ATTACHMENT C
Biological Opinion/Conference Opinion
Section 7 ESA

REGION 4
INTRA-SERVICE SECTION 7 BIOLOGICAL EVALUATION FORM

Originating Person: Nancy Finley

Telephone Number: 305/872-2239 **E-Mail:** nancy_finley@fws.gov

Date: 2014

Section 7 Consultation No.:

PROJECT NAME (Grant Title/Number): Mosquito control pesticide applications on lands of the Lower Keys Refuges

I. **Service Program:**

_X_ Refuges

II. **State/Agency:** Florida

III. **Station Name:** Florida Keys National Wildlife Refuges

IV. **Description of Proposed Action:**

The Florida Keys Mosquito Control District (District) is requesting the continued use of the adulticides Trumpet (active ingredient Naled), Pythrethroids, and larvacidal Bti (*Bacillus thuringiensis israelensis*) as proposed in alternative B of the draft mosquito control management plan (MCMP) to control mosquitoes on the Lower Keys Refuges (Figure 1). Adulticide treatments are conducted in association with a surveillance and monitoring program and larvicide treatments on refuge lands in both National Key Deer Refuge and adjacent islands within the Great White Heron National Wildlife Refuge. The District asserts that: "Use of chemicals of different classes, with different modes of action, is important in the prevention of resistance to insecticides in the mosquito population."

The use of mosquito controls adulticides is necessary in some areas to protect the general public from the threat of mosquito-borne diseases (Mulrennan 1991). Due to the diversity of the mosquito fauna in the Keys, the subtropical climate, and the proximity of the Keys to the Caribbean, where active transmission of several disease organisms is ongoing, the District believes that a potential exists for the transmission, and spread, of mosquito-borne diseases. These diseases include malaria, St. Louis encephalitis, eastern equine encephalitis, and West Nile virus. Mosquito pools tested positive for West Nile Virus in the Florida Keys in 2001 (seven pools of mosquitoes were virus positive, and in 2003, ten pools were positive (FKMCD 2006). Seven of the seventeen mosquito pools

found positive for WN in the Florida Keys were from *Ochlerotatus taeniorhynchus*, the black salt marsh mosquito (Hribar et al. 2003, 2004).

Mosquito adulticides have been used in the Keys for approximately 30 years. The areas to be treated will consist of Fish and Wildlife Service (Service) lands adjacent to human development. Excluding no-spray zones, the treatment area includes Refuge lands on Big Pine, No Name, Middle Torch, Big Torch, and Little Torch Keys, consisting of approximately 6,000 acres (Figure 2) spraying programs for public versus private areas. To control mosquitoes in developed areas of these Keys requires that many Refuge lands also be treated, with exceptions including certain relatively large tracts of public land located some distance from subdivision developments. No-spray zones were previously established for Watson Hammock and Cactus Hammock on Big Pine Key, and portions of No Name Key to protect the Stock Island tree snail (Figure 2). Additional no-spray zones to protect large tracts of butterfly habitat in publicly-owned pine rockland on Big Pine Key was implemented in 2007 (Figure 2). The District installed new mosquito count stations in these areas to monitor adult mosquitoes. Application rates and no-spray zones will be re-evaluated upon completion of Service-funded studies by Mote Marine Laboratory and Florida International University to develop a probabilistic analysis of applications of mosquito control adulticides on butterfly and other non-target species to supplement the Ecological Risk Assessment for non-target species. On Cudjoe Key, Sugarloaf Key, Saddlebunch Keys, and Boca Chica Key, only developed areas will be sprayed so Refuge properties on these islands are not directly affected by mosquito adulticide spraying; however, these areas may be impacted by drift to some degree. These and aforementioned islands have additional no-spray areas on State lands, typically with interspersed Refuge lands. Application of adulticides will not be allowed on any refuge islands not connected to US 1 by roads and does not include or imply approval to spray over coastal waters. The Refuge is not permitting or evaluating affects to coastal water species under the purview of NMFS, as the Refuge does not have jurisdiction over those waters. It is the direct responsibility of the Florida Keys Mosquito Control District (District) to initiate consultation for coastal species managed by NMFS. The Refuge will not permit application of adulticide, which may drift to coastal waters without a completed Section 7 consultation with NMFS by the District. The District can provide a letter from the NMFS stating a consultation is not necessary should that be the determination of NMFS. See alternative B of the MCMP for further details of the proposed action.

V. Pertinent Species and Habitat:

A. Listed Species and/or Critical Habitat within the Action Area: Key deer, Lower Keys marsh rabbit, rice rat, key tree cactus, Garber's spurge, Cape Sable thoroughwort, semaphore cactus, and eastern indigo snake. The Miami blue butterfly was listed as endangered in a final rule effective April 6, 2012 (77 FR 20948). With the final rule, the Service included a special rule listing the cassius blue butterfly, ceraunus blue butterfly and nickerbean blue butterfly as threatened in portions of their natural ranges due to their similarity of appearance to the Miami blue.

B. **Proposed Species and/or Critical Habitat within the Action Area**: Florida leafwing, Bartram's hairstreak
C. **Candidate Species within the Action Area:** Wedge Spurge, Sand Flax, Big Pine Partridge Pea
D. **Species Habitat Occurrence:** See figures 1-7.

SPECIES	CRITICAL HABITAT	STATUS
Listed Species		
Stock Island Tree Snail (*Orthalicus reses*; not incl. *nesodryas*)		
Key Deer (*Odocoileus virginianus clavium*)		Threatened
Rice Rat (*Oryzomys palustris natator*)		Endangered
Lower Keys Marsh Rabbit (*Sylvilagus palustris hefneri*)		Endangered
Eastern Indigo Snake (*Drymarchon corais couperi*)		Endangered
Key Tree-Cactus (*Pilosocereus robinii*)		Threatened
Garber's Spurge (*Chamaesyce garberi*)		Endangered
Miami blue butterfly *(Cyclargus thomasi bethunebakeri)*		Threatened
Cassius blue butterfly *(Leptotes cassius theonus)*		Endangered
Ceraunus blue butterfly *(Hemiargus ceraunus antibubastus)*		Threatened
Nickerbean blue butterfly *(Cyclargus ammon)*		Threatened
Cape Sable Thoroughwort (Chromolaena frustrata)		Threatened
Florida Semaphore Cactus (Consolea corallicola)		Endangered
Candidate Species		Endangered
Florida leafwing butterfly (*Anaea troglodyta floridalis*)		
Bartram's hairstreak butterfly (*Strymon acis bartrami*)		Proposed Endangered
Big Pine partridge pea (*Chamaecrista lineata keyensis*)		Proposed Endangered

Wedge spurge (*Chamaesyce deltoidea serpyllum*) Sand flax (*Linum arenicola*)		Candidate Candidate Candidate

VI. **Location** (See Figure 1). Location of National Key Deer Refuge and Great White Heron NWR in the Lower Keys, Monroe County, Florida.

A. **Ecoregion Number and Name:** 53; Southeast Region

B. **County and State:** Monroe County, Florida

C. **Section, township, and range (or latitude and longitude):** Township 65-68 South, Range 25-30 East.

D. **Distance (miles) and direction to nearest town:** 10-30 miles northeast of Key West, Florida.

E. **Species/habitat occurrence:** Thirteen endangered and threatened species and seven candidate species indicated above potentially could be affected by the proposed action.

VII. **Determination of Effects:**

A. **Explanation of effects of the action on species and critical habitats:**

Ecological Risk Assessment and Hazard Quotient (HQ) for Naled

An Ecological Risk Assessment was completed in 2002 by the Service's South Florida Ecological Services Office to assess the impacts of Naled on listed species, specifically the Key deer, Lower Keys marsh rabbit, rice rat, and indigo snake. The assessment and exposure model, prepared by URS Corporation (2004), was used to determine total applications and application intervals to the species listed in Table V.B., excepting the Lepidopterans and plants.

Naled belongs to the class of insecticides referred to as organophosphates. These chemicals act by interfering with the activities of acetylcholinesterase (AChE), an enzyme that is essential for the proper working of the nervous systems in animals. Naled is toxic through ingestion, inhalation, and dermal absorption. Naled does not affect the physiology of plant life.

Inhalation of Naled appears to be more acutely toxic than by oral or dermal exposure. The range in inhalation LD_{50}s (lethal dose 50 percent) for mammals is from 3.2 to 7.7 mg/kg-BW (body weight), while oral Naled exposure LD_{50}s for mammals range from 92 to 409 mg/kg-BW and dermal mammalian LD_{50}s range from 360 to 1234 mg/kg-BW (URS, 2002). These differences in toxicity based on exposure route are likely due to the relative absorption of Naled into the blood stream.

Mice dosed by a single oral exposure of 103 mg/kg-BW experienced an immediate 68 percent inhibition of AChE. By 48 hours after exposure, AChE inhibition was 88 percent. AChE activity had returned to normal by 96 hours post exposure (Berteau and Deen 1978).

Chronic and subchronic exposures to Naled have produced chronic (long-lasting) AChE inhibition, tremors, histopathological effects in the lung and liver, decreased weight gain, mineralization of the lumbar spinal cord, mortality, and effects on reproduction which include reduced fetal implants, fetal resorption, and decreased offspring body weights and survival.

A 13-week subchronic inhalation study in rats documented inhibition of AChE at doses as low as 0.17 mg/kg-BW per day, with clinical signs and histopathological changes occurring at 0.99 mg/kg/day (USEPA 1999). Under these exposure conditions, normal AChE activity took more than six weeks to recover following cessation of exposure. This indicates a relationship between length of exposure and recovery time, since AChE activity following a single exposure recovered within 96 hours (Berteau and Deen 1978).

For oral exposures, both rats and rabbits showed adverse effects on reproduction when exposed chronically or during gestation at doses around 20 mg/kg-BW/day. Rabbits exposed during gestation showed marked cholinergic signs at a dose of 10 mg/kg-BW/day, and both rats and dogs showed AChE reduction after chronic exposures to 2 mg/kg-BW/day. Both clinical signs and AChE activities were unaffected in rats and dogs chronically exposed to 0.2 mg/kg/day of Naled (USEPA 1999).

Dermal studies are available for rats, rabbits, and sheep. The LD_{50}s for dermally exposed rats were 800 and 1234 mg/kg-BW (HSDB 2002, Pupysheva 1971). Dermal LD_{50}s for rabbits were slightly lower at 360 (females) to 390 (males) mg/kg. Only one assay of Naled dermal toxicity utilizing multiple exposures was obtained (USEPA 1999). The exposure duration was subchronic (4-weeks) in rabbits and revealed a significant, adverse effect where reduced weight gain and extreme dermal irritation were observed at a dose of 3.57 mg/kg-BW/day. The No Observed Adverse Effects Level (NOAEL) for this assay was 0.179 mg/kg-BW/day (USEPA 1999).

All three exposure routes are likely pathways for some listed species in the Lower Keys to be exposed to Naled. Therefore, this analysis focuses on the potential effects of Naled exposure through these pathways. Laboratory assays discussed above are used to identify Toxicity Reference Values (TRVs) for each exposure route (Table 1), for use in the risk assessment model produced by URS (2002). Toxicity values used in this analysis are presented and discussed in detail in the ecological risk assessment document (URS 2002).

Naled breaks down over time into dichlorvos (DDVP), which is another organophosphate that can be highly toxic to wildlife. The acute oral LD50 for dichlorvos is 56-80 mg/kg-BW in rats; its acute dermal LD50 in rats is 75-107 mg/kg-BW. These contaminants are more persistent in the atmosphere than in surface water. Persistence in the atmosphere may be contributed to by loss from leaves and other substrates that occurs between 24 and 48 hours after application. Loss from soils is highly variable. Bioconcentration and bioaccumulation of Naled and/or dichlorvos are minimal (URS 2002).

This assessment will consider TRVs for the Lowest Observed Adverse Effects Level (LOAEL) and the NOAEL. The LOAEL represents the lowest level at which adverse effects have been observed in the lab. The NOAEL represents the highest level at which no adverse effects have been observed. For an activity to be considered not likely to adversely affect a given species, the dose to a species likely should be at or below the NOAEL for the species. The risk assessment model produces a Hazard Quotient (HQ) that is a ratio of the predicted dose divided by the NOAEL or LOAEL. This represents the relative likelihood that the NOAEL or LOAEL would be reached at given application rates and intervals. To calculate the HQ, the lowest TRVs for all exposure pathways were used (Table 1). In other words, this analysis uses the TRVs for the most sensitive species for each exposure pathway to ensure that the analysis errs on the side of the species. When the HQ meets or exceeds 1.0, the risk of reaching one of these effects levels exists. When the HQ is less than one, the risk is insignificant or discountable. For risk of adverse effects to listed species from the application of Naled to areas inhabited by these species to be not likely to adversely affect, the HQ for the NOAEL must be at or below 1.0.

Table 1. Naled toxicity to mammals.

Exposure Pathway		
Acute exposure	Lowest LD_{50}	
Inhalation	3.2 mg/kg	
Dermal	360 mg/kg	
Oral	92 mg/kg	
Subchronic exposure	LOAEL	NOAEL
Inhalation	0.99 mg/kg-BW-day	0.17 mg/kg-BW-day

Dermal	3.57 mg/kg-BW-day	0.179 mg/kg-BW-day
Oral	10 mg/kg-BW-day	1 mg/kg-BW-day
Chronic exposure	LOAEL	NOAEL
Inhalation	0.99 mg/kg-BW-day	0.17 mg/kg-BW-day
Dermal	3.57 mg/kg-BW-day	0.179 mg/kg-BW-day
Oral	2 mg/kg-BW-day	0.2 mg/kg-BW-day

Ecological Risks of Pyrethoids

Most toxicological data for pyrethroids is associated with permethrin, thus for practical purposes permethrin will be used to assess risks to pyrethroids in the absence of formulation specific data. Permethrin is a persistent pyrethroid in the environment, and was immobile in several soils tested, both sterile and viable (USEPA 2009). It is relatively stable to hydrolysis at pHs ranging from 3 to 7 when stored in the dark at 25°C; at pH 9, permethrin degraded very slowly with a half-life of 125–350 days (USEPA 2009). The half-life reported for permethrin in an anaerobic aquatic study ranged greatly (113-175 days), which suggests that the degradation in soil and water is slower as the oxygen levels are reduced when compared to land surface values (USEPA 2009).

Table 2. Toxicity Reference Values for Mammals and Birds for Permethrin.

Exposure Scenario	Species	Exposure Duration	Toxicity Reference Value	Toxicity Category/ Effect
Mammals				
Acute	Rat	Single dose	LD_{50} = 8,900 mg/kg /day	Practically non-toxic
Chronic	Rat	Developmental Toxicity	NOAEC = 1,000 ppm (50 mg/kg/day) LOAEC = 3,000 ppm (150 mg/kg/day)	Decreased mean fetal bodyweight
Birds				
Acute	Mallard duck	5-day dietary	LC_{50} > 10,000 ppm	Practically non-toxic
Chronic	Mallard duck	Reproduction study	NOAEC = 500 ppm	Slight decrease in egg production

USEPA (2009)

Non-Target Insects

Permethrin toxicity data show that the compound is highly toxic to honeybees, as well as beneficial insects (USEPA 2009). A hazard assessment shows that permethrin exposure may result in acute toxicity to honeybees and therefore is considered to be highly toxic on both a contact and an oral basis (contact LD_{50} = 0.13 ug/bee; oral LD_{50} = 0.024 ug/bee) (EPA 2009). Permethrin was also found to be highly toxic to honeybees exposed to foliage that had been sprayed with a permethrin formulation (USEPA 2009). Several field studies were submitted that showed the effects of permethrin formulations on non-target insects (USEPA 2009). Studies show that

applications of permethrin products are likely to reduce the numbers and possibly eliminate populations of beneficial insects (USEPA 2009).

Non-Target Plants

Toxicity data are not available for terrestrial plants (USEPA 2009). Therefore, the potential for risk to terrestrial plants from exposure to permethrin cannot be assessed (USEPA 2009). Permethrin is a specific neurotoxin and as such poses no known threat to plant physiology. This lack of measurable direct effects to plant physiology indicates candidate and listed plant species are unlikely to be affected by application of Permethrin. However, indirect effects could be seen as some percentage of available pollinators may be affected.

The most common pyrethroids are the synthetic pyrethroids, permethrin, resmethrin, and sumethrin. Both pyrethroids and pyrethrins are usually combined with the synergist piperonyl butoxide, which interferes with an insect's detoxifying mechanisms (Tomlin 1994). Non-target toxicity from pyrethroids may occur in either terrestrial or aquatic habitats as a result of deposition, runoff, inhalation, or ingestion (Appendix G). In general, pyrethroids have lower toxicity to terrestrial vertebrates than the organophosphates. Pyrethroids, although less toxic to birds and mammals, are toxic to fish and aquatic invertebrates (Anderson 1989, Siegfried 1993, Tomlin 1994, Milam et al. 2000). The actual toxicity of pyrethroids in aquatic habitats; however, is less than may be anticipated because of the propensity of these pesticides to adsorb to organic particles in the water (Hill et al. 1994). There are also data that indicate synthetic pyrethroid degradates have endocrine disrupting properties (Tyler et al. 2000).

Ecological Risks of Larvacides

Larvicides are materials that affect the four larval stages of mosquitoes known as instars. They can be applied through a wide variety of methods including hand application and backpack sprayers, amphibious tracked vehicle, truck-mounted equipment and aerial sprayers. Mosquito larvicides relevant to this EA include Bti (*Bacillus thuringiensis* var.*israelensis) and Bacillus sphaericus* (Bsp). Larvicides may be reviewed and approved/disapproved through a PUP by the Project Leader of the Florida Keys NWR Complex. Bti (*Bacillus thuringiensis* var. *israelensis*) is a very common larvicide. Refer to Appendix F for a more detailed account of non-target effects of this larvicide in mosquito control.

Bacillus thuringiensis (Bt) is a natural soil bacterium that acts as a larval stomach poison. Bt must be ingested by the larval form of the insect in order to be effective. Bt contains crystalline structures containing protein endotoxins that are activated in the alkaline conditions of an insect's gut. These toxins attach to specific receptor sites on the gut wall and, when activated, destroy the lining of the gut and eventually kill the insect. The toxicity of Bt to an insect is directly related to the specificity of the toxin and the receptor sites. Without the proper

receptor sites, the Bt will simply pass harmlessly through the insect's gut. Several varieties of Bt have been discovered and identified by the specificity of the endotoxins to certain insect orders. *Bacillus thuringiensis* var. *kurstaki*, for example, contains toxins that are specific to Lepidoptera (butterflies and moths), while Bti (var. is specific only to certain primitive dipterans (flies), particularly mosquitoes, black flies, and some chironomid midges. Bti is the form used on the Refuge. Bti is not known to be directly toxic to non-dipteran insects.

Because Bti must be ingested to kill mosquitoes, it is most effective on first-, second-, and early third-instar larvae than on late third and fourth instars since the earlier instars feed at a faster rate (fourth instar larvae feed very little) and require ingestion of fewer crystals to induce mortality. The pesticide is ineffective on pupae because they do not feed at all. Formulated products may be granular or liquid, and potency is expressed in International Toxicity Units (ITU), usually ranging from 200-1200 ITU. The concentrations of Bti in water necessary to kill mosquito larvae vary with environmental conditions, but are generally 0.05-0.10 ppm. Higher concentrations (0.1->0.5 ppm) of Bti are necessary when there is a high amount of organic material in the water, late-third and early fourth instar larvae predominate, larval mosquito density is high, or water temperature is low (Nayar et al. 1999). Operationally, Bti is applied within a range of volume or weight of formulated product per acre as recommended on the pesticide label, with the goal to achieve an effective concentration. The label recommended range of application rates under most conditions varies by a factor of 4 for most formulations (e.g., for granular formulations, 2.72-11.12 kg/ha (2.5-10 lb./acre)). For later instar larvae and water with a high organic content, higher application rates are recommended that may reach 8 times the lowest rate (e.g., for granular formulations, the higher rate is 11.1-22.5 kg/ha (10-20 lb./acre)). Mosquito control agencies use the recommended label rates, along with previous experience, to administer an effective dose.

Bti has practically no acute or chronic toxicity to mammals, birds, fish, or vascular plants (U.S. EPA, 1998). Extensive acute toxicity studies indicated that Bti is virtually innocuous to mammals (Siegel and Shadduck 1992). These studies exposed a variety of mammalian species to Bti at moderate to high doses and no pathological symptoms, disease, or mortality were observed. Laboratory acute toxicity studies indicated that the active ingredient of Bti formulated products is not acutely toxic to fish, amphibians or crustaceans (Brown et al. 2002, Brown et al. 2000, Garcia et al. 1980, Lee and Scott 1989, and Wipfli et al. 1994). However, other ingredients in some formulated Bti products are potentially toxic. The acute toxicity response of fish exposed to the formulated Bti product Teknar® HPD was attributed to xylene (Fortin et al. 1986, Wipfli et al. 1994). Teknar® HPD is not a standard product of the District, nor is planned to be in the future. Field studies indicated no acute toxicity to several fish species exposed to Bti (Merritt et al. 1989, Jackson et al. 2002); no detectable adverse effects to breeding red-winged blackbirds using and nesting in Bti treated areas (Niemi et

al. 1999, Hanowski 1997); and no detectable adverse effects to tadpole shrimp 48 hours post Bti treatment (Dritz et al. 2001).

In addition to mosquitoes (Family Culicidae), Bti affects some other members of the suborder Nematocera within the order Diptera. Also affected are members of the Family Simuliidae (black flies) and some chironomids midge larvae (Boisvert and Boisvert 2000, Garcia et al. 1980). The most commonly observed Bti effects to non-target organisms were to larvae of some chironomids in laboratory settings when exposed to relatively high doses (Boisvert and Boisvert 2000, Lacey and Mulla 1990, Miura et al. 1980). In field studies, effects to target and susceptible non-target invertebrates have been variable and difficult to interpret. Field study results are apparently dependent on the number, frequency, rate and aerial extent of Bti applications; the Bti formulation used; the sample type (e.g. benthic, water column or drift); the sampling interval (e.g. from 48 hrs to one or more years after treatment); the habitat type (e.g. lentic or lotic); the biotic (e.g. aquatic communities), and abiotic factors (e.g. suspended organic matter or other suspended substrates, temperature, water depth); the mode of feeding (e.g. filter feeder, predator, scraper or gatherer); the larval development stage and larval density (Ali 1981, Boisvert and Boisvert 2000, Lacey and Mulla,1990). Bti activity against target and susceptible non-target invertebrates is also related to Bti persistence and environmental fate, which are in turn affected by the factors associated with field study results (Dupont and Boisvert 1986, Mulla 1992). Simulated field studies resulted in the suppression of two unicellular algae species, *Closterium* sp. and *Chlorella* sp. resulting in secondary effects to turbidity and dissolved oxygen of aquatic habitats, with potential trophic effects (Su and Mulla 1999). For these reasons, Bti effects to target and susceptible non-target organisms, and potential indirect trophic impacts in the field are difficult to predict. *Bti* does not persist in the environment after application and studies of activity after application indicate a decline in efficacy within days and little residual activity after several weeks (Glare and O' Callaghan, 1998).

Bacillus sphaericus (Bsp)

Bsp has slight to practically no acute mammalian toxicity, practically no acute avian toxicity, slight to practically no acute fish toxicity, and slight aquatic invertebrate toxicity (USFWS 1984, and FCCMC 1998). Insecticidal activity may persist longer than 20 days because Bsp can reproduce and sporulate in larval cadavers (Becker et al, 1995) and can retain its larvicidal properties after passing through the gut of a mosquito. Bsp is insoluble in water. Spores and toxin become suspended in the water column and retain insecticidal activity in water with high organic matter content and suspended solids. Because Bsp is a more recently developed larvicide than Bti, there are fewer studies that have examined the non-target effects of this pesticide. The data available; however, indicate a high degree of specificity of Bsp for mosquitoes, with no demonstrated toxicity to chironomid larvae at any mosquito control application rate (Mulla, 1984, Ali, 1986, Lacey, 1990, and Rodcharoen, 1991). Therefore risks to sensitive wildlife resources

resulting from direct exposure to a single Bsp application and indirect food chain effects are expected to be negligible. However, the ability for a population to re-colonize a wetland following multiple larvicide treatments would depend on the intensity and frequency of applications at different spatial scales.

Pupicides *(Surface Oils and Films):* Surface oils and films are applied to mosquito breeding sites to kill mosquito larvae and pupae. The products create a barrier to the air-water interface and suffocate insects, which require at least periodic contact with the water surface in order to obtain oxygen. The oils are mineral oil based and are effective for 3-5 days. Surface films are alcohol based and produce a monomolecular film over the water surface. Both oils and the films are potentially lethal to any aquatic insect that lives on the water surface or requires periodic contact with the air-water interface to obtain oxygen. Studies have demonstrated significant negative effects on water surface-dwelling insects from applications of oils (Mulla and Darwazeh 1981; Lawler et al. 1998). Surface oils may also adversely affect wildlife by wetting the feathers of young waterfowl. This may be of particular concern at low temperatures when the oil could affect thermoregulation (Lawler et al. 1998).

Golden Bear 1111. Golden bear 1111 is a petroleum product registered for larval mosquito control. It is considered an effective control agent that acts on the pupal stage of mosquitoes to prevent adult mosquito emergence. This surface oil is effective against all immature stages through suffocation. It disrupts the surface tension of water by preventing female mosquitoes from landing to lay eggs. In some cases control with this material has been demonstrated for up to two weeks (Mulla and Darwazeh 1981). The use of petroleum distillate products is prohibited on the Refuge although it is a pesticide that is used within the local area. Oil-based pupicides are currently used by the District off Refuge lands, only.

CocoBear. CocoBear is a mineral oil product registered for larval and pupal mosquito control. It is considered an effective control agent that acts on the pupal stage of mosquitoes to prevent adult mosquito emergence. Cocobear contains petroleum distillates. Compared to older generations of larvicidal oils, CocoBear contains only 10% petroleum distillates as opposed to the more than 98% found in older formulations. This surface oil is effective against all immature stages through suffocation. It disrupts the surface tension of water by preventing female mosquitoes from landing to lay eggs. The use of petroleum distillate products is prohibited on the Refuge although it is a pesticide that is used within the local area. Oil-based pupicides are currently used by the District off Refuge lands, only.

Agnique Monomolecular Film (MMF) is a non-ionic surfactant that has an alcohol base. The film produced by MMF reduces the surface tension of the water making it difficult for mosquito larvae and pupae to attach and causes them to drown. Emerging adult mosquitoes or midges are unable to fully emerge and will drown. The film produced by Agnique is not visible on the water surface and should not

be used in areas that are subject to unidirectional winds greater than 10 mph or where surface water overflow or runoff is an issue.

Key Deer

The Key deer is present in all areas proposed for application of Larvacides, Naled and Permethrin (Figure 5). It frequents most vegetative community types found in the Lower Keys, including hardwood hammock, pine rocklands, mangrove forest, freshwater marsh, and saltmarsh. Its habits are crepuscular, placing it actively feeding during ideal periods for Naled and Permethrin application. The exposure pathways for Naled for this mammal would be through dermal, oral, and inhalation exposure (URS 2004).

Data on toxicity of Naled to deer species are sparse. The only LD_{50} available for an oral dose is for the mule deer (*Odocoileus hemionus*) and was approximately 200 mg/kg-BW (Hudson et al. 1984). No chronic, subchronic, or reproductive toxicity values are available for deer. Therefore, all HQs are based on TRVs derived from common laboratory species. Key deer would be exposed to Naled through direct application, contact with contaminated vegetation or soil, ingestion of the product on vegetation, and inhalation.

The interval between applications as well as the total number of applications changes the HQ. The model results indicate that no application frequency yielded an HQ for the LOAEL of greater than 1.0. However, applications of Naled at an average frequency of 7 days or less resulted in HQs for the chronic NOAEL above 1.0. Application intervals of 8 days or greater (i.e., 23 applications per year or less) resulted in HQs of less than 1.0 for both the LOAEL and NOAEL. Based on these results, the proposed application rate (nine applications per year) would be unlikely to adversely affect the Key deer.

Permethrin generally exhibits low mammalian and avian toxicity (Ware 1994). Permethrin concentrations from drift into the Refuge were found at >.50mg/kg within 100 meters and up to .25mg/kg within 250 meters of Permethrin truck applications (Pierce 2012). At such low field measured concentrations, the Key deer is unlikely to be impacted.

Rice Rat

The rice rat is an omnivorous mammal that prefers salt marsh habitat in the Lower Keys (Figure 7). Critical habitat has been designated for this species. The rice rat is predominantly nocturnal, which may reduce the likelihood of exposure to the highest concentrations of Naled and potentially Permethrin drift onto Refuge property which will occur during the early morning hours.

Rats are commonly used in laboratory assays, meaning that data on the effects of Naled on a related species are relatively abundant compared to the other species addressed in this document. Rice rats would be exposed to Naled through inhalation, oral exposure, and dermal exposure. The oral exposure for the rice rat is different than that of the Key deer or Lower Keys marsh rabbit since the rice rat is an omnivore. Rice rats may ingest dead or dying invertebrates following Naled applications, a compounding factor that may or may not increase the level of exposure.

As noted for the Key deer, the HQ for the LOAEL (both chronic and subchronic) for the rice rat did not exceed 1.0 for any of the application intervals modeled. The HQ for the chronic NOAEL, however, exceeded 1.0 at application intervals of 4, 5, and 8 days. Chronic HQs for the rice rat did not drop below 1.0 until the average application interval was at least 14 days (13 applications per year).

Subchronic, dermal exposures may present a risk to the rice rat with short application intervals. Application frequencies of every two to four days resulted in HQs greater than 1.0 even for short term (subchronic) exposure. The HQs dropped to 1.0 or less at exposure intervals of five or more days. These results indicate that the proposed application rate of nine applications per year spaced at least five days apart would be unlikely to adversely affect the rice rat (URS 2004).

Rats are commonly used in laboratory assays, meaning that data on the effects of Permethrin on a related species are relatively abundant compared to the other species addressed in this document. Rice rats would be exposed to Permethrin through inhalation, oral exposure, and dermal exposure. The oral exposure for the rice rat is different than that of the Key deer or Lower Keys marsh rabbit, since the rice rat is an omnivore. Rice rats may ingest dead or dying invertebrates following Permethrin applications, a compounding factor that may or may not increase the level of exposure.

For Permethrin, acute oral toxicity in rats was found at 2280 mg/kg for females and 3580 mg/kg in males (USEPA 2009). An apparent data gap exists for acute inhalation. Acute neurotoxicity in rats found a LOAEL=75 mg/kg based on observations of clinical signs such as abnormal or decreased movement (USEPA 2009). Permethrin concentrations from drift into the Refuge were found at >0.50 mg/kg within 100 meters and up to 0.25 mg/kg within 250 meters of permethrin truck applications (Pierce 2012). Based on this, Permethrin is not likely to adversely impact the rice rat.

Lower Keys Marsh Rabbit

The Lower Keys marsh rabbit is a small, herbivorous rabbit found only in the Lower Florida Keys (Figure 6). This species prefers herbaceous cover and inhabits transition zone habitats with cordgrass (*Spartina spartinae*) and sea oxeye daisy (*Borrichia frutescens*). It is crepuscular / nocturnal in nature and will

be foraging at optimal Naled application times. Life history information for this species indicates it will be exposed to Naled through dermal, oral, and inhalation exposure pathways.

The Lower Keys marsh rabbit appears to be the most sensitive of all species examined to potential adverse effects from Naled application. The chronic LOAEL HQ did not exceed 1.0 for any of the application intervals modeled. However, the chronic NOAEL HQ exceeded 1.0 for 4, 5, 8, and 14 day intervals (Table 2). An application interval of 21 days or greater (nine applications per year) was required to reduce the HQ to less than 1.0.

In addition to being the species most sensitive to chronic Naled exposure, the Lower Keys marsh rabbit may be affected by shorter term (subchronic) exposures. The subchronic NOAEL HQ for combined dose from all exposure routes exceeded 1.0 at an application interval of four days or less. The HQ dropped below 1.0 at five day intervals (Table 2). Therefore, the results of the model indicate that the proposed application rate of nine applications per year no more frequently than every five days is unlikely to adversely affect the Lower Keys marsh rabbit.

It is crepuscular and nocturnal in nature and will be foraging at the optimal Permethrin application time. Life history information for this species indicates it will be exposed to Permethrin through dermal, oral, and inhalation exposure pathways.

Permethrin was found to be virtually non-toxic after 21 days of oral dosage from 0.1 to 1 g/kg of weight showed no signs of toxicity but some skin irritation (WHO 1994). Permethrin is considered to be low toxcicity for dermal exposure also (LD50 >2000 mg/g) (USEPA 2009). Permethrin concentrations from drift into the Refuge were found at >.50mg/kg within 100 meters and up to 0.25mg/kg within 250 meters of permethrin truck applications (Pierce 2012). Because of this, incidental drift of Permethrin is not likely to adversely affect LKMR.

Eastern Indigo Snake

The eastern indigo snake is a large, heavy-bodied snake found in nearly all the upland and wetland plant communities in Florida. Historically, the eastern indigo snake ranged throughout the upland habitats of the Lower Keys; however, its present distribution is uncertain (Figure 8). Indigo snakes have not been documented in the mainline Florida Keys connected to U.S.1 since 1991 and road mortalities have not been recorded. It is diurnal in nature and commonly uses burrows or tree root holes as refugia during dry conditions. This species has a large home range, making censusing difficult. The indigo snake is not a constrictor, but ambushes prey items. Prey items include small mammals and birds as well as reptiles and amphibians. Exposure routes for this species would

primarily be through inhalation and ingestion. It is believed that the scales on the snake's skin would impede absorption of Naled and Permethrin through the dermis.

No data are available on the toxicity of Naled to reptiles. The lowest TRV values for both the LOAEL and NOAELs presented for any of the species studied were used. These values are 2.0 and 0.2 for the oral LOAEL and NOAEL, respectively (URS 2002). Since the only other complete exposure pathway for this species is inhalation, the lowest TRV LOAEL and NOAEL of 0.17 and 0.033 were used as were applied to all species. Concentrations on the animal post-application were modeled based on data available for racers.

Model results indicate that risks to the indigo snake are insignificant and discountable at all the application rates modeled. These results indicate that the proposed application rate would be unlikely to adversely affect this species.

Exposure routes for this species would primarily be through inhalation and ingestion. It is believed that the scales on the snake's skin would impede absorption of Permethrin through the dermis.

Much paucity exists in the data regarding snakes and Permethrin toxicity. One study, Brooks et al. (1998), suggests that pyrethroids produced mortality at doses of 40 mg/kg. Permethrin concentrations from drift into the Refuge were found at >0.50 mg/kg within 100 meters and up to 0.25 mg/kg within 250 meters of permethrin truck applications however, exposure dose and residue concentration cannot be directly compared (Pierce 2012).

Permethrin will not be applied on any island known or suspected to be occupied by indigo snake. Currently, indigo snakes are only known to occur outside the proposed spray areas, thus there is no exposure to this species and therefore no risk.

Stock Island Tree Snail

The Stock Island tree snail eats algae and lichens found on hardwood hammock flora. During the dry season, the snail aestivates by sealing its outside shell to the tree trunk or branch. This strategy prevents dessication. Within the area to be treated, the Stock Island tree snail only occurs on No Name Key.

Studies have shown that Naled is highly toxic to freshwater and estuarine/marine invertebrates; acute LC_{50} values were 0.3 8.8, 0.79, and 0.019 ppb, respectively (EPA 1999; EPA 2006b). For the purposes of this analysis, we assume that Naled is equally toxic to the Stock Island tree snail. Stock Island tree snails may be especially susceptible to aerial Naled application due to their location in the tree canopy and their exposed mucosa, which provide little barrier to the absorption of

chemicals. Three small populations on No Name Key could be adversely affected by the application of Naled for the control of mosquitoes.

Given the potential exposure to Naled and the high level of toxicity to invertebrates, a risk assessment model was not necessary to analyze the potential effects of the proposed action on this species. Any direct application of Naled could result in mortality of individual Stock Island tree snails. To avoid adverse impacts to Stock Island tree snails, all occupied habitat of this species will be designated as a no spray zone (Figure 2).

Studies have shown that Permethrin is highly toxic to freshwater and estuarine/marine invertebrates; acute LC50 values were 0.79, 8.8, 0.79, and 0.019 ppb, respectively (USEPA 1999, USEPA 2006b). Three small populations on No Name Key could be adversely affected by the application of Permethrin for the control of mosquitoes. Any direct application of Permethrin could result in mortality of individual Stock Island tree snails. However, no actions are planned to occur proximate to occupied Stock Island Tree Snail habitat, and conditions of the permit such as wind direction and speed will likely eliminate any significant impacts (Figure 2).

Florida leafwing and Bartram's hairstreak, Miami blue and other imperiled butterflies

Both the Florida leafwing butterfly and Bartram's hairstreak butterfly are endemic to south Florida and the Keys, and occur only within pine rocklands that retain their sole host plant, pineland croton (Figure 10). Once locally common in south Florida, the Florida leafwing and Bartram's hairstreak now occur only within the pine rocklands in and adjacent to Everglades National Park (ENP) and Big Pine Key. Salvato (pers. comm. 2007) estimated that the population of Bartram's hairstreak collectively at Big Pine Key, Long Pine Key within ENP, and within relict pine rocklands adjacent to ENP may range from roughly 100–800 adults. Surveys of both species on Big Pine Key have indicated a decline over the last decade.

Based on the results of historic (Baggett 1982, p. 80; Schwartz 1987, p. 16; Hennessey and Habeck 1991, pp. 117-119; Worth et al. 1996, pp. 62-65; Schwarz et al. 1996, pp. 59-61) and recent (Salvato 1999, p. 1; 2001, p. 8; 2003, p. 53; Salvato and Hennessey 2004, p. 223; Salvato and Salvato 2010a, p. 154) surveys and natural history studies, the Bartram's hairstreak is extant in ENP and on Big Pine Key, while occurring only sporadically in pineland fragments in mainland Miami-Dade County. Hennessey and Habeck (1991, pp. 49-50) reported an estimate of 3.9 and 1 adult Bartram's hairstreaks per ha (1.6 and 0.4 per ac) during 1988-1989 survey transects on Big Pine Key and Long Pine Key, respectively. During 1997-1998, Salvato (1999, p. 52) recorded an estimated 4.3

adults per ha (1.7 per ac) at survey transects across Big Pine Key. However, Salvato (1999, p. 52; 2001, p. 8) failed to find stable numbers in either the Watson's Hammock on Big Pine Key or in Long Pine Key. The lower densities in Watson's Hammock and Long Pine Key reported by Salvato (1999, p. 52; 2001, pp. 8-14; pers. comm. 2006) and Salvato and Hennessey (2004, p. 224) during the late 1980s and 1990s have been attributed to a lack of prescribed fires necessary to maintain host plants. Through 2010, Salvato and Salvato (unpublished data) indicate the total number of adults observed annually on Big Pine Key has varied considerably from 9 to 278, based on monthly (1999-2006) or quarterly (2007-2010) surveys conducted from 1999 to 2010. The number of adults observed on Big Pine Key has declined precipitously over the duration of the Salvato's studies. The Salvato transects were fixed locations.

Based on results of all historic (Baggett 1982, p. 78; Schwartz 1987, p. 22; Hennessey and Habeck 1991, p. 17; Worth et al. 1996, p. 62; Schwarz et al. 1996, p. 59) and recent surveys and natural history studies (Salvato 1999, p. 1; 2001, p. 8; 2003, p. 53; Salvato and Hennessey 2003, p. 243; Salvato and Salvato 2010a, p. 91), the Florida leafwing is extant in ENP and, until recently, had occurred on Big Pine Key and rarely in pineland fragments in mainland Miami-Dade County (Salvato and Salvato 2010a, p. 91; 2010b, p. 139). Schwartz (1987, pp. 1-19), Hennessey and Habeck (1991, pp. 1-75), Emmel et al. (1995, p. 7), and Salvato (1999, pp. 1-168), searched the lower Florida Keys extensively for the Florida leafwing, only encountering the species on Big Pine Key. In the Everglades, Hennessey and Habeck (1991, p. 1-75) and Salvato and Salvato (2010a, pp. 91-97) reported the species from Long Pine Key. Hennessey and Habeck (1991, pp. 40, 42) reported an estimate of 3.7 adults per ha (1.5 per acre) during 1988-1989 from survey transects at both Watson's Hammock at NKDR on Big Pine Key and on Long Pine Key in ENP. During 1997-1998, Salvato (1999, p. 52) estimated 3.1 and 2.4 adults per ha (1.2 and 1.0 per acre) at Watson's Hammock at NKDR and the Gate 4 nature trail of Long Pine Key in ENP, respectively; these were at higher densities than what was found on survey transects elsewhere in his study. During 1999-2002, Salvato (pers. comm. 2009) recorded an average of 10.9 adults per ha (4.4 per acre) in the Watson's Hammock area, while other locations on Big Pine Key have yielded an average of 0.3 to 6.5 adults per ha (0.1 to 2.6 per acre). The higher densities in Watson's Hammock have been attributed to the fact that this is the only pine rockland area on Big Pine Key restricted from chemical pesticide applications for mosquito control (Hennessey and Habeck 1991, p. 1; Hennessey et al. 1992, p. 715; Salvato 2001, p. 8; Salvato and Salvato 2010b, pp. 139-140). However, analysis of survey data collected from 2003 through 2006 indicate a substantial decline in leafwing numbers on Big Pine, even within Watson's Hammock (Salvato and Salvato 2010b, pp. 139-140). During 2003-2006, Salvato (pers. comm. 2009) recorded an average of 1.5 adults per ha (0.6 per acre) in Watson's Hammock; other locations on Big Pine Key yielded an average of 0 to 1.3 per ha (0 to 0.5 per acre). Salvato and Salvato (2010b, p. 139) recorded three larvae and one adult in 2006. No leafwings were recorded on Big Pine Key after 2006 (Salvato and Salvato 2010b, p. 139).

In short, the Florida leafwing butterfly is considered to have a low population size, roughly several hundred or fewer (M. Salvato, pers. comm. 2011a). Its overall status is tenuous. On the mainland, it is found in ENP and only sporadically in other locations in Miami-Dade County near ENP. It may be extirpated from Big Pine Key, since it has not been found at that location since 2006 (Salvato and Salvato 2010b, pp. 139-140). Similarly, Marc Minno (pers. comm. 2007, 2008) believed that the Florida leafwing was either extremely rare or extirpated from the Keys based on surveys since August 2006. Minno indicates that this species was common on Big Pine Key in the 1980s, but the habitat has changed dramatically in recent years. Enumerations of habitat change were not provided.

In addition, several other tropical butterflies in the Keys have declined sharply in the last 20 years (Minno and Minno 2007a, 2007b). Two of the most imperiled are the rockland grass skipper (*Hesperia meskei pinocayo*) and zestos skipper (*Epargyreus zestos zestos*) (Minno and Minno 2007a, 2007b). During the early 1990s, the rockland grass skipper could still be found in pine rockland habitat of southern Miami-Dade County and on Big Pine Key in Monroe County (Minno and Minno 2007a). More recently, it was believed to still occur in very low abundance in one small area of Big Pine Key (Minno and Minno 2007a). Marc Minno (pers. comm. 2008) indicates that the rockland grass skipper has not been found since 2000. Similarly, the zestos skipper was once locally common in southern Miami-Dade County and the Keys (Minno and Emmel 1993), but is currently extremely rare or may even be extirpated from Florida and the U.S. (Minno and Minno 2007b). Minno (pers. comm. 2008) indicates that the zestos skipper has not been found since 2002. Despite targeted survey efforts initiated in September 2006, neither the rockland grass skipper or the zestos skipper have been observed on Big Pine or elsewhere in their ranges (Minno and Minno 2007a, 2007b; M. Minno, pers. comm. 2008).

Known threats to the Florida leafwing and the Bartram's hairstreak include the destruction, modification, or fragmentation of their habitat or range, and specimen collection. Habitat loss has occurred due to residential and commercial development on private lands. Habitat fragmentation continues to occur even on public lands due to unnatural or altered fire regimes. A natural fire regime is an important process for maintaining pineland croton populations in pine rocklands.

Mosquito control operations are another potential contributor to the decline of butterflies. Mosquito control adulticides are broad-spectrum insecticides. As such, they are potentially lethal to various life stages of most insects (Higgins 2005). They have been implicated as a likely contributing factor to the decline of several lepidopteran species in the Florida Keys (Baggett 1982, Emmel and Tucker 1991, Eliazar 1992, Calhoun et al. 2000, Salvato 2001).

Efforts to control salt marsh mosquitoes, *Aedes taeniorhynchus*, among others,

have increased as human activity and population have increased in south Florida. To control mosquito populations, second-generation organophosphate (naled) and pyrethroid (permethrin) adulticides are applied by mosquito control districts throughout south Florida. Both of these compounds have been characterized as being highly toxic to nontarget insects by the U.S. Environmental Protection Agency (2002, p. 32; 2006a, p. 58; 2006b, p. 44). The use of such pesticides (applied using both aerial and ground-based methods) to control mosquitoes presents a potential risk to nontarget butterfly species.

The aerial application of mosquito control pesticides has occurred for nearly 30 years in the Keys, including nearly all public lands in the National Key Deer Refuge. Since most of the remaining suitable habitat for both the Florida leafwing and Bartram's hairstreak in the Keys is located within National Key Deer Refuge, aerial and ground application of mosquito adulticides may present a risk to their continued existence in the Keys.

A comparison of Naled toxicity to five butterfly species with Naled toxicity to honey bees demonstrates that the U.S. EPA's standard hazard assessment protocol for assessing risk to non-target terrestrial invertebrates (toxicity testing with honey bees) significantly underestimates the potential risk for butterflies (Table 2). Accordingly, Naled is potentially hazardous to non-target lepidopteran species. Any direct application of Naled could result in mortality of individual Florida leafwing butterflies and Bartram's hairstreak butterflies. Aerial application of adulticides may also result in negative sub-lethal effects to these butterflies.

Table 2. Naled Toxicity (LD50 [ug/g bw]) to selected butterfly species and honey bees.

	3rd Instar	4th Instar	5th Instar	Adult
H. cresphontes [1]	0.9	0.966	0.384	0.19
V. cardui [1]			0.417	0.541
A. vanillae [1]			0.717	
U. proteus [2]	0.0699	0.0439	0.0296	0.1892
P. oileus [2]		1.021	0.304	0.0823
Honey bee [3]				4.528

[1] Eliazar 1992.
[2] Salvato 2001
[3] U.S. EPA 2006

The potential for mosquito control chemicals to drift into nontarget areas and persist for varying periods of time has been well documented. Hennessey and Habeck (1989, pp. 1–22; 1991, pp. 1–68) and Hennessey et al. (1992, pp. 715–721) illustrated the presence of mosquito spray residues long after application in

habitat of the Schaus swallowtail and other imperiled species in both the upper (Crocodile Lake NWR, North Key Largo) and lower Keys (NKDR). Residues of aerially applied naled were found 6 hours after application in a pineland area that was 820 yards (750 meters) from the target area; residues of fenthion (an adulticide no longer used in the Keys) applied via truck were found up to 55 yards (50 meters) downwind in a hammock area 15 minutes after application in adjacent target areas (Hennessey et al. 1992, pp. 715–721). Hennessey and Habeck (1989), Hennessey and Habeck (1991), and Hennessey et al. (1992) (the three references detail activities of one field study) did not provide conclusive findings regarding the effects of mosquito control spraying on the two butterfly species examined (Florida leafwing and Bartram's hairstreak). A greater number of adult Florida leafwing butterflies were observed in untreated areas during one year of the study, but this difference was not observed in the second year of the study (Hennessey and Habeck 1991, p. 14). Additionally, the study revealed that one of the reference locations received adulticide deposition through aerial drift, thus compromising the utility of the location to be used as a reference site and making it difficult to discern pesticide effects (Hennessey and Habeck 1991, pp. 29–30). Mosquito control spraying technology and strategies have advanced in recent years. Despite these advances, recent research (Pierce 2009, pp. 2–15; Zhong et al. 2010, pp. 1966–1967; Pierce 2011, pp. 6–11; T. Bargar, USGS, pers. comm. 2011) documents quantifiable residues of mosquito control chemicals on filter pads and foliage in nontarget areas.

Pierce (2009, pp. 1–17) monitored naled and permethrin deposition following application in and around NKDR from 2007 to 2009. Permethrin, applied by truck, was found to drift considerable distances from target areas with residues that persisted for weeks. Naled, applied by plane, was also found to drift into nontarget areas but was much less persistent, exhibiting a half-life of approximately 6 hours. To expand this work, Pierce (2011, pp. 6–11) conducted an additional deposition study in 2010 focusing on permethrin drift from truck spraying and again documented measurable amounts of permethrin in nontarget areas. In 2009, Tim Bargar (pers. comm. 2011) conducted two field trials on NKDR that detected significant naled residues at locations within nontarget areas on the Refuge that were up to 440 yards (402 meters) from the edge of zones targeted for aerial applications.

In addition to mosquito control chemicals entering nontarget areas, the toxic effects of mosquito control chemicals to nontarget organisms have also been documented. Lethal effects on nontarget Lepidoptera have been attributed to fenthion and naled in both south Florida and the Keys (Emmel 1991, pp. 12–13; Eliazar and Emmel 1991, pp. 18–19; Eliazar 1992, pp. 29–30). In the lower Keys, Salvato (2001, pp. 8–14) suggested that declines in populations of the Florida leafwing (now a Federal candidate) were also partly attributable to mosquito control chemical applications. Salvato (2001, p. 14; 2002, pp. 56–57) found populations of the Florida leafwing (on Big Pine Key within NKDR) to increase during drier years when adulticide applications over the pinelands decreased,

although Bartram's hairstreak did not follow this pattern. It is important to note that vulnerability to chemical exposure may vary widely between species, and current application regimes do not appear to affect some species as strongly as others (Calhoun et al. 2002, p. 18; Breidenbaugh and De Szalay 2010, pp. 594–595; Rand and Hoang 2010, pp. 14–17, 20; Hoang et al. 2011, pp. 997–1005).

Dose-dependent decreases in brain cholinesterase activity in great southern white butterflies (*Ascia monuste*) exposed to naled have been measured in the laboratory (T. Bargar, pers. comm. 2011). An inhibition of cholinesterase, which is the primary mode of action of naled, prevents an important neurotransmitter, acetylcholine, from being metabolized, causing uncontrolled nerve impulses that may result in erratic behavior and, if severe enough, mortality. From these data, it was determined that significant mortality was associated with cholinesterase activity depression of at least 27 percent (T. Bargar, pers. comm. 2011). In a subsequent field study on NKDR, adult great southern white and Gulf fritillary (*Agraulis vanillae*) butterflies were placed in field enclosures at both target and nontarget areas during aerial naled application. The critical level of cholinesterase inhibition (27 percent) was exceeded in the majority of butterflies from the target areas, as well as in a large proportion of butterflies from the nontarget areas (T. Bargar, pers. comm. 2011). During the same field experiment, great southern white and Gulf fritillary larvae were also exposed in the field during aerial naled application and exhibited mortality at both target and nontarget sites (T. Bargar, pers. comm. 2011).

In a laboratory study, Rand and Hoang (2010, pp. 1–33) and Hoang et al. (2011, pp. 997–1005) examined the effects of exposure to naled, permethrin, and dichlorvos (a breakdown product of naled) on both adults and larvae of five Florida native butterfly species: common buckeye (*Junonia coenia*), painted lady (*Vanessa cardui*), zebra longwing (*Heliconius charitonius*), atala hairstreak (*Eumaeus atala*), and white peacock (*Anartia jatrophae*). The results of this study indicated that, in general, larvae were slightly more sensitive to each chemical than adults, but the differences were not significant. Permethrin was generally the most toxic chemical to both larvae and adults, although the sensitivity between species varied.

The laboratory toxicity data generated by this study were used to calculate hazard quotients (concentrations in the environment/concentrations causing an adverse effect) to assess the risk that concentrations of naled and permethrin found in the field pose to butterflies. A hazard quotient that exceeds one indicates that the environmental concentration is greater than the concentration known to cause an adverse effect (mortality in this case), thus indicating significant risk to the organism. Environmental exposures for naled and permethrin were taken from Zhong et al. (2010, pp. 1961–1972) and Pierce (2009, pp. 1–17), respectively, and represent the highest concentrations of each chemical that were quantified during field studies in the Keys. When using the lowest median lethal concentrations from the laboratory study, the hazard quotients for permethrin were greater than

one for each adult butterfly, indicating a significant risk of toxicity to each species. In the case of naled, significant risk to the zebra longwing was predicted based on its hazard quotient exceeding one.

In a recent study, Bargar (2012, pp. 1–7) conducted a probabilistic risk assessment for adult butterflies using published acute toxicity data in combination with deposition values for naled that were quantified at eight locations within NKDR. The published toxicity data were used in conjunction with morphometric data (total surface area and weight) for 22 butterfly species and the NKDR naled deposition values to estimate the probability that field exposure to naled will exceed butterfly effect estimates (quantity of naled per unit body weight associated with mortality in adult butterflies). From the field deposition measurements, the probability that the effect estimate for 50 percent of the examined butterfly species will be exceeded ranged from 70 (lowest butterfly surface area to weight ratio) to 95 percent (highest surface area to weight ratio) based on filter paper deposition results and 33 to 87 percent based on yarn sampler results. As the surface area to weight ratio increases, the probability that a greater quantity of naled per unit body weight will be delivered increases. These results suggest that significant impacts on butterfly survival may result from aerial naled application.

From 2006 to 2008, Zhong et al. (2010, pp. 1961–1972) investigated the impact of single aerial applications of naled on Miami blue larvae in the field. The study was conducted in North Key Largo in cooperation with the Florida Keys Mosquito Control District (FKMCD) and used experimentally placed Miami blue larvae that were reared in captivity. The study involved 15 test stations: 9 stations in the target zone, 3 stations considered to be susceptible to drift (2 stations directly adjacent to the spray zone and 1 station 12 mi (19.3 km) southwest of the spray zone), and 3 field reference stations (25 mi (40.2 km) southwest of the spray zone). Survival of butterfly larvae in the target zone was 73.9 percent, which was significantly lower than both the drift zone (90.6 percent) and the reference zone (100 percent), indicating that direct exposure to naled poses significant risk to Miami blue larvae. The vitality of the larvae used in the study is confirmed by the fact that no larval mortality was observed in the control zone (Zhong et al. 2010, p. 1969). In addition to observing elevated concentrations of Naled at test stations in the target zone, 9 of 18 samples in the drift zone also exhibited detectable concentrations, once again exhibiting the potential for mosquito control chemicals to drift into nontarget areas. The mortality trend observed in mosquitoes placed in the spray, drift, and control zones also followed a clear dose-response similar to that of the butterfly caterpillars (Zhong et al. 2010, p. 1969).

Based on these studies, it can be estimated that even with 50 meter buffer zones for Critical Habitat and 250 meter buffers from occupied areas that mosquito control activities that involve the use of both aerial and ground-based spraying methods have the potential (while greatly minimized) to deliver pesticides in

quantities sufficient to cause adverse effects to nontarget species in both target and nontarget areas. The likelihood of significant drift, in terms of measurable effect on the Bartram's Hairstreak or the Florida Leafwing, will be largely mitigated with the establishment of a larger and fully inclusive no spray area. (Figure 2). Furthermore, the number of applications and conditions under which treatment is permitted will be limited. The District will not be permitted to spray Permethrin if winds are forecasted to be more than 12 mph and gusts should not exceed 20 mph during any operational period. If conditions deviate from forecasted conditions while the mission is in progress, the mission will be suspended until such time as the aforementioned acceptable conditions occur again. Applications will occur at no less than 90 hour intervals between treatments on Big Pine Key. It should be noted that many of the studies referenced above dealt with single application scenarios and examined effects on only one to two butterfly life stages. Under a realistic scenario, the potential exists for exposure to all life stages to occur over multiple applications in a season. For this reason, buffer zones become needed to reduce the potential for exposure. In the case of a persistent compound like permethrin where residues remain on vegetation for weeks, the potential exists for nontarget species to be exposed to multiple pesticides within a season (e.g., permethrin on vegetation coupled with aerial exposure to naled).

Aspects of the Miami blue's natural history may increase its potential to be exposed to and affected by mosquito control pesticides and other chemicals. For example, host plants and nectar sources are commonly found at disturbed sites and often occur along roads in developed areas, where chemicals are applied. Ants associated with the Miami blue (see Interspecific relationships) may be affected in unknown ways. Host plant and nectar source availability may also be indirectly affected through impacts on pollinators.

Although there is no evidence of mosquito control impacts on wild Miami blue populations, potential impacts over the subspecies' historical range have never been examined. Recent research has shown that exposure to mosquito control chemicals in sufficient quantities can impact various butterfly species, including captive-bred Miami blue (Zhong et al. 2010 pp. 1967–1968; Hoang et al. 2011 pp. 1000–1002). Based on these findings, the Service determined that mosquito control pesticides can be a threat to the Miami blue.

No mosquito control pesticides are used within KWNWR. Mosquito control practices currently pose no risk to the Miami blue within KWNWR. However, mosquito control activities, including the use of larvicides and adulticides, are being implemented within suitable and potential habitat for the Miami blue elsewhere in its range (Carroll and Loye 2006, pp. 14–15). The findings of Zhong et al. (2010, pp. 1961–1972) and Pierce (2009, pp. 1–17) along with other studies suggest that aerial or truck-based applications of mosquito control chemicals may pose a threat to the Miami blue, if the butterfly exists in other, unknown locations. Additionally, mosquito control practices potentially may limit expansion of

undocumented populations or colonization of new areas. If the Miami blue colonizes new areas or if additional populations are discovered or reintroduced, adjustments in mosquito control (and other) practices may be needed to help safeguard the subspecies.

Garber's Spurge

Historically, Garber's spurge occupied pine rocklands and hammock edge from south Florida through the Florida Keys. Although surveys in 2005 and 2006 indicated that certain Garber's spurge populations were extirpated on Big Pine Key (Green et al. 2006), some extant populations were found on southern Big Pine Key more recently (Green et al. 2008). Garber's spurge is believed to be wind pollinated (Keith Bradley, The Institute for Regional Conservation [IRC] pers. comm. 2007). The lack of phytotoxicity of Naled and lack of reliance on insect pollination for reproduction indicates that the application of Naled and Permethrin in the Florida Keys is not likely to adversely affect this Garber's spurge.

Key Tree-Cactus

The Key tree-cactus is present in Cactus Hammock on Big Pine Key. Populations formerly located in Key West and Boca Chica Key have been extirpated. The closest population to the Big Pine Key population is located on Long Key (Service 1999). Although Cactus Hammock is a no-spray zone, this species could be adversely affected by long distance drift of mosquito spraying through a reduction in the population of potential pollinators, thus producing a reduction in the reproductive potential of this endangered plant species. The likely pollinator of the Key tree-cactus appear to be moths and or bats.

While flowering occurs year round, the peak reproductive months for the Key tree-cactus are July through September, which coincides with the peak period for mosquito spraying in the Florida Keys (Adams and Lima 1994). A study on the reproductive biology of this species revealed that bagged and non-bagged blooms of the Key tree-cactus in Naled application areas set fruit and produced viable seed (Hennessey and Habeck 1994). This indicates that the Key tree-cactus can employ self-pollination in reproduction at least to some extent. While this does not entirely eliminate the potential adverse effects to the species, it does minimize them to the extent they become insignificant or discountable.

Plants

Big Pine partridge pea, wedge spurge, and sand flax are three candidate plants that occur within pine rocklands in the Keys and have a large proportion of their habitat within the NKDR. Most of the range of the Big Pine partridge pea is within the NKDR. Ross and Ruiz (1996) estimated that about 90 percent of the plants on Big Pine Key are within NKDR. Wedge spurge is known only from Big

Pine Key in the Keys, and most of its range is encompassed within the NKDR. Sand flax has a larger range, but the largest population in Monroe County is located on Big Pine Key within NKDR. The status of sand flax in the Keys is of particular concern. Updated monitoring information from TNCs Terrestris Preserve on Big Pine Key indicates that no sand flax was found on transects in any of the management units in 2006 (Slapcinsky and Gordon 2007; D. Gordon, TNC, pers. comm. 2008). Slapcinsky and Gordon (2007) generally found density of sand flax declined to zero in all three burn units (burns were conducted from 1994 – 2003) in 2006, although Gordon (pers. comm. 2008) attributed the response to the damaging effects of Hurricane Wilma in 2005.

Pollinator limitation is identified as a threat for the Big Pine partridge pea within wildland-urban interface areas on Big Pine Key where fewer seeds per fruit were produced than those well within solid blocks of preserved pine rockland (Liu and Koptur 2003). Buzz-pollinating bees (*Xylocopa micans* and *Melissodes* spp.) were the only functional group observed to be effective in pollinating partridge pea. The species composition of visits to partridge pea by those bees was altered in urban edge (fewer visits by *Melissodes* spp.) as compared to visits in the more pristine pine rockland areas, and overall visits were reduced on the urban interface. Liu and Koptur (2003) suggested that aerial mosquito spraying may exacerbate the existing pollinator limitation suffered by Big Pine partridge pea by reducing the number of visits by the buzz-pollinating bees. The protection of all pine rockland habitat as Critical Habitat for the Bartram's Hairstreak and Florida leafwing offer significant protection for this species. Therefore, potential adverse effects to the species are expected to be insignificant or discountable.

Bradley (2006) indicated that pesticide spraying is common on Big Pine Key and its suppression of pollinator populations may also have a long term impact on reproduction rates of sand flax and wedge spurge. However, the lack of pollinator information makes assessing the effects of mosquito spraying in the Keys on sand flax and wedge spurge difficult if not impossible (Hodges and Bradley 2006). Furthermore, the protection of all pine rockland habitat as Critical Habitat for the Bartram's Hairstreak and Florida leafwing offer significant protection for this species. Therefore, potential adverse effects to the species are expected to be insignificant or discountable.

The Cape Sable thoroughwort (*Chromolaena frustrata*) was proposed as Endangered with critical habitat on October 11, 2012 (77 FR 61836). Currently unoccupied critical habitat for the species is proposed for Refuge lands on Big Pine Key, however the species does not currently occur on Big Pine Key. Furthermore, pollinator limitation is not identified as a threat to the Cape Sable thoroughwort. Therefore, potential adverse effects to the species are expected to be insignificant or discountable.

The Florida semaphore cactus (*Consolea corallicola*) was proposed as Endangered on October 11, 2012 (77 FR 61836). The species occurs on Little

Torch Key and Lower Saddlebunch Key, but no wild plants are located on Refuge lands. Furthermore, pollinator limitation is not identified as a threat to the Florida semaphore cactus. Therefore, potential adverse effects to the species are expected to be insignificant or discountable.

SPECIES/ CRITICAL HABITAT	IMPACTS TO SPECIES/CRITICAL HABITAT
Stock Island tree snail	Not likely to adversely affect with specified application rates, frequencies, intervals, no spray zones, and restrictions on wind speed and direction. No critical habitat.
Key deer	Not likely to impact via truck fogging and aerial applications likely to adversely affect with specified application rates, frequencies, and intervals. Barrier treatments are likely to adversely impact. No critical habitat.
Rice rat	Not likely to adversely affect with specified application rates, frequencies, and intervals. No adverse effects to critical habitat are anticipated as a result of the proposed action.
Eastern indigo snake	Not likely to adversely affect with specified application rates, frequencies, and intervals. No critical habitat and not thought to occur in action area.
Key tree-cactus	Not likely to adversely affect with specified application rates, frequencies, and intervals. No critical habitat. A no spray zone for Permethrin and Naled is in place in Cactus Hammock
Lower Keys marsh rabbit	Not likely to adversely affect with specified application rates, frequencies, and intervals. No critical habitat.
Garber's spurge	Not likely to adversely affect with specified application rates, frequencies, and intervals. No critical habitat.
Florida leafwing butterfly	Likely to adversely affect with currently specified application rates, frequencies, no spray zones and intervals.
Bartram's hairstreak butterfly	Likely to adversely affect with currently specified application rates, frequencies, no spray zones and intervals.
Miami blue butterfly	Not likely to adversely affect with specified application rates, frequencies, and intervals. No critical habitat, not known to occur in action area.
Big Pine partridge pea	Not likely to adversely affect with currently specified application rates, frequencies, no spray zones and intervals.
Wedge spurge	Not likely to adversely affect with currently specified application rates, frequencies, no spray zones and intervals.

SPECIES/ CRITICAL HABITAT	IMPACTS TO SPECIES/CRITICAL HABITAT
Sand flax	Not likely to adversely affect with currently specified application rates, frequencies, no spray zones and intervals.

B. Explanation of actions to be implemented to reduce adverse effects:

The Proposed Action is to develop and implement a mosquito management plan (plan) that would allow the Refuge to respond to public health issues due to mosquitoes on the Refuge as identified by a current monitoring data by a public health agency or their designated authorized representative. The mosquito management plan would consist of a phased approach to mosquito management and is consistent with the principles of integrated pest management. The Proposed Action emphasizes design, and management of Refuge lands in a manner beneficial to wildlife consistent with the mission of the Refuge and so as to minimize mosquito production and specifically the public health threat due to Refuge mosquitoes. Monitoring and surveillance will be the first front to identify mosquito source areas and status.

District activities should be focused on identifying changes in hydrology, weather and vegetation that form mosquito habitat and develop improvements in monitoring and use advances in pesticide methods to reduce the potential for exposure to non-target species. The methods employed should minimize chemical control measures and to decrease mosquito production, seeking the least invasive approach given the current environmental conditions. This alternative is consistent with an integrated pest management (IPM) approach. IPM is a sustainable approach to managing pests by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks. When practical, the approach may include compatible actions that reduce mosquito production and do not involve pesticides. We consider the procedures described below as long-term practices to reduce persistent potential mosquito-associated health threats that Federal, State, and/or local public health authorities have identified.

While the emphasis of this alternative is to minimize chemical control measures and protect non-target resources, it also includes monitoring, surveillance and the potential application of pesticides. Application of pesticides would be approved based on the phased (threshold based) approach outlined below. The principle goal of a phased approach to mosquito management is to minimize effects on refuge resources to fulfill the Refuge mission while addressing legitimate human health concerns and complying with Service regulations and policy. The implementation of a phased-response program represents a standardized approach that would result in a consistent mosquito management program that adheres to Service and District guidelines. Because occurrences of human health issues

resulting from mosquitoes are sporadic, phases of mosquito management implemented on the Refuge would vary through time.

The Refuge and the District would work jointly in the implementation of a mosquito management program. The District would have the lead for monitoring, disease surveillance, and pesticide applications; however, the evaluation of monitoring data and approval for the management actions proposed would be the responsibility of the Refuge. While this would require additional staff time, it is necessary to ensure that the conditions for compatibility are met and the program is implemented so as to avoid or minimize effects on Refuge resources.

Mosquito Threshold Treatment Levels. Human and wildlife treatment threshold levels (e.g., numbers per sample) are determined by considering several factors unique to an area. Factors for the Florida Keys are presented in Table . These factors, in conjunction with sheer abundance of biting mosquitoes, including allergic response, potential magnification of disease in mosquito and host populations, and potential passage of disease even if mosquitoes have not yet been determined to contain a pathogen are considered.

Although treatment thresholds vary according to several factors, most districts across the country have an established baseline threshold treatment level for larval and adult forms.

Table 1. Factors that may affect thresholds for application of chemical pesticides.

Factor	Description	Consideration
Proximity to human populations	The distance from potential mosquito habitat on the refuge to population centers (numbers and density).	The potential to produce large numbers of mosquitoes in close proximity to population centers may result in less tolerance or lower thresholds for implementation of mosquito control on the Refuge.
Seasonality and weather patterns	Seasonal changes in prevailing wind patterns, precipitation, and temperatures.	Prevailing wind patterns that carry mosquitoes from Refuge environments to population centers may require lower thresholds. Inclement weather conditions may prevent mosquitoes from moving off-refuge resulting in higher thresholds.

Cultural mosquito tolerance	The tolerance of different populations within proximity of the Refuge varies.	The Refuge lies within a highly populated area that exhibits lower thresholds (relative to other areas of the country) and a general intolerance to mosquitoes. Number of mosquito complaints is a factor.
Adults harbored, but not produced , on-refuge	Refuge provides resting areas for adult mosquitoes produced in the surrounding landscape	Threshold for mosquito management on the Refuge should be high with an emphasis for treatment of larval development habitat off Refuge
Spatial extent of mosquito larval development habitat on and off the refuge	The relative availability of mosquito habitat within the landscape that includes the Refuge.	If the Refuge is a primary larval development area for mosquitoes that likely affect human health, thresholds may be lower. If refuge mosquito habitats are insignificant in the context of the landscape, thresholds may be higher.
Tidal cycles	The tides rise and fall twice daily in areas within the tidal zone. Spring tides bring higher than normal tide levels and result in flooding of the marsh plain.	Much of the land base of the Refuge lies within the tidal zone where spring tides can flood the marsh plain. Where lower elevation swales exist, water ponds and creates mosquito habitat
Natural predator populations	Balanced predator-prey populations may limit mosquito production.	If Refuge vertebrate and invertebrate prey populations are adequate to control mosquitoes, threshold for treatment should be high.
Water quality	Water quality influences mosquito productivity.	High organic content in water may increase mosquito productivity, lower natural predator abundance, and may require lower thresholds.
History of mosquito borne diseases in area	Past monitoring of wildlife, mosquito pools, horses, sentinel chickens, and humans have documented mosquito-borne diseases.	Thresholds in areas with a history of mosquito-borne disease(s) are lower.

Alternative B will include monitoring driven, site specific management of treatment units based on thresholds and resource risk. Treatments will be done in

the temporally and spatially strategic fashion to maximize reductions of mosquito populations and reduce damage to natural resources. The intent of this alternative will be to integrate the buffer zone from critical habitat of the Bartram's hairstreak and Florida leafwing butterflies and require site specific adaptive management based on phased approach (Figure 4) for control applications given a demonstrated necessity for treatments based on thresholds. Additional buffers (250 meters) will be designated around occupied Bartram's hairstreak or Florida leafwing areas as determined by regular monitoring of all currently known and newly discovered sites (Figure 5) and are layered over the existing "no spray zones" designated under Alternative A (protecting hammock and other sensitive areas). The thresholds, or trigger points, will relate to densities of mosquitoes that pose a threat to human health. Depending on the magnitude of the health threat associated trigger points this alternative would allow for different treatment application methods and products. Refuge Islands will be divided into treatment areas, such as neighborhoods or Keys (in the case of the Back Country Islands). The types of mosquito control products, treatment methods, and environmental conditions permitted will be based on site specific issues such as natural resource concerns and Wilderness status.

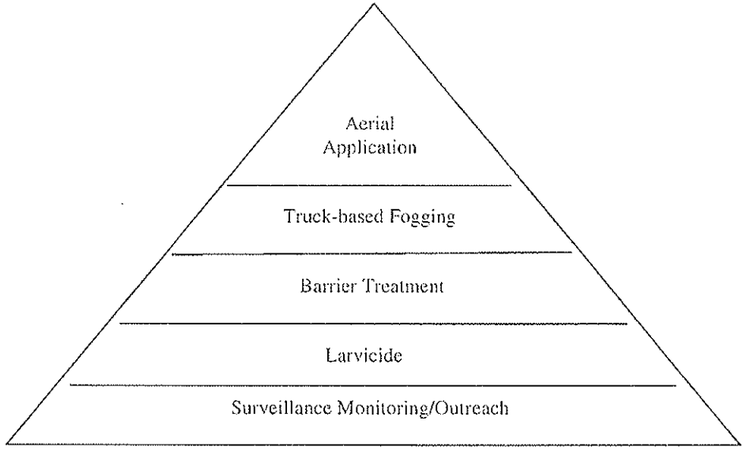

Illustration of Phased Approach to Mosquito Control indicating that higher risk approaches will represent the least utilized methods.

Level 1

In Level 1, a health threat has not been identified and mosquito management

issues have not been recorded by District Monitoring, reported, or identified by the appropriate public health authority. To avoid any possible mosquito management issues, artificial mosquito larval development habitat throughout the Refuge, such as tires, open containers, and other equipment or objects that pool water where mosquitoes may breed, should be eliminated. To aid in this effort, outreach efforts shall be conducted to homes inside the acquisition boundaries of the Refuge by both the District and the Refuge. Mosquito larvae are not detected in larval development sites. Community education will be a key component to ensure understanding the balance of use of mosquito intervention methods vs. preventative measures. In addition, under this phase, innocuous control measures including use of native *Gambusia* (mosquito fish) for control of larval mosquitos would be continued.

Level 2

Larvicide will be the primary tool for reducing mosquito populations using the methods described in Alternative C. Larvicide operations will be triggered by the presence of larval instars at larval development sites as observed in field monitoring, as well as historical presence of larvae should the District perform a pre-treatment application. Larvicides used will be Bti or Bsp. Larvicide is applied at the earliest period provided so as to reduce the quantity of larvicide deployed to minimize cost and improve effectiveness (as early instars are more vulnerable to the action of the larvicide than later instars). Larvicide applications are made to eliminate potential emergence of adult mosquitoes. Applications will be made as described in Alternative A; however, additional areas may be identified as larval development locations and may be added as appropriate, based on monitoring. Additional larvicide treatments are preferable to initiating adulticide operations.

Level 3

Ground application of pyrethroid products at maximum label concentrations as a "barrier treatment" may be used when adult mosquito thresholds exceed 3.0 mosquitoes/minute for two consecutive days on private property or in public areas (i.e. park, school) but not on Refuge managed areas. Positive samples collected during surveillance monitoring for invasive mosquito species can also trigger this method of application. Applications will be done using either a backpack or trailer-mounted mist blower, depending upon the area (Barrier treatments typically consist of applying the product at approximately 300-350 um to non-flowering vegetation in a vertical band of 3-6 feet; effectiveness lasts approximately 1 week in the Keys ecosystem) (FKMCD, unpublished data). Barrier treatment will be applied to suitable vegetation and extreme care will be used to avoid direct treatment to refuge lands. "Extreme care" is defined as stopping the barrier application at least 15 feet from non-target areas (e.g., buffer areas in the refuge).

Barrier treatments will be done by the application of the pyrethroid insecticide

bifenthrin. Barrier treatments for mosquito control are useful when applied as part of an integrated mosquito control program (Cilek 2008). They can outperform truck sprays and can give significant cost savings (Qualls et al. 2012). Barrier treatments are differentially effective due to sex, parity status, blood-fed status, and time after application (Doyle et al. 2009). Not all mosquito species are impacted equally by the barrier treatment (Hurst et al. 2012). Exposure to rain and sunlight also affect bifenthrin barrier sprays, with residual effects most pronounced in shady areas protected from rainfall (Allen et al. 2009). Avoidance of damage to bees can be attempted by making barrier applications late in the day, as the active ingredient would have time to break down overnight (Qualls et al. 2010). Hoffman et al. (2009) tested 5 sprayers for droplet size, deposition on both surfaces of the leaf (top and bottom) and depth of penetration into the canopy. Larger droplet size was better for barrier treatments, and sprayers with higher wind velocity at nozzle discharge performed better than did those with lower wind velocity. All sprayers tested gave maximum deposition of bifenthrin at 1 meter into the treated vegetation.

Barrier treatments will be applied at a distance of 5 feet from the vegetation canopy with a sprayer angle of 60 degrees. Target height of spray is 6 to 9 feet high, and the concentration of droplets in this target area is 30 to 180 droplets / cm2. Droplet size in this scenario is 300 – 350 μm.

Level 4

When monitoring results indicate one or more neighborhood(s) adjacent to Refuge are displaying adult mosquito numbers that exceed the threshold of 3.0 mosquitoes/minute average, Pyrethroid application could be initiated outside of excluded areas and buffer zones. Positive samples collected during surveillance monitoring for invasive mosquito species can also trigger this method of application. Two separate options would be available. Both options are intended to minimize exposure of this pesticide to non-target species including listed butterflies. In addition, residents experiencing a high level of mosquito activity in a limited and localized area (i.e., within their property limits) could request application of Pyrethroid via handheld applications by trained District staff, as appropriate. Monitoring would need to confirm this localized effect and all efforts will be made by District staff to limit applications near Refuge boundaries so as to avoid any localized drift (Given the localized control of the handheld application method, 50 ft buffer is recommended based on expected drift distances from application techniques used and no application direct to refuge lands permitted. This would be restricted to low wind periods as described below).

Option A: Truck based fogging of Pyrethroid products will be applied in

neighborhood(s) adjacent to Refuge. Applications will not occur within the proposed Critical Habitat or designated Critical Habitat (50 meters) or occupied Bartram's hairstreak butterfly and Florida Leafwings buffers (250 meters) (see Figure 5). Buffer zones are based on expected drift distances to protect sensitive habitats. Drift at a level of concern has been measured under 250 m from truck routes (Pierce 2010, Rand and Hoang 2010). Therefore, no permethrin applications shall occur within 250 m of known occupied Florida leafwing or Bartram's hairstreak proposed critical habitat (Figure 5). Buffer areas may be refined with additional study, in an iterative process as needed, and with the approval of the Service. These applications will not reoccur unless the thresholds are met following a monitoring period and no more than 96 hours following a previous treatment to prevent accumulation of product. Treatments will otherwise follow the methods outlines in Alternative A. Permethrin applications shall not occur when sustained winds exceed 10 mph, or gusts exceeding 15 mph during the entire operational period. Wind direction shall be considered in all application scenarios where excluded areas or buffer zones are not established, but Refuge is present.

Option B: Truck based fogging of Pyrethroid products will be applied to neighborhood(s) adjacent to Refuge. Applications may occur within the proposed Critical Habitat (50 meters) but never within the occupied Bartram's hairstreak butterfly and Florida Leafwings buffers (250 meters) (see Figure 5). This would be a one-time application based on an isolated mosquito occurrence rate that exceeds the 10.0 mosquitoes/minute average threshold. Only one neighborhood that is within the critical habitat area designation could be fogged in this manner at any one time so as to provide refuge for animals within other areas. Applications will not reoccur unless the thresholds are met following a monitoring period and no more than 1 month following a previous treatment. Treatments will otherwise follow the methods outlines in Alternative A. Pyrethroid applications shall not occur when sustained winds are forecasted to exceed 10 mph, or gusts exceeding 15 mph. Wind direction shall be considered in all application scenarios to ensure that drift does not penetrate critical or occupied habitat for the listed species. Buffer zones are in place to ensure that added protective buffer to those sensitive habitats.

Level 5

Aerial application of naled products will be broadcast when mosquito levels reach 10.0 mosquitoes/minute average on any given treatment area on Big Pine Key, or mosquito levels reach 40 mosquitoes/minute average on any given treatment area

on No Name Key. Positive samples collected during surveillance monitoring for invasive mosquito species can also trigger this method of application. These applications shall follow the methods outlined in Alternative A. Every effort will be made to avoid occupied butterfly habitat within the Refuge. Aerial application will not be permitted when sustained winds are forecasted to exceed 10 mph, or gusts exceed 15 mph.

If a Miami blue is detected on Refuge lands, naled and pyrethroid applications will be suspended at the associated habitat patch or patches. And consultation will be reinitiated immediately to determine next actions.

Recommendations made by the Imperiled Species Subcommittee of the Florida Coordinating Council on Mosquito Control, include requiring buffers for known Miami blue populations, allowing for incidental take in areas receiving mosquito control, and supporting additional research into nontarget impacts from mosquito control.

The level of detail for reporting on naled was further increased, and extensively increased for Permethrin, as of this (2012) season, also to include retroactive data. Application reporting for all materials (naled, permethrin, *Bti*) will include dates, times, spatial coordinates, concentrations, weather, and volumes for all application segments of each type. Volumes and coverage areas will also be reported, by individual island, subdivision and month (including corresponding values from earlier years, to the extent available).

Additional research will be helpful in developing a more thorough understanding of impacts from mosquito control, fire ants, and other threats. Recently completed and ongoing research was outlined above. Research studies examining the lethal and sublethal effects of adulticides on butterflies are currently underway; these data will increasingly inform the assessment of impacts to candidate, listed, and other lepidopterans. The Service continues to support research to characterize drift from truck-based and aerial spraying methods. The data from these studies will aid in better determining actual residue values, and appropriate buffer distances around sensitive areas.

Central to these efforts is the Services goal of moving beyond hazard-based assessments of potential injury for assessing pesticide impacts to species. Instead, we and partners seek to establish spatially explicit, risk-based assessments to address the probability of injury, based on actual field exposure. Risk-based assessments that take into account actual field exposure scenarios are an effective way to evaluate risk to threatened and endangered species. For example, in a recent study, field deposition values for naled on the National Key Deer Refuge (NKDR), Big Pine Key, were incorporated into a probabilistic risk assessment that predicted significant risk to common butterflies (Bargar 2012, pp. 1–7). Such risk assessments would examine direct effects on individual organisms, but would also be interpreted at the population level. This could be used to quantitatively

estimate take and incidental take under the Act, and do so under average conditions and relevant alternative scenarios. The Refuge and the District will seek out and conduct studies to refine management studies. The District's roles and responsibilities to minimize impacts based on new research will be stipulated in the annual Special Use Permit (see MCMP for further details).

SPECIES/ CRITICAL HABITAT	ACTIONS TO MITIGATE / MINIMIZE IMPACTS
Stock Island Tree Snail	A naled no spray zone with buffer was established around the snails and their habitat on No Name Key. Pesticide applications on No Name Key should occur only when winds are < 10 mph and from the south, southeast, or southwest. Permethrin fog routes and conditions extensively adjusted to eliminate or minimize drift to Refuge lands (numerous small segments and substantial long, continuous segments previously sprayed, e.g., Torch Keys, Big Pine Key, No Name Key).
Key deer	No additional actions needed beyond the specified landing rate counts, application rates, intervals, and frequencies.
Rice rat	No additional actions needed beyond the specified landing rate counts, application rates, intervals, and frequencies.
Eastern indigo Snake	No additional actions needed beyond the specified landing rate counts, application rates, intervals, and frequencies.
Key tree-cactus	No additional actions needed beyond the specified landing rate counts, application rates, intervals, and frequencies. No spray zone for Naled and Permethrin is currently in place for Cactus Hammock
Lower Keys marsh rabbit	No additional actions needed beyond the specified landing rate counts, application rates, intervals, and frequencies.
Garber's spurge	No additional actions needed beyond the specified landing rate counts, application rates, interintervals, and frequencies.
Florida leafwing butterfly	Previously established zones and new "phased approach" in combination with naled no spray zones and buffer imparted to pine rockland areas on Big Pine Key. Permethrin fog routes and conditions extensively adjusted to eliminate or minimize drift to Refuge lands (Critical habitat, occupied habitat, numerous small segments and substantial long, continuous segments previously sprayed, e.g., Torch Keys, Big Pine Key, No Name Key). Science based management efforts include research and focus on attaining ability to derive risk-based assessments of the probability of injury based on actual field exposure.

SPECIES/ CRITICAL HABITAT	ACTIONS TO MITIGATE / MINIMIZE IMPACTS
Bartram's hairstreak butterfly	Previously established zones and new "phased approach" in combination with naled no spray zones imparted to pine rockland areas on Big Pine Key. Permethrin fog routes and conditions extensively adjusted to eliminate or minimize drift to Refuge lands (Critical habitat, occupied habitat, numerous small segments and substantial long, continuous segments previously sprayed, e.g., Torch Keys, Big Pine Key, No Name Key). Science based management efforts include research and focus on attaining ability to derive risk-based assessments of the probability of injury based on actual field exposure.
Miami blue butterfly	None needed. Consolation will occur if new populations are found.
Cassius blue butterfly	None needed.
Ceraunus blue butterfly	None needed.
Nickerbean blue butterfly	None needed.
Big Pine partridge pea	Previously established and new naled no spray zones and buffer zones imparted to pine rockland areas on Big Pine Key. Permethrin fog routes and conditions extensively adjusted to eliminate or minimize drift to Refuge lands.
Wedge spurge	Previously established and new naled no spray zones and buffer zones imparted to pine rockland areas on Big Pine Key. Permethrin fog routes and conditions extensively adjusted to eliminate or minimize drift to Refuge lands.
Sand flax	Previously established and new naled no spray zones and buffer zones imparted to pine rockland areas on Big Pine Key. Permethrin fog routes and conditions extensively adjusted to eliminate or minimize drift to Refuge lands.

VIII. **Effect Determination and Response Requested:**

SPECIES/ CRITICAL HABITAT	DETERMINATION[1]			[1]RESPONSE REQUESTED
	NE	NA	AA	
Stock Island Tree Snail		X		Concurrence
Key deer		X		Concurrance
Rice rat		X		Concurrence
Eastern indigo Snake		X		Concurrence
Key tree-cactus		X		Concurrence
Lower Keys marsh rabbit		X		Concurrence
Garber's spurge		X		Concurrence
Florida leafwing butterfly			X	Conference
Bartram's hairstreak butterfly			X	Conference
Miami blue butterfly		X		Concurrence
Cassius blue butterfly		X		Concurrence
Ceraunus blue butterfly		X		Concurrence
Nickerbean blue butterfly		X		Concurrence
Candidates				
Big Pine partridge pea		X		Concurrence
Wedge spurge		X		Concurrence
Sand flax		X		Concurrence

[1]DETERMINATION/ RESPONSE REQUESTED:
NE = no effect/no adverse modification.
NA = not likely to adversely affect.
AA = likely to adversely affect.

_____ _____
signature (originating station) **date**

Refuge Manager
Title

signature (originating station) 4-10-14 date

Refuge Manager
Title

IX. Reviewing Ecological Services Office Evaluation:

A. Concurrence ___X___ Nonconcurrence _____

B. Formal consultation required _____

C. Conference required X____

D. Informal conference required _____

E. Remarks (attach additional pages as needed): Concurrence is for listed species. Conference is for candidate butterflies and plants.

signature date

title office

IX. Reviewing Ecological Services Office Evaluation:

A. Concurrence ___X___ Nonconcurrence _____

B. Formal consultation required _____

C. Conference required X____

D. Informal conference required _____

E. Remarks (attach additional pages as needed): Concurrence is for listed species. Conference is for candidate butterflies and plants.

signature	5/25/14 date
Acting Field Supervisor title	South Florida Ecological Services Office office

Figures

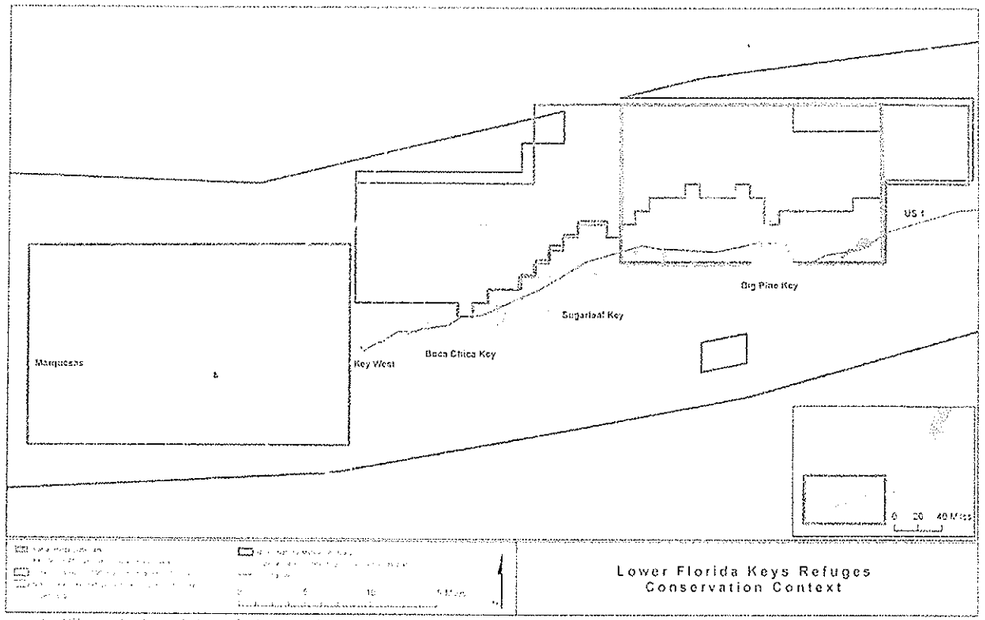

Figure 1. Boundaries of the National Key Deer Refuge and Great White Heron National Wildlife Refuge in the Lower Florida Keys.

U.S. Fish & Wildlife Service

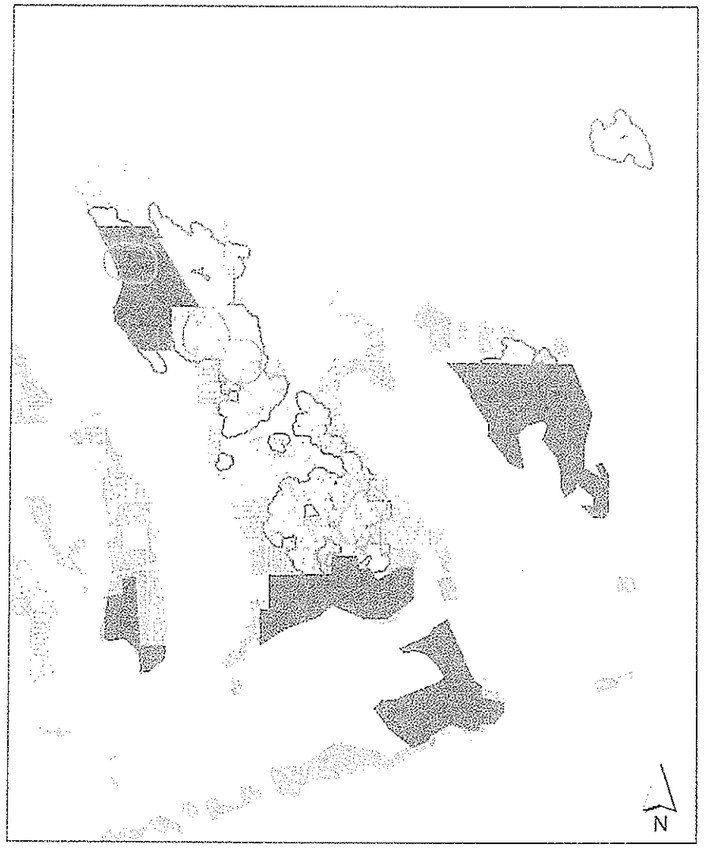

Figure 2. Big Pine and No Name Keys with land ownership and no spray areas represented.

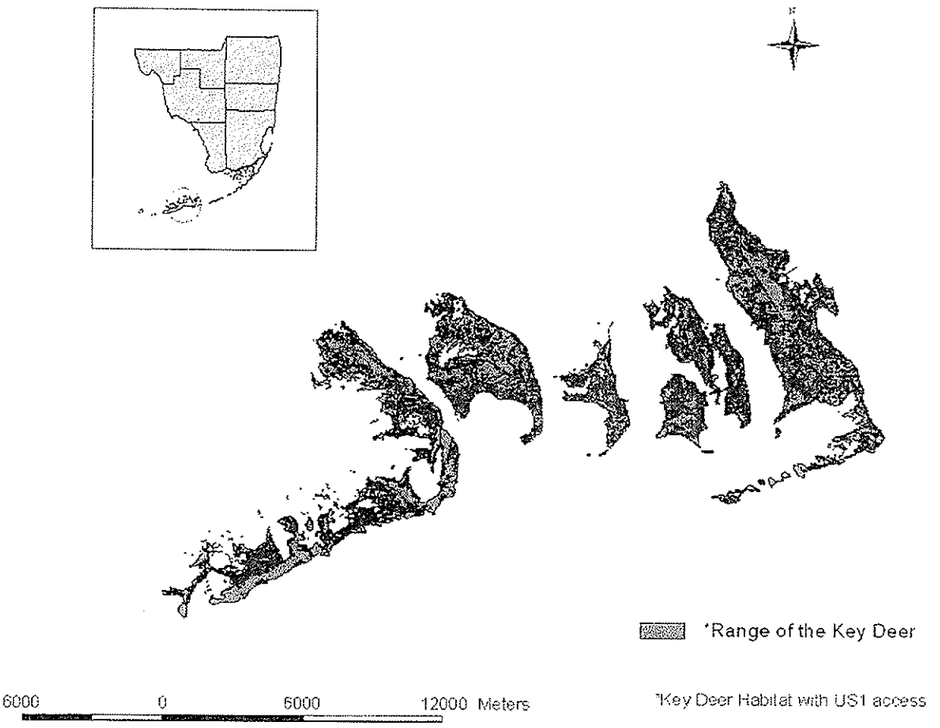

Figure 3. Current range of the Key deer in the Lower Florida Keys.

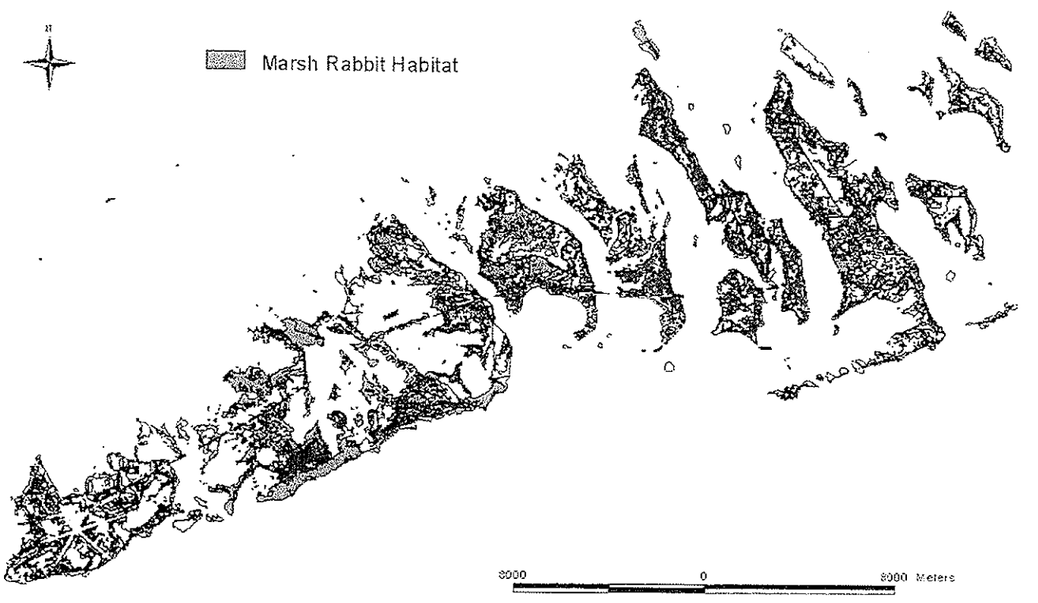

Figure 4. Current range of the Lower Keys marsh rabbit in the Lower Florida Keys.

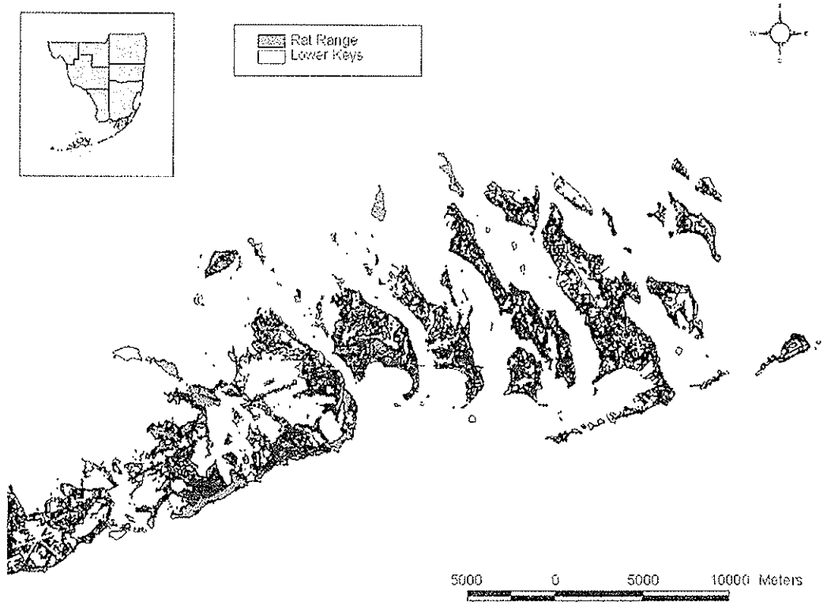

Figure 5. Current range of the silver rice rat in the Lower Florida Keys.

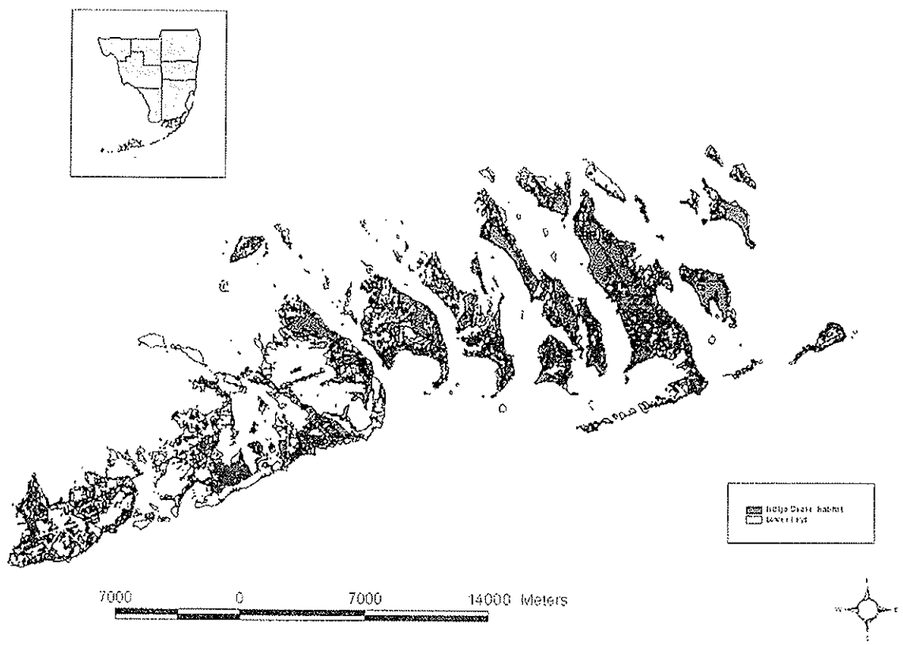

Figure 6. Potential range of the Eastern indigo snake in the Lower Florida Keys. Based on a lack of sightings of this animal in the mainline Lower Keys connected to U.S. 1 in the last decade, it is probable that this species has become extirpated on those islands.

'am's hairstreak butterflies on

Literature Cited

Acropora Biological Review Team. 2005. Atlantic Acropora Status Review Document. Report to National Marine Fisheries Service. Southeast Regional Office.

Baggett, H. D. 1982. Order Lepidoptera. In R. Franz (ed.), Invertebrates. In P.C. Pritchard (ed.). Rare and Endangered Biota of Florida. 6:78-81. University Press. Gainesville, Florida. 131 pp.

Bargar, T.A. 2012. Risk assessment for adult butterflies exposed to the mosquito control pesticide naled. Environmental Toxicology and Chemistry 31:1-7.

Berteau, P. E., W. A. Deen, and R. L. Dimmick. 1978. Effect of particle size on the inhalation toxicity of Naled aerosols. Toxicology and Applied Pharmacology 41:183.

Bradley, K. A. 2006. Distribution and population size for three pine rockland endemic candidate plant taxa on Big Pine Key, Florida. The Institute for Regional Conservation. Report submitted to U.S. Fish and Wildlife Service. Vero Beach, Florida.

Breidenbaugh, M.S. and F.A. De Szalay. 2010. Effects of aerial applications of naled on nontarget insects at Parris Island, South Carolina. Environmental Entomology 39(2):591-599.

Calhoun, J. V., J. R. Slotten, and M. H. Salvato. 2000. The rise and fall of tropical blues in Florida: Cyclargus ammon and Cyclargus thomasi bethunebakeri (Lepidoptera: Lycaenidae). Holarctic Lepidoptera 77:13-20.

Calhoun, J.V., J.R. Slotten, and M.H. Salvato. 2002. Rise and fall of tropical blues in Florida: Cyclargus ammon and Cyclargus thomasi bethunebakeri (Lepidoptera: Lycaenidae). Holarctic Lepidoptera 7(1):13-20.

Carroll, S.P. and J.E. Loye. 2006. Invasion, colonization, and disturbance; historical ecology of the endangered Miami blue butterfly. Journal of Insect Ecology 10:13-27.

Eliazar, J.E. 1992. Impact of two mosquito adulticides, naled and fenthion, on selected nontarget lepidopteran species. A thesis presented to the graduate school of the University of Florida in partial fulfillment of the requirements for the degree of Master of Science. University of Florida; Gainesville, Florida.

Emmel, T. C., and J. C. Tucker (eds.). 1991. Mosquito control pesticides: ecological importance and management alternatives. Scientific Publishers, Inc., Gainesville, Florida.

Florida Coordinating Council on Mosquito Control. 1998. Florida Mosquito Control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. University of Florida, Gainesville.

Florida Fish and Wildlife Conservation Commission. 2010. Miami blue butterfly management plan. Cyclargus thomasi bethunebakeri. Adopted: October 31, 2003. Revised: June 2010. Florida Fish and Wildlife Conservation Commission; Tallahassee, Florida.

Florida Keys Mosquito Control District. 2006. Efficacy of larvicidal treatment of wilderness islands for reduction of adulticidal treatment of areas adjacent to Key Deer National Wildlife Refuge, and Great White Heron National Wildlife Refuge, Big Pine Key, Florida. Progress report. Submitted on December 31, 2006 to U.S. Fish and Wildlife Service. Florida Keys Mosquito Control District; Key West, Florida.

Florida Keys Mosquito Control District. 2009. Efficacy of larvicidal treatment of wilderness islands for reduction of adulticidal treatment of areas adjacent to Key Deer National Wildlife Refuge, and Great White Heron National Wildlife Refuge, Big Pine Key, Florida. Progress report. Submitted on December 31, 2009 to U.S. Fish and

Wildlife Service. Florida Keys Mosquito Control District; Key West, Florida.

Florida Keys Mosquito Control District. 2011. Efficacy of larvicidal treatment of wilderness islands for reduction of adulticidal treatment of areas adjacent to Key Deer National Wildlife Refuge, and Great White Heron National Wildlife Refuge, Big Pine Key, Florida. Progress report. Submitted on December 31, 2011 to U.S. Fish and Wildlife Service. Florida Keys Mosquito Control District; Key West, Florida.

Green, S.E., K.A. Bradley, and S.W. Woodmansee. 2008. Status Survey of the Federally Threatened Chamaesyce garberi in South Florida. Grant Agreement #401816G055 Final Report submitted by The Institute for Regional Conservation to the U.S. Fish and wildlife Service, Vero Beach, Florida.

Hennessey, M.K. and D.H. Habeck. 1989. Effects of mosquito adulticides on non-target arthropods in the Florida Keys. Annual report 1988. Submitted to reviewers. University of Florida, Institute of Food and Agricultural Sciences; Homestead, Florida.

Hennessey, M.K. and D.H. Habeck. 1991. Effects of mosquito adulticiding on populations of non-target, terrestrial arthropods in the Florida Keys. Final report of research results. Submitted on February 1, 1991 to U.S. Fish and Wildlife Service and University of Florida Cooperative Wildlife Research Unit. United States Department of Agriculture - Agricultural Research Service; Miami, Florida.

Hennessey, M.K., H.N. Nigg, and D.H. Habeck. 1992. Mosquito (Diptera: Culicidae) adulticide drift into wildlife refuges of the Florida Keys. Environmental Entomology 21(4):715-721.

Hoang, T.C., R.L. Pryor, G.M. Rand, R.A. Frakes. 2011. Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. Environmental Toxicology and Chemistry 30(4):997-1005.

Hodges, S. R., and K. A. Bradley. 2006. Distribution and population size of five candidate plant taxa in the Florida Keys: Argythamnia blodgettii, Chamaecrista lineata var. keyensis, Indigofera mucronata var. keyensis, Linum arenicola, and Sideroxylon reclinatum subsp. austrofloridense. The Institute for Regional Conservation. Final Report Contract Number 401815G011, submitted to U.S. Fish and Wildlife Service, Vero Beach, Florida.

Hribar, L.J., J.J. Vlach, D.J. DeMay, L.M. Stark, R.L. Stoner, M.S. Godsey, K.L. Burkhalter, M.C. Spoto, S.S. James, J.M. Smith, and E.M. Fussell. 2003. Mosquitoes infected with West Nile virus in the Florida Keys, Monroe County, Florida, USA. J. Med. Entomol. 40: 361-363.

Hribar, L.J., L.M. Stark, R.L. Stoner, D.J. DeMay, A.L. Nordholt, M.J. Hemmen, J.J. Vlach, & E.M. Fussell. 2004. Isolation of West Nile virus from mosquitoes (Diptera: Culicidae) in the Florida Keys, Monroe County, Florida. Carib. J. Sci. 40: 362-367.

Hudson, R. H., R. K. Tucker, and M. A. Haegele. 1984. Handbook of Pesticides to Wildlife: Second Edition. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C. Resource Publication 153.

Liu, H., and S. Koptur. 2003. Breeding system and pollination of a narrowly endemic herb of the lower Florida Keys: impacts of the urban-wildland interface. American Journal of Botany 90(8):1180–1187.

Matley, A. 2003. West Nile shows up in the Keys. Florida Keys Keynoter. August 20, 2003.

Minno, M. and M. Minno. 2007a. Status survey for the rockland grass skipper (Hesperia meskei pinocayo) in southern Florida, 2006-2007. Interim Report for August – October 2007 to U.S. Fish and Wildlife Service, South Florida Ecological Services Office. Grant Agreement No. 401816G139. Gainesville, Florida.

Minno, M. and M. Minno. 2007b. Status survey for the zestos skipper (Epargyreus zestos) in southern Florida, 2006-2007. Interim Report for August – October to U.S. Fish and Wildlife Service, South Florida Ecological

Services Office. Grant Agreement No. 401816G139. Gainesville, Florida.

Minno, M. and M. Minno. 2009. A plan to conserve rare butterflies in the Florida Keys. Submitted on July 1, 2009 to Florida Keys Mosquito Control District. Eco-Cognizant, Inc; Gainesville, Florida.

Mulrennan, J. A. 1991. Benefits of mosquito control. In Mosquito Control Pesticides: Ecological Impacts and Management Alternatives. T.C. Emmel and J.C. Tucker (eds.). Scientific Publishers, Inc., Gainesville, Florida. pp. 15-16.

Pierce, R.H. 2009. Determine direct and indirect effects of mosquito control pesticides on listed species inhabiting DOI managed lands in the Florida Keys. Progress report October 1, 2007 to September 30, 2009. Submitted on October 12, 2009 to U.S. Fish and Wildlife Service. Mote Marine Laboratory; Sarasota, Florida.

Pierce, R.H. 2011. Indirect effects of mosquito control pesticides on listed species inhabiting DOI managed lands in the Florida Keys: assessment of permethrin drift and accumulation in the National Key Deer Refuge. Final report for 2010 applications. Submitted on April 12, 2011 to U.S. Fish and Wildlife Service, Florida Keys National Wildlife Refuges Complex and Florida Keys Mosquito Control District. Mote Marine Laboratory; Sarasota, Florida.

Rand, G.M. and T. Hoang. 2010. Exposure and biological effects of the adult mosquito control agents naled and permethrin in the Key Deer National Wildlife Refuge, Big Pine Key: an ecological risk characterization for beneficial non-target organisms (butterflies). Progress report. Submitted December 2010 to U.S. Fish and Wildlife Service. Florida International University; North Miami, Florida.

Ross, M., and P. Ruiz. 1996. A study of the distribution of several south Florida endemic plants in the Florida Keys. Report to U.S. Fish and Wildlife Service, Jacksonville, Florida.

Salvato, M.H. 2001. Influence of mosquito control chemicals on butterflies (Nymphalidae, Lycaenidae, Hesperiidae) of the lower Florida Keys. Journal of the Lepidopterists' Society 55(1):8-14.

Salvato, M.H., and H.L. Salvato. 2010. Notes on the status and ecology of Anaea troglodyta floridalis in Everglades National Park. Journal of the Lepidopterists' Society. 64: 91-97.

Slapcinsky, J., and D. Gordon. 2007. Monitoring report 2007 - Pine Rocklands.

URS. 2004. Naled risk assessment for the endangered and threatened species of Lower Keys. Prepared for the U.S. Fish and Wildlife Service. URS, Franklin, Tennessee.

USFWS. 1999. South Florida multi-species recovery plan. U.S. Fish and Wildlife Service, Ecological Services, Vero Beach, Florida.

U.S. Environmental Protection Agency. 1999. Naled: addendum to EFED's registration chapter. United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C.

U.S. Environmental Protection Agency. 2002. Interim reregistration eligibility decision for naled. EPA 738-R-02-008. January 2002. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. Washington, D.C.

U.S. Environmental Protection Agency. 2006a. Reregistration eligibility decision (RED) for malathion. EPA 738-R-06-030. July 2006. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. Washington, D.C.

U.S. Environmental Protection Agency. 2006b. Reregistration eligibility decision (RED) for permethrin. EPA 738-R-06-017. April 2006. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic

Substances. Washington, D.C.

Zhong, H., L.J. Hribar, J.C. Daniels, M.A. Feken, C. Brock, and M.D. Trager. 2010. Aerial ultra-low-volume application of naled: impact on non-target imperiled butterfly larvae (Cyclargus thomasi bethunebakeri) and efficacy against adult mosquitoes (Aedes taeniorhynchus). Environmental Entomology 39(6):1961-1972.

United States Department of the Interior

FISH AND WILDLIFE SERVICE
South Florida Ecological Services Office
1339 20th Street
Vero Beach, Florida 32960

May 29, 2014

Memorandum

To: Nancy Finley, Refuge Manager, Lower Keys National Wildlife Refuge Complex

From: Craig Aubrey, Field Supervisor, South Florida Ecological Services Office

Subject: Intra-Service Section 7 Review for the Adoption of a Mosquito Management Plan for the Use of Mosquito Control Pesticides by the Florida Keys Mosquito Control District on Lands of the Lower Keys National Wildlife Refuge Complex and Corresponding Conference Opinion

Attached is a signed intra-Service section 7 Biological Evaluation (BE) for the adoption of the Mosquito Management Plan (Plan) for the Lower Keys National Wildlife Refuge Complex (Refuge). The Refuge requested concurrence on determinations that the adoption of the Plan is not likely to adversely affect the Stock Island tree snail (*Orthalicus reses*), Key deer (*Odocoileus virginianus clavium*), rice rat (*Oryzomys palustris natator*), Key tree cactus (*Pilosocereus robinii*), eastern indigo snake (*Drymarchon corais couperi*), Lower Keys marsh rabbit (*Sylvilagus palustris hefneri*), Garber's spurge (*Chamaesyce garberi*), Miami blue butterfly (*Cyclargus thomasi bethunebakeri*), cassius blue butterfly (*Leptotes cassius theonus*), ceraunus blue butterfly (*Hemiargus ceraunus antibubastus*), nickerbean blue butterfly (*Cyclargus ammon*), Big Pine partridge pea (*Chamaecrista lineata keyensis*), wedge spurge (*Chamaesyce deltoidea serpyllum*), and sand flax (*Linum arenicola*). In addition, the Refuge determined that the adoption of the Plan is likely to adversely affect the proposed listed species Florida leafwing butterfly (*Anaea troglodyta floridalis*) and Bartram's hairstreak butterfly (*Strymon acis bartrami*) and their proposed critical habitats and requested conference.

We concur with the determinations that the proposed action is not likely to adversely affect the species referenced above. The Conference Opinion for the proposed butterfly species and their proposed critical habitats is described below.

CONFERENCE OPINION

This document transmits the U.S. Fish and Wildlife Service's (Service) conference opinion based on the Service's review of the proposed adoption of the Mosquito Management Plan (Plan) for the Lower Keys National Wildlife Refuge Complex and its potential effects on the proposed endangered Florida leafwing butterfly (*Anaea troglodyta floridalis*), the proposed endangered Bartram's hairstreak butterfly (*Strymon acis bartrami*), and proposed critical habitat for both

butterfly species in accordance with section 7 of the Endangered Species Act of 1973, as amended (Act) (16 U.S.C. 1531 et seq.). Please note this Conference Opinion evaluates the potential effects of pesticide application by the Florida Keys Mosquito Control District (District) where the application affects species both on and off Refuge lands. Should the species covered in this Conference Opinion be subsequently listed pursuant to the Act and this conference opinion be adopted, the District will not be exempted from prohibition against take for listed species affected off Refuge lands.

This Conference Opinion is based on information provided in the Intra-Service Section 7 Biological Evaluation Form received by email on April 10, 2014, and the associated Plan. A complete administrative record of this consultation is on file in the South Florida Ecological Services Office.

DESCRIPTION OF THE PROPOSED ACTION

The Refuge has proposed to adopt the Plan, which details mosquito control activities conducted by the District on and adjacent to the National Key Deer Refuge (Refuge) lands over a 5-year period beginning in 2014. The action area is defined as all areas to be directly or indirectly affected by the Federal action and not merely the immediate area involved in the action. The Service has described the action area for this activity to be the Refuge and adjacent communities on Big Pine Key and No Name Key (Figure 1).

Four alternatives are presented in the Plan. This conference opinion is based on the preferred alternative: "Alternative B, Implement Phased Mosquito Management Plan Alternative." Alternative B consists of a phased approach that dictates thresholds that must be met before the application of pesticides is initiated.

The first phase involves surveillance monitoring and outreach. Personnel from both the District and Refuge will conduct outreach exercises aimed at reducing artificial larval mosquito refugia in the community (*e.g.*, old tires, open containers, etc.). Innocuous mosquito control methods, such as the use of native mosquito fish, will be conducted under this first phase.

The second phase of Alternative B will utilize the larvicides *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bsp), which are both soil-dwelling bacteria. Field monitoring will identify the presence of larval mosquitoes; thus initiating the application of larvicide to standing water in areas accessed by primary and secondary roads, as well as backcountry islands.

The third phase of Alternative B, which includes the application of a barrier treatment, will be initiated when District monitoring identifies a mosquito landing rate that exceeds 3.0 mosquitoes/minute for two consecutive days on private property or public land not managed by the Refuge. The barrier treatment involves the application of the pyrethroid insecticide bifenthrin at a distance of 5 feet(ft) from the vegetative canopy with a sprayer angle of 60 degrees. The target height of the spray will be 6-9 ft, droplet concentration in the target area will be 30-180 droplets/cm^2, and droplet size is expected to be 300-350 μm. Application will not occur on Refuge land.

The fourth phase of Alternative B, which includes truck-based application of pyrethroid insecticides, will be initiated when one or more neighborhoods adjacent to the Refuge exhibit a landing rate that exceeds 3.0 mosquitoes/minute under Option A and 10 mosquitoes/minute under Option B.

Option A dictates truck-based application of pyrethroids will not occur within 50 meters (m) of proposed critical habitat for the Bartram's hairstreak and Florida leafwing butterflies or within 250 m of what is referred to in the Plan as "occupied" habitat for the Bartram's hairstreak and Florida leafwing (Figure 2). Additional treatment at a site will not occur for a minimum of 96 hours. Application shall not occur during sustained winds exceeding 10 miles per hour and gusts exceeding 15 miles per hour. Wind direction will also be considered to minimize drift onto the Refuge.

Option B of the fourth phase allows application within 50 m of proposed critical habitat, but not within 250 m of "occupied" Bartram's hairstreak and Florida leafwing habitat. This option will be initiated when a mosquito landing rate exceeding 10 mosquitoes/minute is observed. Only one neighborhood within the proposed critical habitat would be treated at any one time and additional treatment at a site will not occur for a minimum of 1 month. The wind stipulations described above for option A will apply.

Under the fourth phase, residents may request localized applications of pyrethroids be conducted by District staff using handheld sprayers, with a 50ft buffer around Refuge boundaries.

The fifth phase of Alternative B, which includes aerial application of the organophosphate insecticide naled, would be conducted on Big Pine Key when mosquito landing rates exceed 10 mosquitoes/minute as an average of all monitoring stations and on No Name Key when mosquito landing rates exceed 40 mosquitoes/minute as an average of all monitoring stations. In addition to meeting the landing rate thresholds, detection of invasive mosquito species during surveillance monitoring may also trigger this treatment. Aerial application will not be permitted when sustained winds exceed 10 miles per hour or gusts exceed 15 miles per hour. No buffer distances around critical habitat and/or "occupied" areas are specified in the Plan. The Refuge has agreed to implement an additional 400-m buffer around "occupied" areas for the Bartram's hairstreak in addition to the existing aerial no-spray zones (Figure 3).

In the Plan, areas referred to as "occupied" represent locations where the Bartram's hairstreak butterfly can be reliably found in the greatest numbers. The Bartram's hairstreak may occur elsewhere on the Refuge and use habitat outside of the "occupied" areas. For this reason, the "occupied" areas will be referred to as core areas throughout the remainder of this document.

STATUS OF THE SPECIES AND CRITICAL HABITAT RANGEWIDE

Florida Leafwing Butterfly

Species/critical habitat description

The Florida leafwing butterfly is a medium-sized butterfly approximately 76 to 78 millimeters (mm) in length with a forewing length of 34 to 38 mm and has an appearance characteristic of its genus (Comstock 1961; Pyle 1981; Opler and Krizek 1984; Minno and Emmel 1993). The

upper-wing (or open wing) surface color is red to red-brown, the underside (closed wings) is gray to tan, with a tapered outline, cryptically looking like a dead leaf when the butterfly is at rest. The Florida leafwing exhibits sexual dimorphism, with females being slightly larger and with darker coloring along the wing margins than the males. The species also has seasonal forms (Salvato and Hennessey 2003). Comstock (1961) employed the terms "summer" and "winter" morph to differentiate between seasonal forms within the genus. The length of photoperiod exposure experienced by fifth-instar larvae (several days prior to pupation), as well as the influence of seasonal moisture have been identified as key factors in determining the seasonal forms within members of the *Anaea* genus of leafwing butterflies (Riley 1980; 1988a; 1988b; Salvato and Hennessey 2003). The summer form (wet-season or long-day form), occurring in late May to September, tends to have forewing margins that are blunt and a hind-wing with a less pronounced tail; colors also tend to be brighter. The winter form (dry-season or short-day form), occurring in October to early May, tends to have the opposing characters, with pronounced tails and crescent-shaped forewings (Comstock 1961; Salvato 1999; Salvato and Hennessey 2003). Eggs are spherical and light cream-yellow in color (Worth et al. 1996). The first three instars begin what continues throughout the larval development to be a remarkable cryptic mimicry of the hostplant, pineland croton (*Croton linearis*) (Euphorbiaceae). These stages appear like dead leaves, with a brown color and resting on a dead part of the plant during the day (Salvato 1999; 2003). These instars tend to eat the leaves to the mid-vein and then dangle from them in camouflage. In addition, the first two instars make a frass chain for protection from predators (Salvato and Salvato 2008). Briefly, a frass chain is created when *Anaea* larvae attach their fecal pellets to the mid-vein of a partially eaten croton leaf with silk (Minno et al. 2005). The larvae then crawl to the terminus of this strand to avoid predation. The two later instars are light green in color, with a tapering body from the cephalad (head capsule) to the caudal end, so that when at rest, it also appears like a croton leaf in the spiral fashion of the terminal end (Worth et al. 1996). The head capsule during all stages bears many tiny setae, presenting the granular appearance of croton seeds (Worth et al. 1996).

The Service proposed to list the Florida leafwing as endangered and designate critical habitat on August 15, 2013. The Service has subsequently modified the proposed critical habitat based on comments received on the rules. The four areas proposed as critical habitat are: (1) FLB1 Everglades National Park, Miami-Dade County, Florida, (2) (FLB2) Navy Wells Pineland Preserve, Miami-Dade County, Florida, (3) (FLB3) Richmond Pine Rocklands, Miami-Dade County, Florida, and (4) (FLB4) Big Pine Key, Monroe County, Florida. Land ownership within the proposed critical habitat consists of Federal (85 percent), State (3 percent), and private and other (12 percent). Table 1 shows these units by land ownership, area, and occupancy.

Primary Constituent Elements (PCEs) are considered to be specific elements of the physical or biological features (PBFs) that provide for a species' life-history processes and are essential to the conservation of the species. The Florida leafwing is dependent upon functioning pine rockland habitat to provide its fundamental life requirements, such as pineland croton for larval development, food sources and roosting areas required by adult butterflies. Based on our current knowledge of the PBFs and habitat characteristics required to sustain the butterfly's life-history processes, we determine that the PCEs for the Florida leafwing are:

1. Areas of pine rockland habitat, and in some locations, associated rockland hammocks and hydric pine flatwoods.
    a. Pine rockland habitat contains:
        i. Open canopy, semi-open subcanopy, and understory;
        ii. Substrate of oolitic limestone rock; and
        iii. A plant community of predominately native vegetation.
    b. Rockland hammock habitat associated with the pine rocklands contains:
        i. Canopy gaps and edges with an open to semi-open canopy, subcanopy, and understory; and
        ii. Substrate with a thin layer of highly organic soil covering limestone or organic matter that accumulates on top of the underlying limestone rock; and
        iii. A plant community of predominately native vegetation.
    c. Hydric pine flatwood habitat associated with the pine rocklands contains:
        i. Open canopy with a sparse or absent subcanopy, and dense understory;
        ii. Substrate with a thin layer of poorly drained sands and organic materials that accumulates on top of the underlying limestone or calcareous rock; and
        iii. A plant community of predominately native vegetation.
2. Competitive nonnative plant species in quantities low enough to have minimal effect on survival of the Florida leafwing.
3. The presence of the butterfly's hostplant, pineland croton, in sufficient abundance for larval recruitment, development, and, food resources, and for adult butterfly roosting habitat, and reproduction.
4. A dynamic natural disturbance regime or one that artificially duplicates natural ecological processes (*e.g.,* fire, hurricanes or other weather events, at appropriate intervals) that maintains the pine rockland habitat and associated hardwood hammock and hydric pine flatwood plant communities.
5. Pine rockland habitat and associated hardwood hammock and hydric pine flatwood plant communities that are sufficient in size to sustain viable Florida leafwing populations.
6. Pine rockland habitat and associated hardwood hammock and hydric pine flatwood plant communities with levels of pesticide low enough to have minimal effect on the survival of the butterfly or its ability to occupy the habitat.

The Florida leafwing occurs only within pine rocklands that retain its hostplant, pineland croton. Pineland croton, a subtropical species of Antillean origin, is the only known hostplant for the leafwing (Opler and Krizek 1984; Schwartz 1987; Minno and Emmel 1993; Smith et al. 1994). Once occurring throughout the pine rocklands of the lower Florida Keys (Dickson 1955; Hennessey and Habeck 1991; Salvato 1999), pineland croton now occurs only on Big Pine Key. The last reports of the hostplant from other keys were from No Name in 1992 (Carlson et al. 1993) and from Little Pine in 1988 (Hennessey and Habeck 1991). Recent surveys of relict pineland throughout the lower Keys by Salvato (2008) failed to locate the plant from any island other than Big Pine. Hennessey and Habeck (1991) and Salvato (1999) estimated approximately 198 acres of croton-bearing pine rockland habitat occur on Big Pine Key. More recently, Chad Anderson (pers. comm. 2010a), biologist at the Refuge on Big Pine Key, estimated roughly 600 acres of croton on Big Pine Key, based upon Bradley and Saha (2009) pine rockland data and personal observations.

Life history

Adults are rapid, wary fliers. The subspecies is extremely territorial, with both sexes flying out to pursue other butterflies (Baggett 1982; Worth et al. 1996; Salvato and Hennessey 2003; Salvato and Salvato 2010a). Minno (pers. comm. 2009) and Salvato and Salvato (2010a) note males are generally more territorial. The Florida leafwing is multivoltine (*i.e.*, produces multiple generations per year), with an entire life cycle of about 60 days (Hennessey and Habeck 1991) and maintains continuous broods throughout the year (Salvato 1999). The precise number of broods per year remains unknown, but the leafwing has been recorded in every month (Baggett 1982; Opler and Krizek 1984; Minno and Emmel 1993; Salvato and Hennessey 2003; Salvato and Salvato 2010a; 2010b). Females lay eggs singly on both the upper and lower surface of the host leaves, normally on developing racemes (Baggett 1982; Hennessey and Habeck 1991; Worth et al. 1996; Salvato 1999). Worth et al. (1996) and Salvato (1999) visually estimated females might fly more than 30 m in search of a suitable host and usually require less than a minute to oviposit each egg.

Population dynamics

The Florida leafwing has been observed during every month within the Everglades and formerly on Big Pine Key; however the exact number of broods appears to be sporadic from year to year (Baggett 1982; Opler and Krizek 1984; Minno and Emmel 1993; Salvato and Hennessey 2003; Salvato and Salvato 2010a; 2010b). Salvato and Salvato (2010a) and Land (pers. comm. 2012) encountered the subspecies throughout the year, but the majority of observations occurred from late fall to spring in ENP. By contrast, when extant on Big Pine Key, Salvato and Salvato (2010c) reported finding the subspecies abundantly throughout the year, particularly during the summer months.

Status and distribution

Based on results of all historic (Baggett 1982; Schwartz 1987; Hennessey and Habeck 1991; Worth et al. 1996; Schwarz et al. 1996) and recent surveys and natural history studies (Salvato 1999; 2001; 2003; Salvato and Hennessey 2003; Salvato and Salvato 2010a), the Florida leafwing is extant in ENP and, until recently, had occurred on Big Pine Key and rarely in pineland fragments in mainland Miami-Dade County (Salvato and Salvato 2010a; 2010b). Schwartz (1987), Hennessey and Habeck (1991), Emmel et al. (1995), and Salvato (1999), searched the lower Florida Keys extensively for the Florida leafwing, only encountering the species on Big Pine Key. In the Everglades, Hennessey and Habeck (1991) and Salvato and Salvato (2010a) reported the species from Long Pine Key. Hennessey and Habeck (1991) reported an estimate of 1.5 adults per acre during 1988-1989 from survey transects at both Watson's Hammock on Big Pine Key and on Long Pine Key in ENP. During 1997-1998, Salvato (1999) estimated 1.2 and 1.0 adults per acre at Watson's Hammock and the Gate 4 nature trail of Long Pine Key in ENP, respectively; these were at higher densities than what was found on survey transects elsewhere in his study. During 1999-2002, Salvato (pers. comm. 2009) recorded an average of 4.4 adults per acre in the Watson's Hammock area, while other locations on Big Pine Key have yielded an average of 0.1 to 2.6 adults per acre. The higher densities in Watson's Hammock have been attributed to the fact that this is the only pine

rockland area on Big Pine Key restricted from chemical pesticide applications for mosquito control (Hennessey and Habeck 1991; Hennessey et al. 1992; Salvato 2001; Salvato and Salvato 2010b). However, analysis of survey data collected from 2003 through 2006 indicate a substantial decline in leafwing numbers on Big Pine, even within Watson's Hammock (Salvato and Salvato 2010b). During 2003-2006, Salvato (pers. comm. 2009) recorded an average of 0.6 adults per acre in Watson's Hammock; other locations on Big Pine Key yielded an average of 0 to 0.5 adults per acre. Salvato and Salvato (2010b) recorded three larvae and one adult in 2006. No leafwings were recorded on Big Pine Key after 2006 (Salvato and Salvato 2010b).

Salvato and Salvato (2010a) have found the density of leafwings at ENP within Long Pine Key to be highly variable ranging from 4 to 54 individuals observed annually at Gate 4 based on monthly studies from 1999 to 2010. In addition, Salvato and Salvato (2010a) have monitored populations of the leafwing elsewhere within Long Pine Key as well as within adjacent habitats (Palma Vista Hammock and several former agricultural and military lands) during 2005-2010 and encountered similar, variable densities throughout the survey period. Similarly, Perry (pers. comm. 2007) has observed only small, scattered occurrences within the spatially extensive pineland area of Long Pine Key. She notes that counts were typically only in the single digits during her survey efforts.

Salvato and Salvato (2010a) have generally found about 0.4 leafwing per acre during recent surveys of ENP during select seasons and none on Big Pine Key (Salvato and Salvato 2010b). Salvato (pers. comm. 2011) indicates the current population size ranges from several hundred or fewer, although it varies greatly depending upon season and other factors. However, Minno (pers. comm. 2009) estimated the population size at less than 100 per day on 20 to 30 acres within ENP. In ENP, the species is most often encountered from late fall through spring, and less abundantly during the summer (Salvato and Salvato 2010a). However, the species appeared to maintain a consistent year-round phenology when it occurred on Big Pine Key (Salvato and Salvato 2010a; 2010b).

Minno (pers. comm. 2007, 2008) believes that the Florida leafwing is either extremely rare or extirpated from the Keys and the Navy Wells site based on surveys since August 2006. Minno indicates this species was common on Big Pine Key in the 1980s, but the habitat, due to lack of fire, has changed dramatically in recent years. Minno (pers. comm. 2009) believes the butterfly is now less common than the endangered Schaus swallowtail (*Heraclides aristodemus ponceanus*). In short, Minno (pers. comm. 2007, 2008) believes that this species has declined greatly since the 1980s and is not likely to survive without special efforts.

In short, the Florida leafwing butterfly is considered to have a low population size, roughly several hundred or fewer (M. Salvato, pers. comm. 2011). Its overall status is tenuous. On the mainland, it is found in ENP. Outside of ENP, it is only sporadically found in locations such as Navy Wells and other fragments in Miami-Dade County near ENP. It may be extirpated from Big Pine Key, since it has not been found at that location since 2006 (Salvato and Salvato 2010b).

The leafwing has a rounded global status of T1, critically imperiled because of extreme rarity (*i.e.*, 5 or fewer occurrences of less than 1,000 individuals) or because of extreme vulnerability to extinction due to natural or manmade factors (NatureServe 2010). The basis for this ranking

stems from its very limited remaining range, overall threats (e.g., destruction of habitat on Big Pine Key, pesticide application, fire, lack of fire, stochastic events), and decline, which make it highly vulnerable to extinction (NatureServe 2010). FNAI (2011) places the butterfly's State rank at "S1", critically imperiled in Florida because of extreme rarity (5 or fewer occurrences or less than 1,000 individuals) or because of extreme vulnerability to extinction due to some natural or man-made factor. The leafwing is also considered threatened by the Florida Committee on Rare and Endangered Plants and Animals (Deyrup and Franz 1994). However, these designations provide no legal authority or protection. The leafwing is recognized in Florida's Comprehensive Wildlife Conservation Strategy as one of Florida's species of greatest conservation need, with status "low" and trend "declining" (FWC 2005). The Florida leafwing is not listed as endangered or threatened in Florida, and there is no wildlife management plan for this species.

The Florida leafwing is endemic to south Florida and the lower Keys. The species was locally common within pine rockland habitat that once occurred within Miami-Dade and Monroe Counties and was less common and sporadic within croton-bearing pinelands in Collier, Martin, Palm Beach, and Broward Counties (Baggett 1982; Smith et al. 1994; Salvato 1999; Salvato and Hennessey 2003). There is little recent evidence that the Florida leafwing ventured further north than southern Miami-Dade to make use of localized, relict populations of hostplants that still persist as far north as Martin County (Salvato 1999; Salvato and Hennessey 2003). Furthermore, although the leafwing was widely reported from several locations in southern Miami until the mid-20th century, Salvato (1999) has found few documented field sighting records or museum collection specimens from areas north of Monroe and Miami-Dade Counties, suggesting it may not have been common further north historically (Salvato and Hennessey 2003).

Populations of Florida leafwing have become increasingly localized as pine rockland habitat has been lost or altered through anthropogenic activity (Baggett 1982; Hennessey and Habeck 1991; Schwarz et al. 1996; Salvato and Hennessey 2003). Long Pine Key contains the largest remaining coverage of pine rockland habitat (8,029 ha) (19,840 acres) on the mainland (Salvato 1999; Service 1999; Salvato and Hennessey 2004). Hennessey and Habeck (1991) and Salvato (1999) estimated approximately 1,068 ha (2,638 acres) of appropriate hostplant-bearing pine rockland habitat occur within Long Pine Key. This figure may underestimate the amount of actual croton-bearing pine rockland within ENP, but it is the best estimate at this time. More information on the distribution of croton within Long Pine Key is needed (J. Sadle, pers. comm. 2007, 2011; S. Perry, pers. comm. 2007).

In Miami-Dade County, outside of ENP, there are approximately 375 pine rockland fragments remaining totaling approximately 4,398 acres (Service 1999). Although several of these fragments, particularly those adjacent to ENP, such as Navy Wells Pineland Preserve and Camp Owaissa Bauer Hammock, appear to maintain small, localized populations of croton, Salvato and Hennessey (2003) and Salvato (pers. comm. 2008) have generally failed to observe the leafwing in these or other mainland areas outside ENP. During June 2007, one leafwing was observed within Navy Wells (M. Salvato, pers. comm. 2008), but none have been recorded outside of ENP since that time. A GIS analysis conducted by the Service using data collected by The Institute for Regional Conservation (IRC) in 2004 indicates that 65 pine rockland fragments containing

pineland croton remain in private ownership in Miami-Dade County totaling approximately 470 acres (IRC 2006). Another 12 fragments totaling 446 acres contain croton and are in public ownership (IRC 2006).

In the lower Keys, Big Pine Key retains the largest undisturbed tracts of pine rockland habitat totaling an estimated 1,732 acres (Folk 1991; Hennessey and Habeck 1991; Salvato and Hennessey 2004). The 2004 land cover data from South Florida Water Management District shows 1,276 acres of pine rockland on Big Pine Key (M. Minno, pers. comm. 2009). Although relict pine rocklands can still be found on several other islands within the Refuge, only Big Pine Key maintains pineland croton (Salvato 1999; Salvato and Hennessey 2003). Hennessey and Habeck (1991) and Salvato (1999) estimated approximately 198 acres of croton-bearing pine rockland occur on Big Pine Key. More recently, Anderson (pers. comm. 2010a) suggested an estimate of 600 acres of croton on Big Pine Key. However, the butterfly has not been seen in the Keys since 2006 (M. Minno, pers. comm. 2009, 2011; Salvato and Salvato 2010b).

The reduction in range and limited distribution for this butterfly is of serious concern. Minno (pers. comm. 2009, 2011) and Salvato and Salvato (2010b) believe the Florida leafwing is now extant and breeding only in ENP and nowhere else. Similarly, staff at ENP are concerned because it may now have the only population remaining (S. Perry, pers. comm. 2008; J. Sadle, pers. comm. 2010b, 2011).

Bartram's Hairstreak Butterfly

Species/critical habitat description

The Bartram's hairstreak is a small butterfly approximately 25 mm in length with a forewing length of 10.0 to 12.5 mm and has an appearance (i.e., color, size, body shape) characteristic of the genus (Pyle 1981; Opler and Krizek 1984; Minno and Emmel 1993). Despite its rapid flight, this hairstreak is easily observed if present at any density as it alights often, and the brilliance of its grey underside marked with bold, white postdiscal lines beneath both wings provides an instant flash of color against the foliage of its host plant, pineland croton (Smith et al. 1994; Salvato 1999). The Bartram's hairstreak does not exhibit sexual or seasonal dimorphism, but does show some sexual differences. The abdomen of the male is bright white, while females are gray (M. Minno, pers. comm. 2009). Eggs are laid singly on the flowering racemes of pineland croton (Worth et al., 1996; Salvato and Hennessey 2004). First and second instars remain well camouflaged amongst the white croton flowers, while the greenish later stages occur more on the leaves. Salvato and Hennessey (2004) reported approximate body lengths of 2, 4, 6, and 11 mm for Bartram's hairstreak for the second through fifth instar larvae, respectively.

The Service proposed to list the Bartram's hairstreak as endangered and designate critical habitat on August 15, 2013. The Service has subsequently modified the proposed critical habitat based on comments received on the rules. The seven areas proposed as critical habitat are: (1) BSHB1 Everglades National Park, Miami-Dade County, Florida, (2) BSHB2 Navy Wells Pineland Preserve, Miami-Dade County, Florida, (3) BSHB3 Camp Owaissa Bauer, Miami-Dade County, Florida, (4) BSHB4 Richmond Pine Rocklands, Miami-Dade County, Florida, (5) BSHB5 Big Pine Key, Monroe County, Florida, (6) BSHB6 No Name Key, Monroe County, Florida, and

(7) BSHB7 Little Pine Key, Monroe County, Florida. Land ownership within the proposed critical habitat consists of Federal (80 percent), State (5 percent), and private and other (15 percent). Table 2 summarizes these units. Proposed critical habitat for the Florida leafwing occurs entirely within Bartram's scrub-hairstreak units BSHB1, BSHB2, BSHB4, and BSHB5.

The Bartram's hairstreak is dependent upon functioning pine rockland habitat to provide its fundamental life requirements, such as pineland croton for larval development, food sources and roosting areas required by adult butterflies. Based on our current knowledge of the PBFs and habitat characteristics required to sustain the butterfly's life-history processes, we determine the PCEs for the Bartram's hairstreak are:

1. Areas of pine rockland habitat, and in some locations, associated rockland hammocks and hydric pine flatwoods.
    a. Pine rockland habitat contains:
        i. Open canopy, semi-open subcanopy, and understory;
        ii. Substrate of oolitic limestone rock; and
        iii. A plant community of predominately native vegetation.
    b. Rockland hammock habitat associated with the pine rocklands contains:
        i. Canopy gaps and edges with an open to semi-open canopy, subcanopy, and understory;
        ii. Substrate with a thin layer of highly organic soil covering limestone or organic matter that accumulates on top of the underlying limestone rock; and
        iii. A plant community of predominately native vegetation.
    c. Hydric pine flatwood habitat associated with the pine rocklands contains:
        i. Open canopy with a sparse or absent subcanopy, and dense understory;
        ii. Substrate with a thin layer of poorly drained sands and organic materials that accumulates on top of the underlying limestone or calcareous rock; and
        iii. A plant community of predominately native vegetation.
2. Competitive nonnative plant species in quantities low enough to have minimal effect on survival of Bartram's scrub-hairstreak butterfly.
3. The presence of the butterfly's hostplant, pineland croton, in sufficient abundance for larval recruitment, development, and food resources, and for adult butterfly nectar source and reproduction;
4. A dynamic natural disturbance regime or one that artificially duplicates natural ecological processes (*e.g.*, fire, hurricanes, or other weather events, at appropriate intervals) that maintains the pine rockland habitat and associated hardwood hammock and hydric pine flatwood plant communities.
5. Pine rockland habitat and associated hardwood hammock and hydric pine flatwood plant communities that allow for connectivity and are sufficient in size to sustain viable populations of Bartram's scrub hairstreak butterfly.
6. Pine rockland habitat and associated hardwood hammock and hydric pine flatwood plant communities with levels of pesticide low enough to have minimal effect on the survival of the butterfly or its ability to occupy the habitat.

The Bartram's hairstreak occurs only within pine rocklands that retain its hostplant, pineland croton. Pineland croton, a subtropical species of Antillean origin, is the only known hostplant for the hairstreak (Opler and Krizek 1984; Schwartz 1987; Minno and Emmel 1993; Smith et al. 1994). Once occurring throughout the pine rocklands of the lower Florida Keys (Dickson 1955; Hennessey and Habeck 1991; Salvato 1999), pineland croton now occurs only on Big Pine Key. The last reports of the hostplant from other keys were from No Name in 1992 (Carlson et al. 1993) and from Little Pine in 1988 (Hennessey and Habeck 1991). Recent surveys of relict pineland throughout the lower Keys by Salvato (1999, pers. comm. 2008) failed to locate the plant from any island other than Big Pine. Hennessey and Habeck (1991) and Salvato (1999) estimated that approximately 80 ha (198 acres) of croton-bearing pine rockland habitat occur on Big Pine Key. More recently, Chad Anderson (pers. comm. 2010a) estimated roughly 600 acres of croton on Big Pine Key, based upon Bradley's pine rockland data and personal observations.

Life history

The Bartram's hairstreak is rarely encountered more than 5 m from its host plant (Schwartz 1987; Worth et al. 1996; Salvato and Salvato 2008). Females oviposit on the flowering racemes of pineland croton (Worth et al. 1996; Salvato and Hennessey 2004). Eggs are laid singly on developing flowers.

Population dynamics

The Bartram's hairstreak has been observed during every month on Big Pine Key and ENP; however the exact number of broods appears to be sporadic from year to year (Salvato and Hennessey 2004; Salvato and Salvato 2010b). Baggett (1982) indicated the Bartram's hairstreak seemed most abundant in October-December. Salvato and Salvato (2010b) encountered the subspecies most often during March to June within ENP. Land (pers. comm. 2012) has noted the subspecies to be most abundant in the spring and summer months. One of the earliest reports of *S.a. bartrami* phenology from Big Pine Key was provided by Schwartz (1987) who encountered the subspecies only during April, November and December, despite an extensive annual survey. Subsequent research by Hennessey and Habeck (1991), Emmel et al. (1995), and Minno and Minno (2009) reported occurrences of *S.a. bartrami* on Big Pine throughout the year with varying peaks in seasonal abundance. Salvato (1999) recorded 92 and 36 adult Bartram's hairstreak on Big Pine Key during 1-week periods in July 1997 and January 1998, respectively, suggesting the species can occur in high numbers during any season if suitable habitat and conditions are present. Since 2010 on Big Pine Key, Anderson has found them most active when the average temperature is consistently near 80°F which can occur at any time of year (Anderson, pers. comm. 2012).

Status and distribution

Based on the results of historic (Baggett 1982; Schwartz 1987; Hennessey and Habeck 1991; Worth et al. 1996; Schwarz et al. 1996) and recent (Salvato 1999; 2001; 2003; Salvato and Hennessey 2004; Salvato and Salvato 2010a) surveys and natural history studies, the Bartram's hairstreak is extant in ENP and on Big Pine Key, while sporadically occurring in pineland fragments in mainland Miami-Dade County. Hennessey and Habeck (1991) reported an estimate

of 1.6 and 0.4 adult Bartram's hairstreaks per acre during 1988-1989 survey transects on Big Pine Key and Long Pine Key, respectively. During 1997-1998, Salvato (1999) recorded an estimated 1.7 adults per acre at survey transects across Big Pine Key. However, Salvato (1999; 2001) failed to find stable numbers in either the Watson's Hammock on Big Pine Key or in Long Pine Key. The lower densities in Watson's Hammock and Long Pine Key reported by Salvato (1999; 2001; pers. comm. 2006) and Salvato and Hennessey (2004) during the late 1980s and 1990s have been attributed to a lack of prescribed fires necessary to maintain host plants. Through 2010, Salvato and Salvato (unpublished data) indicate the total number of adults observed annually on Big Pine Key has declined precipitously from 278 to 9, based on monthly (1999-2006) or quarterly (2007-2010) surveys conducted from 1999 to 2010.

In March 2009, a high concentration of the butterfly and its host plant was recorded at one area on Big Pine Key (C. Anderson, pers. comm. 2009). However, as of April 28, 2010, Anderson (pers. comm. 2010b) had only observed two adults on the Refuge in 2010. Anderson initiated more intensive surveys for hairstreaks at one-hectare pine rockland transects (n = 6) across Big Pine Key during 2010. These studies noted an average of up to approximately 2.4 hairstreaks per acre during each weekly sampling period (C. Anderson, pers. comm. 2010c). Minno (pers. comm. 2007) reported observing less than 10 individuals after a day of sampling on Big Pine. Additional surveys on Big Pine Key during April 2011 produced only low numbers of the hairstreak across Big Pine Key (C. Anderson, pers. comm. 2011; M. Minno, pers. comm. 2011; M. Salvato, pers. comm. 2011).

Salvato (1999; 2003) noted that the Bartram's hairstreak had either been extirpated or greatly reduced across the majority of Long Pine Key in ENP at the time of his 1997-1998 studies. However, due in large part to an effective and systematic burn plan, Salvato and Salvato (2010a) encountered as many as 15 adult Bartram's hairstreak annually at Gate 4 from 1999 to 2008. In addition, Salvato and Salvato (2010a) also monitored populations of the Bartram's hairstreak at other gates within Long Pine Key during 2005-2008 and encountered similar densities. Overall, Perry (pers. comm. 2007) has observed only small, scattered occurrences within the spatially extensive pineland area of Long Pine Key. She noted counts are typically only in the single digits during her survey efforts.

Salvato (pers. comm. 2009) estimated the populations collectively at Big Pine Key, Long Pine Key, and within relict pine rocklands adjacent to ENP ranges from several hundred or fewer, although it varies greatly depending upon season and other factors. Despite extensive monitoring, Salvato (pers. comm. 2009) only sporadically observes the species within pine rockland areas adjacent to ENP. However, one such pine rockland fragment, Navy Wells, continues to maintain a consistent population of Bartram's hairstreak (Salvato and Salvato 2010a). During 2008 through 2011, Salvato and Salvato (unpublished data) have frequently encountered adults at Navy Wells, including a high of 12 individuals on May 22, 2010. In recent years, annual winter frost events at Navy Wells have served to temporarily reduce hostplant; however, in each instance both the plant and hairstreak have recovered quickly (M. Minno, pers. comm. 2007, 2009; J. Sadle, pers. comm. 2010a; M. Salvato, pers. comm. 2011). In addition, croton appears to have responded well to prescribed burns conducted throughout the preserve,

with fresh croton growth occurring in many locations (Salvato and Salvato 2010a). Additional pine rockland fragments within Miami-Dade County are known to maintain small, localized populations of pineland croton and sporadic occurrences of Bartram's hairstreak, including: Larry and Penny Thompson Memorial Park, Miami Metro Zoo Preserve, Martinez Pineland Park, and Coast Guard lands in Homestead (Minno and Minno 2009; J. Possley, Fairchild Tropical Botanic Garden, pers. comm. 2010; E. Nuehring, pers. comm. 2011).

Minno (pers. comm. 2007) believes that this species has declined greatly since the 1980s and is not likely to survive without special efforts. Bartram's hairstreak may be at least as rare as the federally endangered Schaus swallowtail (M. Minno, pers. comm. 2009). Minno (pers. comm. 2009) indicated that the current population size is not known, but thought to be declining; he believes it is likely to be less than 100 per day for all locations combined. Minno and Minno (2009) recorded 143 adults on Big Pine Key, 36 adults in Miami-Dade County preserves, and 14 adults in ENP during surveys conducted in the Keys and southern Florida mainland from August 2006 through June 2009.

The Bartram's hairstreak has a rounded global status of T1, critically imperiled because of extreme rarity (*i.e.*, 5 or fewer occurrences of less than 1,000 individuals) or because of extreme vulnerability to extinction due to natural or manmade factors (NatureServe 2010). The basis for this ranking stems from the overall threats of: (1) range being reduced by development, (2) pesticide application, (3) fire (prescribed or otherwise), (4) complete fire suppression, and (5) hurricanes as well as restricted distribution, low abundance, and loss of habitat (NatureServe 2010). FNAI (2011) places the butterfly's State rank at "S1", critically imperiled in Florida because of extreme rarity (5 or fewer occurrences or less than 1,000 individuals) or because of extreme vulnerability to extinction due to some natural or man-made factor. This butterfly is recognized in Florida's Comprehensive Wildlife Conservation Strategy as one of Florida's species of greatest conservation need, with status "low" and trend "unknown" (Florida Fish and Wildlife Conservation Commission [FWC] 2005). Bartram's hairstreak is not listed in Florida, and there is no wildlife management plan for this species.

ENVIRONMENTAL BASELINE

The environmental baseline includes the effects of past and ongoing human and natural factors leading to the current status of the species and their habitats within the action area.

Status of the Species/Critical Habitat within the Action Area

Florida leafwing butterfly

The Florida leafwing is currently known to occur only within the Long Pine Key within Everglades National Park (Miami-Dade County), which is not in the action area. However, Big Pine Key is within the action area and had a population of leafwing as recently as 2006 (Salvato and Salvato 2010b). The Florida leafwing occurs entirely within pine rockland habitat that contains the subspecies' only known larval hostplant, pineland croton. Critical habitat within the action area (critical habitat unit FLB4) has been proposed for this subspecies.

Unit FLB4 consists of 1,382 acres on Big Pine Key. This unit includes Federal lands within the Refuge (901 acres), State lands (223 acres), and property in private or other ownership (104 ha (258 acres). State lands are interspersed within Refuge lands and managed as part of the Refuge.

This unit was historically occupied by the Florida leafwing. This unit is not currently occupied but is essential to the conservation of the Florida leafwing because it serves to protect habitat needed to recover the subspecies, reestablish wild populations within the historical range of the subspecies, and maintain populations throughout the historic distribution of the subspecies in the Lower Florida Keys, and it provides area for recovery in the case of stochastic events if the butterfly is extirpated from the one location where it is presently found. In the Lower Florida Keys National Wildlife Refuges Comprehensive Conservation Plan (CCP), management objective number 11 provides specifically for maintaining and restoring butterfly populations of special conservation concern, including the Florida leafwing butterfly.

Bartram's hairstreak butterfly

The Bartram's hairstreak occurs within the action area on Big Pine Key (Salvato and Hennessey 2004; Service 2011). Surveys of the hairstreak within the action area have shown that the population is currently low, with an average of up to approximately 2.4 hairstreaks per acre (C. Anderson, pers. comm. 2010c). This species occurs entirely within pine rockland habitat, and specifically those that retain contiguous levels (at least 100 hectares) of the subspecies' only known larval hostplant, pineland croton. Critical habitat within the action area (critical habitat units BSHB5 and BSHB6) has been proposed for this subspecies.

Unit BSHB5 consists of 1,382 acres on Big Pine Key. This unit includes Federal lands within the Refuge (901 acres), State (223 acres), and property in private or other (258 acres) ownership. State lands are interspersed within Refuge lands and managed as part of the Refuge.

This unit is currently occupied by the Bartram's scrub-hairstreak. This unit contains three of the PBFs, including suitable habitat, hostplant, adult food sources, and breeding sites required by the subspecies, and contains pine rockland and rockland hammock PCEs. The PBFs in this unit may require special management considerations or protection to address threats of disturbance regimes (fire), and pesticide applications, as well as habitat fragmentation, poaching, and sea level rise. However, in most cases these threats are being addressed or coordinated with our partners and landowners to implement needed actions.

Unit BSHB6 consists of 123 acres on No Name Key. This unit includes Federal lands within the Refuge (75 acres), State lands (22 acres), and property in private or other ownership (26 acres). State lands are interspersed within Refuge lands and managed as part of the Refuge. Unit BSHB7 consists of 97 acres on Little Pine Key. This unit is composed entirely of Federal lands. These units are not currently occupied by the Bartram's scrub-hairstreak but are essential to the conservation of the subspecies because they serve to protect habitat needed to recover the subspecies, reestablish wild populations within the historical range of the subspecies, and maintain populations throughout the historical distribution of the subspecies in the Florida Keys, and provide area for recovery in the case of stochastic events that otherwise hold the potential to

eliminate the subspecies from the one or more locations where it is presently found. The Lower Key Refuges, CCP management objective number 11 provides specifically for maintaining and restoring butterfly populations of special conservation concern, including the Bartram's scrub-hairstreak.

Factors Affecting Species Habitat within the Action Area

Habitat loss, fire suppression, and lack of fire management in the past have led to the current fragmentation and degradation of remaining pine rockland habitat and associated population reductions for pine rockland dependent species within the action area. Natural fires are an important part of maintaining an ecosystem's gradual succession and are important in maintaining the herbaceous layer of pine rocklands (Loope and Dunevitz 1981; Carlson et al. 1993; Olson and Platt 1995; Bergh and Wisby 1996). In pine rockland habitat, fires occurred from lightning and as a consequence of use by native Americans. Re-sprouting after burns is the primary mechanism allowing for the persistence of perennial shrubs in pine habitat (Olson and Platt 1995). Without fire, successional climax from tropical pineland to hardwood hammock is rapid, and displacement of native species by invasive exotic plants often occurs. However, due to the proximity of remaining pine rockland habitat to urban areas much of these natural fires have been suppressed, often replaced by inconsistent regimes of managed or prescribed fires. The conversion of pine rockland into hardwood hammock is continuing on northeastern Big Pine and No Name Keys. Pineland croton is now absent from these locations.

The objectives of the current Refuge fire management program are to: (1) protect human life, property, and other resources from unwanted fire; and (2) restore and maintain biological diversity using fire as a viable ecological process (Service 2000). The latter includes maintaining biological diversity in fire-maintained plant communities by prescribed fire and also controlled natural fire under Service guidelines and maintaining habitat for trust resources, including listed plant and animal species, especially the Key deer, through prescribed fire and controlled natural fire (Service 2000). The fire management plan for NKDR mentions the Florida leafwing and its reliance on its fire-dependent hostplant and cites Emmel et al. (1995), who stated "concern has been raised that fire suppression is contributing to the decline of these species as the host plant requires a fire-maintained open pineland to persist" (Service 2000). However, no specific details are provided to enhance habitat or to avoid or mitigate impacts to the Florida leafwing. In addition, management of pine rocklands by the Refuge is made particularly difficult by the pattern of land ownership and development; private homes and light commercial uses are embedded within or in close proximity to the fire-sustained pineland habitat (Service 2000).

Salvato and Salvato (2010b) suggest burns are not being administered as thoroughly within the Refuge as is needed to prevent loss of pine rocklands. As a result, much of the pine rocklands within areas such as northern Watson's Hammock are being compromised by hardwood hammock (Salvato and Hennessey 2004; Salvato and Salvato 2010b). In addition, fire breaks leading into Watson's Hammock have been expanded; these expansions included cutting back and removing large quantities of native vegetation, including croton (M. Salvato, pers. comm.

2008). During 2009, a fire break on the Refuge, running the length of Key Deer Boulevard on Big Pine Key, was mowed by volunteers, thereby cutting back numerous croton plants (M. Salvato, pers. comm. 2010). For over a decade, croton growing within these fire breaks has been actively used by the species (Salvato and Salvato 2010b).

The Refuge is attempting to increase the density of host plants within the pine rockland forests through the use of prescribed fire. These efforts may benefit Florida leafwing populations on Big Pine Key and aid the species in re-establishment within the Refuge. However, there is a backlog of pine rocklands that need to be burned. Of 318 pine rockland plots that were initially assessed on Big Pine Key in 2005, 110 were not burned, 77 were burned once, 55 were burned twice, and 76 were burned either three or four times since 1960 (Bradley and Saha 2009). Complete implementation of a prescribed fire program in the lower Keys has been hampered by an incomplete understanding of the fire ecology in the area, a shortage of resources, and by public opposition to burning. Complicating the issue is that many homes on Big Pine Key have been built in a mosaic of pine rockland, so the use of prescribed fire in many places has become complicated because of potential danger to structures. The Service is working cooperatively with Florida International University in Miami to determine the proper fire frequencies necessary to maintain the pine rockland community on the Refuge (Snyder et al. 2005). Only two burns totaling 10 acres were conducted on the Refuge in 2009 (A. Morkill, pers. comm. 2010). Until more prescribed fires are conducted, fire breaks may provide good habitat on the island because these areas are open, yet mowed or cleared very rarely (C. Anderson, pers. comm. 2010a). For example, Anderson (pers. comm. 2010a) found croton to be at a density of 0.04 plants per m^2 in the forested plots and 0.27 plants per m^2 plot on the fire breaks. Overall, lack of appropriate fire management continues to be a threat for this species on the Refuge and surrounding lands on Big Pine Key. Future actions should avoid mowing of fire breaks with high densities of host plants and implementing more fire in overgrown areas on the Refuge, to the extent possible.

Rare butterflies and moths are highly prized by collectors and an international trade exists in specimens for both live and decorative markets, as well as the specialist trade that supplies hobbyists, collectors, and researchers (Morris et al. 1991; Williams 1996). Consequently, the potential for unauthorized or illegal collection of eggs, larvae, pupae, and adults exists. Even limited collection from the small population on the Refuge could have deleterious effects on reproductive and genetic viability and thus could contribute to its eventual extinction.

The application of mosquito control pesticides has been conducted on Big Pine Key and No Name Key for multiple years and is a potential threat to non-target invertebrates. Pyrethroid adulticides, primarily permethrin, have been applied using truck-based ultra-low volume (ULV) equipment to private and public property. Although Refuge land is not intentionally targeted by the applications, the drift of truck-based pesticides onto Refuge land is unavoidable. Aerial applications of naled, an organophosphate pesticide, have also been conducted routinely on the Refuge using ULV methods. A portion of Refuge lands are targeted by the aerial applications.

Pierce (2009) monitored naled and permethrin deposition following application in and around the Refuge from 2007 to 2009. The deposition values were coupled with laboratory toxicity data generated by Hoang et al. (2011) to conduct a bench-scale risk assessment. Hoang et al. (2011)

exposed larvae and adults of five native Florida butterfly species to permethrin and naled to generate LD50 (the dose sufficient to cause mortality in 50 percent of test subjects) values. The laboratory toxicity data generated by this study were used to calculate hazard quotients (concentrations in the environment/concentrations causing an adverse effect) to assess the risk that concentrations of permethrin found in the field pose to butterflies. A hazard quotient that exceeds one indicates the environmental concentration is greater than the concentration known to cause an adverse effect (mortality in this case), thus indicating significant risk to the organism. Environmental exposures for permethrin were taken from Pierce (2009), and represent the highest concentrations of the chemical that were quantified during the field study. When using the lowest median lethal concentrations from the laboratory study, the hazard quotients for permethrin and naled both indicated a risk of toxicity to non-target butterfly species.

It should be noted many of the studies referenced above dealt with single application scenarios and examined effects on only one to two butterfly life stages. In the case of a persistent compound like permethrin where residues remain on vegetation for weeks, the potential exists for non-target species to be exposed multiple times within a season.

The Service rendered a Biological Opinion (BO), dated April 30, 2010, that evaluates the potential effects of the Federal Emergency Management Agency's (FEMA) implementation of the National Flood Insurance Program (NFIP) on federally threatened and endangered species in the Florida Keys, Monroe County, Florida, in accordance with section 7 of the Endangered Species Act of 1973, as amended (Act) (87 Stat. 884; 16 United States Code [U.S.C.] 1531 *et seq.*). The BO determined the NFIP program could jeopardize four of the nine species that were evaluated and outlined Reasonable and Prudent Alternatives to avoid jeopardy. The Key deer (*Odocoileus virginianus clavium*), rice rat (*Oryzomys palustris natator*), and two of the jeopardized species, the Lower Keys marsh rabbit (*Sylvilagus palustris hefneri*) and the Key tree cactus (*Pilosocereus robinii*), occur on Big Pine and No Name keys. The Florida leafwing and Bartram's scrub-hairstreak had not been proposed and were not addressed in the BO.

A Habitat Conservation Plan (HCP) for Big Pine and No Name Keys was implemented in 2006, but did not address the Florida leafwing and Bartram's hairstreak. The Big Pine/No Name Key HCP addresses development related effects to three of the nine species addressed in the FEMA BO, the Key deer, Lower Keys rabbit, and eastern indigo snake (*Drymarchon corais couperi*). In order to fulfill the HCP's mitigation requirements, Monroe County has been actively acquiring parcels of high-quality pine rockland habitat and placing them into conservation. These conservation actions have benefited the Florida leafwing and Bartram's scrub-hairstreak by protecting habitat.

EFECTS OF THE ACTION

This section includes an analysis of the direct and indirect effects of the proposed action on the proposed species and their proposed critical habitat and the proposed action's interrelated and interdependent activities. The Service and the Refuge have worked in collaboration to develop specific conservation measures/criteria included in this conference opinion. The Service believes

that, as implemented, the conservation measures will minimize to some extent potential adverse effects. However, even with the implementation of the proposed conservation measures, some remaining adverse effects may occur to the covered species as described below.

Factors to be considered

Truck-spraying of pyrethroids, aerial spraying of naled, and the application of barrier treatments may have adverse impacts on the Bartram's hairstreak and the proposed critical habitat for the Bartram's hairstreak and Florida leafwing. The application of the larvicides Bti and Bsp to aquatic habitat is not expected to have an impact on the butterflies or their proposed critical habitat. The Bartram's hairstreak currently resides within pine rockland habitat on the Refuge, as did the Florida leafwing as recently as 2006. For both the Florida leafwing and Bartram's hairstreak, pine rockland habitat with levels of pesticide low enough to have minimal effect on survival or the ability to occupy the habitat has been identified as a PCE.

Analyses for effects of the action

Direct effects: Direct effects are those effects that result from the proposed action (including the effects of interrelated and interdependent actions) and immediately impact the species or its habitat. The direct effects this project may have on the proposed butterfly species and their proposed critical habitat are discussed below.

In order for mosquito control chemicals to be effective, they must make direct contact with mosquitoes. The truck-based and aerial application methods proposed for use on the Refuge utilize ULV technology, which allows for small pesticide droplet sizes to be created. These small droplets remained suspended for a longer period of time; therefore increasing the probably that the pesticide will contact flying mosquitoes. Such an increased suspension time may also lead to the pesticide drifting some distance from the application site. The barrier treatment, using the pyrethroid bifenthrin, may be selectively applied to vegetation in private and public areas, but not on Refuge-managed lands. Resting mosquito contact with the barrier treatment is expected to cause mortality.

Truck-based ULV methods are expected to produce a swath of suspended pesticides approximately 100 m wide (M. Hudon, pers. comm. 2013). The movement of this swath will depend on multiple factors including wind speed and direction. Vegetation density may also influence the movement and penetration of the swath into terrestrial habitat. Pierce (2012) detected permethrin in the Refuge at concentrations determined to be lethal to a surrogate butterfly species in a laboratory exposure study by Hoang et al. (2011) at a distance approximately 227m from the existing truck spray routes (Figure 4). The direct effects of permethrin exposure could include acute toxicity to larval or adult butterflies through dermal or inhalation pathways. Exposure to permethrin may also elicit sublethal impacts, though such impacts are difficult to quantify in a butterfly species.

Aerial ULV application of naled may also lead to the direct exposure of larval or adult butterflies through dermal or inhalation pathways. Little research has been conducted to confidently estimate the expected distance that naled may drift during aerial applications. In 2009, two field

trials on NKDR detected significant naled residues at locations within non-target areas that were approximately 400 m from the edge of zones targeted for aerial applications (Bargar pers. comm. 2011). Aerial application equipment was re-calibrated following these events and significantly less drift was observed in a later trial. The direct effects of naled exposure could include acute toxicity to larval or adult butterflies. Bargar (2012) also documented a sublethal effect, the inhibition of cholinesterase activity, in butterflies exposed to naled following an aerial application on NKDR.

The application of bifenthrin as a barrier treatment may cause mortality in Bartram's hairstreak if the butterfly is present on target vegetation during the application, or if the butterfly lands on vegetation with bifenthrin residues. Bifenthrin will not be applied in core areas for the Bartram's hairstreak, which will minimize the probability that Bartram's hairstreak will be exposed. Because of the highly controlled nature of the barrier application (backpack or trailer-mounted sprayer), proposed critical habitat for the Bartram's hairstreak and the Florida leafwing should not be impacted.

Do to their tenuous population size within the action area, it is unknown exactly how many Bartram's hairstreak butterflies could be directly exposed to pyrethroids, naled, or bifenthrin. Within the action area, there are approximately 1,602 acres of proposed critical habitat for the Bartram's hairstreak butterfly and 1,382 acres of proposed critical habitat for the Florida leafwing. Currently, only 37 acres of this habitat are considered to be core areas for the Bartram's hairstreak. The remaining habitat requires restoration, contains little to no host plant, and does not reliably serve as habitat for the Bartram's hairstreak. Assuming that the 50-m buffer around proposed critical habitat and the 250-m buffer around core areas are employed and that drift of truck-based pesticide ranges from 100 m to 225 m, butterflies residing in 5.7 percent (92 acres) to 26.3 percent (421 acres) of proposed critical habitat in the action area for the Bartram's hairstreak and in 6.3 percent (87 acres) to 28.3 percent (391 acres) of proposed critical habitat in the action area for the Florida leafwing could potentially be directly exposed to truck-based pesticides. Option B of the fourth phase of Alternative B dictates that the 50-m buffer around proposed critical habitat will no longer be recognized, but the 250-m buffer around core areas will still apply. Under this scenario, assuming that truck-based drift ranges from 100 m to 225 m, 25.0 percent (400 acres) to 43.6 percent (699 acres) of proposed critical habitat in the action area for the Bartram's hairstreak and 28.4 percent (392 acres) to 48.0 percent (663 acres) of proposed critical habitat in the action area for the Florida leafwing may receive drift of truck-based pesticides (Table 3). Because the 250-m buffer around core areas for the Bartram's hairstreak will be implemented under all scenarios, the core areas are not expected to be impacted by drift of truck-based pesticides. The Florida leafwing has not been observed on Big Pine Key since 2006 (Salvato and Salvato 2010b) and is considered to be extirpated from its former range within the action area. Without reintroduction efforts, it is unlikely that the Florida leafwing would be directly exposed to truck-based pesticide drift within the action area.

Assuming the existing aerial no-spray zones coupled with the additional 400-m buffer around Bartram's hairstreak core areas are employed, an aerial spray event would directly target 40.5 percent (649 acres) of proposed critical for the Bartram's hairstreak and 37.6 percent (520 acres) of proposed critical habitat for the Florida leafwing (Table 3). These are conservative estimates that

do not account for aerial drift. In the event of a 400 m drift event, a significantly larger portion of the proposed critical habitats would be impacted. The core areas would not be impacted by an aerial spray event drifting 400 m due to the 400-m buffer placed around core areas.

While the estimates above are based on the best available information, additional information regarding drift distance and magnitude for both truck-based and aerial spray events is needed to fully understand the impacts and risks associated with the application of mosquito adulticides within the action area. Additional monitoring data will be required to limit these uncertainties.

Interrelated and interdependent actions: An interrelated activity is an activity that is part of the proposed action and depends on the proposed action for its justification. An interdependent activity is an activity that has no independent utility apart from the action under consultation. There are no interrelated or interdependent actions for this project.

Indirect effects: Indirect effects are those that are caused by or result from the proposed action, are later in time, and are reasonably expected to occur. The indirect impacts evaluated by the Service include the longer term impacts caused by pyrethroid deposition on the butterflies' proposed critical habitat.

Pyrethroids are moderately persistent in the environment. Residues on vegetation or in nectar may be consumed by both larval and adult butterflies for a period of time after application and may cause adverse impacts. Little information exists regarding the risk associated with consumption of contaminated vegetation and nectar by butterflies. The acres of proposed critical habitat in the action area for both butterfly speices subject to potential indirect effects are the same as the potentially impacted acreages described under the direct effects above. Indirect effects of pyrethroid application would be temporary as the pesticides are expected to fully degrade. There would not be a permanent effect on proposed butterfly critical habitat.

Because naled does not persist in the environment, with a half-life on the order of hours, exposure through the consumption of contaminated vegetation or nectar may occur directly after a spray event, but would not be expected to continue chronically. Indirect effects of naled application would be limited as the pesticides are expected to fully degrade in a relatively short period of time. There would not be a permanent effect on proposed butterfly critical habitat.

Species' response to the proposed action

The Bartram's hairstreak occurs within the action area on Big Pine Key. The Florida leafwing has not been observed since 2006 and is considered extirpated from the action area. The potential incidental drift of pyrethroids and naled could result in dermal, ingestion, and/or inhalation exposure to both adult and larval Bartram's hairstreak butterflies. Direct exposure of pyrethroids and naled could be acutely toxic to the Bartram's hairstreak, resulting in death. Pyrethroid residues on larval and adult food sources for the Bartram's hairstreak are likely to be more chronic in nature, with the potential for decreased fitness and eventual take of the species, especially with repeated exposures throughout the spray season. The added buffers around the core areas for the Bartram's hairstreak will provide additional safeguards for butterflies in the core areas.

CUMULATIVE EFFECTS

Cumulative effects include the effects of future State, tribal, local or private actions that are reasonably certain to occur in the action area considered in this conference opinion. Future Federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the Act.

The use of pesticides on private property for the control of mosquitoes and/or additional nuisance species most likely occurs within the action area. Such pesticides could pose a risk to the Bartram's hairstreak or to the host plant, pineland croton, of both the Bartram's hairstreak and the Florida leafwing. The extent of such pesticide use is unknown, but would not be expected to have significant impacts outside of the immediate areas of application in and around private properties and businesses.

CONCLUSION

Proposed and Candidate Species and Proposed Critical Habitat

After reviewing the current status of the Bartram's hairstreak, the environmental baseline for the action area, the effects of the proposed action, and the cumulative effects, it is the Service's conference opinion that the adoption of the Plan, as proposed, is not likely to jeopardize the continued existence of the Bartram's hairstreak, and is not likely to destroy or adversely modify proposed critical habitat for the Bartram's hairstreak or the Florida leafwing. The Refuge made a determination that the proposed action is likely to adversely affect the Florida leafwing. In light of the information previously discussion, the Service cannot concur with this determination and finds the proposed project not likely to adversely affect the Florida leafwing, since the species is considered extirpated from the action area. If the Refuge agrees with the Service's determination, this Conference Opinion may be used as a concurrence, and no further consultation is necessary.

INCIDENTAL TAKE STATEMENT

Section 9 of the Act and federal regulation pursuant to section 4(d) of the Act prohibit the take of endangered and threatened species, respectively, without a special exemption. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Harm is further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavioral patterns such as breeding, feeding, or sheltering. Harass is defined by the Service as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns, which include, but are not limited to, breeding, feeding, or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of this Incidental Take Statement.

The measures described below are non-discretionary, and must be undertaken by the Refuge so that they become binding conditions of any grant or permit issued to the District, as appropriate, for the exemption in section 7(o)(2) to apply. The Refuge has a continuing duty to regulate the activity covered by this incidental take statement. If the Refuge (1) fails to assume and implement the terms and conditions or (2) fails to require the District to adhere to the terms and conditions of the incidental take statement through enforceable terms that are added to the permit or grant document, the protective coverage of section 7(o)(2) may lapse. In order to monitor the impact of incidental take, the District must report the progress of the action and its impact on the species to the Service as specified in the incidental take statement. [50CFR §402.14(i)(3)]

The prohibitions against taking the species found in section 9 of the Act do not apply until the species is listed and critical habitat is designated. However, the Service advises the Refuge to consider implementing the following reasonable and prudent measures. If this Conference Opinion is adopted as a biological opinion following a listing or designation, these measures, with their implementing terms and conditions, will be non-discretionary.

Proposed Species

These incidental take statements address the Florida leafwing and Bartram's hairstreak and their proposed critical habitat. The incidental take statement provided in this Conference Opinion does not become effective until the species is listed or critical habitat is designated and the Conference Opinion is adopted as the biological opinion issued through formal consultation. At that time, the project will be reviewed to determine whether any take of the species has occurred. Modifications of the opinion and incidental take statement may be appropriate to reflect that take. No take of the species may occur between the listing of the Florida leafwing or the Bartram's hairstreak and the adoption of the Conference Opinion through formal consultation, or the completion of a subsequent formal consultation for the Florida leafwing and the Bartram's hairstreak.

Florida leafwing butterfly

The Service does not anticipate incidental take of the Florida leafwing since it is considered to be extirpated within the project area. Based on 100 to 225 m of potential drift from truck-based application, it is estimated that 28.4 to 48.0 percent (392 to 663 acres) of proposed Florida leafwing critical habitat within the action area could be temporarily impacted. An aerial application of naled would directly target 37.6 percent (520 acres) of proposed critical habitat in the action area. Because pesticide residues will degrade with time, the potential adverse impact on proposed critical habitat would not be permanent, and these areas will be suitable for recolonization.

Bartram's hairstreak butterfly

The Service anticipates incidental take of the Bartram's hairstreak will be difficult to detect due to their small body size, their relatively short life cycle, and their tenuous population status. Based on 100 to 225 m of potential drift from truck-based application, it is estimated 25.0 to 43.6 percent (400 and 699 acres) of proposed critical habitat for the Bartram's hairstreak within the action area could be temporarily impacted by the proposed activity. Although the area

potentially vulnerable to drift is outside of the core areas for the Bartram's hairstreak, individual butterflies may occur within this potential drift area and could be taken. The incidental take is expected to be in the form of harm, harassment, and direct mortality. An aerial application of naled would directly target 40.5 percent (649 acres) of proposed critical habitat in the action area. The added 400-m buffer around core areas for Bartram's hairstreak should lessen impacts from an aerial application to the Bartram's hairstreak. Because pesticide residues will degrade with time, the potential adverse impact on proposed critical habitat would not be permanent, and these areas will be suitable for recolonization.

EFFECT OF THE TAKE

In the accompanying Conference Opinion, the Service determined this level of anticipated take is not likely to result in jeopardy to the candidate species or destruction or adverse modification of proposed critical habitat.

REASONABLE AND PRUDENT MEASURES

The prohibitions found in section 9 of the Act against taking a species do not apply until the species is listed. However, the Service advises the Refuge to consider implementing the following reasonable and prudent measures. If this Conference Opinion is adopted as a biological opinion following a listing or designation, these measures, with their implementing terms and conditions, will be nondiscretionary.

1. No-spray zones and buffers shall be established or expanded to prevent drift of pyrethroids and naled into core areas for the Bartram's hairstreak butterfly.
2. Bifenthrin barrier treatments shall not occur on or near host plant for the Bartram's hairstreak.
3. Limits on wind direction and speed during pyrethroid and naled applications shall be specified to limit the potential for drift of pyrethroids and naled into non-target areas.
4. The effects of the proposed spraying on core areas for the Bartram's hairstreak shall be monitored, and immediate steps taken to minimize those effects should monitoring indicate that adverse effects may be occurring.

TERMS AND CONDITIONS

Should this Conference Opinion be adopted as a biological opinion following the listing of the butterfly species and/or the designation of critical habitat, in order to be exempt from the prohibitions of section 9 of the Act, the Refuge must comply with the following terms and conditions, which implement the reasonable and prudent measures described above and outline reporting and monitoring requirements. These terms and conditions are non-discretionary.

1. All proposed critical habitat within the Refuge shall be established as a no-spray zone with a 50-m no-spray buffer when mosquito landing rates are below 10 mosquitoes per minute. All proposed critical habitat is considered potential butterfly habitat, so no truck-based applications of pyrethroids shall be permitted within the 50-m buffer without meeting the appropriate landing rate threshold.

2. Pyrethroid residues have been measured over 225 m from truck routes (Pierce 2012). Therefore, no truck-based applications shall occur within 250 m of core areas occupied by the Bartram's hairstreak at any time. If any new core areas for the Bartram's hairstreak habitat are discovered or if the Florida leafwing is discovered on the Refuge, then truck-based mosquito control operations should immediately cease within 250 m of the identified areas.
3. A monitoring strategy for truck-based pyrethroid applications is to be developed and submitted to the SFESO for approval. The monitoring should serve to confirm the presence/absence of pyrethroid residues in the core areas and to examine drift distances into the proposed critical habitat. As core areas shift over time, it will be important to understand truck-based drift distances as buffers and no-spray zones need to be redrawn. The strategy should be developed during the 2014 mosquito control season and be ready for implementation in 2015.
4. A single aerial application of naled may be administered on the Refuge. Prior to such application, a comprehensive monitoring strategy must be developed and submitted to the SFESO for approval. The monitoring should serve to primarily ensure that drift into core areas is not occurring and to examine aerial drift distances in general. This information will be used in the development of appropriate buffer distances to be placed around core areas during aerial applications. A 400-m buffer is to be established around core areas during this initial aerial event. The results of the monitoring will be evaluated by the District, Refuge, and SFESO to determine if further monitoring is required to adequately designate buffer distances. No additional aerial applications shall be conducted until the results of the initial trial have been evaluated by the SFESO.
5. Truck-based and aerial applications shall not occur when sustained winds are forecasted to exceed 10 mph, or gusts exceeding 15 mph. Wind direction shall be such that drift is carried away from the no-spray zones and buffers.
6. Personnell conducting the barrier treatment shall be trained to field-identify pineland croton. Application of the barrier treatment shall not occur on or within 10 m of pineland croton.
7. Coordination between the District, Refuge, and the South Florida Ecological Services Office (SFESO) will be necessary for determining accurate butterfly core area locations. A yearly evaluation of core areas shall be conducted prior to the issuance of a special use permit for the initiation of mosquito control activities and submitted to the SFESO. New core areas may be added at any time at the discretion of the Refuge and SFESO with added protections even if the Bartram's hairstreak or Florida leafwing are not present.
8. A complete report shall be provided to the SFESO at the end of each mosquito control season clearly stating the number, date, and location of all application events for the preceding season.
9. Reporting and disposition of dead or injured animals (salvage):
    a. Upon locating a dead, injured, or sick federally listed species, initial notification must be made to the referenced project biologist and the nearest Service Law Enforcement Office (U.S. Fish and Wildlife Service; 1339 20th Street, Vero Beach, Florida; 772-562-3909). Secondary notification should be made to the

FWC, South Region; 8535 Northlake Boulevard, West Palm Beach, Florida; 33412-3303; 561-625-5122; 1-888-404-3922.

b. Care shall be taken in handling sick or injured specimens to ensure effective treatment and care, or, in the handling of dead specimens, to preserve biological material in the best possible state for later analysis as to the cause of death. Dead specimens should be placed on ice and frozen as soon as possible. In conjunction with the care of sick or injured specimens or preservation of biological materials from a dead animal, the finder has the responsibility to carry out instructions provided by Law Enforcement to ensure that evidence intrinsic to the specimen is not unnecessarily disturbed.
c. Report all Bartram's hairstreak injuries or deaths, resulting from the proposed action to the referenced project biologist. This report shall contain the location (latitude and longitude), dates, times, prevailing environmental conditions, and the circumstances surrounding all sightings and the disposition of all animals found. A site map with observation locations shall also be included in this report. If no Bartram's hairstreak butterflies are encountered, a report shall be submitted indicating that fact.

The Service believes incidental take as a result of the proposed action will be difficult to detect. The reasonable and prudent measures, with their implementing terms and conditions, are designed to minimize the impact of incidental take that might otherwise result from the proposed action. If, during the course of the action, this level of incidental take is exceeded, such incidental take represents new information requiring reinitiation of consultation and review of the reasonable and prudent measures provided. The Federal agency must immediately provide an explanation of the causes of the taking and review with the Service the need for possible modification of the reasonable and prudent measures.

CONSERVATION RECOMMENDATIONS/CONSIDERATIONS

Section 7(a)(1) of the Act directs Federal agencies to utilize their authorities to further the purposes of the Act by carrying out conservation programs for the benefit of endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help implement recovery plans, or to develop information.

- Implement restoration activities, which may include mechanical or fire-based activities, on portions of the proposed critical habitat that are not currently suitable for the Bartram's hairstreak and Florida leafwing. Restoring habitat in areas that are not in direct conflict with mosquito control activities would benefit the butterfly population without further limiting the ability to conduct mosquito control activities. Additionally, restoring habitat between the existing core areas for Bartram's hairstreak could provide connectivity between the core areas.

In order for the Service to be kept informed of actions minimizing or avoiding adverse effects or benefitting listed species or their habitats, the Service requests notification of the implementation of any conservation recommendations.

REINITIATION NOTICE

This concludes conference on the proposed action. You may ask the Service to confirm the Conference Opinion as a biological opinion issued through formal consultation if the Bartram's hairstreak or Florida leafwing are listed or if critical habitat is designated. The request must be in writing. If the Service reviews the proposed action and finds there have been no significant changes in the action as planned or in the information used during the conference, the Service will confirm the Conference Opinion as the biological opinion on the project and no further section 7 consultation will be necessary.

After listing of the Bartram's hairstreak or Florida leafwing as endangered or threatened and/or designation of critical habitat for the Bartram's hairstreak or Florida leafwing and any subsequent adoption of this conference opinion, the Federal agency shall request reinitiation of consultation if: (1) the amount or extent of incidental take is exceeded; (2) new information reveals effects of the agency action that may affect the species or critical habitat in a manner or to an extent not considered in this conference opinion; (3) the agency action is subsequently modified in a manner that causes an effect to the species or critical habitat that was not considered in this conference opinion; or (4) a new species is listed or critical habitat designated that may be affected by the action.

The incidental take statement provided in this conference opinion document does not become effective until the species is listed and the Conference Opinion is adopted as the biological opinion issued through formal consultation. At that time, the project will be reviewed to determine whether any take of the species has occurred. Modifications of the opinion and incidental take statement may be appropriate to reflect that take. No take of the species may occur between the listing of the Bartram's hairstreak and Florida leafwing, and the adoption of the Conference Opinion through formal consultation, or the completion of a subsequent formal consultation.

If you have any questions, please contact Anthony Sowers at 772-469-4223.

cc: electronic only
Service, Vero Beach, Florida (Dana Hartley, Mark Salvato)

LITERATURE CITED

Alexander, T.R., and J.D. Dickson. 1972. Vegetational changes in the National Key Deer Refuge II. Quarterly Journal of the Florida Academy of Sciences 35:85-96.

Anderson, C. 2009. Email to Anne Morkill. U.S. Fish and Wildlife Service. Florida Keys National Wildlife Refuge Complex. Big Pine Key, Florida. March 23, 2009.

Anderson, C. 2010a. Email to Paula Halupa (comments on Bartram's hairstreak species assessment). U.S. Fish and Wildlife Service, Florida Keys National Wildlife Refuge Complex. Big Pine Key, Florida. February 10, 2010.

Anderson, C. 2010b. Meeting with South Florida Ecological Services Office. U.S. Fish and Wildlife Service, Florida Keys National Wildlife Refuge Complex. Big Pine Key, Florida. April 28, 2010.

Anderson, C. 2010c. Email to Paula Halupa (preliminary data on Bartram's hairstreak surveys). U.S. Fish and Wildlife Service, Florida Keys National Wildlife Refuge Complex. Big Pine Key, Florida. November 30, 2010.

Anderson, C. 2011. Email to Paula Halupa (April 2011 observations of Bartram's hairstreak on Big Pine Key). U.S. Fish and Wildlife Service, Florida Keys National Wildlife Refuge Complex. Big Pine Key, Florida. April 11, 2011.

Anderson, C. 2012. Email to Paula Halupa (comments on Bartram's hairstreak species assessment). U.S. Fish and Wildlife Service, Florida Keys National Wildlife Refuge Complex. Big Pine Key, Florida. February 16, 2012.

Andrejko, M. 2011. Email to Mark Salvato. Homestead Air Reserve Base. Homestead, Florida. August 5, 2011

Baggett, H.D. 1982. Order Lepidoptera. In R. Franz (ed.), Invertebrates. In P.C. Pritchard (ed.) Rare and Endangered Biota of Florida. Vol. 6. Invertebrates, 78-81. University Press, Gainesville, Florida.

Bargar, T.A. 2012. The relationship between total cholinesterase activity and mortality in four butterfly species. Environ. Toxicol. Chem. 31(9):2124-2129.

Bergh, C., and J. Wisby. 1996. Fire history of the lower Keys pine rocklands. Nature Conservancy (publishers). Key West, Florida.

Bradley, K.A. 2006. Distribution and population size for three pine rockland endemic candidate plant taxa on Big Pine Key, Florida. Report submitted to U.S. Fish and Wildlife Service, Vero Beach, Florida. The Institute for Regional Conservation. Miami, Florida.

Bradley, K.A. 2007a. Email to Paula Halupa. The Institute for Regional Conservation. Miami, Florida. September 13, 2007.

Bradley, K. 2007b. Email to Paula Halupa. The Institute for Regional Conservation. Miami, Florida. March 6, 2007.

Bradley, K. 2008. Email to Paula Halupa. The Institute for Regional Conservation. Miami, Florida. November 17, 2008.

Bradley, K.A. 2009. Assessment of rare plant species and pine rockland habitat at proposed U.S. Army Special Operations Command South Headquarters adjacent to the U.S. Air Reserve Base, Homestead, Florida. Contract No. W91278-09-P-0278. The Institute for Regional Conservation. Report submitted to Ralph Etheridge, U.S. Army Corps of Engineers, Mobile District, Mobile, Alabama.

Bradley, K.A. 2011. Email to Paula Halupa. The Institute for Regional Conservation. Miami, Florida. April 12, 2011.

Bradley, K.A., and G.D. Gann. 1999. Status summaries of 12 rockland plant taxa in southern Florida. Report submitted to U.S. Fish and Wildlife Service, Vero Beach, Florida. The Institute for Regional Conservation. Miami, Florida.

Bradley, K.A., and S. Saha. 2009. Post-hurricane responses of rare plant species and vegetation of pine rocklands in the Lower Florida Keys. Report submitted to U.S. Fish and Wildlife Service, Big Pine Key, Florida. The Institute for Regional Conservation. Miami, Florida.

Carlson, P.C., G.W. Tanner, J.M. Wood, and S.R. Humphrey. 1993. Fires in Key deer habitat improves browse, prevents succession, and preserves endemic herbs. Journal of Wildlife Management 57: 914-928.

Comstock, W.P. 1961. Butterflies of the American Tropics. The Genus Anaea, Lepidoptera: Nymphalidae. American Museum of Natural History, New York.

Department of Defense, Special Operations Command South. 2009. Letter to U.S. Fish and Wildlife Service, South Florida Ecological Services Office, Vero Beach, Florida. March 11, 2009.

Deyrup, M., and R. Franz (eds). 1994. Rare and Endangered Biota of Florida. Volume IV. Invertebrates. University Press of Florida, Gainesville, Florida.

Dickson, J.D. 1955. An ecological study of the Key Deer. Florida Game and Freshwater Fish Commission. Pittmann-Robertson Project Technical Bulletin 3.

Ehrenfeld, J. 1979. Pollination of three species of Euphorbia subgenus Chamaesyce (Euphorbiaceae), with special reference to bees. American Midland Naturalist 101(1) 87-98.

Emmel, T.C., R.A. Worth, and K. Schwarz. 1995. The relationships between host plant and habitat for the distribution of three potentially endangered south Florida butterfly species. Report to the National Biological Survey.

Florida Fish and Wildlife Conservation Commission (FWC). 2005. Florida's Wildlife Legacy Initiative. Florida's Comprehensive Wildlife Conservation Strategy. Tallahassee, Florida.

Florida Keys Mosquito Control District. 2006. Efficacy of larvicidal treatment of wilderness islands for reduction of adulticidal treatment of areas adjacent to Key Deer National Wildlife Refuge, and Great White Heron National Wildlife Refuge, Big Pine Key, Florida. Progress report. Submitted on December 31, 2006 to U.S. Fish and Wildlife Service. Florida Keys Mosquito Control District; Key West, Florida.

Florida Natural Areas Inventory. 2011. FNAI Element Tracking Summary. Tallahassee, Florida. April 7, 2011. http://www.fnai.org/PDF/Element_tracking_summary_201103.pdf [Accessed: May 5, 2011]

Folk, M.L. 1991. Habitat of the Key deer. Ph.D. Dissertation, Department of Zoology, Southern Illinois University, Carbondale, Illinois.

Gann, G.D., K.A. Bradley, and S.W. Woodmansee. 2002. Rare plants of south Florida: their history, conservation, and restoration. The Institute for Regional Conservation, Miami, Florida.

Gann, G.D., K.A. Bradley and S.W. Woodmansee. 2001-2010. The floristic inventory of south florida database online. The Institute for Regional Conservation, Miami, Florida.http://regionalconservation.org/ircs/database/plants/PlantPage.asp?TXCODE=Linuaren [Accessed: May 2, 2011].

Gordon, D. 2008. Email to Paula Halupa. The Nature Conservancy. Gainesville, Florida. January 4, 2008.

Gordon, D.R., G.D. Gann, S.E. Green, K.A. Bradley, A.M. Jenkins, and S. Travis. 2007. Mapping of invasive exotic plants and rare native plants on Florida Department of Transportation District 6 right-of-way in Miami-Dade and Monroe Counties, Florida. Final report to the Florida Department of Transportation District 6 under cooperative agreement 404278-1-32-07. University of Florida, Gainesville, Florida.

Hennessey, M.K., and D.H. Habeck. 1991. Effects of mosquito adulticiding on populations of non-target, terrestrial arthropods in the Florida Keys. United States Department of Agriculture - Agricultural Research Service, Miami, Florida.

Hennessey, M.K., H.N. Nigg, and D.H. Habeck. 1992. Mosquito (Diptera: Culicidae) adulticide drift into wildlife refuges of the Florida Keys. Environmental Entomology 21(4):715-720.

Hoang, T.C., R.L. Pryor, G.M. Rand, R.A. Frakes. 2011. Use of butterflies as non-target insect test species and the acute toxicity and hazard of mosquito control insecticides. Environmental Toxicology and Chemistry 30(4):997-1005.

Hodges, S.R., and K.A. Bradley. 2005. Distribution and population size of five candidate plant taxa in the Florida Keys. Status Report for U.S. Fish and Wildlife Service, Vero Beach, Florida. Institute for Regional Conservation, Miami, Florida.

Hodges, S.R., and K.A. Bradley. 2006. Distribution and population size of five candidate plant taxa in the Florida Keys: Argythamnia blodgettii, Chamaecrista lineata var. keyensis, Indigofera mucronata var. keyensis, Linum arenicola, and Sideroxylon reclinatum subsp. austrofloridense. The Institute for Regional Conservation. Final Report Contract Number 401815G011, submitted to U.S. Fish and Wildlife Service, Vero Beach, Florida.

Hribar, L.J., J.J. Vlach, D.J. DeMay, L.M. Stark, R.L. Stoner, M.S. Godsey, K.L. Burkhalter, M.C. Spoto, S.S. James, J.M. Smith, and E.M. Fussell. 2003. Mosquitoes infected with West Nile virus in the Florida Keys, Monroe County, Florida, USA. J. Med. Entomol. 40: 361-363.

Hribar, L.J., L.M. Stark, R.L. Stoner, D.J. DeMay, A.L. Nordholt, M.J. Hemmen, J.J. Vlach, & E.M. Fussell. 2004. Isolation of West Nile virus from mosquitoes (Diptera: Culicidae) in the Florida Keys, Monroe County, Florida. Carib. J. Sci. 40: 362-367.

Hudon, M. 2013. Presentation to South Florida Ecological Services Office. Indian River County Mosquito Control. Vero Beach, Florida. June 6, 2013.

Irwin, H.S., and R.C. Barneby. 1982. The American Cassiinae: a synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World. Memoirs of the New York Botanical Garden 35(1):1-918.

Kabat, C.A., S.M. Kabat, W.S. Judd, and G.M. Ionta. 2006. Floristic inventory of the remnant pinelands of the USDA subtropical horticultural research station at Chapman Field, Miami-Dade County, Florida. Rhodora 108(936):387-399.

Kernan, C. and K. A. Bradley. 1996. Conservation survey of Linum arenicola in Dade County, Florida. Fairchild Tropical Garden. Report to the U.S. Fish and Wildlife Service, Vero Beach, Florida.

Land, A. 2012. Email to Paula Halupa (comments on Bartram's hairstreak species assessment). National Park Service, Everglades National Park. Homestead, Florida. January 26, 2012.

Liu, H. 2003. Population viability analyses of Chamaecrista keyensis (Leguminosae: Caesalpinioideae), a narrowly endemic herb of the lower Florida Keys: effects of seasonal timing of fires and the urban-wildland interface. Doctoral dissertation. Florida International University. Miami, Florida.

Liu, H., and S. Koptur. 2003. Breeding system and pollination of a narrowly endemic herb of the Lower Florida Keys: impacts of the urban-wildland interface. American Journal of Botany 90: 1180-1187.

Liu, H., E. Menges, and P.F. Quintana-Ascencio. 2005a. Population viability analysis of *Chamaecrista keyensis*: effects of fire season and frequency. Ecological Applications 15(1):210-221.

Liu, H., E. Menges, J. Snyder, S. Koptur, and M. Ross. 2005b. Effect of fire intensity on vital rates of an endemic herb of the Florida Keys, USA. Natural Areas Journal 25(1): 71-76.

Loope, L.L., and V.L. Dunevitz. 1981. Impact of fire exclusion and invasion of *Schinus terebinthifolius* on limestone rockland pine forests of southeastern Florida. South Florida Resource Center. Report T-645, National Park Service. Everglades National Park, Homestead, Florida.

Lopez, R.R. 2001. Population ecology of the Florida Key deer. Dissertation, Texas A&M University, College Station, Texas.

MacAulay, G.M., T.J. Leary, F.J. Sargent, M.M. Colby, E.J. Prouty, and C.A. Friel. 1994. Advanced Identification of Wetlands in the Florida Keys, Final Report. Florida Department of Environmental Protection, Division of Marine Resources, Marathon, Florida.

Maschinski, J. 2007. Email to Paula Halupa. Fairchild Tropical Botanic Garden. Miami, Florida. March 12, 2007.

Maschinski, J. 2011. Email to Marilyn Knight. Fairchild Tropical Botanic Garden. Miami, Florida. April 19, 2011.

Matley, A. 2003. West Nile shows up in the Keys. Florida Keys Keynoter. August 20, 2003.

Minno, M. 2007. Email to Paula Halupa. Eco-Cognizant, Inc. Gainesville, Florida. March 8, 2007.

Minno, M. 2008. Presentation. Survey for butterflies in the Florida Keys 2006-2007. Eco-Cognizant, Inc. Gainesville, Florida. March 1, 2008.

Minno, M. 2009. Email to Paula Halupa. Eco-Cognizant, Inc. Gainesville, Florida. February 16, 2009.

Minno, M. 2011. Presentation. Status of butterflies in the Florida Keys: continued declines and losses. Eco-Cognizant, Inc. Gainesville, Florida. Presentation given to the Imperiled Butterflies of Florida Workgroup, annual meeting. Homestead, Florida. April 22, 2011.

Minno, M.C., and T.C. Emmel. 1993. Butterflies of the Florida Keys. Scientific Publishers, Inc., Gainesville, Florida.

Minno, M., and M. Minno. 2009. A plan to conserve rare butterflies in the Florida Keys. Submitted to: Edsel M. Fussel, Director, Florida Keys Mosquito Control District. Eco-Cognizant, Inc. Gainesville, Florida.

Minno, M. C., J. F. Butler, and D. W. Hall. 2005. Florida Butterfly Caterpillars and Their Hostplants. University Press of Florida, Gainesville, Florida.

Morkill, A. 2010. Meeting with South Florida Ecological Services Office. U.S. Fish and Wildlife Service, Florida Keys National Wildlife Refuge Complex. Big Pine Key, Florida. April 28, 2010.

Morris, M.G., N.M. Collins, R.T. Vane-Wright, and J. Waage. 1991. The utilization and value of non-domesticated insects. Pages 319-347 in Collins, N.M. and J.A. Thomas (eds), The Conservation of Insects and Their Habitats, Academic Press, London.

Mulrennan, J. A. 1991. Benefits of mosquito control. *In* Mosquito Control Pesticides: Ecological Impacts and Management Alternatives. T.C. Emmel and J.C. Tucker (eds.). Scientific Publishers, Inc., Gainesville, Florida. pp. 15-16.

Muir, A., and H. Liu. 2003. Conservation action plan: Chamaecrista lineata var. keyensis. Unpublished report. Fairchild Tropical Garden, Miami, Florida.

NatureServe. 2010. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. http://www.natureserve.org/explorer. [Accessed: May 5, 2011].

Nuehring, E. 2011. Email to Dennis Olle. North American Butterfly Association, Miami Blue Chapter. Coral Gables, Florida. February 26, 2011.

Olson, M.S., and W.J. Platt. 1995. Effects of habitat and growing season fires on resprouting of shrubs in longleaf pine savannas. Vegetatio 119:101-118.

Opler, P.A., and G.O. Krizek. 1984. Butterflies east of the Great Plains. The John Hopkins University Press, Baltimore, Maryland.

Perkins, K. 2007. Email to Keith Bradley. University of Florida Herbarium, Florida Museum of Natural History. Gainesville, Florida. August 27, 2007.

Perry, S. 2007. Email from Sue Perry, Everglades National Park, to Paula Halupa, transmitting comments from the biological staff of Everglades National Park on the update of the species assessments for Bartram's hairstreak and Florida leafwing butterflies. Homestead, Florida. December 18, 2007.

Perry, S. 2008. Email to Paula Halupa. Everglades National Park. Homestead, Florida. December 15, 2008.

Pierce, R.H. 2009. Determine direct and indirect effects of mosquito control pesticides on listed species inhabiting DOI managed lands in the Florida Keys. Progress report October 1, 2007 to September 30, 2009. Submitted on October 12, 2009 to U.S. Fish and Wildlife Service. Mote Marine Laboratory; Sarasota, Florida.

Pierce, R.H. 2012. Supplemental Report for the 2010 Permethrin Drift Study: Assessment of Permethrin Drift and Accumulation in the National Key Deer Refuge. Submitted on January 24, 2012 to the U.S. Fish and Wildlife Service. Mote Marine Laboratory; Sarasota, Florida.

Possley, J. 2010. Email to Paula Halupa. Fairchild Tropical Botanic Garden. Coral Gables, Florida. December 27, 2010.

Possley, J. 2011. Email to Paula Halupa. Fairchild Tropical Botanic Garden. Miami, Florida. January 11, 2011.

Pyle, R.M. 1981. The Audubon Society field guide to North American butterflies. Alfred A. Knopf, New York.

Riley, T.J. 1980. Effects of long and short day photoperiods on the seasonal dimorphism of *Anaea andria* (Nymphalidae) from central Missouri. Journal of the Lepidopterists' Society 34(4):330-337.

Riley, T.J. 1988a. Effect of photoperiod on incidence of adult seasonal forms in *Anaea andria* (Lepidoptera: Nymphalidae). Journal of Kansas Entomology Society 61(2):224-227.

Riley, T.J. 1988b. Effect of larval photoperiod on mating and reproductive diapause in seasonal forms of *Anaea andria* (Nymphalidae). Journal of the Lepidopterists' Society 42(4):263-268.

Ross, M., and P. Ruiz. 1996. A study of the distribution of several south Florida endemic plants in the Florida Keys. Report to U.S. Fish and Wildlife Service, Jacksonville, Florida. Florida International University, Miami, Florida.

Sadle, J. 2007. Email from Sue Perry, Everglades National Park, to Paula Halupa, transmitting comments from the biological staff of Everglades National Park on the update of the species assessments for Bartram's hairstreak and Florida leafwing butterflies. Homestead, Florida. December 18, 2007.

Sadle, J. 2010a. Email to Paula Halupa (comments on species assessment [Bartram's hairstreak]). Everglades National Park. Homestead, Florida. January 28, 2010.

Sadle, J. 2010b. Email and telephone conversation with Dana Hartley and Paula Halupa. Everglades National Park. Homestead, Florida. February 16, 2010.

Sadle, J. 2011. Conversation with South Florida Ecological Services staff at Everglades National Park fire meeting. Everglades National Park. Homestead, Florida. March 21, 2011.

Salvato, M.H. 1999. Factors influencing the declining populations of three butterfly species in South Florida and the lower Florida Keys. M.S. Thesis. University of Florida, Gainesville, Florida.

Salvato, M.H. 2001. Influence of mosquito control chemicals on butterflies (Nymphalidae, Lycaenidae, Hesperidae) of the lower Florida Keys. Journal of the Lepidopterists' Society 55(1):8-14.

Salvato, M.H. 2003. Butterfly conservation and host plant fluctuations: the relationship between *Strymon acis bartrami* and *Anaea troglodyta floridalis* on *Croton linearis* in Florida (Lepidoptera: Lycaenidae and Nymphalidae). Holarctic Lepidoptera 10(1-2):53-57.

Salvato, M.H. 2006. Comments on species assessment. U.S. Fish and Wildlife Service, South Florida Ecological Services Office. Vero Beach, Florida. February 8, 2006.

Salvato, M.H. 2008. Comments on species assessment. U.S. Fish and Wildlife Service, South Florida Ecological Services Office. Vero Beach, Florida. March 13-16, 2008.

Salvato, M.H. 2009. Email to Paula Halupa. U.S. Fish and Wildlife Service, South Florida Ecological Services Office. Vero Beach, Florida. April 16, 2009.

Salvato, M.H. 2010. Comments on species assessment. U.S. Fish and Wildlife Service, South Florida Ecological Services Office. Vero Beach, Florida. May 16 – June 10, 2010.

Salvato, M. H. 2011. Comments on species assessment. U.S. Fish and Wildlife Service, South Florida Ecological Services Office. Vero Beach, Florida. March 1-31, 2011.

Salvato, M.H., and M.K. Hennessey. 2003. Notes on the historic range and natural history of *Anaea troglodyta floridalis*. Journal of the Lepidopterists' Society 57(3):243-249.

Salvato, M.H., and M.K. Hennessey. 2004. Notes on the status and fire-related ecology of *Strymon acis bartrami*. Journal of the Lepidopterists' Society 58(4):223-227.

Salvato, M.H., and H.L. Salvato. 2008. Notes on the feeding ecology of *Strymon acis bartrami* and *Anaea troglodyta floridalis*. Florida Scientist. 71: 323-329.

Salvato, M.H., and H.L. Salvato. 2010a. Notes on the status and ecology of *Anaea troglodyta floridalis* in Everglades National Park. Journal of the Lepidopterists' Society. 64: 91-97.

Salvato, M.H., and H.L. Salvato. 2010b. Notes on the status of *Anaea troglodyta floridalis* on Big Pine Key. News of the Lepidopterists' Society. 52: 139-140.

Salvato, M.H., and H.L. Salvato. 2010c. Parasitic mite larvae (Acari) on adult *Strymon acis bartrami* (Lycaenidae). News of the Lepidopterists' Society. 52: 71.

Schwartz, A. 1987. The butterflies of the Lower Florida Keys. Milwaukee Public Museum, Contributions in Biology and Geology 73:1-34.

Schwarz, K.A., R.A. Worth, and T.C. Emmel. 1996. Conservation of two threatened south Florida butterflies and their host plant (Lepidoptera: Lycaenidae, Nymphalidae). Holarctic Lepidoptera 3:59-61.

Slapcinsky, J.L., and D.R. Gordon. 2007. Monitoring report 2007: Pine rocklands. The Nature Conservancy. Gainesville, Florida.

Smith, D.S., L.D. Miller, and J.Y. Miller. 1994. The butterflies of the West Indies and South Florida. Oxford University Press, New York.

Snyder, J.R., A. Herndon, and W.B. Robertson, Jr. 1990. South Florida rocklands. Pages 230-277 in R.L. Myers and J.J. Ewel, eds. Ecosystems of Florida. University of Central Florida Press, Orlando, Florida.

Snyder, J.R., M.S. Ross, S. Koptur, and J.P. Sah. 2005. Developing ecological criteria for prescribed fire in South Florida pine rockland ecosystems. USGS Open File Report OF 2006-1062.

The Institute for Regional Conservation. 2006. Mapping of the Natural Forest Community System and Endangered Plants of Miami-Dade County, Florida. The Institute for Regional Conservation. Miami, Florida.

The Institute for Regional Conservation. 2011. Chamaecrista lineata (Sw.) Greene var. keyensis (Pennell) H.S. Irwin and Barneby. IRC online Floristic Inventory of South Florida, http://regionalconservation.org/ircs/database/plants/PlantPageFK.asp?TXCODE=Chamlinekeye. [Accessed March 25, 2011].

U.S. Environmental Protection Agency. 2006. Reregistration eligibility decision (RED) for permethrin. EPA 738-R-06-017. April 2006. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. Washington, D.C.

U.S. Fish and Wildlife Service. 1999. South Florida multi-species recovery plan. U.S. Fish and Wildlife Service, Atlanta, Georgia.

U.S. Fish and Wildlife Service. 2000. Fire management plan, National Key Deer Refuge. U.S. Fish and Wildlife Service, Big Pine Key, Florida.

U.S. Fish and Wildlife Service. 2011. Biological opinion on the proposed construction of the Special Operations Command Center (SOCSOUTH) in Homestead, Florida. U.S. Fish and Wildlife Service, Vero Beach, Florida.

Williams, T. 1996. The great butterfly bust. Audubon (March-April): 30-37.

Worth, R.A., K.A. Schwarz, and T.C. Emmel. 1996. Notes on the biology of *Strymon acis bartrami* and *Anaea troglodyta floridalis* in south Florida. Holarctic Lepidoptera 3(2): 62-65.

Wunderlin, R.P., and B.F. Hansen. 2003. Guide to the vascular plants of Florida, 2nd ed. University Press of Florida, Gainesville, Florida.

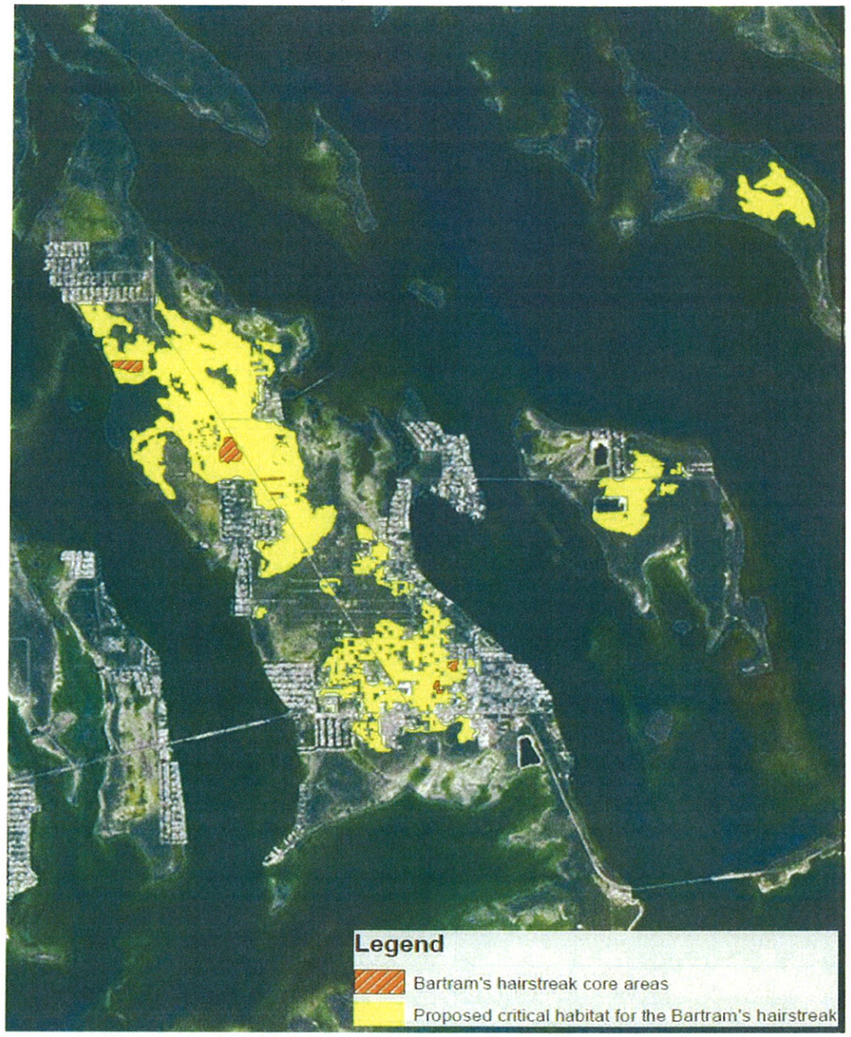

Figure 1. Proposed critical habitat and core areas for the Bartram's hairstreak in the action area. Proposed critical habitat for the Florida leafwing is encompassed by the proposed critical habitat for the Bartram's hairstreak, but does not include the critical habitat unit on No Name Key.

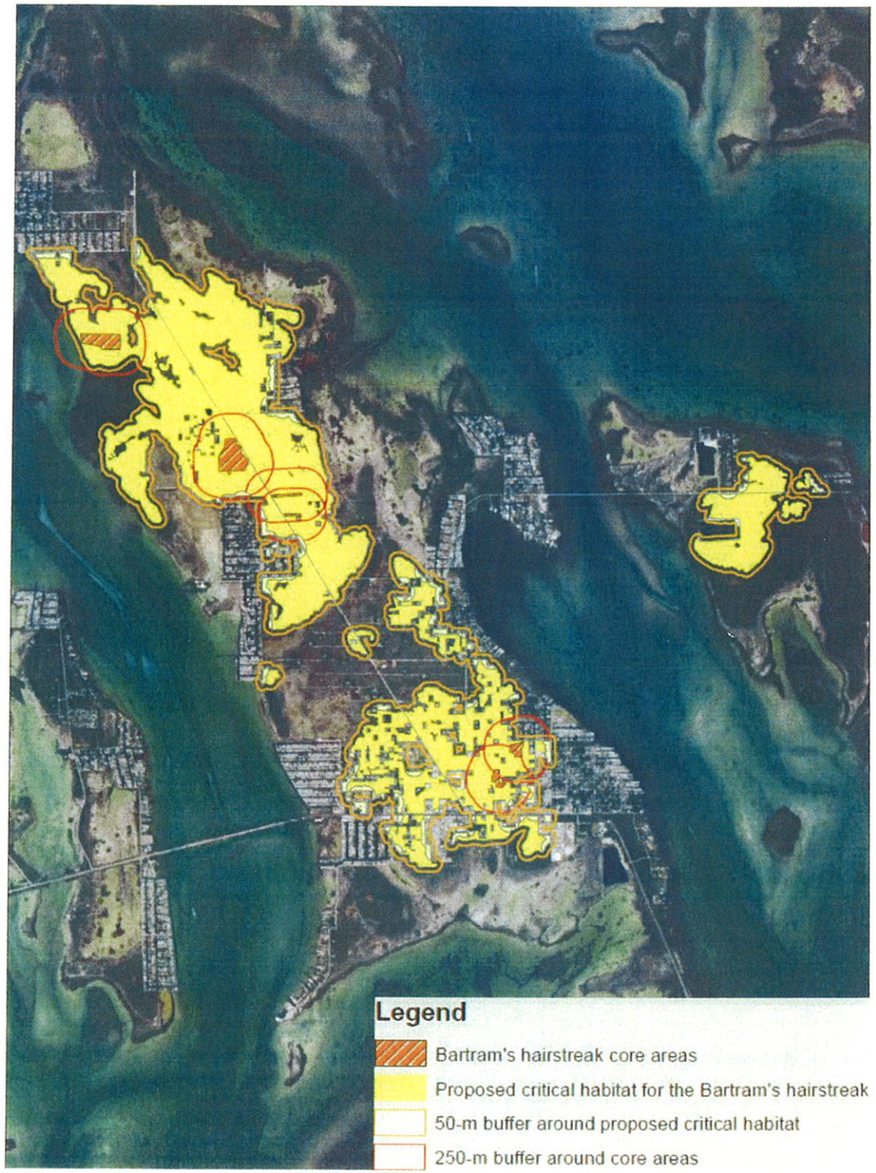

Figure 2. No-spray buffers on Big Pine and No Name Keys for truck-based applications.

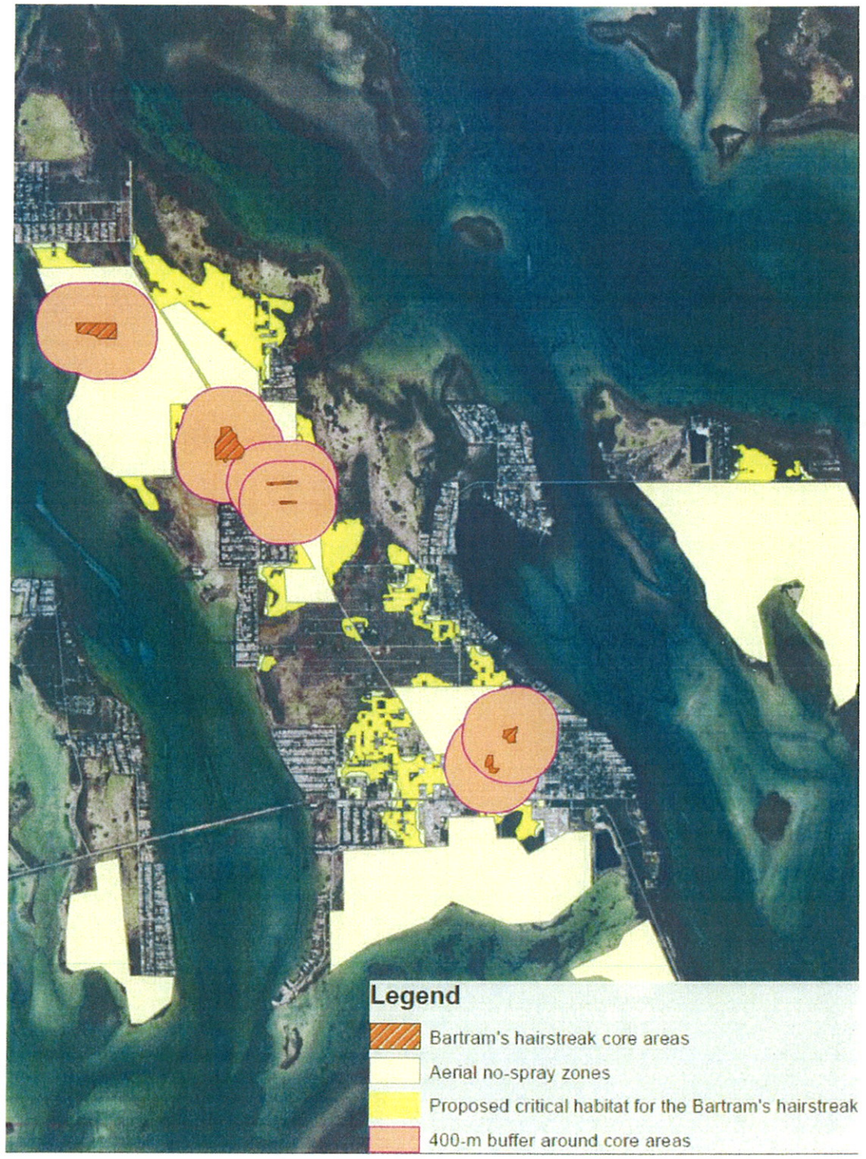

Figure 3. No-spray buffers on Big Pine and No Name Keys for aerial applications.

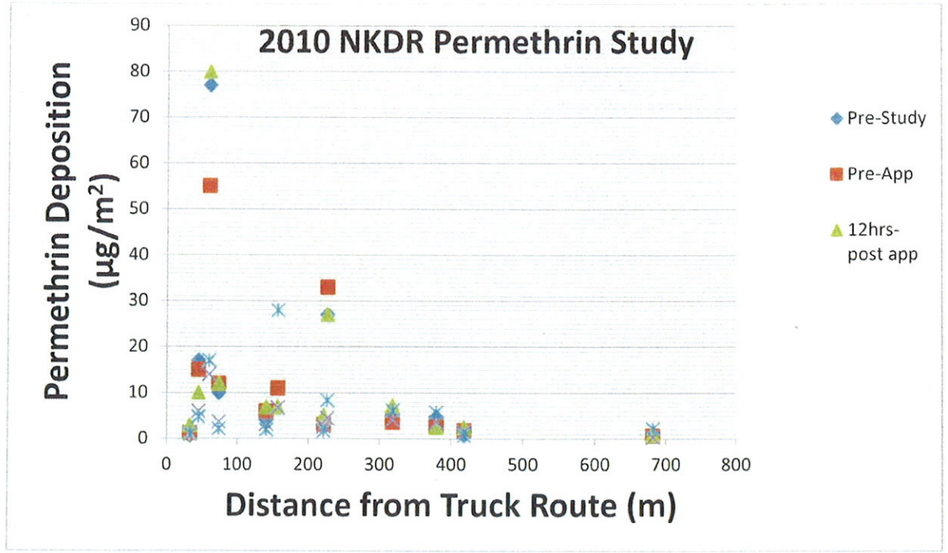

Figure 4. Permethrin drift deposition on vegetation ($\mu g/m^2$) from National Key Deer Refuge (Pierce 2012).

Table 1. Florida leafwing proposed critical habitat units.

Unit No.	Unit name	Ownership	Percent	Hectares	Acres	Occupied
FLB1	Everglades National Park	Federal	100	3,235	7,994	yes.
		Total	*100*	*3,235*	*7,994*	
FLB2	Navy Wells Pineland Preserve	State	29	35	85	no.
		Private-Other	71	85	211	
		Total	*100*	*120*	*296*	
FLB3	Richmond Pine Rocklands	Federal	14	50	122	no.
		Private-Other	86	309	767	
		Total	*100*	*359*	*889*	
FLB4	Big Pine Key	Federal	65	365	901	no.
		State	16	90	223	
		Private-Other	19	104	258	
		Total	*100*	*559*	*1,382*	
Total All Units		Federal	85	3,650	9,017	
		State	3	125	308	
		Private-Other	12	498	1,236	
		All	100	4,273	10,561	

Table 2. Bartram's hairstreak proposed critical habitat units.

Unit No.	Unit name	Ownership	Percent	Hectares	Acres	Occupied
BSHB1	Everglades National Park	Federal	100	3,235	7,994	yes.
		Total	*100*	3,235	7,994	
BSHB2	Navy Wells Pineland Preserve	State	30	62	153	yes.
		Private-Other	70	141	349	
		Total	*100*	*203*	*502*	
BSHB3	Camp Owaissa Bauer	State	20	29	71	yes.
		Private-Other	80	117	288	
		Total	*100*	*146*	*359*	
BSHB4	Richmond Pine Rocklands	Federal	11	50	122	yes.
		State	7	32	79	
		Private-Other	82	356	881	
		Total	*100*	*438*	*1082*	
BSHB5	Big Pine Key	Federal	65	365	901	yes.
		State	16	90	223	
		Private-Other	19	104	258	
		Total	*100*	*559*	*1,382*	
BSHB6	No Name Key	Federal	75	30	75	no.
		State	18	9	22	
		Private-Other	7	11	26	
		Total	*100*	*50*	*123*	
BSHB7	Little Pine Key	Federal	100	39	97	no.
		Total	*100*	*39*	*97*	
Total All Units	Federal		80	3,719	9,189	
	State		5	222	548	
	Private-Other		15	729	1,802	
	All		100	4,670	11,539	

Table 3. Acreage values for direct and indirect effects to proposed critical habitat for the Bartram's hairstreak butterfly and the Florida leafwing within the action area.

	Acres	Percent of proposed critical habitat in action area	Percent of total proposed critical habitat
Bartram's hairstreak			
Truck-based application impacts from drift (assuming 225-m drift, 250-m buffer around core areas)	699	44	6.1
Aerial application impacts on targeted proposed critical habitat	649	41	5.6
Florida leafwing			
Truck-based application impacts from drift (assuming 225-m drift, 250-m buffer around core areas)	663	48	6.3
Aerial application impacts on targeted proposed critical habitat	520	38	4.9