MEMORANDUM OF UNDERSTANDING BETWEEN THE FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION AND THE DEPARTMENT OF ENVIRONMENTAL PROTECTION'S DIVISION OF RECREATION AND PARKS REGARDING THE RELEASE AND STUDY OF MIAMI BLUE BUTTERFLIES IN BILL BAGGS CAPE FLORIDA STATE PARK

WHEREAS:

- The Miami blue butterfly (*Cyclargus thomasi bethunebakeri*, or MBB) is listed by the Florida Fish and Wildlife Conservation Commission (FWC) as endangered due to habitat loss and other causes;

- The FWC's 2003 MBB management plan includes as a conservation strategy to "Determine and implement methods for the release of captive bred Miami blues into existing populations or unoccupied suitable habitat";

- FWC-permitted efforts by University of Florida McGuire Center for Lepidoptera and Biodiversity (UF) biologists to reintroduce MBB to its historic range have been unsuccessful thus far, and more information about how to successfully release and monitor MBB is still being sought;

- Ongoing habitat management activities at Bahia Honda State Park have been compatible with the conservation of that park's naturally occurring MBB population;

- Bill Baggs Cape Florida State Park, managed by the Department of Environmental Protection's Division of Recreation and Parks (DRP), is within the historic range of the MBB and has appropriate larval food plants, nectar plants, and generally compatible habitat management programs to support the survival of MBB, but does not currently have a naturally occurring MBB population;

THEREFORE, the FWC and DRP (or "the Parties") agree to the following:

1. As part of an FWC-permitted study to help determine how to successfully reintroduce MBB to its historic range, the Parties will discuss and work out to their mutual satisfaction the operational issues involved with the release and monitoring of the species in Bill Baggs Cape Florida State Park. The study will be considered successful when MBB populations persist on site, proliferate through reproduction in the wild, and are determined through monitoring to be self-perpetuating without augmentation through additional releases. Additional background and details for the proposed study are provided in Appendix 1, "Proposed 2010-2011 Miami Blue Butterfly Research," prepared for FWC by UF.

2. FWC will coordinate work days and site visits with the Park Manager well in advance. Once operational issues have been worked out by the Parties, DRP will provide necessary permits or other permissions to allow MBB captive-raised by UF or wild-caught from other areas to be released and monitored in Bill Baggs Cape Florida State Park.

3. DRP, while conducting normal habitat and visitor management activities in the state park where the study of released MBB is ongoing, will not deliberately or intentionally do harm to MBB but does acknowledge that normal management activities will affect habitat. Such activities could include, among others: prescribed burning; invasive plant control; trimming of vegetation including nickerbean, blackbead, and other larval host or nectar plants; authorizing specified mosquito larviciding and adulticiding; vegetation removal and other restoration-related activities; nonnative animal control; storm-related preparation, damage assessment, and clean-up activities; and construction projects. DRP will not be expected to prevent natural plant succession, which may out compete larval host and nectar source plants.

4. If requested by DRP, FWC will provide an incidental take permit that covers any and all activities or circumstances where take of MBB might occur for the duration of the study.

5. DRP will provide free access to Bill Baggs Cape Florida State Park for appropriately designated persons for project-related purposes, including conducting park-wide surveys of butterflies, host plants, and nectar plants, assessing appropriate sites for MBB releases, releasing MBB, and monitoring of the fate of released MBB and subsequent generations. In addition to DRP personnel, appropriately designated persons may include personnel associated with FWC, UF, and the North American Butterfly Association (NABA). Free access will not exempt said personnel from normal DRP permitting requirements for collecting plants and animals within state parks.

6. The number and life-stage form (eggs, larvae, pupae, adults) for each MBB release event will be dependent upon captive and/or wild stock availability, project goals, and other factors, and may differ according to each MBB release event. See Appendix 1 for specific information.

7. Representatives of the Parties will correspond or meet, as necessary and appropriate, to discuss issues relating to this agreement and to resolve any questions or problems encountered.

8. This agreement will become effective when it has been signed by both Parties, and will remain in effect until or unless terminated in writing by either Party with at least 30 days prior notice.

9. Nothing in this agreement shall be construed to place financial commitment upon either Party. Actions taken and funds expended to implement this agreement are contingent upon appropriations, priorities, and other constraints.

IN WITNESS WHEREOF, the Parties hereto have caused this agreement to be executed through their duly authorized signatories on the day and year last written below.

DEPARTMENT OF
ENVIRONMENTAL PROTECTION
DIVISION OF RECREATION
AND PARKS

By _____
Mike Bullock
Director, Division of Recreation
And Parks

Date 3-15-10

APPROVED as to form and legality

_____
DEP Attorney

FLORIDA FISH AND WILDLIFE
CONSERVATION COMMISSION

By _____
Timothy A. Breault
Director, Division of Habitat and
Species Conservation

Date 3-22-10

APPROVED as to form and legality:

_____
Commission Attorney

Appendix 1. Proposed 2010-2011 Miami Blue Butterfly Research

Overview:

Captive propagation and organism reintroduction are regularly used conservation techniques to help promote the recovery of imperiled taxa. The methods employed to accomplish organism reintroduction are often based on protocols developed for other similar organisms despite possible differences in life history, habitat, climate, ecology and behavior. Few studies have addressed reintroduction methodology or post release organism fate in a more experimental approach. The following provides a summary of the proposed field-based research projects aimed at enhancing our understanding of the variables and methods impacting organism reintroduction and successful colony establishment for imperiled organisms, with a focus on the state-endangered Miami blue butterfly (*Cyclargus thomasi bethunebakeri*) in south Florida.

A. Overview

1. Proposed Research Location: Bill Baggs Cape Florida State Park
2. Research time frame: 2010-2011 field seasons
3. Time of Year: May – October
3. Suggested management units to be utilized may include 1, 5, 10, 15 (see aerial photo, Fig. 2); these units were agreed upon by local park staff at our site visit with FWC and UF personnel in 2008. We will work closely with park staff to confirm management units to be used and for the coordination of all research efforts.
4. Estimated number of North America Butterfly Association (NABA) volunteers per research trial: 4-15 individuals depending on experiment, research protocol and volunteer availability.
5. Hours of the day: All research/survey efforts will take place during daylight hours from approximately 8:00 am to sunset on scheduled/designated days. After hour surveys or night work is not anticipated.
6. Number of days per trial/post release: Typically 1-3 intensive monitoring days following organism release/trial with periodic follow-up monitoring for a period of 1-3 months as needed.

B. Research Plan

1. *Post-Release Monitoring - Evaluating the Fate of Reintroduced Organisms*

A. Resighting Probability in the Field: Determining the success or failure of reintroduction programs for endangered organisms requires understanding the processes that affect resighting of released individuals. Whereas recaptures clearly demonstrate survival, failure to recapture individuals does not necessarily indicate that the reintroductions have been unsuccessful. The probability of recapturing a released individual (including resighting as part of a monitoring program) can be divided into several distinct processes: mortality, movement out of the monitoring area or failure of the observers to find the organism. However, these processes and their associated probabilities are often conflated as mortality (i.e., failure) in monitoring programs and require explicit examination to estimate their relative importance.

Current reintroduction protocols for the Miami blue butterfly include the release of both larvae and adult organisms into apparently appropriate habitat. Resighting frequency of released individuals generally has been quite low. However, both laboratory and field observations suggest that larvae are difficult to find when on the host plant and that adult butterflies have some, if limited, ability to disperse. As such, it is far from certain that the low frequency of resighting is solely due to mortality.

We therefore propose to experimentally assess the resighting probability of Miami blue butterfly larvae in the field to determine the rates of mortality, movement and failure of observers to find the individuals following initial release. To distinguish among the processes responsible for not resighting released individuals, we will place a set number of Miami blue butterfly larvae on potted (1-3 gal) nickerbean plants in or adjacent to suitable habitat within the park. Plants will originate from UF nursery. See Figure 1 below:

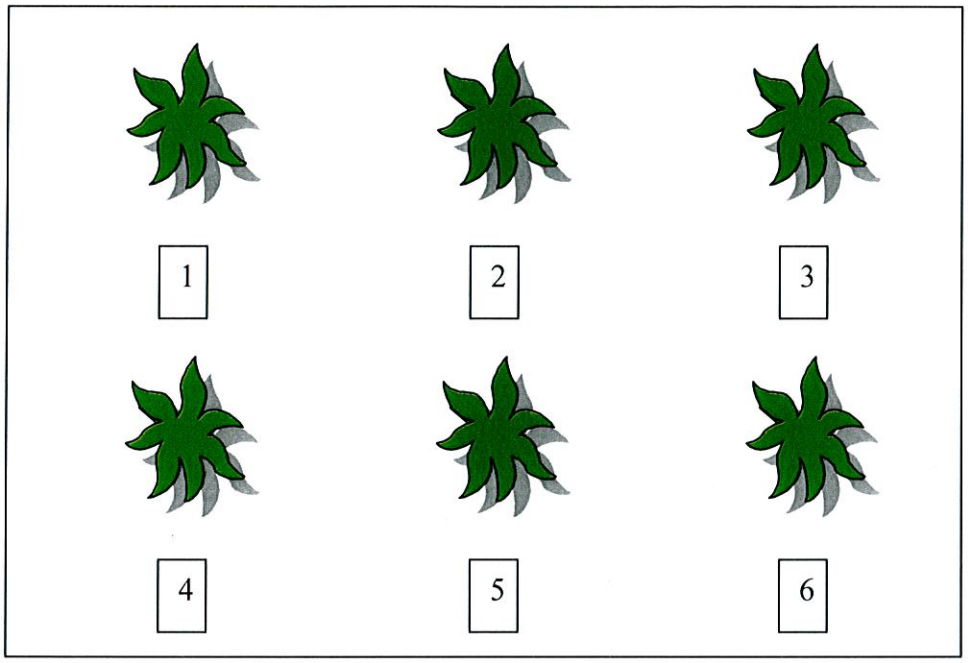

Fig. 1. Example resighting field test plot with six 1-3 gallon gray nickerbean plants. Each plant will receive 0-5 Miami blue butterfly larvae and be sequentially surveyed following initial organism release.

Specifically, all trials will have a semi-blind design. Each plant will receive a known number of larvae (ranging from 0-5) but the observers will not have prior knowledge of the exact organism numbers. Individual organism numbers will depend on availability from the captive colony but will range between 30-50 larvae per trial. We expect 2-3 replications. Single observers will be asked to spend a set time (< 3 minutes) searching each plant for larvae before moving to the next plant in sequential fashion. Observer surveys will be conducted at set intervals approximately 2h, 6h, 24h and 48h following initial larval release. The exact number of larvae per plant and location on plant/pot will be recorded by each observer. 4-6 volunteer observers will used. All larvae will be released early in the day to maximize observer time. All plants will be placed on a large tarp or on mowed grass. All larvae and plants will be removed following each trial.

B. Movement of Released Larvae

Following normal larval release protocols, existing nickerbean patches within the park will receive mature late fifth instar larvae within 24-48 hr of pupation. Volunteer observers will be asked to observe/watch all larvae following initial release for a period of 4-6 hrs the first day and to revisit each patch 24h and 48h post release. Larval behavior, plant location, ant tending,

predation, mortality, pupation location, etc. will all be recorded for each organism. One or more locations within the park will be chosen. The exact research areas within the management units will correspond to those previously agreed upon by local park staff at our site visit in 2008 with FWC personnel. A minimal number of Miami blue butterfly larvae (~10) will be used for each replication. 2-4 replications will be conducted. Total volunteer observers required will be between 10-15 individuals.

Need/objective: Better understanding the accuracy of organism resighting and the fate of released larvae is essential to effectively evaluate post release monitoring data. The proposed projects outlined above are intended to inform post release monitoring protocol and/or reintroduction methods.

2. *Reintroduction Methodology*

A. Predator Exclusion: Survivorship of reintroduced Miami blue butterflies will be evaluated in the field by comparing larvae in caged versus free (un-caged) organism releases. Portable netting cages (multiple dimensions from ~2ft x 2ft x 4ft to 12ft x 12ft x 12ft) will be used depending on the trial. Cage dimensions would be consistent per trial/site. Two specific trials will be evaluated: single host plant and multiple (grouped) host plant larvae releases. Individual host plant trials will consist of 5 Miami blue larvae (late fifth instar originating from the UF captive colony) placed on single 1-3 gallon potted nickerbean plants originating from UF nursery (1 contained in small mesh cage and other in natural park setting adjacent to or within suitable habitat). Multiple (group) trials will consist of 5 or more potted nickerbean plants each receiving up to 5 larvae (one group contained in a large mesh cage and the other grouped in natural park setting adjacent to or within suitable habitat). Volunteer observers will survey each plant for larvae at regular intervals (likely 2hr, 4hr, 6hr, 8hr, 24hr, 36hr, and 48hr) following initial organism release and record the number of larvae per plant. Larval behavior, plant location, ant tending, predation, mortality, pupation location, etc. will be recorded. All larvae and plants will be removed following each trial.

B. Ant Presence: Miami blue butterfly larvae are regularly found in association with various native and nonnative ant taxa (Saarinen and Daniels, 2007; Trager and Daniels, 2009). Lycaenid larvae in such relationships generally emit semiochemicals from a pair of tentacular organs and secrete a sugar-rich solution from a dorsal nectary organ to attract and retain their ant guards (Axén et al. 1996, Axén 2000, Daniels et al. 2005), which then protect the larvae from predators and parasitoids. The potential importance of ant presence/tending on reducing larval predation/parasitism will be examined in the field by establishing experimental test plots similar to 1A above. Specifically, a set number of late instar larvae (~5 larvae) will be placed on 8 potted (1-3 gal) nickerbean plants in or adjacent to suitable habitat within the park. Plants will originate from UF nursery. Individual organism numbers will depend on availability from the captive colony but will range between 30-50 larvae per trial. We expect 2-3 replications. Tanglefoot will be used on half of the plant pots to exclude ant foraging and presence. All plants will be placed on a large tarp or on mowed grass. *Camponotus floridanus* and *C. planatus* are known from the park and should locate larvae relatively quickly following initial field placement. Single observers will be asked to regularly survey all plants following initial organism release. Observer surveys will be conducted at set intervals approximately 1h, 2h, 3h, 6h, 8h, 24h and 48h following initial larval release. The exact number of larvae per plant and location on plant/pot will be recorded by each observer along with larval behavior, observed ant tending, predation, mortality, pupation location, etc. will all be recorded for each organism. Photos of observed ants will be taken to facilitate identification. 4-8 volunteer observers will used. All larvae will be released early in the day to maximize observer time. All larvae and plants will be removed following each trial.

C. Adult Release Technique: The influence of initial adult butterfly experience prior to release will be evaluated. Pupae from the UF captive colony will be transported to and maintained in south Florida. Following successful adult eclosion, adults will be sexed and randomly separated into two groups (each with an approximately 50:50 sex ratio). All adults from each group will be distinctively marked with a Sharpie permanent ink marker. Group A adults will be placed in small 8oz Dixie cups (2-3 per cup), fed with Gatorade and maintained in the individual cups until eventual release in the environment. Group B adults will be temporarily housed in a large (12'x12'12') portable mesh flight cage placed within suitable park habitat. The cage will contain potted nickerbean and nectar plants. All butterflies will be maintained under the specific assigned conditions (cups or cage) for up to 48hrs at which time adults from both groups will be simultaneously released into the same suitable field site. The exact number of organisms used will depend on availability but will range between 25-50 adults per group. One to two replications will be conducted. Following release, the adult organisms will be surveyed visually along established short transects within the habitat area at regular intervals of 2 hrs between 9:00 am and 7:00 pm for up to 3 days following release. Up to 10 volunteers will be utilized.

D. Genetic History: The influence of genetic history (acclimation/adaptation to captivity) on adult behavior, survival and resighting will be evaluated. Pupae of third generation (3 generations in captivity following egg collection from Bahia Honda) and first generation (resulting from egg collection at Bahia Honda) will be transported to and maintained in south Florida. Following successful adult eclosion, adults from both groups will be sexed, distinctively marked with a Sharpie permanent ink marker, and simultaneously released into the same suitable field site. The exact number of organisms used will depend on availability but will range between 25-50 adults per group. One to two replications will be conducted. Following release, the adult organisms will be surveyed visually along established short transects within the habitat area at regular intervals of 2 hrs between 9:00 am and 7:00 pm for up to 3 days following release. Up to 10 volunteers will be utilized.

Need/objective: Successful colony establishment is assumed to be enhanced by increased organism survivorship. The proposed projects outlined above are intended to evaluate reintroduction methods thereby improving overall organism reintroduction survivorship and ultimately enhance colony establishment.

3. *Translocation of individuals from Bahia Honda State Park.*

Adult Miami blue butterflies from Bahia Honda State Park will be collected and transported to an identified recipient park site where they will be subsequently released. Prior to release, all adult butterflies will be marked, data (such as sex, FW length, initial capture location) collected, and a small wing fragment collected for genetic analysis. All adult butterflies will be confined in small containers with available nectar resources and maintained in a cooler during transport. Up to 40 adult butterflies (gravid females) may be collected throughout the 2010 field season. Organism collection (number and frequency –if at all) from Bahia Honda will depend on various factors including population numbers, environmental conditions and time of year. In no case will all 40 individuals be removed at the same time. Following release, the adult organisms will be surveyed visually along established short transects within the habitat area at regular intervals of 2 hrs between 9:00 am and 7:00 pm for up to 3 days following release. Up to 10 volunteers will be utilized. The corresponding site will be periodically (approximately once per month for three months) revisited and surveyed to determine organism reproduction and/or establishment. This research component will only be conducted if time and organism availability permits.

2010 Activities	Jan-March	April-Jun	July-Sept	Oct-Dec
Project Planning	X			
Coordination with Park Staff	X	X	X	X
Volunteer Selection and Initial Training	X	X		
Field Trial Set-up		X		
Additional Volunteer Training		X	X	X
Conduct Research Trials		X	X	
Field Trial Materials Removed				X
Trial Data Analysis				X
2011 Activities				
Coordination with Park Staff	X	X	X	X
Follow-up Field Site Monitoring		X	X	X?

Literature Cited

Axén, A.H. 2000. Variation in behavior of lycaenid larvae when attended by different ant species. *Evolutionary Ecology* 14: 611-625.

Axén, A.H., Leimar, O., and V. Hoffman. 1996. Signalling in a mutualistic interaction. *Animal Behavior* 52: 321-333.

Daniels, H., Gottsberger, G., and K. Fiedler. 2005. Nutrient composition of larval nectar secretions from three species of myrmecophilous butterflies. *Journal of Chemical Ecology* 31: 2805-2821.

Saarinen, E.V. and J.C. Daniels. 2006. Miami Blue butterfly larvae and ants: new information on the symbionts of an endangered taxon. *Florida Entomologist* 89(1):69-74.

Trager, M.D. and J.C. Daniels. 2009. Ant tending of Miami blue butterfly larvae (Lepidoptera: Lycaenidae): partner diversity and effects on larval performance. *Florida Entomologist* 92(3):474-482. 2009

Fig. 2. Aerial photo of Bill Baggs Cape Florida State Park showing numbered burn units. Proposed management unit numbers to be utilized for this project include: 10, 5, 15, and 1.