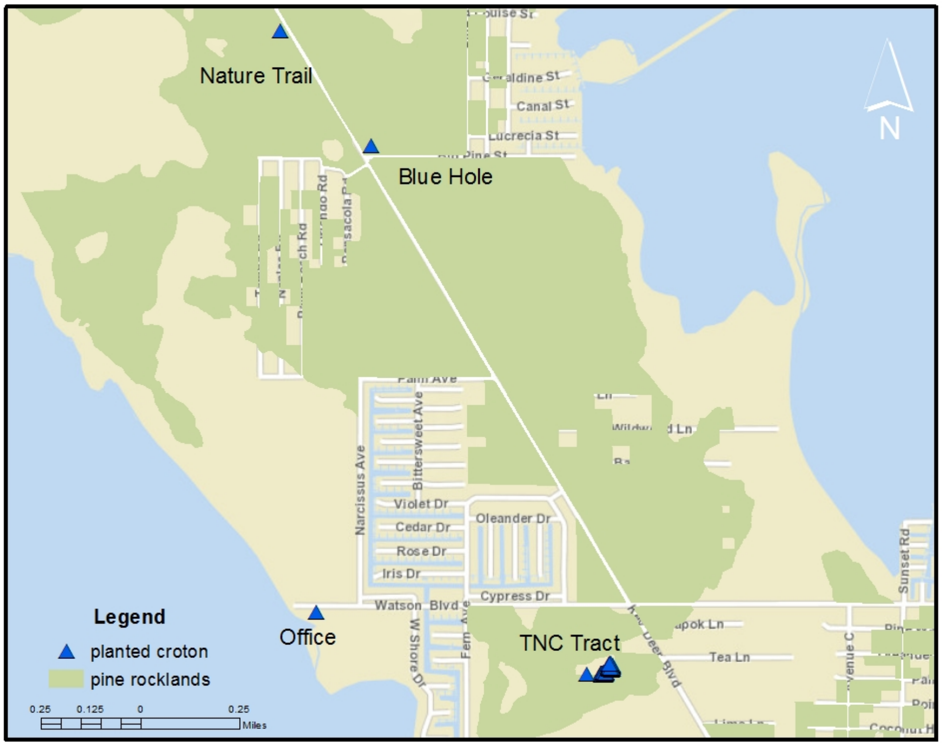
	# croton plants	Avg. plant height (cm)	Average plant size (cm^3)
Original planting	82	38.7	4695
1 yr post planting	59	20.5	3127
Difference	*-23*	*-18.2*	*-1568*

In August 2014, a prescribed fire occurred in the area of the Blue Hole planting. Two weeks post burn, the site was monitored. It was found that 32% of plants were dead, 25.5% of the plants were scorched but alive, 11% of the plants were unburned, 21.4% of the plants were resprouting, and 9 (9%) new seedlings (untagged) germinated. As found in other studies of croton, the plant may continue to re-sprout and re-seed for some time post burn. For this reason, this site should be monitored over time to determine long-term outcomes.

By far the most significant site, the old "TNC tract," has 329 plants, all tagged and fenced. This area was planted in 3 stages beginning in 2012, with one new fenced area planted each subsequent growing season (Fig. 9, Table 2). In the first two seasons, 155 plants were tagged. An additional 173 were planted and tagged in 2014. The 79 plants planted in 2012 were monitored in 2013 and 2014 (not sampled initially after planting.) Overall survival rate for the 2012 planting was 66% over the first two years. The 2012 plants were reduced in average height from (32cm to 26cm) but total area increased over the first two years from 9491.4 cm^3 to 13582 cm^3. Similarly to the first planting, the survival rate for the 76 plants tagged in 2013 was 60%. Plants installed in the 2013 season also decreased in height (23.2 cm to 18 cm) and total area increased dramatically from 3797.4 m^3 to 6805.9 m^3 in the first year post. In 2014, another 173 croton plants were installed and tagged with an average height of 14 cm (Table 2).

Table 2: Summary of TNC tract planting site

	# tagged plants	Survival rate	Average plant height (cm) in 2014	Average plant size (cm^3) in 2014
Planted 2012	79	66%	30.9	14603
Planted 2013	76	60%	20.6	6975
Planted 2014	173	n/a	14.3	4122



Biology\Projects\la_croton\spatial_info\planted croton

Figure 5: Planting sites of Croton linearis.

In-situ Seeding

We designed a pilot experiment to test the effect of prescribed burning and duff raking on croton germination using seeds collected from mature plants on Big Pine Key. We used a randomized block design with two blocks each in recently burned and un-burned pine rockland. Our burn blocks were located in the Blue Hole fire just west of Key Deer Blvd. The Blue Hole fire burned in summer 2011, one year prior to seeding. Un-burned blocks were located on the east side of Key Deer Blvd in pine rockland that has not burned since 2004. In each block we had four ¼ meter-square replicates of the following four treatments: raked, un-raked, raked and seeded, un-raked and seeded. In each seeded plot we scattered 30 croton seeds. All blocks were fenced to discourage deer from eating germinating seedlings.

For the first 6 weeks, we returned weekly to look for croton germination and then reduced our visits to one per month for the next six months. Across all plots, only one new croton germinant was located. We found this germinant in a burned/raked/seeded plot. It is possible that we saw such limited germination because seeds were carted away by seed predators. Another possibility is that croton seeds will not germinate when simply scattered on the ground in the field. Clearly, a more controlled experiment could help to understand why our seeding experiment was unsuccessful.

Management Implications

Direct seeding a raking may very well be an effective method of increasing croton populations. However, this small scale experiment was not able to demonstrate any meaningful results to support the method. Fire, or fire and mechanical treatments combined, are most likely the most beneficial methods for restoring croton populations. Seeding would likely need to occur with thousands of seeds in a recently burned area to expect significant recruitment.

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