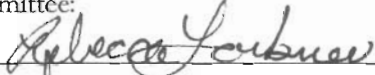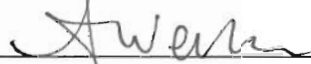ASSESSING THE CONSERVATION STATUS OF THE ZEBRA SWALLOWTAIL, *EURYTIDES MARCELLUS* CRAMER (LEPIDOPTERA: PAPILIONIDAE), IN NORTHERN VIRGINIA FORESTS

by

Craig R. Beatty
A Thesis
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
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Environmental Science and Policy
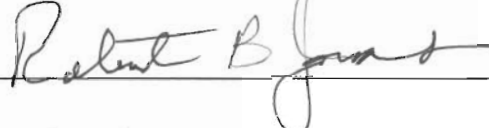
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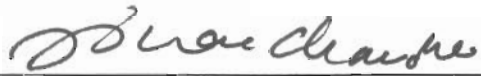
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Date: _December 8th 2011_   Fall Semester 2011
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Assessing the Conservation Status of the Zebra Swallowtail, *Eurytides marcellus* Cramer (Lepidoptera: Papilionidae), in Northern Virginia Forests

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

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Dedication

To all the little things

Acknowledgements

Table of Contents

List of Tables

List of Figures

Abstract

ASSESSING THE CONSERVATION STATUS OF THE ZEBRA SWALLOWTAIL, *EURYTIDES MARCELLUS* CRAMER (LEPIDOPTERA:PAPILIONIDAE), IN NORTHERN VIRGINIA FORESTS

Craig R. Beatty, M.S.

George Mason University, 2011

Thesis Director: Dr. Rebecca E. Forkner

Critical assessments of niche requirements have been made for less than 1% of endangered insects and insufficient data may exist to allow protection of even highly charismatic butterflies. The goals of this study were 1) to assess the conservation status of *Eurytides marcellus* Cramer (Lepidoptera: Paplionidae) in Northern Virginia through larval and adult population surveys in four protected forest areas, 2) to identify suitable habitat for *E. marcellus* populations through a combination of field studies of potential habitat quality factors correlated to larval or adult densities and the spatial analysis of land-use and habitat requirements using geographic information systems (GIS), 3) to model adult dispersal among suitable sites via Euclidean and least-cost distance methods in order to identify barriers to or corridors promoting dispersal across the landscape, and 4) To design and implement a spatially explicit individual-based model capable of testing the effects of habitat parameters on a Zebra Swallowtail population throughout its complex life cycle.

Larval and adult surveys reveal that *E. marcellus* currently exists in extremely low density in Northern Virginia, warranting a revision of its secure conservation (S5) status for this area. Density of caterpillars was most strongly correlated with habitat area, but was also influenced by other habitat

factors, including leaf toxicity. GIS analysis identified 35 suitable locations in the Fairfax County region of Northern Virginia as possible locations for robust larval *E. marcellus* populations. A Euclidean distance dispersal model suggested that there are three unconnected adult butterfly dispersal networks in Fairfax County. Further analysis of dispersal using a least-cost distance model found that Fairfax County exhibits severe impediments to butterfly dispersal. An analysis of population responses to changes in habitat factors through an individual-based model found that populations of *E. marcellus* are most dependent on access to abundant populations of their host plant, pawpaw (*Asimina triloba*), rather than to adult nectaring sources.

Eurytides marcellus may exemplify the decline of a formerly common species, and may act as an indicator for the conservation status of other imperiled species in the region. The integration of geographic analysis techniques and computer modeling applications provided a necessary avenue to explore the conservational integrity and status of this species in a non-intrusive manner. These applications combined with field studies will be integral to the assessment of the conservation status of many species.

Evolution, Ecology, and Conservation of Papilionidae

Introduction

Swallowtail Evolution

Of all the insect orders, the Lepidoptera have the most well elucidated evolutionary history (Grimaldi and Engel 2005). The basal relationships of Lepidoptera are probably the best understood: Lepidoptera diverged from the Trichoptera (Caddisflies), a relationship supported by common morphology (Kristensen 1991, Kristensen *et al.*. 2007), genetics (Whiting 2002), and fossil evidence (Durdon and Rose 1978). This order of insects represents the largest lineage of herbivores worldwide, and their evolutionary radiation closely parallels that of angiosperm plants during the Cretaceous (Grimaldi and Engel 2005). The largest group within the Lepidoptera is the Dytrisia, which contains 98% of described Lepidopteran species. The macrolepidoptera includes those lineages of Dytrisia whose body size is relatively large, and support exists for their overall monophyly (see Minet 1991). However, recent research by Mutanen *et al.* (2010) shows that the evolutionary relationships within the macrolepidoptera are not monophyletic as previously thought, and they attribute the paraphyletic or polyphyletic nature of most clades within the monophyletic Dytrysia to the extremely quick evolutionary radiation of this lineage.

Within the macrolepidoptera is the Rhopalocera, which contains three superfamilies: Papilionoidea (butterflies), Hesperoidea (skippers), and Hedylidae (moth butterflies). Superfamily Papilionoidea contains roughly 14,500 described species of "true" butterflies, those that are diurnal and possess clubbed antennae, and accounts for about 6% of the total diversity within the Lepidoptera (Grimaldi and Engel 2005). Swallowtail butterflies (Family Paplionidae) are the most

conspicuous members of the Papilionoidea. Collectors and scientists alike prize them for their intricate patterns and often astounding colors. However, despite the human fascination with these insects, the details of their evolutionary history beyond the highest taxonomic levels remain relatively unexplored. What is clear is that the Papilionidae are a monophyletic group whose evolutionary history dates to roughly 50–80 million years ago (the most recent of all insects) and is influenced by coevolutionary partnerships with flowering plants (Miller 1987).

Within the Papilionidae, three major subfamilies are recognized: Baroniinae, Parnassiinae, and Papilioninae. The Baroniinae are comprised of a single species, *Baronia brevicornis* Salvin, which occurs throughout Southern Mexico. *Baronia brevicornis*, resembles one of the oldest know butterfly fossils (*Praepapilio colorado*, 48 mya, Durdon and Rose 1978) and has been described as a "living fossil" (Collins and Morris 1985). It has been generally accepted that this species represents the sister taxon to all species of swallowtail (Munroe, 1961; Hancock, 1983). However, a recent phylogenetic reconstruction based on combined molecular data (Nazari *et al.* 2007) and a comprehensive morphological analysis of butterfly phylogeny (De Jong *et al.* 1996) suggest that Baroniinae are not ancestral to all extant butterfly lineages. Regardless of its official position within the Papilionidae, *Baronia brevicornis* represents one ancestral state of the heavily diversified swallowtail clade.

The Parnassiinae are represented by roughly 50 species, mostly distributed throughout the Palearctic. Recent analysis by Nazari *et al.* (2007) has identified three distinct tribes within the Parnassiinae: Zerynthiini, Parnassiini, and Luehdorfiini; however, DNA evidence suggests the Parnassiinae may be paraphyletic (Yagi *et al.* 1999, Caterino *et al.* 2001, Nazari *et al.* 2007). Despite this, members of the Parnassiinae are united by the development of a true, silk cocoon (Grimaldi and Engel 2005). Generally, this clade is most known for the Apollo butterflies, which can usually be found at high altitudes in the Palearctic and western Nearctic (Ackery 1975).

Subfamily Papilioninae is the largest of the three Papilionidae subfamilies and contains over 480 species of swallowtail butterflies, including some of the most charismatic and colorful species (Scott 1986). The Papilioninae were divided by Munroe (1961) into the three tribes Leptocircini, Troidini, and Papilionini; however, no stable classification currently exists within these diverse tribes (Vane-Wright 2003). Briefly, the three tribes are primarily separated on the basis of wing morphology. The Leptocircini (formerly Graphiini) or Kite Swallowtails are composed of 144 species and have a fold of scent scales on the hind margin of the rear wing. Leptocircini often possess long tails from the hind wings and a striped or "zebra" pattern. The Troidini also possess the aforementioned fold of scent scales and feed exclusively on plants is the *Aristolochia* family, making them toxic to vertebrates. Consequently, the 136 species of Troidini are often involved in mimicry complexes. The Papilionini, also known as the fluted swallowtails, comprise 203 species. They are characterized by the "fluted" or downward bent margin of the hind wing (Scott 1986). An extensive review of the characters that unite these tribes can be found in Miller (1987), and a molecular assessment of higher level taxonomic relationships in the Papilioninae are provided by Morinaka *et al.* (1999), Yagi *et al.* (1999), and Caterino *et al.* (2001).

Swallowtail Distribution

The importance of Papilionoidea to ecological research parallels that of the importance of *Drosphila melanogaster* in genetics and *Caenorhabditis elegans* in neurology (Ehrlich 2003). For example, the concept of coevolution arose from studies demonstrating parallels between major taxa of butterflies and the defensive chemistries of their host plants (Ehrlich and Raven 1964). Color perception (Briscoe and Chittka 2001), developmental resources allocation (Boggs 1981), and mechanisms of local adaptation have all been clarified by butterfly studies (Warren *et al.*. 1999, Parmesan 2006). Unlike many organisms, butterflies may be monitored with relative ease and low cost. As a result, they have been instrumental in elucidating population and metapopulation biology

and habitat fragmentation principles (Ehrlich and Gilbert 1973, Tilman and Kareiva 1997, Hanski 1998, Saccheri *et al.* 1998, Ricketts 2001). Perhaps most importantly, butterflies were some of the first species whose distributional changes were directly attributed to climate change (Parmesan *et al.* 1999, Parmesan 2006). They have also been instrumental in documenting both the rate and extent of biodiversity loss, as well as in the development of techniques for mitigation of species extinction (Koh *et al.* 2004). Consequently, they have been considered appropriate indicators of ecological health for many ecosystems (Kremen 1992, Brereton *et al.* 2010). As model organisms, butterflies are poised to assist scientists exploring the effects of land-use change and habitat conversion on biodiversity (Ricketts 2001, Rossi and van Halder 2010).

Papilionids are among the most well documented and studied butterflies. Swallowtails are found on every continent except Antarctica, but are most concentrated in the tropics, primarily in the rainforests of East and Southeast Asia (New and Collins 1991). Despite their near global ubiquity, the distribution of swallowtails appears largely determined by the availability of host plants upon which their larvae feed and on an adequate supply of adult nectar sources. Few species can be considered widespread, and many are extremely localized and rare. In addition to predation, these host plant constraints are major factors that limit the distribution of swallowtails in space and time. These limitations may be compounded by the complex metamorphosis swallowtails undergo as holometabolous insects. The distribution of eggs, larvae, pupae, and adults are all quite different, each evolving under separate selective forces. As a result, factors determining swallowtail distribution must be considered for each life stage. Moreover, parameters in each stage may interact to affect the distribution of a species as a whole.

Factors dictating how adult swallowtails are distributed locally in space include their tolerances for different abiotic factors; their ability to perceive their environment, including their reception of chemical stimuli from plants and mates; and the physiological costs of their flight. For

example, cold tolerance limits the distribution of the Tiger Swallowtail, *Papilio glaucus glaucus*, when compared to its northern relative *P. g. canadensis* (Kukal *et al.* 1991). With respect to both abiotic limitations and costs of flight, lowland desserts may form an effective barrier to butterfly dispersal, especially for montane species (Schweitzer 2001). Lekking, hill-topping, patrolling, perching, and signaling with pheromones are courtship strategies that limit distribution. For example, males of the Black Swallowtail (*Papilio polyxenes*) form and defend leks that are consistently located in the same areas year to year and are not uniformly distributed throughout the landscape. *Papilio polyxenes* tend to aggregate based on topographic distinctness and maximum elevation, such that an area of 6 km² contained only 11 consistently defended territories (Lederhouse 1982). This behavior confines the distribution of this species to only a few main areas throughout the breeding season and illustrates that adult butterflies may modify their distribution based on seasonally timed courtship rituals. These three factors will vary depending on the focal species but will also be constrained by evolutionary history, particularly host-plant associations, which are often taxonomically conserved (Ehrlich and Raven 1964).

The availability of adult nectar sources is paramount for successful reproduction and will also dictate distributional ranges. The interaction of two competing energetic demands, flight and reproduction, on butterfly populations has long been a focus of research. Karlsson and Johansson (2008) showed that there is a physiological trade-off between flight ability and egg output in species of peirid butterfly, leading to a larger investment in reproduction for early spring individuals but a larger investment in flight muscles for later generations. Spring-emerging phenotypes may disperse shorter distances and have more restricted distributions, but show a higher evolutionary investment in reproduction through an increased number of eggs laid by females. Late season individuals, on the other hand, may be physiologically poised to disperse further and cover more territory than their early season counterparts, but display lower fecundity.

In addition, distributions of adult varieties may be influenced by predation, as evidenced by extensive mimicry complexes. The most notable papilionid example is the African Mocker Swallowtail (*Papilio dardanus*). The males of this species do not exhibit any wing polymorphisms across their entire sub-Saharan range; however, females have been documented to express 14 different polymorphic forms, many of which are Batesian mimics of several distasteful species of Danaidae and Acraeidae (Nijhout 2003). This pattern holds for swallowtail mimicry complexes throughout the world; males are identical throughout their range and females exhibit wing polymorphisms based on simple allelic mutations that mimic distasteful species (Ffrench-Constant and Koch 2003). In North America, the female *P. glaucus* often displays a dark form that has been considered a Batesian mimic of the poisonous Pipevine swallowtail (*Battus philenor*) where their ranges overlap (Scott 1986).

Important factors in the distribution of immature life stages include the location of eggs oviposited by the gravid female, the gregarious habits of eclosed larvae, the behavioral or physiological responses of larvae to plants, and the optimal location and environmental conditions for successful pupal development. These factors may operate on much smaller spatial scales than those relevant to adult butterflies. For instance, *P. glaucus* females preferentially lay eggs below a height of 3 meters on branch tips exposed to sunlight facing west. The eggs laid by *P. glaucus* females usually develop individually, not in groups, and those individuals exposed to higher temperatures as a result of maternal microhabitat selection grow 15-35% quicker than their counterparts (Grossmueller and Lederhouse 1985). The success of immature swallowtails can also be affected by their feeding behavior. For example, females of the Pipevine Swallowtail in Northern California (*Battus philenor hirsute*) deposit clusters of eggs on Dutchman's pipe (*Aristolochia californica*) and larvae feed gregariously in groups of up to 300 individuals per square meter (Fordyce and Agrawal 2001). This feeding strategy has been suggested to play a large role in the thermoregulation and defense of larva,

6

often leading to quicker growth and higher pupation rates (Stamp 1980). The environmental conditions as larvae approach pupation also determine their distribution and survival. For instance, photoperiod and temperature are abiotic cues during diapause that direct pupae to emerge or remain dormant (Sims and Shapiro 1983). Swallowtail larvae have also been shown to display distinct differences in their preference for pupations sites, including preferences for specific heights above the ground, types of pupation substrate, and even the widths of pupation substrates (West and Hazel 1996).

Predation also appears to be an important factor in the survival of immature swallowtails. In addition to the mimicry complexes present in adult swallowtails, many species display aposematic coloration as caterpillars. In some cases early instar larva do not possess aposematism, but develop warning coloration as they molt to later instars. Papilionidae in general are also characterized by a larval osmaterium, a structure that everts from behind the head and acts as a vehicle for chemical compounds used to deter predation (Eisner *et al.* 1970). In many species the osmateria also display aposematic coloration. In addition, the eggs of the Chinese Windmill Swallowtail (*Atrophaneura alcinous*) contain defensive aristolochic acids in both the egg yolk and egg coating (Nishida and Fukami 1989). Although studies linking larval predation to species distributions are largely lacking for butterflies, the existence of these predator deterrents in the morphology of immature swallowtails suggests that deterring predation may be a strong selective force capable of spatially limiting populations.

The distributional maps for most swallowtail species are primarily based on the presence of their larval host plants (Scott 1986), but larvae may be temporally, as well as spatially, restricted. The temporal distribution of swallowtails, particularly for the larvae of temperate species, depends heavily on plant phenology. Many species undergo winter diapause and emerge in early spring so that their larvae overlap with the availability of newly emerged leaves. For many species, such as the Zebra

Swallowtail (*Eurytides marcellus*), early spring emergence is critical to survival, as larvae may be unable to consume host plant tissue due to changes in chemistry or leaf toughness as the season progresses. In particular, Sims and Shapiro (1983) have shown that the spring emergence of the Pipevine Swallowtail (*Battus philenor*) from winter diapause peaks with the spring growth of its obligate host plant, *Aristolochia californica,* during approximately one week in April. As the season progresses, leaves of *A. californica* become increasingly indigestible, limiting larval consumption and subsequently limiting their distribution in space and time.

In summary, multiple selective forces, both biotic and abiotic, limit the spatial and temporal distribution of each life stage. Each of these constraints interacts in many different ways to determine the success of a complete generation for each species. From this, it should be apparent that the successful conservation of a species does not solely depend on the habitat requirements of one life stage, but must take into account how the habitat requirements of several life stages interact to support the complete life cycle. Therefore, conservation actions that fail to address the complex and interacting requirements of immature life stages on a species by species basis will fail to adequately conserve swallowtail species.

Swallowtail Conservation Status

Numerous anthropogenic factors threaten swallowtail populations. As is often the case, human attention plagues the largest and most visually appealing animals. Queen Alexandra's Birdwing (*Ornithoptera alexandrae*) is the largest of all butterflies, and populations of this swallowtail have declined due to an over-collection of specimens combined with host plant loss through the conversion of habitat to oil palm cultivation (New and Collins 1991). *Baraconia brevis*, as discussed above, is the only extant species of an ancestral lineage of swallowtail butterflies. Already rare

throughout its geographic range, *B. brevis* is further threatened by habitat degradation and destruction (León-Cortés *et al.* 2004). In addition, lesser known species such as Harris' Mimic Swallowtail (*Eurytides lysithous*), the Jamaican Kite Swallowtail (*Eurytides marcellinus*), and the Yellow Kite Swallowtail (*Eurytides iphitas*) have been identified as vulnerable to extinction due to the extirpation of their host plants and the loss of available habitat (New and Collins 1991), and current assessments of the existence of these species are not available. Having sibling species at risk is a correlate of extinction risk (Van Dyke 2008), suggesting that within swallowtails the genus *Eurytides* may be particularly at risk.

The International Union for the Conservation of Nature (IUCN) Red List is the international standard for documenting the threats faced by species throughout the world. Of the 77 IUCN evaluated Papilionidae species, 56 are considered to be at least vulnerable to extinction or to lack sufficient data to evaluate a threat classification (IUCN Red List 2011). Translated, while only 12% of swallowtail species have been evaluated, 73% of those have been identified as threatened or data deficient. However, the status of these 56 species, which includes the ten species listed as endangered or critically endangered, has not been updated in over twenty years. Not since New and Collins (1991) has a thorough assessment and conservation action plan been suggested for swallowtail butterflies and no information exists on the progress of their assessment or the current geographic distribution, habitat needs, or ecology of any of the swallowtail species most vulnerable to extinction.

The remaining 21 species present in the IUCN Red List database are classified as either as least concern, lower risk/least concern, or lower risk/near threatened. Of these, two species (*Zerynthia cretica* and *Papilio hospiton*) are classified as least concern, all others being granted some level of extinction risk. However, with the exception of these two species, no other species have had their conservation status evaluated since 1996 (IUCN Red List 2011). For two listed species: *Papilio*

9

carolinensis from the Philippines and *P. esperanza* from Mexico, no records exist, but they are given "vulnerable" IUCN Red List classification, apparently based on the limited range and overall rarity of the species. Of the twelve species of birdwing swallowtail butterflies (*Ornithoptera* spp.), eight are threatened with extinction.

Apart from the IUCN global assessment of conservation status, North America relies on the Natureserve network of Natural Heritage Programs for information on population density and conservation status. This organization compiles information on North American species that include assessments of overall conservation status, species range, ecology and life history, research needs, and justifications for delineating population occurrences and distributions. The assessments made by Natureserve are often more thorough and detailed than those of the IUCN and include nearly all North American species of swallowtails. However, similar to the IUCN's Red List, Natureserve has not updated the conservation status of most swallowtail species in North America in over a decade (Natureserve Explorer 2011). In addition, the distributional data are based on occurrences within political boundaries such as states and provinces. This presence-absence method of displaying distributions often overestimates the actual distribution of swallowtails because a single local sighting results in the species being considered present throughout the state or province. Moreover, entries regarding the justification of population occurrences and distributions are based on a general synthesis of *all* members of the family Papilionidae, without regard for species-specific life history idiosyncrasies. While these entries are occasionally based on expert opinion regarding the proposed distribution or on occurrences compiled from accumulated museum specimens over the past 100 years, most assessments of swallowtail status are no more than an affirmation of the various butterfly guidebooks available, which are often based on surveys completed more than 50 years ago. These methods may not accurately represent the current occurrence, density, and distribution of swallowtails throughout the world. Furthermore, they may hinder reliable conservation assessment of

swallowtail species by portraying the group as largely unaffected by current habitat losses, when some species with more restricted habitat requirements, such as *E. marcellus*, may be considerably at risk.

The Zebra Swallowtail

Characteristics and subspecies

Eurytides marcellus Cramer is a charismatic medium-sized kite swallowtail species of the tribe Leptocircini. In the Northern Virginia region, this species exhibits as smaller spring form (*E. m. marcellus*, Figure 1a) that over-winters and emerges near the end of March. These individuals have short forewings with the outer wing margin at or near 90 degrees with the lower wing margin, an average wingspan of 3.3 to 3.7 cm and a conspicuous tuft of hair on the thorax (Clark 1932). This physiology contributes to the quick and direct flight patterns of this early spring form. The margin of the hind wings is deeply scalloped with small tails extending from the caudal tips. On the top of the forewings, a large dark band running from the costal margin to the lower border includes a white stripe that crosses the wing cell. The tails of the hind wings are quite short and are white only at the very tips. Clark (1932) also has documented a larger, intermediate form that displays longer and more extensive black markings and a more conspicuous white border to the tails extending from the hind wing. This form (*E. m. telemonides*, Figure 1b) appears in the beginning of April and flies through the end of May.

The true summer form (*E. m. lecontei,* Figure 1c) appears in early June. These individuals are larger than their spring counterparts with an average wingspan between 3.9 and 4.8 cm and no conspicuous tuft of hair on the thorax (Clark 1932). The angle between the outer and lower margins of the forewing forms an obtuse angle, and the hind wings are not as deeply scalloped. The tails of the summer form are much longer than the spring form with white borders that extend almost to the

11

base and the black markings on the hind wings extend across all or most of the outer half. The white stripe intersecting the large dark band in the spring form is absent or much reduced in the summer form. This form flies from late July to October and is composed of the largest individuals.

Life history

All adult forms of *E. marcellus* are nectivorous, and Clark (1932) reports that their usual shyness toward people disappears while feeding. The flowers most available to the spring forms (*E.m. marcellus* and *E. m. telemonides*) in the Northern Virginia region are redbud (*Cercis canadensis*), spring beauty (*Claytonia virginica*), delicate white (*Viola striata*) and coarse purple (*V. hirsuta*) violets, star chickweed (*Stellarla yubera*), saxifrage (*Saxifraga virginica*), and Virginia bluebell (*Mertensia virginica*). Individuals feeding on these flowers are often seen darting from one flower to another in the forest understory, flying between 4 and 5 feet above the forest floor. Some of the only available flowers for individuals that emerge very early in the spring are blueberries (*Vaccinium spp.*), and butterflies can be seen feeding on flowers very near the ground. Mid-season nectar sources include a number of flowering plants, such as lilac (*Syringea sp.*), verbena, dogbane (*Apocynum cannabinum*), and common milkweed (*Asclepias syriaca*) and are most often located in open fields or stream edges, as the leaf flush has closed the forest canopy. Late season individuals (form *E. m. lecontes*) rely primarily on Goldenrod (usually *Solidago canadensis*) and New Engand aster (*Aster novaeangliae*), which are both widespread and common until the first frost (Clark 1932).

In general, the adult Zebra Swallowtail can be found in woods and open fields throughout most of its range, which extends along the Eastern United States, south through Florida, west through Texas and north to Nebraska and New York State. This species may be found where there are abundant populations of its larval host plant, pawpaw (*Asimina spp.*: Annonaceae) and is not believed to be migratory. Pawpaw is generally restricted to wooded, riparian areas throughout the Southeastern United States. Despite the large geographical range of its host plant, *E. marcellus* appears

especially vulnerable to extirpation along the northern edge of its range and county sightings. While records have generally lacked new information on distribution for several decades, the vulnerability of *E. marcellus* to extinction is recognized in 13 of the 27 states where it occurs (Natureserve Explorer 2011).

Eurytides marcellus is one of the first butterfly species in the Virginia region to emerge from winter diapause, and it persists throughout the summer in at least two generations throughout its temperate range and as many and five generations in its subtropical range (Damman 1989). Maximum population size in flight occurs in the last half of July when the intermediate (*E. m. telemonides*) and the true summer form (*E. m. lecontes*) overlap (Clark 1932). Following this, populations begin to decline for the season; although, adults can be seen flying through October. Adult male butterflies emerge from winter diapause before females and begin patrols of adjacent waterways in search of mates. This strategy, known as protandry, suggests that females mate only once before oviposition (Wiklund 2003); although, the validity of this claim remains to be tested for *E. marcellus*. Once mated, females begin oviposition on *Asimina* species, most commonly *Asimina triloba* (Common pawpaw) as this is the only available species throughout the majority of the range of *E. marcellus*. In early spring, mating and oviposition occur prior to the expansion of leaf buds on *A. triloba*. Pawpaw leaves produce a contact ovipositional stimulant (3-caffeoyl-*muco*-quinic acid) that is strongly responsible for increasing female *E. marcellus* ovipositional likelihood (Haribal and Feeny 1998). Further work by Haribal and Feeny (2003) demonstrated that females are able to assess the suitability of a host plant before they land on a leaf and that once alighting the female is able to compare the ratio of contact stimulant to leaf flavonoids (ovipositional deterrents) to qualitatively and quantitatively assess the quality of the plant for her progeny.

Eggs are laid, usually no more than one egg per plant, and usually eclose in 5 days. Eggs are approximately 8mm in diameter and a pale shade of green or yellow when laid. *Eurytides marcellus*

larvae can take either a light green or a dark form (Figure 2a and b) and once emerged immediately

begin to feed on the young, expanding leaves. Damman (1989) has suggested that the multivoltine

nature of *E. marcellus* is dependent on mid-season defoliation of *A. triloba* by the pyralid moth

Omphalocera munroei; although, preliminary data collected in Northern Virginia conflicts with this

hypothesis (see Chapter 2). Nonetheless, it is clear that larvae prefer to eat young leaves and that as

the season progresses leaves become tougher and less palatable. It has also been shown that the

concentration of ovipositional contact stimulants declines sharply as the season progresses, while the

concentration of leaf flavonoids, considered anti-herbivore defenses (Feeny 1970), increases in *A.*

triloba (Haribal and Feeny 2003).

Both larval forms of *E. marcellus* display aposematic coloration, indicating the possibility that

they sequester predator deterrent chemicals. In addition to possessing osmateria, covered in predator

deterrent chemicals (isobutyric and 2-methyl butyric acids) (Eisner *et al.* 1970)(Fig. 2c), larvae of *E.*

marcellus also sequester in their body tissue the annonaceous acetogenins present in the leaves of

pawpaw (Martin *et al.* 1999). The concentration and incorporation of acetogenins in body and wing

tissue may confer protection against predation; although, this remains to be experimentally tested for

this species. Despite potential protection afforded by sequestered acetogenins, larvae are still quite

susceptible to parasitoids. The wasp *Trogus pennator* (Hymenoptera: Ichneumonidae) is a larval-pupal

parasitoid of swallowtail butterflies, and the combination of osmaterial glands and sequestered toxins

does not completely deter parasitism (Damman 1986). Rates of parasitism upwards of 15% have

been reported (Sime 2005).

Following the successful completion of the sixth instar, larvae pupate on the underside of

green or brown leaves close to the ground (West and Hazel 1996). Pupae are generally 2.5 to 3.5cm

in length and characterized by their triangular shape and resemblance to a pawpaw leaf (Figure 2d).

Some of these individuals will remain pupae throughout the summer and winter to emerge as the first

spring generation the following year. The remaining individuals will emerge as adults in 6 to 10 days

as either *E. m. telemonides* or *lecontei* forms.

Scope of this study

The single greatest threat facing swallowtail species is the loss of suitable habitat (Pimm *et al.*

1995). This includes the habitats of the host plants on which swallowtails rely to complete their life

cycle; appropriate spaces for adult courtship, such as hilltops, riparian areas, and open fields;

sufficient locations for egg laying and pupation; and access to flowering plants that produce nectar or

pollen food resources. General knowledge regarding the swallowtail butterflies is detailed compared

with the limited knowledge about nearly all other arthropods. However, most of this knowledge

focuses on the adult forms of the most colorful and intriguing species. Paul Ehrlich (2003) suggests

that scientists must coordinate their efforts regarding the phenetics of pre-adult stages and more

accurately evaluate larval host plant and nectar source use for a larger sample of species. Such

evaluations for *E. marcellus* are one of aim of this study. The following chapters address this shortfall

for the *E. marcellus* in Northern Virginia through field surveys and analysis of larval and adult habitat

and populations.

It has been generally assumed that this species is widespread and common; however,

preliminary data combined with the paltry assessments of other swallowtail species suggest otherwise.

The North American Butterfly Association (NABA) conducts a continent-wide survey of butterfly

populations each July, and although not a survey of scientific rigor, it does elucidate population

trends of *E. marcellus* in the Northern Virginia region. Figures 3 and 4 show the Zebra Swallowtail

individuals counted near Arlie, Virginia from 1996 to 2011 and in Calvert, MD in the previous

decade. Although most butterfly counts tend to measure adult populations, larval surveys are also an

important aspect of a comprehensive evaluation of *E. marcellus* populations because different life-

stages may be threatened by different natural or anthropogenic factors. To date, NABA July counts have not involved assessments of larval population density.

Critical to both adult and larval populations is the availability of high quality habitat. The requirements for larval habitat differ from those for adults, as discussed above, and this study seeks to quantify aspects of habitat quality for both larva and adults. Specifically, I assessed canopy cover, proximity to watercourses and human habitats, and *A. triloba* patch density. Moreover, I assayed leaf quality across a range of sites to determine to toxicity of leaves consumed by caterpillars, as well as the potential of those leaves to provide acetogenins for sequestration. In the first field season, I also measured the late-season interspecific interactions between *A. triloba* and another of its obligate herbivores, *Omphalocera muroei* (Pyralidae) to determine if defoliation by this species is responsible for the *E. m. lecontes* generation of *E. marcellus* as it causes a late-season leaf reflush upon which late-season larvae may feed (Damman 1989).

I employed transect counts and bait traps to quantify adult *E. marcellus* in Northern Virginia in a more rigorous fashion than the previously mentioned NABA butterfly counts. An additional component of adult habitat quality, the presence or absence of readily available adult nectar sources proximal to *A. triloba* populations, was assessed by counting the available nectar sources throughout the flight season of *E. marcellus* for several sites in the Northern Virginia region.

Finally, I geographically analyzed habitat requirements of both larvae and adults to show the possible centers of *E. marcellus* populations in Northern Virginia and to predict patterns of individual movement throughout the area. Although non-migratory, it is assumed that *E. marcellus* individuals can disperse across large areas. Spatial analysis of available habitat in Fairfax County, Virginia will be key to identifying the areas most prone to deleterious effects from human perturbations, such as increased fragmentation, habitat loss and degradation, and pollution. These analyses will provide

specific recommendations to land managers regarding the conservation of this and other butterfly species in an increasingly urbanized area.

Following the spatial analysis of butterfly movement, I integrated the habitat requirements of larvae and adults into an individual-based model used to predict population dynamics. The purpose of this model was to visualize the interactions between *E. marcellus*, its adult nectar sources, and the larval food source *A. triloba*. An additional aim of this model was to simulate the potential population dynamics of *E. marcellus* depending on several modeled factors. These include the seasonal growth habits of *A. triloba*, the affinity of *E. marcellus* larvae to consume young leaves, and the availability of adult nectar sources proximal to *A. triloba*. This model seeks to explain the crucial balance between adult and larval survival as a function of plant growth and seasonality. Specifically, it addressed the question "to what degree does the concentration of nectar and larval food sources impact the ability of *E. marcellus* adults to successfully oviposit and reproduce?"

Worldwide, nearly all swallowtail populations are likely in decline. The conservation assessments of the IUCN and Natureserve do not present current data. Species that have not been evaluated in 15 years may have declined to the brink of extinction. This thesis is aimed at updating the species account of *E. marcellus* for Natureserve and the IUCN. Amateur collectors the world over can be a vital resource in determining the presence or absence of a particular species, but thorough scientific assessments of larval and adult populations combined with a spatial analysis of these swallowtail species are necessary and long overdue. Scientific studies of swallowtail species will not only contribute to the knowledge of these incredible animals, but also provide the critical knowledge to monitor these beautiful creatures and ensure their continued existence.

Figure 1. Phenological variation in adult morphology of *Eurytides marcellus* a) Spring form, *E. m. marcellus*, photo credit: Bob Moul, b) Late spring form, *E. m. telemonides*, photo credit: Bob Moul, c) Summer form, *E. m. leconti*, Photo credit: University of Florida.
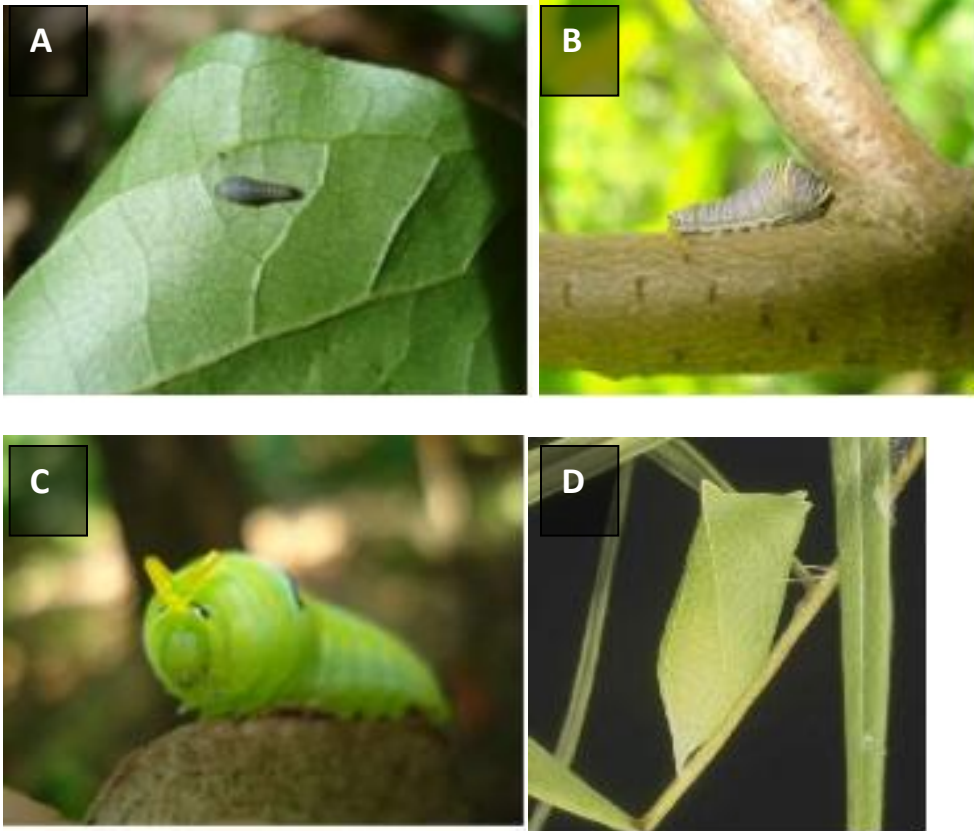
Figure 2. Developmental variation in *Eurytides marcellus* immature stages, a) larva, 2nd instar dark form, b) larva, 5th instar dark form, c) green form, 6th instar with osmateria protruding, d) *E. marcellus* chrysalis, photo credit: University of Florida.
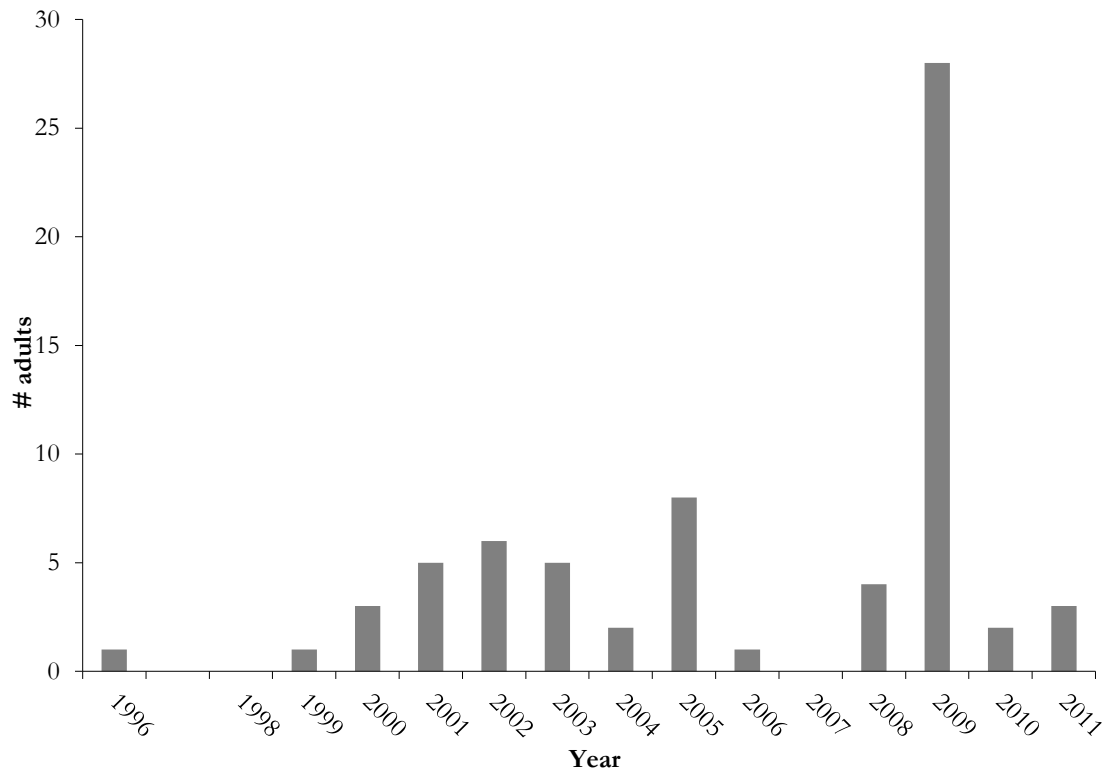
Figure 3. Total observed individuals of *Eurytides marcellus* Cramer from 1996 to 2011 near Arlie, VA. Data are from the North American Butterfly Association's annual butterfly count. Counts have not been standardized for number of observers or observer effort and do not represent a standardized population count from year to year. No data were available for 1997.
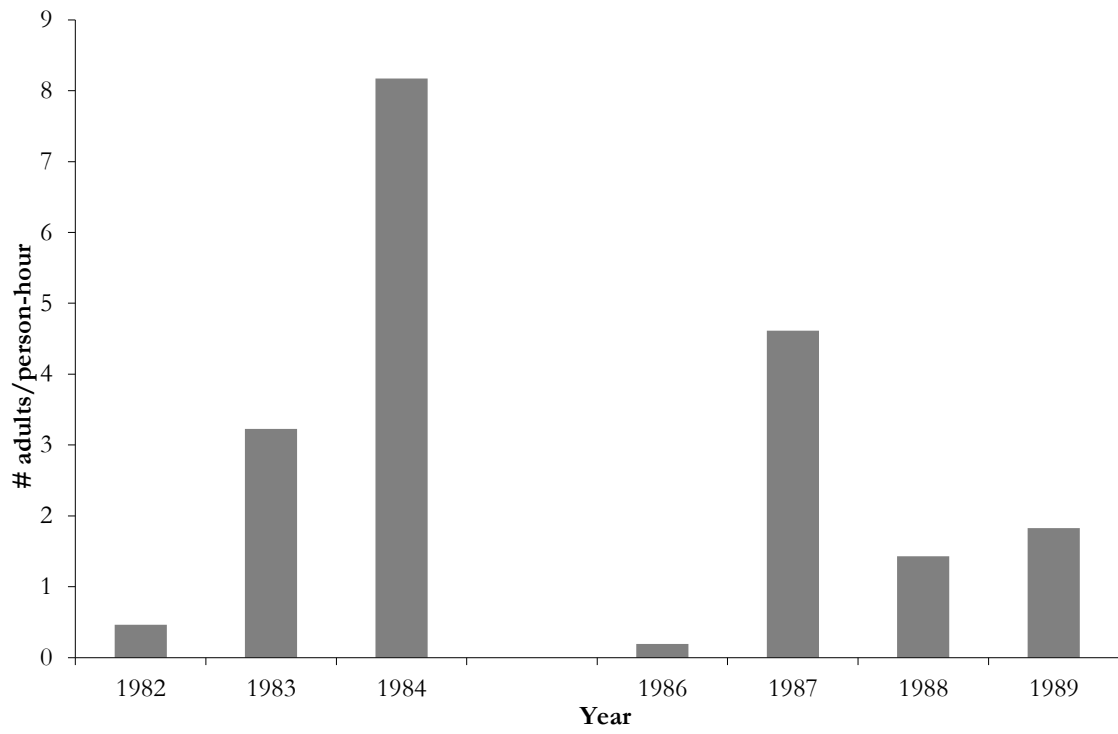
Figure 4. Density of *Eurytides marcellus* near Calvert, MD from 1982 to 1989. Data are from the North American Butterfly Association's annual butterfly count and have been standardized to represent the number of *E. marcellus* observed by each observer for each hour of observation. No data were available for 1985.

The Status of *Eurytides marcellus* in Northern Virginia

Introduction

Anthropogenic habitat destruction is a primary cause of extinction for most species, including insects (Wilson 1988), and anthropogenic disturbances may reduce habitat quality for animal species in numerous and interacting ways. For example, fragmentation, which often occurs concomitantly with habitat destruction, can reduce populations of insects that prefer interior forest habitat by creating edge effects and reducing the total amount of interior habitat (Bender *et al.* 1998, Groom *et al.* 2006). While some insects, including most butterfly species, prefer open, weedy areas, secondary habitat, or forest edges (Ehrlich and Daily 1993), insects with more restricted niche requirements face greater extinction risk as a result of fragmentation (Spitzer *et al.* 1997). These species include Lepidoptera with strict temperature or elevation regimes, commensal or mutualistic interactions with other species, high canopy cover requirements, and monophagy (Akino *et al.* 1999). For example, approximately 75% of described species of Blues (Lepidoptera: Lycaenidae) associate with ants (Pierce *et al.* 2002), and survival of members of the lycaenid genus *Maculinea* is dependent on access to ants of different species, each with its own habitat requirements for a specific grass height (Thomas *et al.* 1989). The impacts of habitat loss and/or fragmentation on populations will depend on the niche requirements of the focal insect species, as well as on those of any species on which it depends. Therefore, successful conservation of an insect not only requires determining its abiotic niche requirements but also assessing population responses to changes in biotic habitat factors.

If recent estimates that approximately 57,000 of every one million species of insects may be threatened with extinction are correct (Dunn 2005), then critical assessments of niche requirements

have been made for less than 1% of endangered insects. Some studies indicate that temperate butterfly populations are declining faster than populations of birds and plants (Thomas *et al.* 2004, Conrad *et al.* 2006). This suggests that insufficient data may exist to allow protection of even highly charismatic butterflies. Of the more than 40 North American Lepidoptera listed as at risk of extinction (IUCN 2011), most exist within a narrow range of habitats, often associated with only one host plant. Thus, survival of butterflies of conservation concern is dependent on their access to species-specific host plants for larval feeding, adult oviposition, or adult nectar resources. Consequently, many studies advocate augmentation of plant resources as a means of restoring Lepidoptera populations (e.g., Schultz and Dlugosch 1999). The idea that augmentation of host plant resources as critical to conservation underlies the popularity of "butterfly gardening" programs.

Even a thorough understanding of host plant niche requirements, however, may be inadequate to provide effective conservation. Differing responses of larvae and adults to disturbance often confound insect conservation. This is especially the case for insects whose larvae may be aquatic but adults terrestrial; it is also likely to occur when larvae have a species-specific host plant but migrate to find unique mating or feeding habitats as adults (Wassenaar and Hobson 1998). In short, anthropogenic disturbances may differentially impact insect ontogenetic stages. For example, monarch larvae (*Danaus plexipus*) benefit from augmentation of populations of their host plant, *Asclepias*, but may still go extinct if habitats where adults over-winter are lost to development. Studies that investigate only the responses of adult butterflies and ignore larvae, or vice versa, or that examine only the insect and ignore responses of host plants to disturbance may miss information critical to their protection or re-establishment. The North American Zebra Swallowtail, *Eurytides marcellus*, is a model system in which to investigate these aspects of butterfly conservation.

Zebra Swallowtail populations are constrained by habitat requirements that differ with life stage. Larvae are dependent on access to adequate populations of pawpaw (*Asimina sp.*) and on the

ability of adult females to find suitable pawpaw plants in shaded understory locations. Adults, on the other hand, require sufficient nectar sources to survive and reproduce. Although adults are able to search for mates or patches of larval host plants, this process may be metabolically costly. Therefore, the optimal habitat for Zebra Swallowtails should contain both a significant density of pawpaw as well as the nectar sources on which adult survival depends. Fragmentation may not decrease nectar sources because edge effects generally permit an increase in weedy species (Harper *et al.* 2005), including many flowering plants. Rather, fragmentation may lead to a reduction in the availability of larval host plants or an increase in inhospitable conditions for larvae, negating any possible population increase due to an increase in adult resources. The availability of appropriate habitat for adult nectar sources proximal to patches of host plants for larvae may be necessary for robust population (Grossmueller and Lederhouse 1987, New *et al.* 1995, Pocewicz *et al.* 2009). Such heterogeneous forest cover may exist only in riparian zones that have relatively minimal human impact, fragmentation, or intrusive edge effects. Studies to determine if *E. marcellus* adults disperse across forest edges into anthropogenic habitat matrices have not been conducted.

Additional life history features may also put Zebra Swallowtails at risk. Although *E. marcellus* is considered multivoltine - it has one generation that is timed to exploit rapidly developing spring leaves and another to exploit late-season leaf flush - preliminary data suggest that populations in Northern Virginia are restricted to a univoltine life cycle (see below). In the spring generation, as the leaves mature, concentration of ovipositional attractant stimulants for adult female *E. marcellus* appear to decline sharply and flavonoid anti-herbivore deterrents increase (Haribal and Feeny 2003). This suggests that factors that mitigate leaf-flush, such as increased temperature as a result of increased habitat fragmentation or climate change, may affect population densities. Edge and similar gap effects have been demonstrated to alter the timing of flowering, leaf flush and senescence, and leaf chemistry (Dudt and Shure 1994, Malcom 1994, Herrerías-Diego *et al.* 2006).

Other studies have suggested that the survival of late season larvae is linked to defoliation by a second herbivore, *Omphalocera munroei* (Damman 1987, Damman 1989). This gregarious Pyralidae causes severe defoliation followed by a flush of new leaves late in the growing season. The interaction between *O. munroei* and *E. marcellus* has been suggested to be responsible for the success of the second flight of *E. marcellus* (Damman 1989). However, preliminary data suggest that *O. munroei* herbivory does not induce late-season leaf flush of *A. triloba* in Northern Virginia. Consequently, leaf phenology in the spring represents a key factor upon which each univoltine generation relies throughout most of its temperate range. Therefore, phenological synchrony between flushing leaves and eclosing caterpillars during this critical time may strongly influence larval survival and adult ovipositional preference.

Finally, *E. marcellus* larvae in Northern Virginia currently exist in low density. Damman (1989) found larvae and egg densities of 1 per every 250 leaves searched in forested areas of Northern Florida. Locally, data collected in June 2010 in Northern Virginia yielded an estimate of 1 larva per 55,000 leaves searched, despite the fact that host plant availability of *A. triloba* is thought to be high in this region. However, the density of the host plant is not necessarily an accurate predictor of the success of a butterfly. For example, *Baronia brevicornis*, the only extant Baroniidae swallowtail, is extremely rare despite feeding on *Acacia*, which is both common and widespread (León-Cortés *et al.* 2004). This suggests that there are other factors controlling Zebra Swallowtail populations. These may include host plant patch size (e.g. total available habitat area) and geographic isolation, adult dispersal, and enemy-free space (Bauerfiend *et al.* 2009, Sime 2005).

Total available habitat area is one of the most important factors in determining the persistence of a local population in a heavily fragmented landscape **(**Gaggiotti and Hanski 2004**).** Most remaining forested areas in Northern Virginia are relegated to thin regions surrounding major water bodies such as the Occoquan and Potomac Rivers and the Chesapeake Bay (see Figure 5).

Many of these riparian areas contain *Asimina*; although, small populations of pawpaw also occur in moist, upland sites, such as the Shenandoah Mountains. The riparian forest surrounding the Occoquan River does not exceed two kilometers in width and is less than 100 meters wide in many areas. The eastern bank of the river has been designated as recreational park space but is still threatened by increasing residential development and new agricultural endeavors, such as vineyards. Riparian areas along the western bank have not been classified as green space and may be under threat from economic development. These remnant forests already represent a fraction of the area once available to both *E. marcellus* and *A. triloba*. A study conducted by the Mid-Atlantic Regional Earth Science Applications Center demonstrated that forest lost from 1937 to 1998 in the surrounding Fairfax County region was 80% (Figure 6), and destruction of these forests for metropolitan expansion has occurred primarily within the last 30 years. Moreover, the oblong arrangement of forested corridors on the eastern bank of the Occoquan River may create the strong edge effects discussed above (Van Dyke 2008).

The distribution of *A. triloba* populations within these remnant forests is patchy. Although the density of individual shoots in each patch can be greater than one shoot per square meter, the distance between available patches can be quite far, often exceeding a mile (Beatty, personal obs.). In addition, evidence suggests that pawpaw populations are pollinator limited and have low reproductive success (Willson and Schemske 1980). These factors combined may contribute to a reduction in the overall density of both of plants and butterflies and may contribute to population isolation of adult swallowtails. However, the remaining riparian habitats in Northern Virginia may serve as dispersal corridors for both plants and butterflies.

In addition to reductions in total forest area and connectivity and an increase forest edges, anthropogenic recreation (e.g., hiking, kayaking, horseback riding) and increased understory browsing by vertebrate herbivores can alter the suitability of habitat for *E. marcellus*. With respect to abiotic

factors, Lepidoptera are sensitive to both light and temperature. For example, *Epirrita autumnata* (Geometridae) larvae feeding on birch grew significantly better in shaded treatments as a result of the higher water content present in shaded leaves (Henriksson *et al.* 2003). Adults of at least one swallowtail, *Heraclides aristodemus ponceanus,* will not seek nectar sources in areas of high light (Natureserve 2011). Both nitrogen and water frequently limit herbivore populations (Mattson 1980), and plants found in shady habitats contain higher levels of nitrogen and water content than plants in high light areas (Aide and Zimmerman 1990, Henriksson *et al.* 2003 Muth *et al.* 2008). In addition, one study (Muth *et al.* 2008) demonstrated that swallowtail larvae preferred to feed in shady habitats. *Asimina triloba* patches that are shaded should provide better growing conditions for immature plants (Peterson 1991), and these plants should also contain higher concentrations of water and nitrogen (Herms and Mattson 1992). Hiking and trail running are popular activities that may amplify ambient light levels and temperature by trampling vegetation or widening trails (Cole 2004, Hall and Kuss 1989). Understory browsing by expanding populations of white-tailed deer has reduced herbaceous plants and small trees in Northern Virginia forests (Heckel *et al.* 2010). Thus, deer browsing, like human visitation, may contribute to increasing canopy openness, ambient light levels, and temperature. Combined, these factors may all contribute to increased light levels and may lead to lower population densities of *E. marcellus* larvae.

With respect to biotic factors, butterflies may respond to changes in plant morphology, chemistry, phenology, density and distribution (León-Cortés *et al.* 2004). Changes in light levels that occur with fragmentation or other disturbances may lead to differences in all of these, especially plant defensive chemistry (Dudt and Shure 1994, Hunter and Forkner 1999, Fortin and Mauffette 2001, Spiller and Agrawal 2003, Forkner and Marquis 2004). *Asimina triloba* experiences low levels of herbivory from generalist insects because it is chemically well defended. Its major secondary compounds, acetogenins, are potent, biologically active chemicals found in the tissues of plants in the

Annonaceae (custard apple family), of which *A. triloba* is a member. The ingestion of these compounds by most herbivores results in lethargic or emetic activity and in smaller insects can lead to death (Martin *et al.* 1999, McLaughlin 2008). We might predict, therefore, that larval densities of *E. marcellus* are negatively correlated to host plant defenses. However, acetogenins present in the leaves of *A. triloba* are metabolically sequestered by *E. marcellus* and provide chemical defense against many predators (Martin *et al.* 1999). Foliar acetogenins concentrations can vary three-fold between plants and has been demonstrated to peak concurrently with *E. marcellus* larval development in May and June (Gu *et al.* 1999). Thus, the high concentration of acetogenins in the leaves of *A. triloba* may benefit larval *E. marcellus* via increased chemical protection from predators. Additionally, anti-herbivore metabolites that deter generalist herbivores, like acetogenins, can act as host recognition cues and feeding stimulates for specialist herbivores (Agrawal and Karban 1999). Thus, we might also predict that larval densities will positively correlate to acetogenin content. Regardless, changes in light regimes and temperature are expected to alter plant quality for this herbivore.

The influence of habitat changes on defensive chemicals, and the subsequent consequences for Zebra Swallowtail populations, has not yet been determined for *A. triloba* and are hard to predict. However, changes in habitat quality that alter the ability of *Asimina* to produce acetogenins are likely to impact the abundance of *E. marcellus*, as well as other herbivores that feed on pawpaw. If *A. triloba* responds to increasing sunlight by decreasing concentrations of acetogenins or other host plant recognition cues, we might predict that larval densities will be lower in open canopy locations. If, however, *Asimina* responds similarly to spicebush (*Lindera benzoin* L.) and has higher concentrations of secondary metabolites in high-light areas (Muth *et al.* 2008) then densities of *E. marcellus* may increase if larvae or adults detect stronger chemical cues from these plants. Although the plants in sunlit areas may contain less nitrogen, it may be advantageous for larvae to exploit these habitats if

they are not restricted by a physiological tolerance to acetogenins and if they can increase growth
rates in warmer microhabitats.

Unlike larvae, adults may require sunny habitats or prefer open riparian corridors in order to
find sources of nectar or opportunities to bask or puddle (Grundel *et al.* 1998, Krauss *et al.* 2003).
During oviposition in early spring the concurrence of nectar sources such as blue bells (*Mertensia
virginica*) for adults and newly expanded pawpaw leaves may be critical to adult and larval survival,
respectively. However, apart from oviposition and the occasional nectar source, the presence of adult
E. marcellus individuals in patches of *A. triloba* may be unnecessary for the remainder of the season.
Adult *E. marcellus* density may be related to *A. triloba* patches only insofar as patches are located
proximal to abundant nectar sources, and adult *E. marcellus* are capable of traveling large distances in
search of adequate nectar sources (Walker 2001). The thermal profile of a patch as a result of light
level may play a significant role in the use of forest patches by adults for feeding if there are available
nectar sources (Grundel and Pavlovic 2007). Increasing canopy openness as a result of human
perturbations has led to increases in some butterflies as a result of a greater abundance of weedy
nectar sources (Koh and Sodhi 2004, Grossmueller and Lederhouse 1987). In this respect, responses
of adult *E. marcellus* may contradict patterns seen in larvae in that they may increase in areas with
greater human visitation or canopy openness.

A comprehensive review of the conservation status of swallowtail butterflies has not been
published since 1991 (New and Collins 1991), and no published assessment of *E. marcellus* exists. In
general, biologists have assumed that populations of Swallowtails are robust, but thorough
population surveys have not been conducted. Preliminary data suggest that *E. marcellus* populations
may be much lower than other Papilionidae. Indeed, adult population surveys compiled by the North
American Butterfly Association (NABA) over the past 15 years appear to show strong fluctuations in
population density and a general decline in *E. marcellus* populations in the mid-Atlantic region

(unpublished data, see Figure 3 and Figure 4, Chapter 1). The goals of this study were to assess the conservation status of *E. marcellus* through larval and adult population surveys and through the assessment of suitable habitat in four protected forest areas. Specifically, I 1) assessed the density of adult *E. marcellus* populations, 2) assessed host-plant quality of *A. triloba* populations for larval Zebra Swallowtails and estimated larval densities, and 3) determined the potential impact of anthropogenic land use and disturbance on *E. marcellus* in human-impacted regions of the Occoquan watershed of Northern Virginia. I predicted that higher abundances of *E. marcellus* larvae would be found in shaded instead of sunny, open-canopy habitats, and I expected high numbers of larvae in riparian areas with less human visitation, higher amounts of canopy cover and understory vegetation, and greater densities of host plants. Moreover, I expected that adults would be less sensitive to reduced habitat quality compared to larvae, and because of their high mobility, I predicted that adult densities will be largely determined by the abundance of available nectar sources for each of the four regions. If these predictions are supported by field research, areas that contain high densities of pawpaw within shaded, closed-canopy habitats and that are adjacent to intact, less disturbed waterways and open areas containing sufficient adult nectar sources should be considered of high conservation priority for this butterfly.

Methods

Study Species

Annonaceae, of which *Asimina triloba* is a member, is a pantropical family of plants primarily dispersed by mammals (Richardson *et al.* 2004). Nine species of *Asimina* (Linnaeus) occur in the Eastern United States; *Asimina triloba* is the sole temperate representative with eight smaller subtropical species established in Florida. The tropical origins of *A. triloba* dictate that the habitat for optimal seed germination is warm (25°– 30°C) and moist (Peterson 1991). In addition, seedlings and young plants are extremely sensitive to sunlight, which can cause mortality in as little as one day of

exposure. Although seedlings will not survive in full sun, clonal plants produced from root suckers are characteristic of well-lit forest edges, disturbed areas, and stream banks. Clonal propagation has been suggested to be the primary means of local population growth, accounting for 90% of new shoots in some areas (Hosaka *et al.* 2005). Despite being generally common in eastern states, *A. triloba* is currently listed by Natureserve as "imperiled" in New York and Iowa, and "critically imperiled" in New Jersey. In addition, the conservation status of *A. triloba* has not been assessed throughout the majority of its reported range (Natureserve 2011).

Eurytides is primarily a tropical genus of Kite Swallowtails (Papilionidae: Leptocircini), which are widely distributed through the New World tropics and Caribbean. *Eurytides marcellus* extends from southern New England west to southern Minnesota and south to eastern Texas and Florida. Areas containing *E. marcellus* in Northern Virginia are mixed deciduous, secondary forests primarily populated by upland populations of beech (*Fagus grandifolia*), red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*), and various species of oak (*Quercus* spp.). *Eurytides marcellus* usually completes two flights during the summer in this northern region (Wagner 2005), with active adults present from early April to late September (Watson and Hyatt 1988). Adults are reported to gather nectar from common flowers, including blue bells (*Mertensia virginica*), blueberry (*Vaccinium*), milkweed (*Asclepias*), dogbane (*Apocynum*), verbena (*Verbena hastata*), goldenrod (*Solidago sp.*), and buttonbush (*Cephalanthus sp.*), as well as flowering understory trees such as redbud (*Cercis canadensis*). Males actively patrol watercourses for females (Wagner 2005). Adult females oviposit one egg per plant on leaf buds when the leaves are about to flush in the spring.

Following eclosion, larvae undergo five instars. Some larvae from the first cohort overwinter, and nearly all larvae in the second generation enter diapause when signaled by the shortened, late-season photoperiod. Individuals pupate on living and dead leaves approximately 5 to 15 cm above the ground (West and Hazel 1996). Because *E. marcellus* larvae prefer new leaves, the

31

second cohort may be dependent on defoliation from a second *Asimina* herbivore species, *Omphalocera munroei* (Martin), to initiate new leaf production in *A. triloba*. *Omphalocera munroei* is a univoltine, gregarious Pyralidae. Female moths may oviposit more than 40 eggs in a batch on a single *A. triloba* leaf in the late summer (Damman 1991). Larvae hatch and feed in groups, and larval aggregations construct a silken web around several leaves and may completely defoliate one or more plants (Damman 1989, 1991). Larvae primarily feed on leaves throughout their seven instars, but may also bore into fruit (Covell 2005). Larvae possess bright orange dorsal and subdorsal coloration, suggesting that they may be aposematic. However, larvae incorporate their own frass in construction of silken nests, which may also serve as a predator or parasitoid deterrent (Damman 1991).

Data collection

Stands of pawpaw for transects, larval surveys, and assessments of habitat quality were identified and georeferenced in April of 2010. Regional land-use data have been inferred through spatial data provided by Fairfax County, VA, and ground-truthed near pawpaw stands in four protected areas in the Bull Run Occoquan watershed (see Table 1 and Figure 5).

Assessments of habitat quality

Within each of the four locations, four patches of *A. triloba* were chosen from naturally occurring patches to represent a dichotomy of habitat quality and human disturbance within that area: two were selected based on their proximity to human disturbances such as foot traffic, roads, and other disturbances, and the remaining two sites were selected based on the relative absence of anthropogenic disturbances (n=16 total patches). In each of these 16 patches, circular 10 m quadrats were established and the following data collected: dominant canopy and understory tree species, plant species diversity, canopy cover, and degree of understory openness (calculated as pawpaw leaf density). Canopy cover was quantified using a spherical densiometer at each of the cardinal

geographic points along the circumference of the quadrat in addition to the center point. These measurements were then averaged to provide an estimate of canopy cover for the quadrat.

  Swallowtail abundance

The density of *E. marcellus* adults was assessed using line-transects surveys in 2010 and 2011, and bait traps in 2011. Three times a summer, in the latter half of April, June, and August, four 100 m transect surveys were completed at each of the four areas listed in Table 1. In 2011, bait traps accompanied line-transect surveys. Transects were completed in primarily riparian areas along established trails. The number of adult *E. marcellus* within sight of each transect was recorded. Transects for all sites were completed within one week during similar weather conditions and all measurements were taken between the hours of 10:00 and 17:00, when butterflies are most active. In addition, the presence of adults alighting on leaves for oviposition or flying through the visible area surrounding each of the sixteen designated pawpaw sites was recorded, along with the time spent at each site. Bait traps were used during the peak of the *E. marcellus* flight in late July 2011 to quantify population density. Eight bait traps were set for each of the four sites listed in Table 1. Traps were arranged in pairs and were located either within, or in close proximity to circular quadrats (n=32 traps). Traps were baited in the morning with over-ripe fruit and monitored at one-week intervals for four weeks. Traps were reset with new bait each week. Unfortunately, no adults were trapped using this baiting method. In addition, transect surveys in 2010 and 2011 found no adults. However, during caterpillar surveys, nine adults over two years were observed. However, as these sightings were not part of our systematic search protocol, they were not analyzed. Therefore, analyses of adult densities were not attempted, and statistical analyses described below are for larval densities only.

The density of *E. marcellus* larvae was quantified through field sampling of *A. triloba* leaves within the established circular quadrats along the Occoquan River in Northern Virginia. These assessments were conducted once a year, beginning on approximately May 30, 2010. Each year, 60

plants in 4 locations in each of four parks listed in table 1 (n=960 trees) were surveyed. All leaves on

the plant up to 2 m in height were counted and searched for *E. marcellus* eggs, larvae, or pupae. The

occurrence of additional orders of arthropods, including predators, and the presence or absence of

foliar herbivory on each plant was also recorded during caterpillar surveys. Ideally, estimations of

percent herbivory for each plant would have been gathered, but the bias between field observers

proved great. Therefore, each plant was recorded as possessing or not possessing visual foliar damage

by herbivores.

Leaf area and quality

In mid-June 2010, estimates of leaf quality were taken at each of the 16 sample locations.

One hundred large leaves from randomly chosen plants within each plot were collected into ice and

returned to the lab. Wet weight was taken and leaves scanned on a flatbed scanner and their area

estimated using the ImageJ program (http://rsb.info.nih.gov/ij/). Areas were averaged per plot and

used to estimate larval density per square meter of leaf area. Leaves were then dried at 30°C and

reweighed to estimate water content. Dried leaves were then ground individually in a Wiley Mill and

used to chemically assay the acetogenin concentration using the Brine Shrimp Toxicity (BST) assay.

Previous work has demonstrated that the BST assay correlates directly to levels of biologically active

acetogenins (Gu *et al.* 1999). Dried, ground plant material was extracted using methanol for 24 hours.

Crude extracts were then used in a modification of the BST protocol described in Gu *et al.* (1999) to

estimate the acetogenin concentration of the leaf material for each collected leaf, expressed as LD_{50}

(see appendix). Assays were originally completed over a 24-hour period, as per Gu *et al.* (1999),

however, survival of *Artemia* in control treatments was low over 24 hours, and assays were shortened

to a 12-hour period.

Omphalocera munroei surveys

In mid-August 2010, the presence of *O. munroei* was assessed at the Elizabeth Hartwell

Mason Neck Wildlife Refuge. Sixty plants inhabited and showing extensive defoliation by *O. munroei*

and 60 plants without *O. munroei* were identified and marked. Any caterpillars present on the plants were removed. The number of caterpillars removed was noted and the number of undamaged leaves remaining was recorded, along with the approximate sizes/instars of the caterpillars. Collected caterpillars were then relocated randomly to the 60 undamaged plants, and the plants were marked to monitor future reflush and defoliation. This was done to control for any pre-existing differences in plant quality that might affect host plant choice by *E. marcellus* larvae or adults. An additional 60 undamaged plants were marked and used as controls. All plants were then resurveyed every two weeks until mid-October and the re-growth of leaves and the presence of any *E. marcellus* larvae was recorded. These data were used to create a model of leaf availability for second-generation *E. marcellus* larvae in Northern Virginia and to test the assumption by Damman (1989) that defoliation by *O. munroei* is responsible for a late season generation of *E. marcellus*.

Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to determine if measurements of canopy cover, pawpaw density, plant quality variables, and herbivory and population densities of arthropods differed among the four sites and across years. Summed for each plot, binomial presence/absence of herbivory was converted to a proportion of plants per plot showing herbivore damage. Counts of arthropods and herbivores were standardized to reflect the number of herbivores present per leaf area searched. Canopy cover and proportion of herbivory were transformed using the arcsine square-root transformation to normalize the data. Population count data for larval *E. marcellus* was correlated to each variable measured at each plot, including distance to nearest waterway, distance to park edge, LD_{50} acetogenin concentration (for 2010), leaves per plant, canopy openness, and density of pawpaw. Larval densities and acetogenin concentrations (LD_{50}) could not be transformed to fit a normal distribution and so were analyzed using a non-parametric one-way ANOVA (separately by year for larval densities). Analyses were conducted in SAS 9.2

Results

The total estimated leaf area searched for larvae in both years was 5226 m^2 (n=201,032 leaves). The total number of leaves surveyed declined by 7160 leaves between 2010 and 2011. However, as the same plants were surveyed in each year, this may represent a decrease in the productivity of these plants between 2010 and 2011. A repeated measures ANOVA of leaf density per plot for each year found that there was no difference among sites or years ($F_{3,12}$=6.77, $P = 0.006$). Densities of leaves per m^2 were highest in Bull Run Regional Park (131.61 ± 10.60), followed by Hemlock Overlook Regional Park (85.90 ± 14.46), Mason Neck State Park (53.90 ± 9.85), and Fountainhead Regional Park (48.53 ± 2.57) (see Figure 7).

A linear regression between canopy cover in 2010 and 2011 indicated that while canopy cover was significantly correlated across years ($R^2 = 0.625$, $P = 0.0002$), cover differed enough to expect differences in shading of understory between years. Thus, a repeated measures ANOVA was completed and indicated that canopy cover did not differ significantly between sites but did differ significantly among years ($F_{3,12} = 13.10$, $P = 0.004$). The between subject effects of this analysis indicated that there was a marginally significant difference between canopy cover at Hemlock Overlook Regional Park in year two ($F_{3,12} = 2.82$, $P = 0.08$) (Figure 8).

Although sites were selected based on the existence of larval host plants, only 3 caterpillars were found during surveys in 2010 and 11 caterpillars were found in 2011. Over two years, caterpillar density was 0.0027/m^2 leaf area, which is much lower than all previously reported values (Damman 1987). A non-parametric one-way analysis of variance was performed separately for larval densities in 2010 and 2011. During 2010 there was not a significant difference between sites (Kruskal-Wallis X^2 = 1.187, df = 3, $P = 0.756$). Caterpillar densities were marginally different between sites in 2011 (Kruskal-Wallis $X^2 = 6.00$, df = 3, $P = 0.077$, Figure 9).

Herbivores recorded feeding on *Asimina* included species of Coleoptera, Orthoptera, Hemiptera, Homoptera, Diptera, Geometridae, Trichoptera, and other Lepidoptera larvae. Repeated measures ANOVA showed no significant difference between sites in herbivore abundance in 2010 and 2011 ($F_{3,12}$ = 3.06, P = 0.069). However, there was a significant interaction between year and site (time × site, $F_{3,12}$ = 3.58, P = 0.047, Figure 10), as the density of herbivores declined between years at Mason Neck State Park. Proportion of plants with herbivory did/did not differ significantly among sites ($F_{3,12}$ = 1.11, P = 0.384, Figure 11).

Predatory species belonged to several taxonomic groups including, Arachnidae, Formica and other hymenoptera, Reduviidae, Mantoidea, and Chilopoda. Repeated measures ANOVA results were similar to those of herbivores per leaf area in that they indicated that there was no significant difference in the abundance of predators per leaf area between years ($F_{3,12}$= 2.86, P = 0.117), but that there was a significant interaction of site and time in predator density ($F_{3,12}$ = 9.42, P = 0.002), with predator densities declining in 2011 in Fountainhead Regional Park (Figure 12).

There was no significant correlation between canopy cover and caterpillar density per plot area (r = -0.187, P = 0.489) or caterpillar density per leaf area (r = 0.015, P = 0.957). Similarly, there was no significant correlation between caterpillar density per plot area and pawpaw leaf density (r = -0.074, P =0.785) and no significant correlation between caterpillar density per leaf area and distance to the nearest waterway (r = -0.056, P =0.837). However, there was a significant positive correlation between caterpillar density per leaf area and the distance to the edge of the park (r =0.663, P = 0.005).

Finally, there was no significant difference in acetogenin concentration of leaves between sites (Kruskal-Wallis X^2 = 3.397, df = 3, P = 0.334) (Figure 13). Additionally, caterpillar densities in 2010 were too low to correlate with leaf acetogenin concentrations and leaf samples from 2011 were

not assayed. However, leaf toxicity from 2010 negatively correlated to caterpillar density in 2011 (r = 0.572, P =0.02).

Site differences were also analyzed geographically by determining the proximity of the 16 monitored habitat areas to the park edge, as well as their distance from a major waterway. A site level ANOVA determined that there was a significant difference between both the distance to the edge of the conservation area ($F_{3,12}$ = 9.04, P = 0.002) and the distance to the nearest waterway ($F_{3,12}$ = 4.04, P = 0.033). Specifically, plots at Mason Neck were furthest from park edges and furthest from waterways. These subplots within this site included more interior forest habitat, while sites at Hemlock Overlook were closest to a waterway and closest to the park edge (Figure 14)

Finally, tests of Damman's (1989) assertion that late season herbivory by the gregarious pyralid *Omphalocera munroei* contributes to the multivoltine nature of *E. marcellus* did not support this hypothesis for Northern Virginia populations. Transplanted *O. munroei* continued to feed on their new hosts, often completely defoliating the entire plant. However, 98% of the damaged plants (plants to which caterpillars were transplanted) did not produce any late-season leaves, and those that did, produced on average only one new leaf before the first frost and therefore were not observed to have larvae present. None of the control plants in this study incurred any damage from *O. munroei* or any other herbivores, produced no new late-season leaves, and did not recruit zebra swallowtail eggs or larvae.

Discussion

Eurytides marcellus populations in the Northern Virginia region were less abundant than expected. *Eurytides marcellus* is widely assumed not to be endangered; however, data from this study suggests that in Northern Virginia this species is neither persistent nor common. Overall numbers of larvae encountered were less than 15 individuals over both years, despite that fact that 5226 m² of

habitat area were surveyed. This would place regional Zebra Swallowtails populations in the IUCN

Red List category of Critically Endangered under Criterion D, as populations consisted of less than

50 individuals. Densities of adult *E. marcellus* were also lower than expected: despite repeated transect

surveys over two years and the addition of bait traps in the second year, no adult *Eurytides marcellus*

were recorded at any of the study sites. The casual observation of only nine individuals over two

seasons of fieldwork substantiates that overall adult abundance is minimal in this region. Indeed,

adult population surveys compiled by the North American Butterfly Association (NABA) over the

past 15 years appear to show strong fluctuations in population density and a general decline in *E.*

marcellus populations in the mid-Atlantic region (See Chapter 1, Figure 3 and 4). Adult populations,

therefore, meet the IUCN Red List category of Critically Endangered on the basis of Criterion C –

populations consist of fewer than 250 individuals, are declining at an unspecified rate and show

considerable fluctuations in density. Rates of localized decline in adult density may be difficult to

measure because adults are highly mobile, but over time a pattern of population density decline might

be expected for the region given projected increases in urbanization in the area. However, adults

were observed at all sites at least once during the duration of this study. Whether this indicates that

there are small but stable populations in each of these areas, or rather, that these individuals had

dispersed from other areas to the study areas would be best addressed by future mark-recapture

studies.

Nonetheless, negative impacts on larval populations through localized changes in quality or

quantity of available resources or landscape-level changes in habitat heterogeneity will lead to an

overall decrease in adult population densities over time. The prediction that larvae would prefer

shaded instead of sunny habitats could not be verified, as neither canopy cover nor understory

pawpaw density varied across sites, suggesting that general microclimates were relatively

homogeneous in areas sampled in this study. However, of the fourteen caterpillars detected, none

were found on plants in open, sunlit habitats. It is likely that the microclimate of larval development plays a greater role than the general light and temperature regime of an area, because assessments did find that larval densities were higher in areas at greater distances from park edges (interior forests). This may also reflect an adult preference for oviposition sites that are in interior forest habitats.

The presence of larvae in 2011 was strongly correlated with low leaf acetogenin concentrations from 2010. If acetogenin concentrations of plants are relatively stable between years, as has been reported for other defensive compounds, then this may indicate that larvae move to less toxic plants, have lower survival on more toxic plants, or ovipositing females preferentially choose less toxic plants (Haribal and Feeny 2003). Analyses indicate that while sites did not differ in acetogenin concentrations, Mason Neck State Park had subplots with consistently the lowest leaf toxicity. This may be the result of a genotype unique to these plots, or the result of micro-scale habitat factors. Mason Neck is the largest and most intensely managed (protected) of all of the research sites. It also has the largest forested area and is surrounded by waterways.

The effects of human disturbance on adult and larval densities were difficult to measure. Distance to park edge was used as a proxy for human disturbance, as smaller distances indicated increased encroachment by incompatible forms of human land-use. However, other measures such as average trail width and visitation rates proved unreliable for an estimate of human disturbance. Trail width varied widely within each site and appeared more a function of drainage patterns than human intervention. With respect to visitation rates, apart from Mason Neck State Park, none of the areas studied had any form of visitor estimation count. From a conservation standpoint, this makes the effective monitoring of human disturbance on species in this area quite difficult.

The results of this study indicate that *E. marcellus* may differ from other swallowtail species that seem to increase in response to increasing edge habitats. *Eurytides marcellus*, an apparently widely distributed and common species, exists in such low density in this area that the estimation of adult

densities over two years through proven methods was unable to garner data. Additionally, larval densities were more than 50 times lower than reported estimates of one caterpillar for every 250 leaves searched (Damman 1989). Densities reported by Damman (1989) were for populations in Florida, where *E. marcellus* larvae feed on at least 4 species of pawpaw and adults are multivoltine. In Virginia, at such low density, this species may be at highly, locally vulnerable to any future habitat disturbance.

Eurytides marcellus is currently classified secure both globally and nationally. Within each state its conservation status varies (Figure 15) from S1 (critically endangered) to S5 (secure). Its status in Virginia is currently S5; however, the jurisdictions surrounding Northern Virginia show conservation classifications of S4 (Maryland) and S3 (Delaware and Pennsylvania), which indicates that there are concerns about the security of this species and it exhibits vulnerability to extinction in Delaware and Pennsylvania. This study illustrates that *E. marcellus* is threatened or critically endangered in Northern Virginia. According to the IUCN Red List Categories and Criteria (3.1), a species can be listed as critically endangered if it meets any of several requirements, one of which is a population of less than 250 individuals (IUCN 2011). Although the Red List classification scheme is to be used from a global perspective, it indicates that from the perspective of the Fairfax County jurisdiction, *Eurytides marcellus* is probably endangered.

Eurytides marcellus may exemplify the decline of a formerly common species. As a result of habitat fragmentation and destruction, population levels in Northern Virginia are extremely low. How adults may respond to declining regional populations and the effects of changes in habitat quality on *A. triloba* foliar acetogenin concentration would both be prudent avenues of future research. Moreover, the management and conservation priorities in this area should be realigned to address the biotic integrity and diversity of this areas remaining forests.

Table 1. *Eurytides marcellus* conservation assessment study sites.

	Bull Run	Hemlock Overlook	Fountainhead	Mason Neck
Lat, Long	38.8060°N -77.4779°W	38.8003°N -77.4771°W	38.7251 °N -77.3293 °W	38.6524 °N -77.1899 °W
Elevation	49 meters	52 meters	47 meters	20 meters
Visitation rates	Moderate	High	Moderate	Restricted Moderate
Forest Type	Riparian	Riparian	Riparian	Floodplain
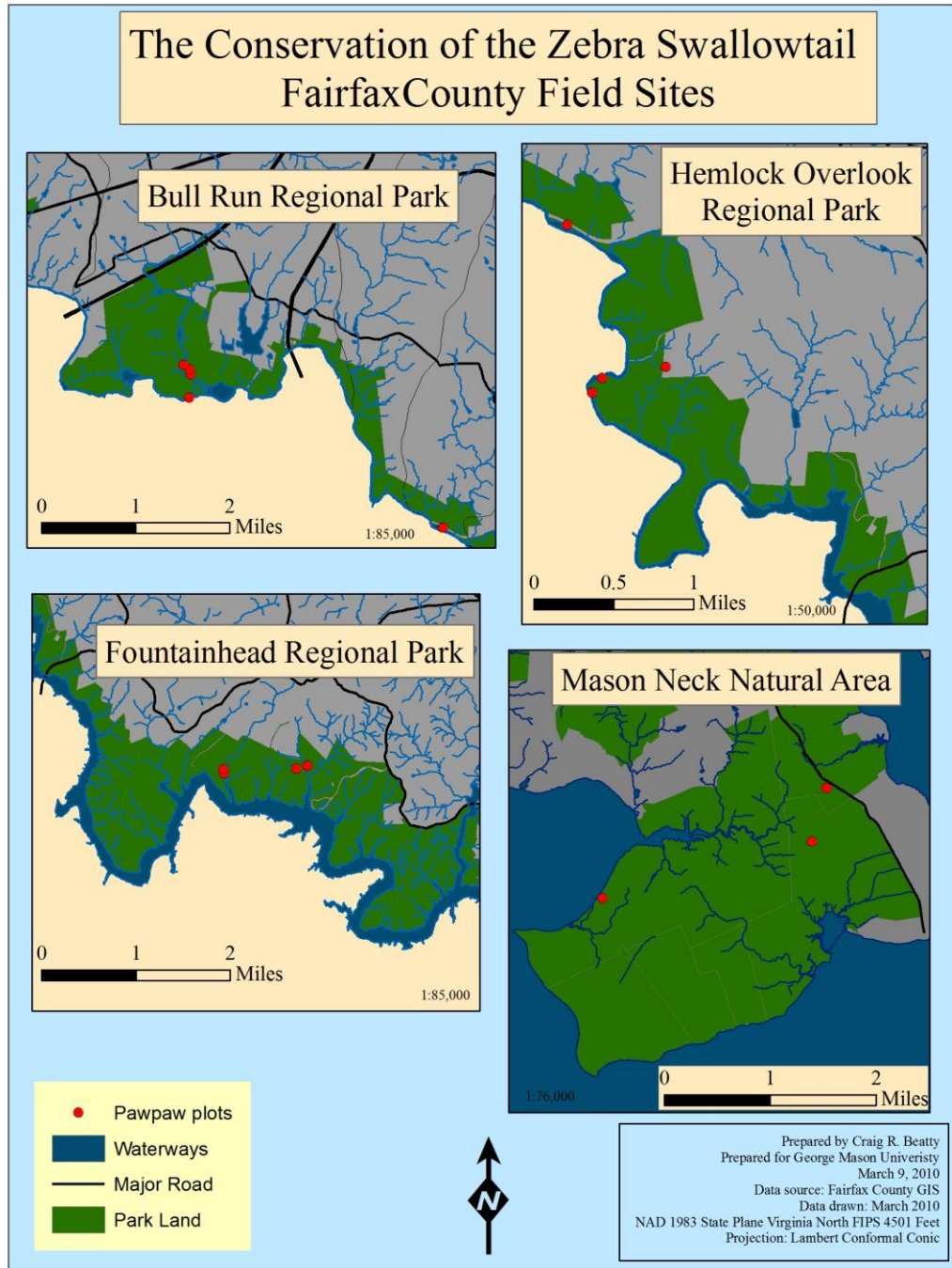
Figure 5. Location of study sites along the Occoquan River in Fairfax County, Virginia.
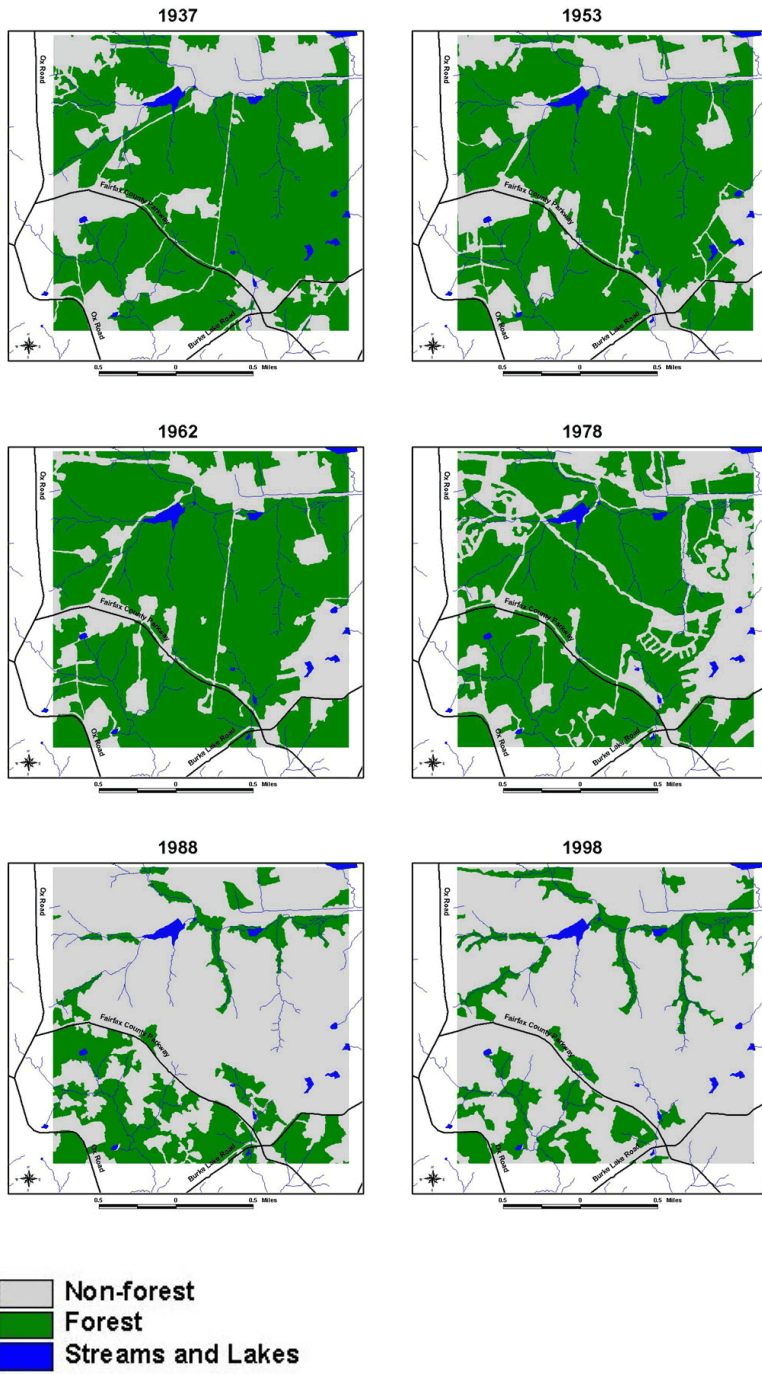
Figure 6. Land cover change in Fairfax County 1937-1998
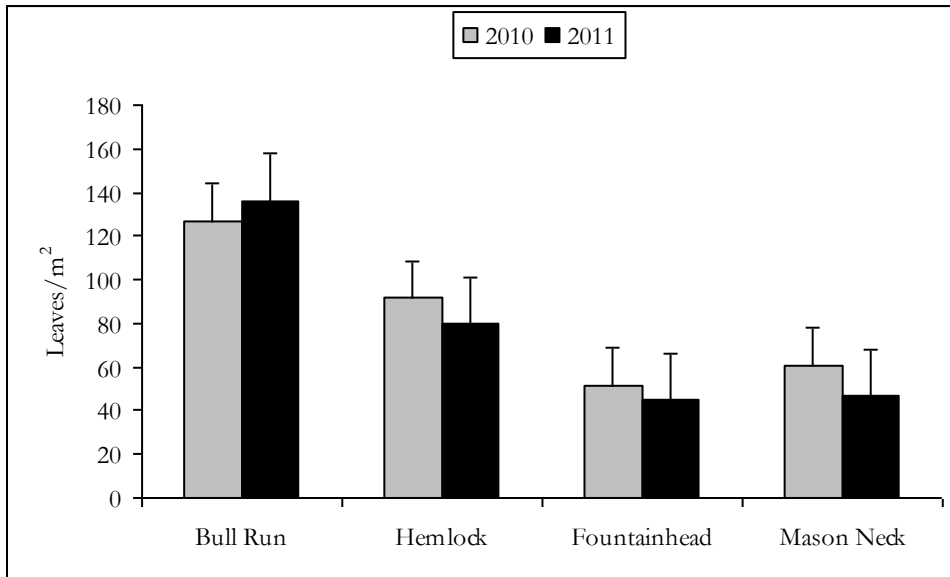
Figure 7. Average number of Pawpaw leaves per square meter in each location in 2010 and 2011.
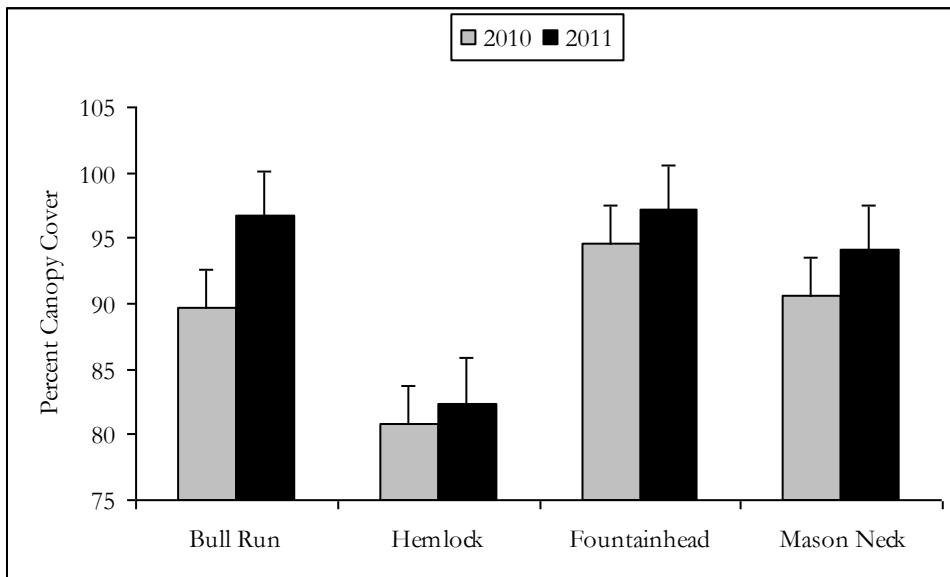
Figure 8. Average percent canopy cover for each site in 2010 and 2011.
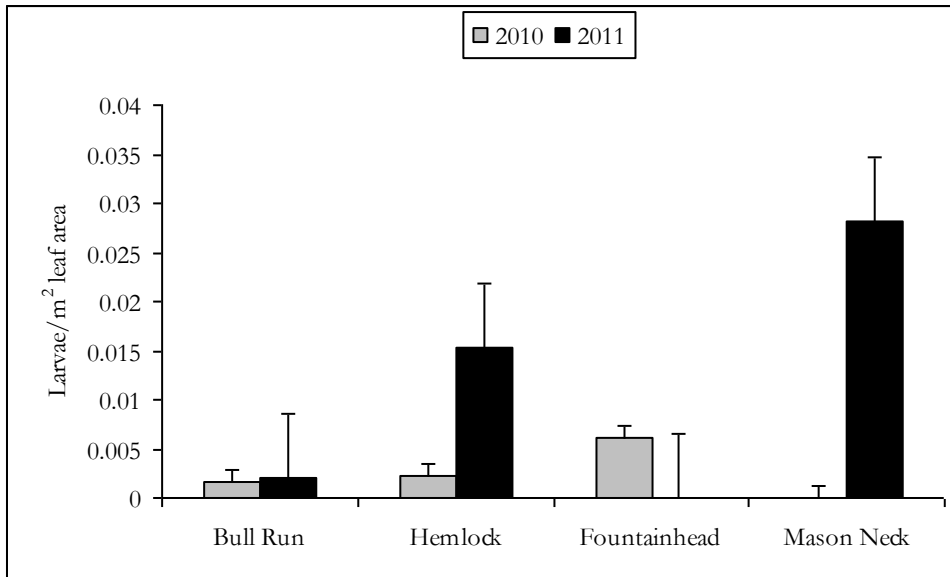
Figure 9. *Eurytides marcellus* **larvae per leaf area during 2010 and 2011**
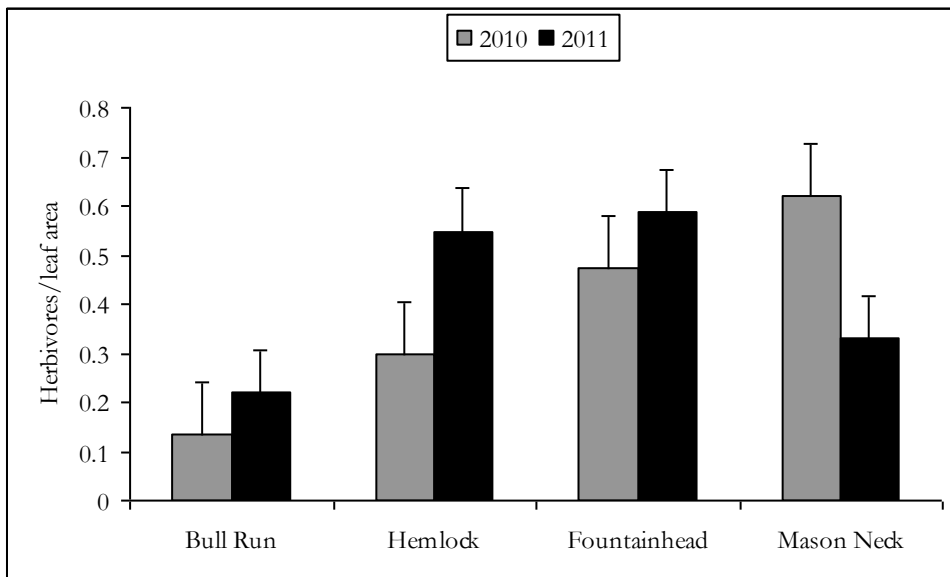
Figure 10. Average herbivore density per square meter of leaf area in 2010 and 2011. Herbivore guild includes Coleoptera, Orthoptera, Diptera, Hemiptera (not including reduviidae), Homoptera, and Lepidoptera.
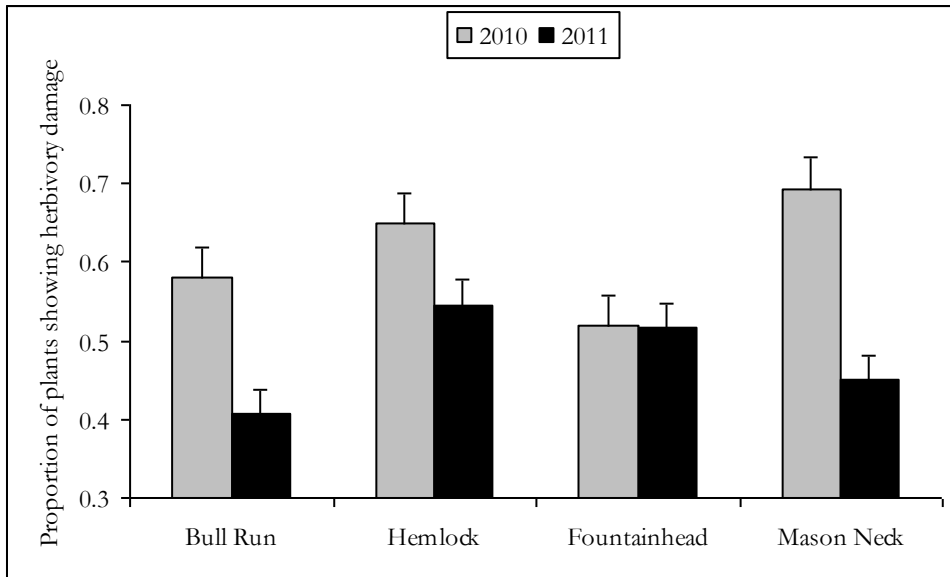
Figure 11. Proportion of plants showing herbivore damage in 2010 and 2011
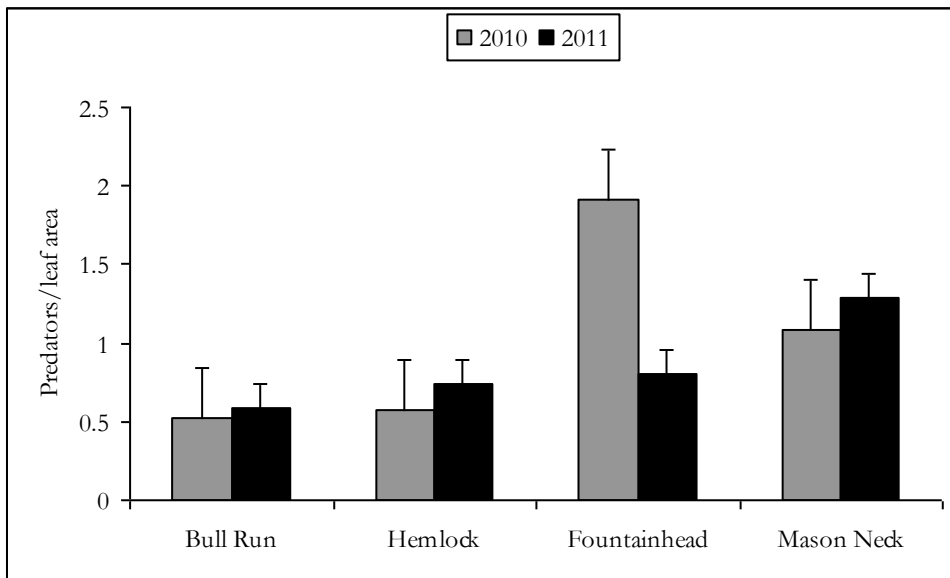
Figure 12. Average arthropod predator density per square meter of leaf area in 2010 and 2011 . Predator guild includes Hymenoptera, Arachnidae, Reduviidae, Mantoidea, and Myriapoda.
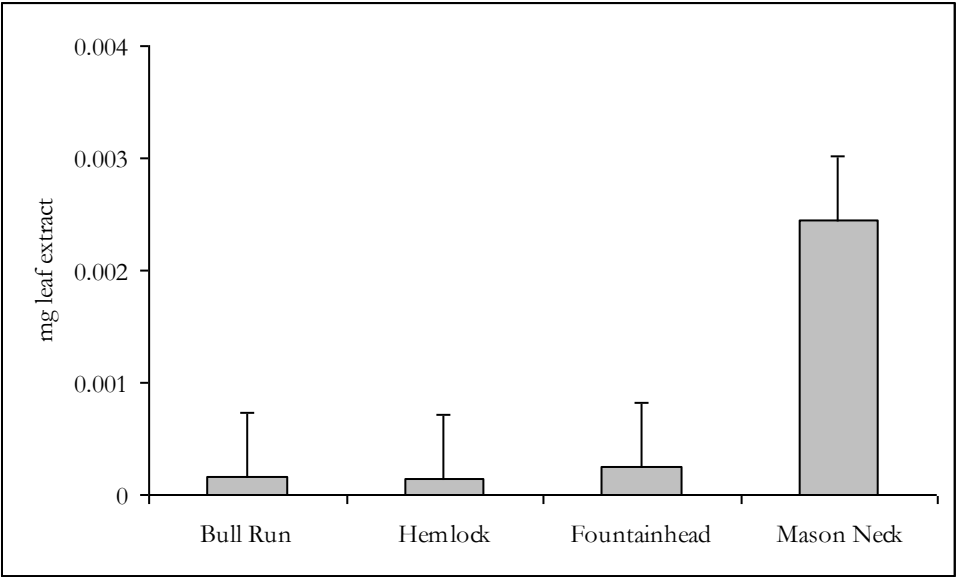
Figure 13. Average mass (mg) *Asimina triloba* extract resulting in 50% lethality for *Artemia*.
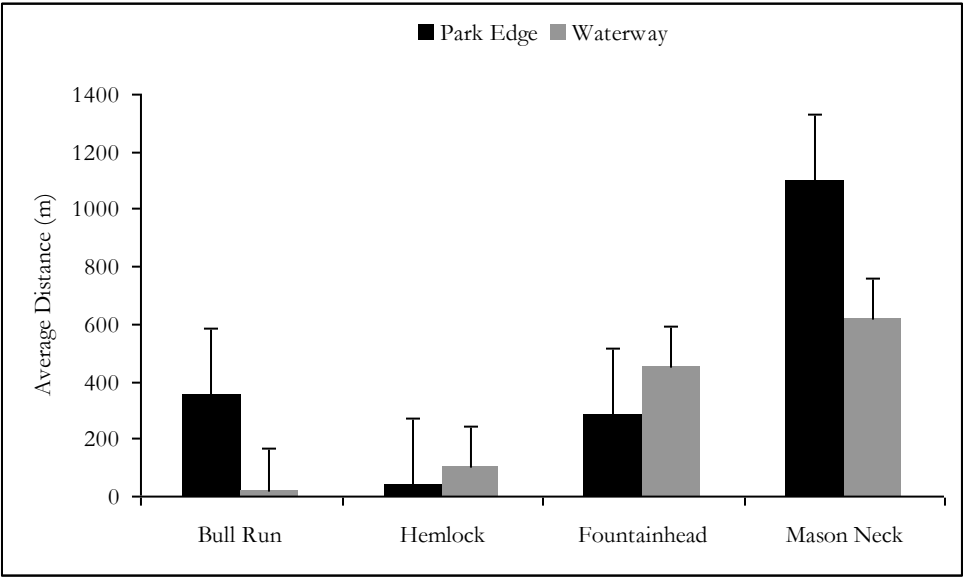
Figure 14. Average distance (m) of subplots from the nearest park edge and the nearest waterway.
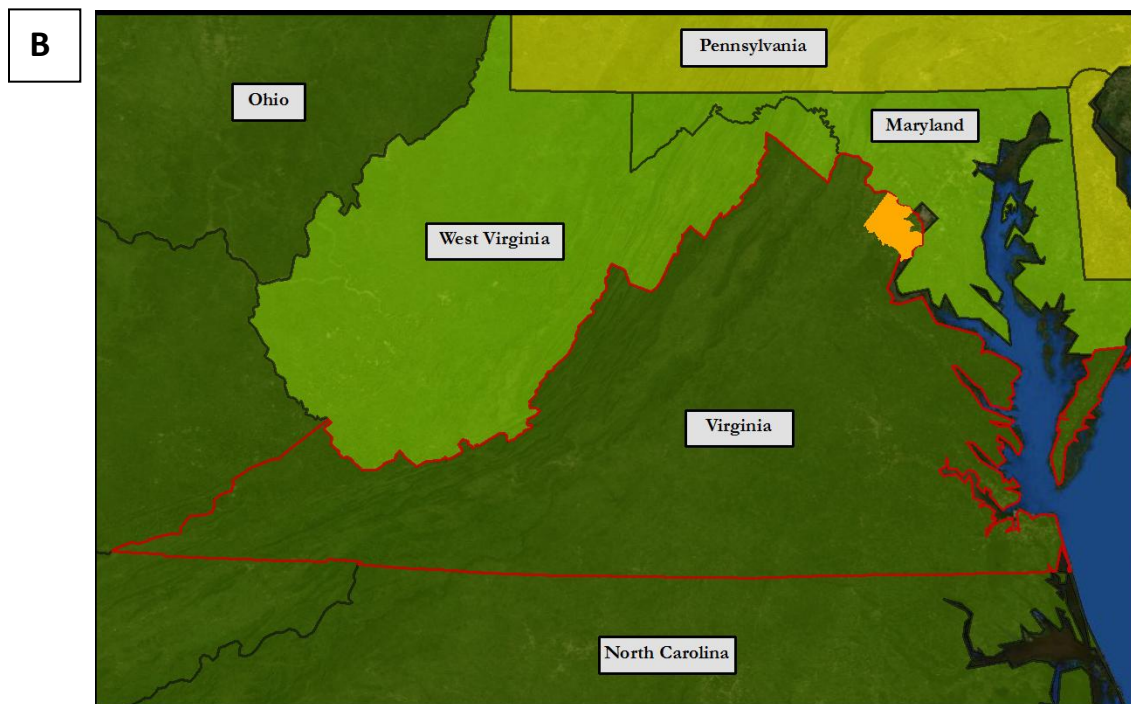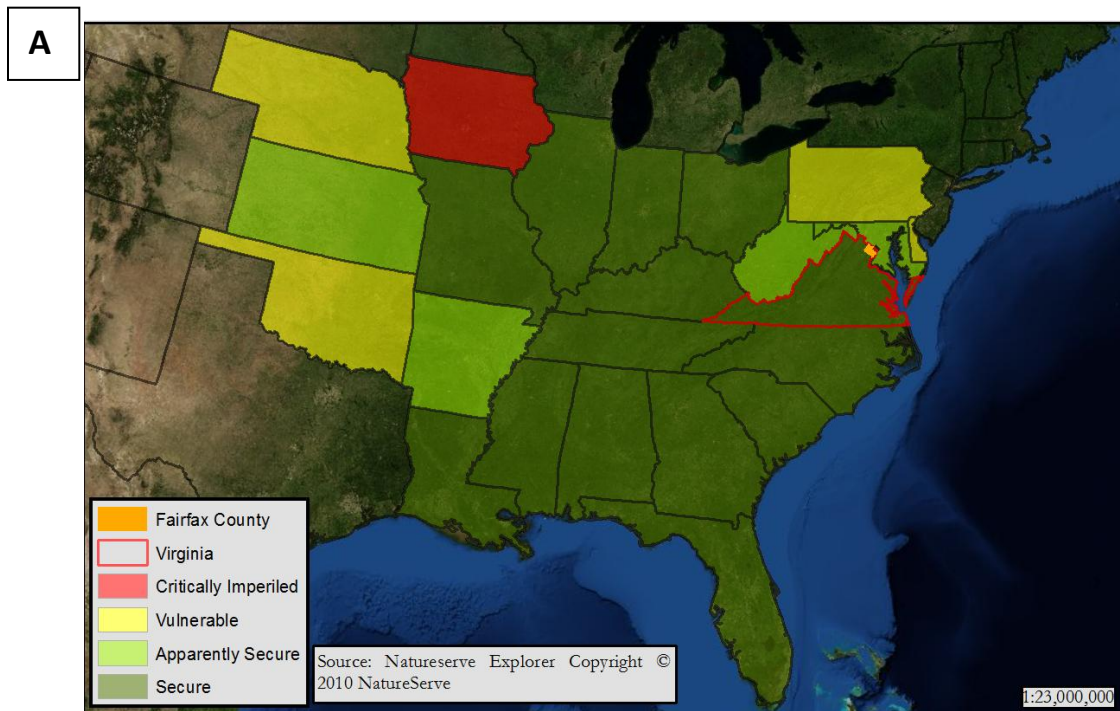
Figure 15. Conservation status of *Eurytides marcellus* in each state throughout its range (A) and the location of study sites (Fairfax County) in orange (B).

Geographic Analysis of Optimal *Eurytides marcellus* Habitat and Patch Connectivity in Northern Virginia

Introduction

Accurately modeling the dispersal and aggregation of individuals within a landscape is a central

challenge to effective species protection (Chanell and Lomolino 2000). A species' spatial aggregation

depends on the quantity and quality of taxon-specific environmental factors that create an acceptable

range of habitat. Landscape fragmentation substantially alters both the quality and quantity of

available habitat (Van Dyke 2008). Heavily fragmented landscapes display biological attrition and

isolate populations (Dover and Settele 2009). However, species may persist in landscapes consisting

of isolated habitat fragments provided that intrinsic (Ehrlich 1961) barriers to dispersal do not exist

and fitness costs of dispersing among patches are not high (Bowler and Benton 2005).

When modeling dispersal among isolated patches in fragmented landscapes, ecologists often

use a graph theoretic approach (Harary 1969) in which spatially explicit, remotely sensed data on the

distribution of habitat patches are combined with information about species dispersal abilities.

Traditionally, in these models the connectivity between habitat patches has been considered a

function of Euclidian distance (Hanski 1999, Moilanen and Hanski 2001, Moilanen and Mieminen

2002). Studies have modeled dispersal predominantly for mammals, which represent the taxonomic

minority of threatened and endangered species, and models often focuses only on the habitat

requirements and dispersal distances of adults. Thus, these models may have limited utility for

insects, for which many species may have fundamentally different habitat requirements at different

life stages. For example, insects such as Odonata (dragonflies and damselflies) may have aquatic

larval stages but persist in terrestrial habitat as adults. Furthermore, dispersal abilities may vary considerably between life stages even for insect species in which adults and larvae have similar habitat requirements. This is particularly true of Lepidoptera for which the larvae of many rarely migrate off of the host plant on which they hatch. Models that incorporate only adult dispersal and habitats may overestimate population persistence by overlooking threats to other life history stages.

These models are additionally limited in that Euclidian distance may fail to capture the idiosyncrasies of movement behavior of the species under study (Bowler and Benton 2005). In fact, landscapes provide a wide spectrum of dispersal barriers depending upon a number of factors, including a species' locomotive capacity (Roland *et al.* 2000) and size (Minor and Lookingbill 2010). Incorporating additional factors beyond Euclidian distance into models of habitat connectivity is especially important in fragmented landscapes, where suitable habitat may be scarcely distributed among unsuitable habitat matrix. More accurate measures of species dispersal and patch connectivity account for the permeability of the landscape surrounding acceptable habitat. For instance, roads form effective, physical barriers to dispersal for many species (reviewed by Debinski and Holt 2000). In addition, incorporating the costs associated with movement of an individual of a species across a landscape provides better insight into actual movement and dispersal patterns (Chardon *et al.* 2003). The least cost-distance measurement (Halpin and Bunn 2000) is a common modeling approach that incorporates costs of dispersal. These models are based on a graph theoretic approach (Harary 1969, Pickett and Cadenasso 1995), where the landscape is divided into a raster and each cell is given an impedance value based on the metabolic cost associated with traversing that cell (Minor and Urban 2008). Because metabolic costs are often estimated from population averages, these models are not considered agent (or individual) based models, and again may not accurately represent individual-based dispersal decisions. Patch connectivity has also been addressed, however, through a combination of graph theory and individual-based dispersal models. For example, Lookingbill et al.

(2010) used a network of existing habitat patches combined with an individual-based dispersal model to define the existing connectivity between patches and to identify areas of greatest conservation concern for the Delmarva fox squirrel.

This study focuses on a charismatic insect, the Zebra Swallowtail, which is experiencing direct pressures from urbanization in the eastern United States. The objective was to compare Euclidean, least-cost distance, and individual-based models incorporating both larval habitat requirements and adult dispersal for a potentially threatened forest insect species. Comprehensive reviews of the conservation status of swallowtail butterflies from twenty years ago (New and Collins 1991) deduced that swallowtail populations were robust. Common U.S. swallowtails, such as *P. glaucus*, prefer open fields or forest edges as adults, consume a wide range of plant species as larvae, including many weedy species and are strong fliers that may disperse long distances, often exceeding 5km (Grossmueller and Lederhouse 1987). Their densities, therefore, may be robust to fragmentation and may respond to easily promoted conservation efforts, such as butterfly gardening. The Zebra Swallowtail, *Eurytides marcellus*, however, requires closed forest habitat, and its larvae are monophagous on Common Pawpaw (*Asimina triloba*), which is restricted to wet, riparian forest understories. Adult population surveys compiled by the North American Butterfly Association (NABA) over from 1982 to 2011 (Chapter 1, Figure 3 and 4) show strong fluctuations in population density and a general decline in *E. marcellus* populations in the mid-Atlantic region. Moreover, *E. marcellus* populations are endangered in parts of their range, including Iowa and vulnerable to extinction in Oklahoma, Nebraska, Pennsylvania, and Delaware (Natureserve Explorer 2011,Chapter 2, Figure 15). Adult *E. marcellus* flights are uniquely timed to exploit nectar of spring ephemeral flowers before the forest floor becomes shaded in mid-summer (Clark 1932). Thus, spatially and phenologically restricted larval and temporally restricted adult food resources make this butterfly

52

more representative of at-risk species rather than the more visible, potentially disturbance-adapted butterflies that are the focus of most monitoring efforts.

To identify suitable habitat for *E. marcellus*, to ascertain potential barriers to dispersal using multiple modeling approaches, and to determine strategies for connecting potential dispersal corridors, we first conducted field surveys of larval populations to provide an estimate of density in appropriate habitat throughout Fairfax County, Virginia. Next, we compiled geographic information for the region into GIS data layers identifying suitable areas of larval Zebra Swallowtail habitat in the region. Using these data, we first modeled network connectivity between these areas by estimating the potential of dispersal between these areas as a function of maximum Euclidian dispersal distance of adult butterflies. Following this, the identified possible larval habitat was used in a cost-distance analysis to determine the impedance to dispersal across the inter-habitat matrix. Finally, following the spatial analysis of butterfly movement, the habitat requirements of larvae and adults were integrated into an individual-based model used to predict population dynamics (see chapter 4).

Methods

Location and Study Species

Eurytides marcellus is the only interior forest species of swallowtail in the U.S. and, as the only kite swallowtail in North America, is a member of a historically tropical genus, giving it a doubly unique status in the biological diversity of North America. Larvae feed exclusively on newly emerged leaves of Common Pawpaw (*Asimina triloba*) populations. Thus, available habitat for this life stage is restricted both spatially and temporally. *Asimina triloba* also has strict habitat requirements that include moist, slightly acidic soils. New plants occur mostly in riparian areas, growing via cloning and vegetative propagation, and new shoots are extremely intolerant of direct sunlight (Peterson 1991).

Therefore, appropriate habitat larval *E. marcellus* also requires closed forest canopy cover. Adult *E. marcellus* eclose from overwintering pupae as early as March and require ambient nectar sources to fuel reproduction and oviposition flights. Adults prefer sunlit, riparian habitat, and are found in highest densities along watercourses (Scott 1986). Indeed, apart from mating and oviposition, the presence of adult *E. marcellus* in shaded, interior forest *A. triloba* patches may be unnecessary, as the larval host plants flower prior to adult emergence (Willson and Schemske 1980), have a "carrion flower" pollination syndrome (Willson and Schemske 1980, Goodrich and Raguso 2009) and do not serve as adult nectar sources for butterflies.

Modeling

The first portion of our geographic analysis identifies areas in Fairfax County that are possible locations for *A. triloba* and larval *E. marcellus* populations. Identification of suitable habitat for *E. marcellus* was accomplished by locating sites meeting three separate criteria. First, the occurrence of riparian understory areas was considered essential to the growth and persistence of pawpaw, and monophagous *E. marcellus* caterpillars will only be found in areas where these plants occur. As a result of the riparian requirements of *A. triloba*, 200 meters was determined to be near the maximum distance of populations from hydrological features. Most locally surveyed populations exist within 50 meters from hydrological features, but there are some upland populations that exceed this figure. Two hundred meters was chosen as a more generous distance to account for these small upland *A. triloba* populations.

In addition to populations of the host plant *A. triloba*, our second model criterion for larval habitat was the occurrence of suitable conservation areas (i.e. parkland). Effective conservation of any species requires authority on the part of land managers to implement conservation protocols. Although *E. marcellus* may occur throughout riparian areas in Fairfax County, conservation efforts may only proceed on areas that have been designated parks or recreation areas and urbanization

pressure is likely to reduce or remove greenspace outside these areas. For this reason, only designated parkland areas were used in the analysis of suitable larval habitat.

Finally, forested areas appropriate for the implementation of conservation protocol also must have adequate spatial habitat extent for the focal species. Previous work (see Chapter 2) indicated that larvae were sensitive to distance to park edge; thus, our third model criterion was suitable patch size for the persistence of local populations of *E. marcellus*. Areas of at least 100 hectares were chosen in these analyses based on result of larval field surveys, which suggested that to support a population of 1000 caterpillars, an estimated area of 17 ha of continuous pawpaw forest is required. However, throughout Fairfax County, pawpaw exists in relatively small, isolated patches of < 1ha along watercourses within parks. An overall conservation area of 100 ha was used as a conservative estimate of the area sufficient to support multiple patches of pawpaw and thus *Eurytides marcellus*. Areas that were less than two hectares were excluded from this study because of the ephemeral nature of butterfly populations in areas less than this, and published studies often use a minimum of two hectares of habitat (Chardon *et al.* 2003, Warren 1992). Butterfly survival, especially closed forest species, is drastically reduced when the available forested area is less than two hectares (Chardon *et al.* 2003) due to the pressures of fragmentation, including but not limited to increased temperature and wind, invasive species propagation, and lower biodiversity. Two hectares represents a minimum boundary where populations of *E. marcellus* will experience intact forest.

The GIS data layers provided for Fairfax County includes dams, ditches and certain sewer lines as hydrological features and also includes water parks and soccer fields as protected county land. Care was taken, therefore, to identify land-use designations within protected land and to use only forested or semi-forested land in habitat analysis. Data for hydrological features was also manipulated to remove unnatural hydrological features such as dams and ditches. To map areas meeting these criteria, hydrological and land-use data were compared using ArcGIS 9.3.1 (ESRI) to determine

available protected riparian areas within 200 meters of an available water source. The remaining

patches were then analyzed to exclude those patches less than 2 ha in total area. The remaining

patches represented the possible locations of *E. marcellus* larval habitat in Fairfax County (Figure 16).

Those patches greater than 100 hectares were identified and selected to include in a county

assessment of possible adult dispersal (Figure 17).

For effective dispersal of adults, *A. triloba* patches must be present within a reasonable

distance of each other, but research on the possible distances *E. marcellus* may travel to acquire

adequate resources has not been conducted. As mentioned above, 5km dispersal is not uncommon

for the *P. glaucus* (Grossmueller and Lederhouse 1987). However, this species is larger and more

robust than *E. marcellus* and may have the ability to travel further. Traveling large distances poses high

metabolic costs and can reduce fecundity (Karlsson and Johansson 2008). To estimate network

connectivity among patches, we calculated Beta index values for dispersal distances of 1.5, 2, 3, 4 and

5 miles, using 5 miles as a generous, upper limit of dispersal for this species. The Beta index is a

numerical representation of the connectivity of a graph and is calculated as the number of links

(connections) divided by the number of nodes (suitable habitat) (Harary 1969). Beta values higher

than one indicate a complex network with many connections, while Beta values less than one indicate

a low level of connectivity between network nodes.

To model the possible dispersal of adult *E. marcellus* between large conservation areas, the

centroid of each of the original 100+ hectare parkland polygons was calculated. Following this, the

boundary lines of each of the park areas were converted from polygons to polylines, which allowed

for the creation of a dispersal network. This representation has 35 network vertices and a varying

number of edges depending on the assumed maximum Euclidean dispersal distance of adult *E.*

marcellus. This method of visualizing dispersal across the landscape gives a broader picture of

connectivity throughout the County among the largest areas available for conservation, irrespective of any environmental impedance to flight.

The quality of intervening habitat matrix, including the existence of temporally concurrent nectar sources near riparian areas, may also affect dispersal and population expansions for this species. To address the possible effect of the habitat matrix on the dispersal capabilities of adult Zebra Swallowtails, cost-distance analysis was completed using the Fairfax County zoning raster and assigning impedance values to each of the land-use types. Fairfax County includes 5 broad categories of land use: residential, planned units, commercial, industrial, and other. Upon further analysis, the "other" category was composed mainly of land outside the jurisdiction of the County, which included Fairfax City, Manassas City, and all major highways whose jurisdiction lies with the Virginia Department of Transportation. Within the cost-distance analysis, we defined "other" raster cells as having an impedance value of 10, which was the highest dispersal cost value. It is reasonable to assume that such a large value is warranted; interstate highways provide substantial barriers to butterfly dispersal for this closed forest species. The remaining impedance values were: planned units 3, residential 4, industrial 5, and commercial 6. Fairfax City and Manassas City values were converted to NO DATA values and were not used in analysis.

Results

All of Fairfax County represents 107,006 hectares of which 13% is parkland greater than two hectares in size. Within this area, spatial analysis using our criteria for *Asimina* and larval populations (protected areas within 200m of a waterway of at least 100 ha extent) identified 35 conservation areas theoretically able to support robust *E. marcellus* populations. The centroids of these areas were used in network analysis, and the conservation areas best suited for *A. triloba* populations appear in Figure

17. The Occoquan and Potomac Rivers, located on the respective southeast and northeast boundaries of the county, provided the largest remaining habitat areas in Fairfax County. The largest single conservation area in Fairfax County is Fountainhead Regional Park, which is 915 hectares of possible larval habitat. However, the Mason Neck State Park and Wildlife Refuge, when combined with the Mason Neck Regional Park, is roughly 1000 hectares of quality habitat for *E. marcellus*.

The network of habitat patches in Fairfax County can be described in several ways. First, if each large conservation area is represented by its centroid, the network density of the entire region is 0.15 links per hectare. However, the more likely scenario looks at the connectivity within each large conservation area at a scale of 100 meters. The area within the boundary of each conservation area was analyzed for possible habitat and within these areas networks were constructed at a 100m resolution. For all of Fairfax County there are 14,868 possible points within large-area parkland, within 100m of each other, where pawpaw and larval *E. marcellus* could theoretically exist. At this scale, the network density is 2.63 links per hectare. Thus, despite a high degree of internal connection within these areas (2.63 links per hectare), geographic analysis shows that the larger habitat patches of suitable parkland are highly fragmented and disconnected (0.15 links). This can be further quantified by examining variation in the ratio of habitat area to habitat perimeter. Most habitat patches in Fairfax County have a small overall area, but a large perimeter. This is indicative of a high potential level of edge effects because the area of the fragments remains relatively small compared to their perimeter.

Analysis also indicates that rarely did acceptable *E. marcellus* habitat occur near industrial, commercial, or planned unit zones in Fairfax County. In fact, zoning data from Fairfax County fails to distinguish between conservation areas and residential areas. Spatially, the best predictors of suitable habitat for *E. marcellus* larvae and adults appear to be the distance from planned residential units or industrial and commercial zones, as well as proximity to hydrological features.

Further network analysis of the connectivity between available habitat areas gave a more accurate representation of the spatial connections and disturbance parameters for the survival of this species in Fairfax County. Among the 35 conservation areas with a land area greater than 100 hectares, two appear most important in connecting dispersal between a large numbers of areas: the Laurel Hill and Meadowlake areas become increasingly important for county-wide dispersal as effective dispersal distance is decreased from a maximum of 5 miles to only 1.5 miles. Table 2 shows the Beta values of connectivity for Euclidean dispersal networks among the 35 conservation area centroids. As dispersal distance is increased from 1.5 to 5 miles, three distinct subgraphs appear (Figure 17). One is located along the Potomac River in Northern Fairfax County, one appears along the Northern Occoquan and the largest is located in the southern part of Fairfax County where the Potomac and Occoquan Rivers converge (Figure 17).

Analysis of cost-distance produced a map of Fairfax County (Figure 18) showing that dispersal costs for *E. marcellus* across the landscape are high. The Fairfax County zoning designations underlie the map and darker grey colors indicate increased barriers to dispersal as a result of land-use type and distance from possible habitat locations. The maximum dispersal distance in this analysis was set to five miles, but the impedance of the habitat matrix often halted dispersal far below this value. In particular, three areas of this map stand out as the darkest spots and indicate strong barriers to adult *E. marcellus* dispersal to these areas. These areas include a residential portion of Northern Fairfax County, the area south of Dulles International Airport along the western boundary of the County, and a residential area that lies between route 123 and the Occoquan River. In addition, there appears to be a large barrier to dispersal northeast of the Occoquan River and south of the Potomac River. Within the remainder of the map are easily identifiable islands of dispersal probability that are often only slightly connected. For example, the area surrounding Manassas City displays optimal

Zebra Swallowtail habitat to the west and six islands of possible larval habitat surrounded by individual areas of dispersal that appear only slightly connected.

Discussion

The optimal habitat for Zebra Swallowtail butterflies in Fairfax County is heavily dependent on life stage. The geographic analysis of this study identified 35 areas comprise possible larval *E. marcellus* habitat in Fairfax County. These areas are primarily along the Potomac and Occoquan Rivers and encompass the last remaining extensive riparian forests in addition to adjacent fields and upland secondary forest. Although potential larval habitat appears abundant in the region, as a result of the extensive major road networks and highly impermeable surfaces characteristic of urbanization, the dispersal ability of adult butterflies may be severely restricted.

Euclidean models identified three critical adult dispersal networks (Figure 17). The first, and smallest, network is along the Potomac River in the northern portion of the county. Comprised of four connected vertices, this subgraph has the potential to support populations of *E. marcellus* by allowing movement up and down the Potomac River and inland. The second subgraph is in the western portion of Fairfax County and comprises the areas of Bull Run Regional Park and the Manassas National Battlefield. Bolstering connectivity between the two arms of this network could drastically improve the capability of species to disperse. Finally, the largest subgraph appears in the southern portion of the county. It is the most extensive network in the county and possibly the greatest avenue for *E. marcellus* dispersal to the other subgraphs (e.g. source habitat (Pulliam 1988)). Critical to the maintenance of this subgraph are the Laurel Hill and Meadowlake areas, both of which facilitate connections between almost all other park centroids. It is the recommendation of this study that land mangers work to create dispersal corridors between the three disjunct population networks in Fairfax County, and that they specifically focus on improving habitat quality in central hubs of this network.

Despite the ease and usefulness of simple Euclidean distance measures, the cost-distance measurements provided additional critical information. As a result of these analyses, we can identify areas in Fairfax County that present the greatest impedance to adult *E. marcellus* dispersal, as well as the areas least likely to contain adults of this species. Specifically, the majority of strong dispersal barriers mentioned above occur in residential areas or along major roadways. Cost-distance analysis indicated that the large subgraph in the southeastern portion of the county is not as well connected as estimated by Euclidean distance approaches. This network may, in fact, consist of three disconnected areas separated by Highways 1, 123, and 95. Moreover, all of the areas least likely to contain dispersing or resident populations of *E. marcellus* are residential. Challenges to improving residential areas for this species may prove insurmountable, as residential areas in the Washington D.C. metropolitan area, which includes the area analyzed in this study, rarely contain sufficient acreage for butterfly gardening or community oriented conservation initiatives.

Within this study, impedance was calculated based on the estimated permeability of the landscape to butterfly dispersal. However, future studies could calculate impedance values based on a host of factors in addition to land use, including elevation, non-permeable ground cover, and nectar source distribution, all of which may influence butterfly dispersal. Furthermore, mark release recapture experiments with adults could provide more accurate estimations of impedance and would assist in determining local population densities. In our models, *A. triloba* patches are the centers of *E. marcellus* populations and it follows that dispersal should occur from one habitat patch to another. Using the large-scale network of butterfly dispersal possibilities identified previously and cost-distance measurements, a shortest path analysis, such as Dijkstra's algorithm (Dijkstra 1959) could be performed to identify the most optimal paths for the dispersal of this species throughout Fairfax County. These optimal paths would help inform conservationists and land managers of specific areas likely to augment the dispersal and persistence of this species.

Butterflies have often been used as model organisms when studying dispersal patterns. In this region *E. marcellus* may be particularly useful as indicative of the responses of other threatened species because of its unique habitat requirements. This may make it more representative of the other species in the region that also have complex life-history parameters and or require large, intact areas of suitable habitat. Land-use change can have drastic effects on the ability of local populations to resist extirpation or extinction and the research and analysis of how these factors impact the biology of one species can inform decisions on species that are less known. Modeling changes in land use and how they may impact local species is an indispensable part of creating regional and national conservation schemata. This study is the first to explore the interaction between land use and habitat quality for the interaction between *A. triloba* and *E. marcellus*, one of few studies to look at the specific paths and costs to butterfly dispersal across a landscape, and should provide baseline dispersal data for this increasingly threatened species.

Table 2. Beta indices of network connectivity for various dispersal distances using 35 vertices, which represent large possible habitat areas of Fairfax County, VA.

Dispersal Distance	# of edges	ß
1.5 miles	4	0.11
2 miles	17	0.49
3 miles	37	1.06
4 miles	56	1.60
5 miles	81	2.31
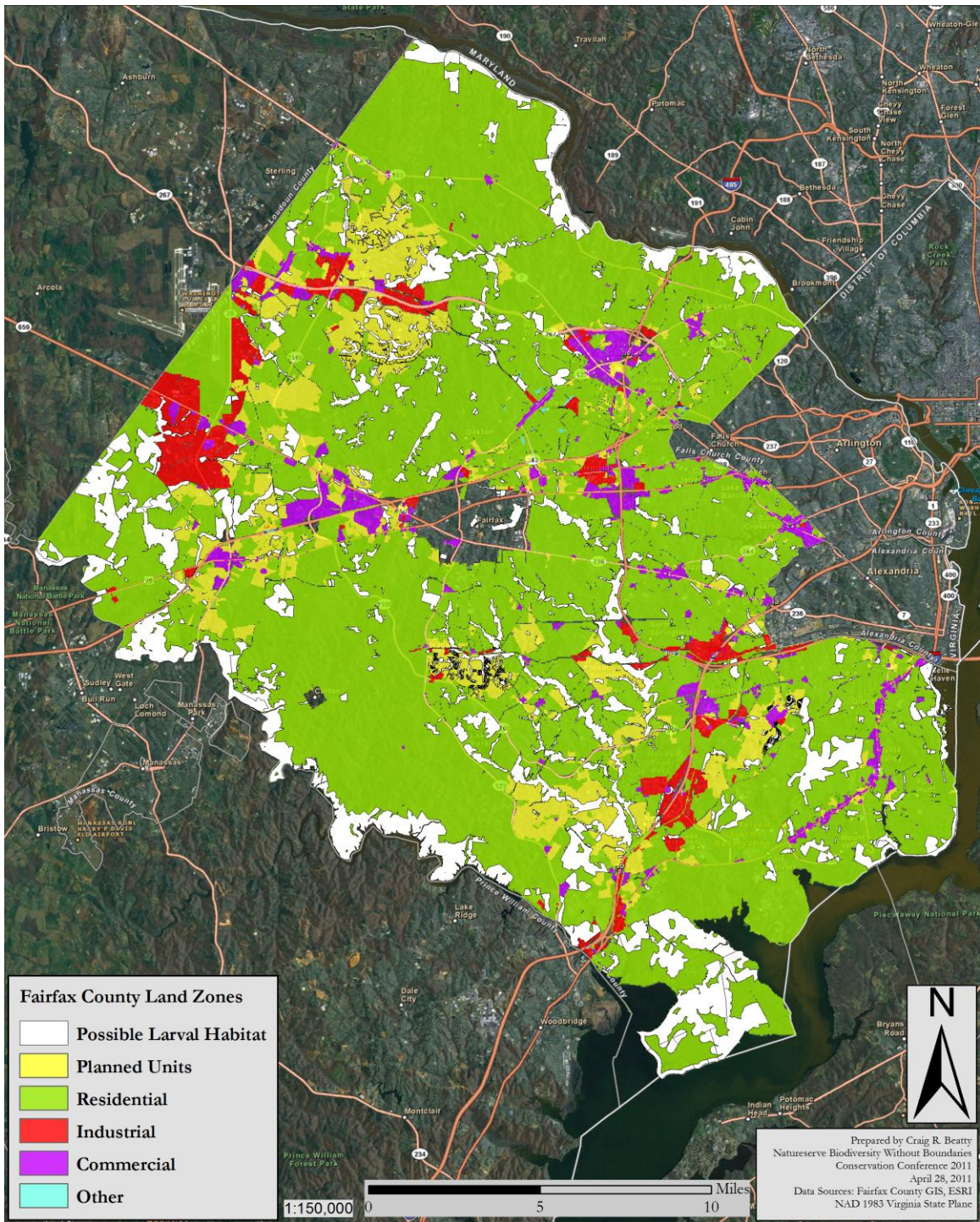
Figure 16. Areas in Fairfax County suitable for populations of _A. triloba_ and that, consequently, should include populations of _E. marcellus_ larvae
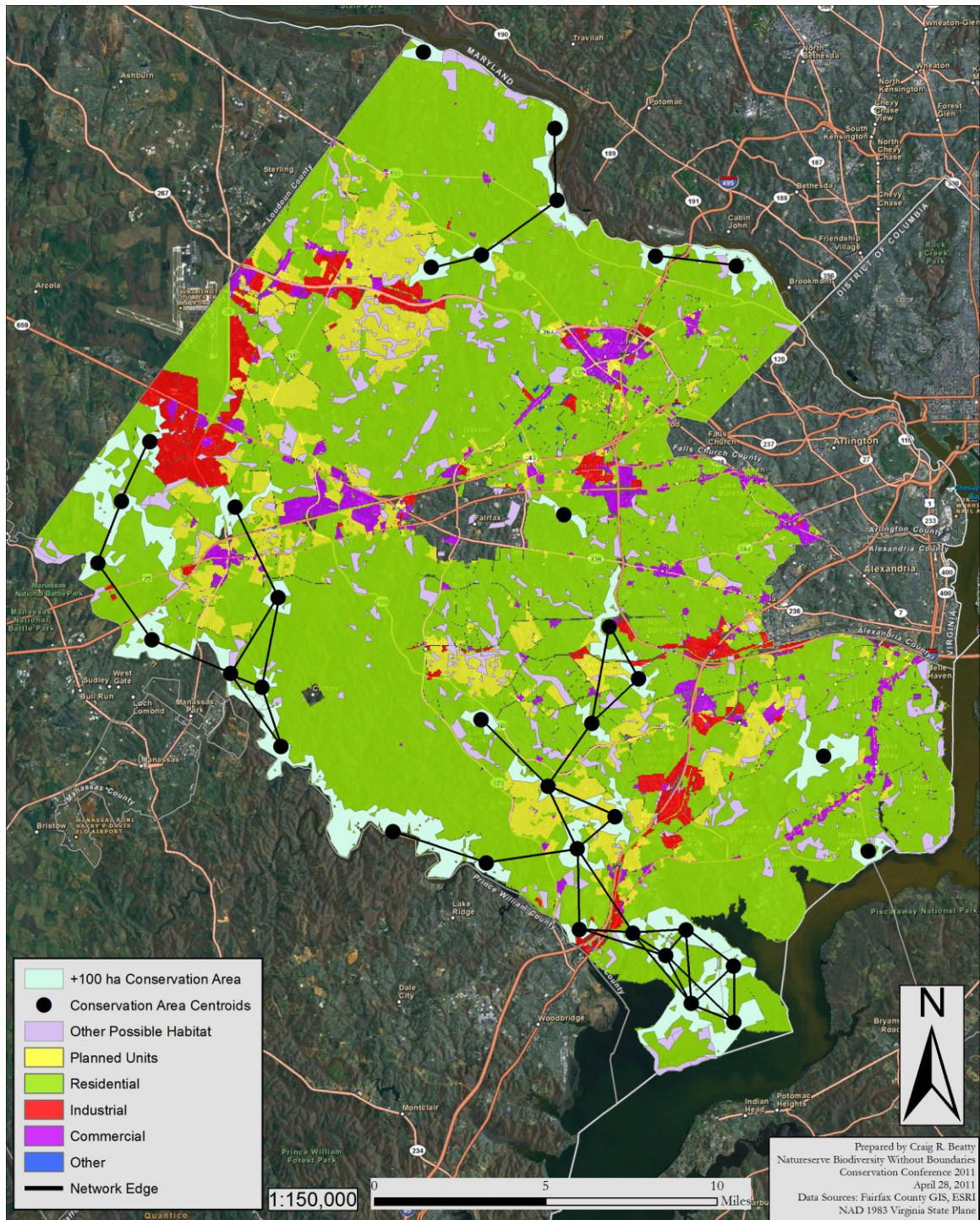
Figure 17. Euclidean dispersal network between possible habitat patches greater than 100 ha in area, created when the dispersal distance of _E. marcellus_ adults is set at a maximum of 3 miles.
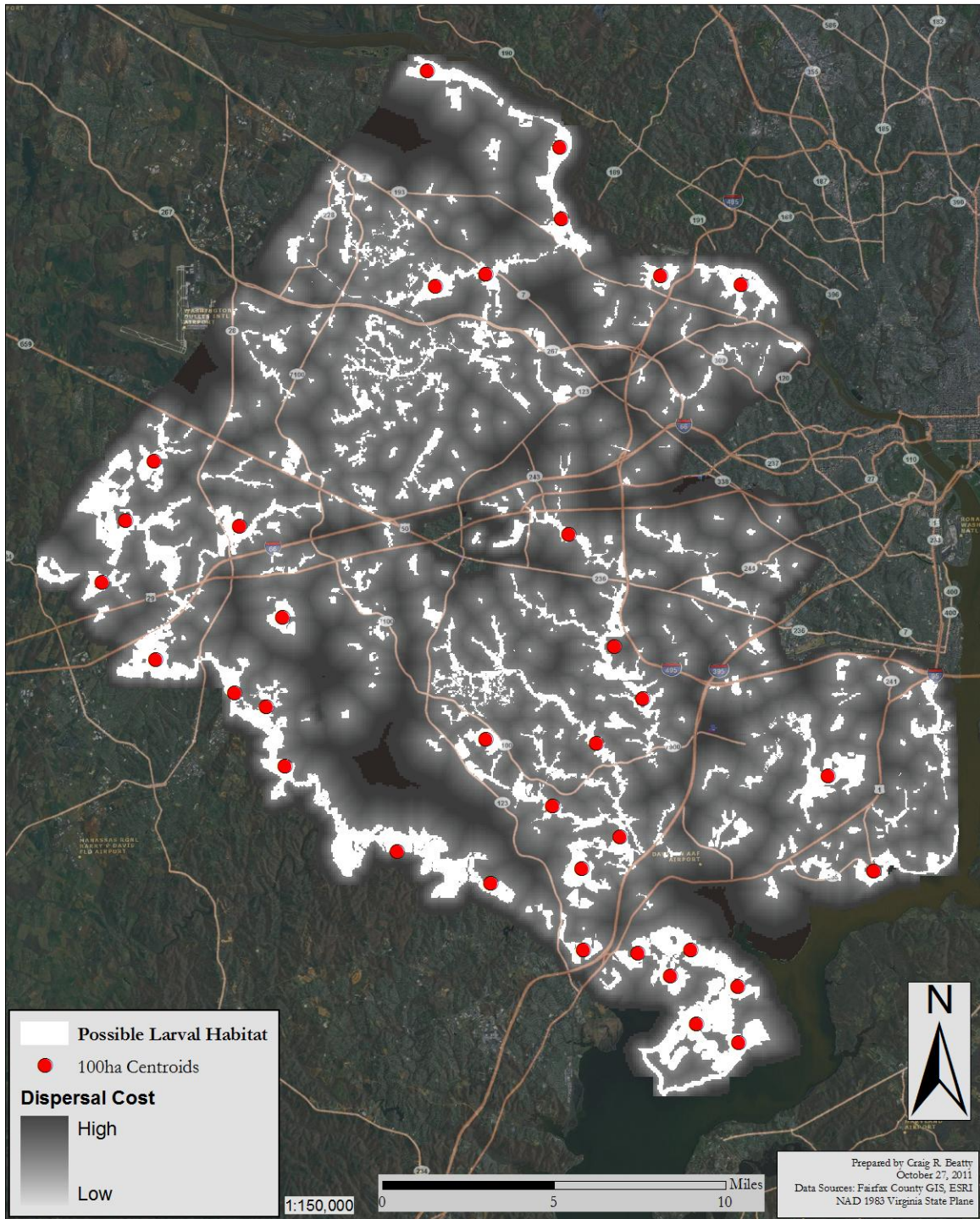
Figure 18. Cost-distance dispersal model. Darker grey areas indicate a low probability of adult dispersal from possible larval habitat.

The Individual Dynamics of Three Butterfly Life Stages: Modeling the Aggregated Individual Zebra Swallowtail (*Eurytides marcellus*).

Introduction

Accurately assessing the viability and dynamics of populations in disturbed habitats is becoming increasingly important in light of habitat destruction and the accelerated extinction rate faced by organisms the world over. Traditionally, ecologists have used mathematical models of population and metapopulation dynamics to explain and predict population viability. These models, such as Lotka-Voltera, and the derivatives thereof, have been instrumental in shaping policy regarding endangered and threatened species (Rockwood 2006). Combined with novel statistical analyses through combinatorial methods such Bayesian analysis, these models are attempting to deconstruct some of the complexity inherent in natural systems and population fluctuations (Cressie *et al.* 2009). However, despite their predictive prowess, these methods are computationally intensive and often subjective by design. In contrast, individual-based models provide a field in which parameters can be quickly shifted and resultant observations evaluated while retaining differing levels of stochasticity in both environmental variables and individual behavior.

The utility of individual-based models as a means for scientific explanations in ecology relies on their ability to include behavioral and stochastically mediated differences among individuals. Classical ecological models such as the logistic and Lotka-Volterra models describe ecological interactions based on the average values aggregated from individuals in a population and in many cases are purely phenomenological (Peck 2004, Rockwood 2006). These models also often fail to take into consideration that many organisms experience different ontogenetic stages throughout their lives

that impact their pre-reproductive lives and post-reproductive mortality rates. The utility of the individual-based approach lies in its ability to include variation at any appropriate step in the model. The outcomes of this variation have led to the emergence of individual-based modeling as a legitimate method of simulating actual populations within or without of the paradigm of ecological equilibrium theory (DeAngelis and Mooij 2005).

To date, there has only been one published study of an individual-based model of population dynamics of a butterfly. Griebler and Sietz (2002) describe an individual-based model of *Maculinea arion* (Lepidoptera: Lycaenidae), an endangered species of Blue butterfly that has very specific habitat requirements and a complex life history. This species briefly requires *Thymus spp.* or *Origanum vulgare* host plants and colonies of red ants in the genus *Myrmica* to support the caterpillars during a prolonged final instar. Caterpillars emit a chemical cue that mimics ant larvae, and worker ants carry the larva into the ant nest and tend to it as if it were an ant. The caterpillar consumes ant larvae until it pupates and emerges as an adult within the nest. Their model focuses on the life of the caterpillar on the initial host plant, the adoption of the caterpillars by *Myrmica spp.* ants, and the life of the caterpillars within host ant nests. They perform sensitivity analysis on their results and conclude that the number of eggs laid per adult and the proportion of queenless nests (those in an increasing state of entropy) were the most sensitive parameters contributing to population variation.

The study by Griebler and Sietz (2002) is specifically focused on identifying the parameters that are most important in the survival of *M. arion*. The charismatic nature of this species parallels that of the Zebra Swallowtail, *Eurytides marcellus*, and both species have complex life histories that depend on access to a limited number of resources. In the case of the *E. marcellus*, the availability of nectar sources combined with *A. triloba* populations limits the distribution of this species (Grossmueller and Lederhouse 1987). Exploring the parameters influencing *E. marcellus* populations through an individual-based model is a valuable avenue considering its potentially threatened status,

the spatial and temporal complexity of its life history, and its exceedingly low densities, which preclude experimentation or statistical analysis of abundance data.

Overview

Following, I present a simple outline of an individual-based model created in Netlogo (Wilensky 1999) based on the Overview, Design Concepts, and Details (ODD) protocol described by Grimm *et al.* (2006). The purpose of this model is to visualize the interactions between *E. marcellus*, their adult nectar sources, and larval *A. triloba* host plants. An additional aim of this model is to simulate the potential population dynamics of *E.marcellus* depending on several modeled factors: the growth habits of *A. triloba* regarding seasonality, the affinity of *E. marcellus* larvae to consume young leaves, and the availability of adult nectar sources proximal to *A. triloba*. This model seeks to explain the crucial balance between adult and larval survival as a function of plant growth and seasonality. Specifically, I ask, "To what degree does the concentration of nectar and amount and timing of larval food sources impact the ability of *E.marcellus* adults to successfully reproduce"?

Within the model there are five agents: *E. marcellus* eggs, *E. marcellus* larvae, *E. marcellus* adults, *A. triloba* plants, and nectar sources. The model space represents a heterogeneous stylized environment focused on patches with associated *A. triloba* populations and randomly distributed nectar sources (Figure 19). The plants are stationary within the model and their population dynamics are not explicitly modeled. The mobile agents are the *E. marcellus* adults, and to a lesser degree the *E. marcellus* larvae. The eggs of *E. marcellus* are stationary and assumed to be distributed randomly by the adults of the previous generation. Below, I first describe the environment in which these agents occur and then I provide an explanation of the behavior of the mobile agents.

Design Concepts

Plants

All patches begin the simulation colored grey, to signify the absence of plant growth. Those patches that remain grey throughout the simulations are not an explicit aspect of the model, but they do serve a purpose in the general spatial arena. In reality, these patches could represent natural areas of little interest to the populations considered in the model. Conversely, they could represent areas of human habitation. Regardless of their meaning, the patches that remain grey through the simulation provide no barrier to butterfly movement, other than the movement itself, which is considered energetically costly for the butterfly.

A yellow "plant" icon signifies patches containing *A. triloba*. The model represents seasonal growth of *A. triloba* through the correlation of leaf age and temporal position. *Asimina triloba* first expresses new leaves at the beginning of the growing season. This condition is represented by the patch under the yellow plant changing from grey to green. The amount of energy available to the agents at this stage is modifiable in the graphical user interface and is an important component of the model. The success of the caterpillar populations depends on the amount of energy from new leaves that they are able to convert into "life-energy." The amount of energy present in the leaves was modeled because nutritional content of leaves can change on a system-wide basis. System-wide nutritional change can result from several factors that affect all plants simultaneously, including changes in climate, soil moisture, environmental chemistry (e.g., CO_2), extensive herbivory from insect outbreaks, and availability of light (Haribal and Feeny 2003, Scriber et al 1995). The patch on which the plant sits changes from green to brown to model leaf maturation. At this point the energy contained by the leaves is modeled as half of the original energy present in new leaves. As the leaf

matures, it becomes tougher, more toxic (due to increases in concentration of annonaceous acetogenins (see chapter 2), and contains less water. At this stage in the development of the plant a caterpillar is unable to digest the leaf material and either starves, moves in search of better quality plants, succumbs to the toxic compounds present in the leaf, or completes its metamorphosis and emerges as a butterfly. In the model, caterpillars that have gained enough energy from new leaves complete their life cycle and emerge as butterflies. *Asimina triloba* finishes its seasonal succession by senescing all leaves. This is represented by the patch changing color from brown to grey. This stage contains no leaf growth and so patch energy is set at zero.

This model also simulates the availability of nectar sources for newly emerged butterflies. Nectar sources are represented as blue plants in the model and are distributed randomly throughout model space. When these plants contain nectar the patch on which they sit turns blue. As butterflies move through the model they come upon nectar plants and consume the nectar. Following the visit of a butterfly to a nectar source the patch changes color from blue to white. This change in color is merely to visualize the pattern of nectar consumption by butterflies. Because nectar sources can continue to produce nectar in reality, in the model the nectar source is not exhausted by the visit of one butterfly. However, it should be noted that the butterflies are programmed to move to a random series of different flowers. This prevents them from simply finding a nectar source and sitting on it, and represents the temporal depletion of nectar that would occur in real settings. Similar to *A. triloba*, the availability of nectar plants to *E. marcellus* is as ephemeral as the growing season. Following the death of the butterflies all nectar plants return to the no-nectar stage indicated by a grey patch.

Butterflies

In the design of this model, the life histories of agents were considered on several levels. First, the emergence of predictable shifts in ontogenetic stages during successive generations of *E. marcellus* in this model is dependent on reported behavioral and life history data of butterflies. The

timing of caterpillar emergence and metamorphosis into butterflies is consistent with published data on this species (Damman 1989). Although all of the specific details, such as energetic requirements, habitat suitability, dispersal distance, survival rates of eggs and larvae and adults, and over-wintering success of pupae are currently unavailable, closely related species served as legitimate proxies for the life history parameters of *E. marcellus* included in the model (Groom *et al.* 2006).

There are three classes of agents representing three of the four life stages of butterfly ontogeny (pupal stages were not modeled). The model is initialized containing a variable number of eggs in the habitat. This variable can only be changed during the setup phase; although, an "immigration" option in the model allows fifty eggs to immigrate to the area (representing new adult oviposition within a season). Eggs are laid at random in the model space by the previous generation of adults. Although a random distribution of eggs is not found in nature - females evaluate *Asimina triloba* quality and choose specific oviposition sites - a random distribution is useful in characterizing the population dynamics of butterfly eggs. In the model, eggs only hatch into caterpillars if they are laid on cells containing *A. triloba*. The growth and development of caterpillars is dependent on their access to new leaves and the energy provided by the leaves. In this sense, the caterpillars are mobile agents. If the energy from the new leaves does not translate into enough energy for the caterpillar to survive, the model directs the caterpillar to move and seek out a new food source. However, movement incurs energetic costs and these are explicitly included in the model. For instance, if the caterpillar moves to another patch and that patch does not contain any *A. triloba*, the "life" of that individual is divided by two. Thus, the survival and metamorphosis of the caterpillar is almost entirely dependent on spatial factors beyond its control, which is a realistic situation. Should the caterpillars gain enough energy from new leaves and avoid the pitfalls of movement within the model, they will emerge as butterflies after the leaves mature. However, if individual caterpillars do not secure the necessary energy requirements, they die and are removed from the population.

As butterflies, the individuals have two goals 1) find a nectar source and 2) reproduce by laying eggs. The number of nectar sources in the model space is a variable that can be modified during the setup process. The probability of the butterflies in finding a nectar source is modeled as much greater than the probability of a caterpillar finding a food source based solely on the greater movement capabilities of adults. The butterflies move through the model space in a random direction in front of them and forward a random number of patches between 1 and 10. The amount of "life" lost in searching for nectar is the square-root of the energy value of the nectar sources. The butterflies also have additional "life" deducted if, after their movement, they land on a patch that does not contain any nectar. If the butterflies secure enough energy from nectar sources they lay 10 eggs. Although it has been demonstrated that some swallowtails can lay several hundred eggs (Feeny *et al.* 1985), the model was calibrated to 10 eggs as a conservative estimate based on field surveys (see Chapter 2). Because the model includes neither the possibility for emigration from the model space nor any estimation of predation or failure to find mates, it was important to choose a number of eggs that produced realistic dynamics. The 'life' of each egg is equal to the life of the adult divided by the square-root of the energy from the nectar sources, except in the initial model setup where an egg is given a life equal to 5. Following the oviposition of eggs, all butterflies die and the model space enters the no-growth phase.

Energy requirements of mobile agents

The energy available to any successful life stage is a function of the energy gained from resources. However, within the model, a semantic distinction was made between "energy" and "life". The resources produced by plants in the model were coded as "energy". This allowed for the separation of first order plant energy from energy as it moved through trophic levels. After energy was consumed as either leaf material in the case of caterpillars, or as nectar in the case of butterflies, it was converted to "life". In the conversion from "energy" to "life" an inverse square relationship

between "energy" and "life" was necessary to account for incomplete metabolism of plant energy. An inverse square relationship was chosen because it most closely approximates the 10% ecological efficiency rule between trophic levels (Slobodkin 1960), while at the same time it introduces stochasticity based on the randomly distributed energy levels of each energy source in the model. As a result, not all individuals gained the same amount of 'life' from consuming identical types of energy sources.

Mobile agent interaction

No attempt was made to show the distinct interaction between individuals of a life stage. Eggs did not interact with other eggs because *E. marcellus* lays only one egg per plant. However, the model accounts for exploitative competition in both larval and adult stages. Caterpillars interacted with each other only if they hatched on the same plant. If energy was exhausted on a plant the caterpillars were forced by their 'life' requirement to search for a new source of food. Adult butterflies indirectly affect each other through consumption of nectar sources.

In addition, it should be noted that, as is common in modeling, all individuals within the model are considered female. Modeling males would require knowledge of energetics and success of mate location, degree and cost of male-male competition, energetics and length of sperm storage by females, numbers of eggs fertilized or reabsorbed, and links between paternal care and larval survival. Such data are not available for this species, nor are they easily modeled. Therefore, the final results of population counts in the model simulations were doubled to reflect a 1:1 sex ratio.

Agent fitness

The 'life' and resulting fitness of each agent in the model is not explicitly modeled. However, as agents reproduce the amount of "life" from the parent is transferred to the egg. Over evolutionary time, it is possible that some "genetic" lines will increase their "life" spans. However, the purpose of

this model was not to explore inherited fitness. Therefore, the model terminates these individuals when they reach "life" level above 1000.

Stochasticity

The variability in individuals and environmental conditions is modeled through the conversion of "energy" to "life" acquired by individuals in the model. In addition, the random movement of caterpillars and butterflies, and the random location of the oviposition of eggs by adults contribute to stochasticity within the model. For instance, the energy from *A. triloba* is a variable in the graphical user interface of the model. However, to account for the variability in plant quality, a random number between zero and five is chosen from a Poisson distribution and added to the set energy level. This embeds metabolic variability in each individual.

Model Details

Initialization.

At the beginning of each simulation, set-up inputs eggs, *A. triloba*, and nectar sources to the model space. All agents are bound within an environmental space that does not wrap. This means that as an individual approaches the edge of model space, it cannot be transported to another area in the model. This provides a framework in which more realistic population dynamics can emerge. The initialization that results in the strongest population dynamics across generations requires that the availability of *A. triloba* and nectar sources be relatively high. All agents at initialization are distributed randomly in model space. The random distribution of eggs leads to the immediate failure of some eggs to hatch, which is the process that accounts for the low hatching success of caterpillars in the model, a proxy for many environmental factors and a necessary constraint on exponential population growth.

Input

Table 3 provides the distributional ranges for each of the model parameters. Initial egg,

caterpillar and adult life parameters were set at 5. Parameters for the number of eggs, pawpaw plants,

and nectar sources are between zero and 1000. These values were chosen as a result of the available

model space, which contains 1089 individual cells where agents and the stylized environment can

interact. Energy values were given possible values of between 0.1 and 10.0. These values were

selected based on their proximity to initial "life" values for agents and they allow for a realistic gain

or loss of energy as a result of the costs of movement. For instance, caterpillars are initialized in the

model at a "life" value of 5. As each caterpillar consumes plant material, it converts the "energy"

from the plant into "life", which is additive to its total "life" value. However, if a caterpillar must

move to a neighboring plant, its "life" level incurs a 50% penalty for moving to an area that contains

no available leaves.

Arrays of behavior space experiments were run in Netlogo 4.1.3 (Wilensky 1999) to

determine the parameters at which *E. marcellus* populations were most successful. All simulations are

comprised of 100 repetitions consisting of 500 time steps in each scenario. Data was compiled and

analyzed using Excel. Each parameter was swept through the range of values provided in Table 3,

while remaining parameters were held constant at a given value, until it had no additional effect on *E.

marcellus* populations in the model. All results in the iterations of this model are reported in terms of

final adult female butterfly populations.

Results

Sensitivity analysis of the model parameters determined that butterfly populations were most

sensitive to changes in the number of *A. triloba* plants in the environment. This sensitivity was not

surprising since the density of *A. triloba* is directly related to the success of eggs hatching and the

subsequent survival of caterpillars in the model. Parameter sweeps revealed that if all variables were

76

at 50% of their maximum, a minimum of 100 eggs was needed to maintain a population for 500 years. This indicates that even a small immigration event by one to two females to an area with high *A. triloba* population density has the potential to establish a population center. Further increasing the number of eggs laid or the number of *Asimina* plants had positive consequences for maintaining population density of butterflies over the 500-year period, as did increasing the energy content of *A. triloba* and nectar sources. However, at 50% parameter levels, increasing the number of eggs above 300 had little effect on the mean population size in model iterations. Average population values remained at approximately 100 individuals despite an increase in eggs laid, and remained at approximate 150 individuals despite an increase in nectar sources.

After determining that 100 eggs was sufficient to maintain *E. marcellus* populations at 50% parameter levels, further analysis of *A. triloba* density was modeled. The density of eggs in these simulations was set at 100 and nectar sources were placed at 100% of parameter value (1000 individuals). The model was run for 10 *A. triloba* densities at 100-individual intervals between zero and one thousand plants. The model shows that below approximately 400 plants, *E. marcellus* populations are unsustainable and quickly become extinct. However, as the population of plants increased in the model, so did the population of *E. marcellus* until population sizes leveled off at approximately 1000 individuals.

Discussion

The relationship between *Eurytides marcellus* and *Asimina triloba* is highly dependent (Erhlich and Raven 1965). The development and fitness of *E. marcellus* is directly dependent on its ability to metabolize the tissue of *A. triloba*; it is no surprise that the population dynamics of a monophagous butterflies are so heavily dependent on the availability of host *A. triloba*. The more pertinent question

posed by the implementation of this model was, "how do populations respond to variability in access to larval host plants versus adult nectar plants"? Answering this question through looking at hypothetical population scenarios shows the utility of an individual-based model approach. Specifically, the model showed that density of *A. triloba* was more important than adult resource in determining population persistence. The result of increasing *A. triloba* density is that the number of larvae hatching into caterpillars increased, and increased eclosion and higher successful ovipositional probability lead to higher population densities of butterflies. However, the model indicated that population sizes were less dependent on nectar sources than anticipated. Increasing nectar plants about 400 individuals did not increase population persistence.

Another interesting aspect of this model was the relationship between agents and the energy they acquired. In the first several versions of the model, butterfly populations would grow exponentially without an upper bound. The mathematical relationship between eggs, caterpillars, and butterflies could not be exponential for the simple observation that we are not buried underneath miles of *E. marcellus* butterflies. Population ecology refers to this check on exponential growth as the environmental carrying capacity. At some point, the resources consumed will out-pace the ability of those resources to be produced. This leads to declines in populations in a strictly economic dance of supply and demand that, for the most part, does not occur in natural systems. There are several reasons why populations are not strictly economic, but the most important in this case is that individuals interact with their environment and each other in varying capacities. Each individual in the model is slightly different and these differences often scale up to produce emergent population dynamics. It is important to note that *E. marcellus* does not occur in high density, even in areas with relatively large *A. triloba* populations (Damman 1989, C. R. Beatty, personal obs.). Within this model, the external causes of mortality at each life stage of *E. marcellus* are not explicitly modeled, but rather combined in the energetic relationships between life stages. If an individual does not acquire the

necessary stores of resources, she is unable to reproduce. The model is programmed such that each

stage in the life cycle of *E. marcellus* will have fewer individuals than its initial number. The exception

to this are the eggs laid by females because there is not a 1:1 relationship between butterfly and eggs

(i.e. butterflies lay many eggs but only 1 egg can become 1 caterpillar). These mathematical

relationships ensure that the environment is not overrun by any stage of butterfly development and

more closely matches population dynamics observed in nature.

In order to further verify and validate this model to natural situations several modifications

should be made in the future. Currently, the space within the model is stylized. Although a random

distribution of *A. triloba* and nectar in model space somewhat accounts for a random distribution of

E. marcellus, future editions of the model should create an environment that is spatially relevant to

real-world situations. For instance, *A. triloba* generally occurs in mostly homogenous, isolated and

dispersed patches in riparian areas. Outside of riparian areas, one would not expect to find *A. triloba*

populations. Although this model is scaled to represent a locally restricted riparian area, future

models should inform the landscape to add differences in elevation and temperature, and more

stringent attention to ecological communities and interactions surrounding *A. triloba* patches.

Another interesting addition to this model would be the population dynamics of *Asimina*

triloba and the nectar sources present in the environment. The model presented here did not take into

account the survival and reproduction of plants upon which butterfly populations depend. Instead, it

treated these populations as static and occurring on a consistent basis. Addition of these factors

would be particularly interesting with regard to the clonal growth patterns of *A. triloba*. Future

models should include sub-models of the population dynamics of *A. triloba* (both clonal and seed

generated populations) and nectar sources over short-term and long-term time scales.

Finally, missing from the ecological processes modeled here were disturbance regimes. One

of the most important aspects of conservation is the extent to which human activities affect natural

populations. Future models should include anthropogenic forces such as residential encroachment, recreational use patterns, or land-use change that would increase edge effects or reduce patch size. The model presented in this paper lays the ecological foundation of *E. marcellus* population dynamics, and the inclusion of anthropogenic disturbance through the conversion of available habitat or the introduction of invasive species would be a logical step in future models.

Conclusion

Butterflies are some of the most intensely studied and scientifically informative creatures in the natural world. They are charismatic, gentle, easy to spot, and, in many cases, have very strict environmental and ecological requirements. A reoccurring challenge for conservation studies are the often fragile conditions faced by species of concern. Although it may be possible to collect field data on a species, biological data collection often must involve collection of individuals for study. For species of conservation concern, population dynamics may be too stochastic or population levels too low to warrant actual field collection of relevant data. A Red List status of endangered or threaten likewise will preclude collection or manipulation of individuals. Individual-based modeling affords scientists the opportunity to carry out population simulations on threatened species without placing them further in jeopardy.

However, despite the advances in computational power and modeling platforms the perspective must continue to hold that models are helpful in explaining natural phenomenon, but they are unable to capture the extent of natural complexity. The model explained in this paper begins to tease apart how *E. marcellus* depends on *A. triloba* as well as ambient nectar sources. It shows that the population dynamics of *E. marcellus* ultimately depend more heavily on access to its larval host plant *A. triloba*, and it suggests that unlike the majority of open-habitat butterfly species, Zebra

Swallowtails may not respond to augmentation of nectar sources. Thus, in contradiction to the widely-held assumption that Zebra Swallowtails are not at risk of extinction, populations may be as vulnerable as smaller, coevolutionarily limited species like ant-tended Blues (Grundel and Pavlovic 1998) or long distance, migratory monarchs (Brower and Malcolm 1991).

Table 3. Ranges of model input parameters

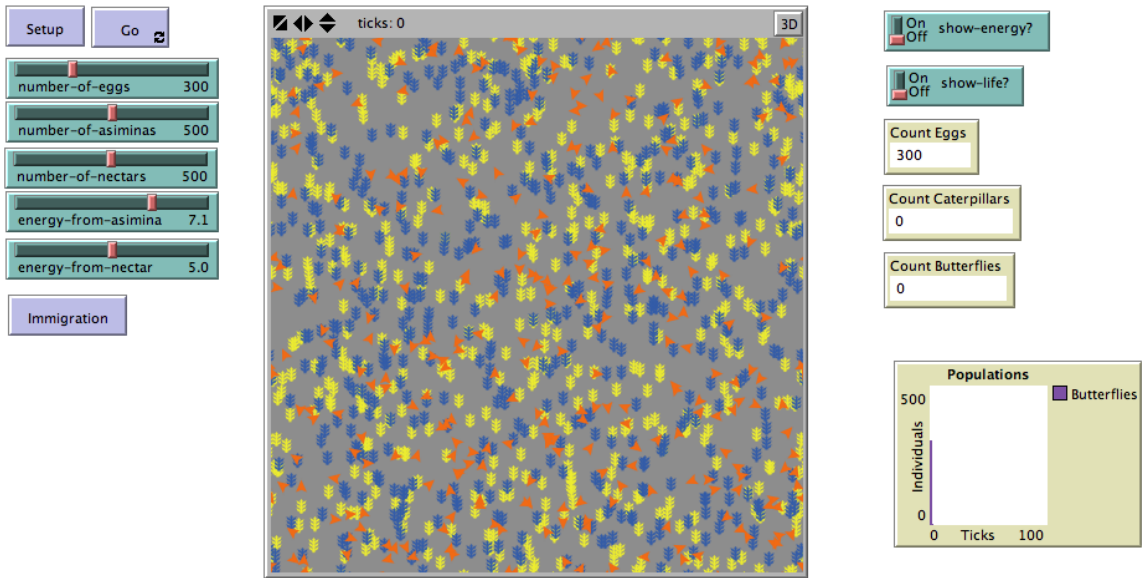| Parameter	Values
Number of Eggs	0 - 1000
Number of *Asimina triloba*	0 - 1000
Nectar sources	0 - 1000
Energy from *A. triloba*	0.1 – 10.0
Energy from nectar	0.1 – 10.0
Initial butterfly life	5
Initial caterpillar life	5
Initial egg life	5
Model Space	1089 patches

Figure 19. Graphical User Interface of the Zebra Swallowtail individual-based model. Orange arrows indicate butterfly adults, yellow plants indicate *A. triloba* individuals, and blue plants indicate nectar sources.

Appendix

Assay Protocol for Brine Shrimp Toxicity of *Asimina triloba* Extracts

<u>Sample Preparation</u>:

1. Collect leaves from field plants into appropriately labeled paper or plastic bags and keep on ice until they are returned to the lab.

2. Record wet weights of leaves following appropriate protocol for using the analytical balance. If leaves are to be used for estimates of leaf area, label each leaf using a Sharpie and scan on the flatbed scanner following the protocol for the ImageJ program.

3. Place leaves in appropriately labeled (site, plot, tree, collection date) paper bags and place them in the oven to be dried at 35 °C.

4. Record dry weight of leaves following appropriate protocol for using the analytical balance

5. Grind leaves into fine powder using Wiley Mill and a 40 grade mesh screen. Follow appropriate procedures for grinding plant material using the Wiley Mill. A portion of each leaf to be ground in the Wiley Mill will need to be set aside to be pulverized as a part of the "bulk" standard.

<u>Solution Preparation and Set up</u>:

Hatching brine shrimp:

Brine shrimp must be hatched 48 hours BEFORE conducting the BST assay because it takes 24 – 36 hours for *Artemia* to hatch, and newly hatched nauplii are too small to count. You will need approximately 5 mL of brine per plant sample, and can conceivably do up to 30 samples per week. Preparing 100 mL of *Artemia* should provide you with extra *Artemia* in the event you need to redo samples; however, since *Artemia* do not live much more than 4 days, if you prepare more than this, you will waste your brine shrimp eggs. In addition, when preparing brine to hatch *Artemia* you will need to reserve some of the brine solution without *Artemia* to add to your final culture tubes

1. Prepare 4% Sodium Chloride solution (brine) by mixing 4g NaCl per 100 mL DDH_2O in large beaker. Place on stir plate with stir bar, stir until all NaCl is dissolved.

2. Add 0.05g dried brine shrimp eggs per 100 mL brine in a large beaker.

3. Swirl **gently** by hand (do not use vortex genie or stir bar as vigorous shaking can kill brine shrimp).

4. Cover the beaker with parafilm, leaving enough room at the spout of the beaker to insert an air tube.

5. Place beaker in incubator and place air tube in brine with eggs. Ensure the temperature remains at 25 C for 24 hours and that the chamber is illuminated.

Extraction of acetogenins:

1. Label a 2 mL centrifuge tube with plant sample number for each plant you plan to assay.

2. Tare each tube and weigh 0.1 g of dried, ground leaf material into the appropriately labeled tube.

3. Using a graduated pipet, add 1 μL methanol for every 0.0001g of ground leaf material.

4. Cap sample and agitate gently on vortex genie.

5. Place vials in floating tube rack and sonicate for 2 hours at 5 Degas. Insure that the solution temperature does not exceed 30°C. Ice may be added to the sonicator to reduce the temperature.

6. Allow samples to extract for 24 hours. Record time extract solution was added to tubes and record exact length of time that samples were extracted before allowing samples to evaporate.

7. Centrifuge samples for 25 minutes at 24 °C and a speed 4500 radians.

8. Decant samples into **pre-weighed** and appropriately labeled 2mL centrifuge tubes.

9. Place sample vials in Speedvac and evaporate for 3 cycles at 30°C and 30 minutes per cycle.

10. Re-weigh vial to determine sample yield. Record sample yield on appropriate data sheet.

11. Recap vial. Place all vials into test tube rack. Place test tube rack inside two plastic bags and place Drierite desiccant in outer bag. Label inner plastic bag with appropriate plant sample information. Store test tube rack in lab freezer.

Brine Shrimp Assay:

1. Label four 2-dram vials with leaf sample number for each leaf sample you plan to assay and each treatment (e.x. HOA001 a, HOA001 b, HOA001 c, HOA001 d).

2. Remove plant extracts from lab refrigerator.

3. Add 1000 µL methanol for each 0.0100 g of extract. Add the appropriate amount of methanol to each tube to retain this ratio of methanol to extract. For example, 0.0025g of extract would require 250 µL methanol. Cap tubes, and vortex gently. Allow samples to sit until extract has dissolved. This is solution A.

4. Add 25 µL of solution A to the second appropriately labeled 2 mL tube and add 975µL methanol, cap tubes, and vortex gently to mix. This is solution B.

5. Transfer the following amounts of solution to each piece of filter paper, allow to air dry

Culture tube	Solution A	Solution B	95% Ethanol	Brine + 10 shrimp	g extract/mL
a	0	0	25 µL	5 mL	0 (control)
b	0	25 µL	0	5 mL	1
c	25 µL	0	0	5 mL	10
d	100 µL	0	0	5 mL	100

6. Allow samples to dry in 2-dram vials for 4 hours

7. Using a clean disposable glass pipette, transfer 5 mL of brine (without *Artemia*!) to each 2-dram vial.

8. Using a disposable glass pipette, transfer 10 brine shrimp from the stock solution to a holding area in a ceramic droplet plate. Make sure to fill the droplet cell completely with brine. Recount to insure you are transferring ONLY 10 brine shrimp. Transfer all *Artemia* from each droplet cell into its respective 2-dram vial. When transferring *Artemia*, you will also transfer all brine in the droplet cell.

9. Place tubes in incubator.

10. After 12 hours, using a hand lens, record the number of dead **and** the number of surviving *Artemia* in each culture tube.

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Curriculum Vitae

Craig R. Beatty immigrated with his immediate family to the United States from Calgary, Alberta, Canada in 1998. He has been a clerk, waiter, bartender, laborer, telemarketer, educator, sound engineer, radio host, musician, political activist, and chocolate maker. He went on to earn a Bachelor of Science from the University of South Florida in 2007 after rediscovering a love for ecology and paleontology. He has involved himself in various forms of ecological research in Florida, Virginia, Mexico, Tazmania, and Greater Australia. For many years, Craig will continue with spatial and conservational aspirations around the globe, and from his home, wherever that may be.