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Cover Page Footnote

Thank you to Nova Southeastern University for providing its campus as the location of study. Dr. Joshua Feingold generously shared his photographs of the studied species and expertise as an advisor.

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I. Abstract

Eumaeus atala is an endangered tropical butterfly native to the Caribbean and some parts of Florida, USA. Following population reductions primarily due to habitat loss, *E. atala* populations are now increasing due to conservation efforts of its cycad host plants, especially *Zamia integrifolia* (coontie). The purpose of this study was to observe, document, and measure the population of wild *E. atala* on the Ft. Lauderdale, Florida campus of Nova Southeastern University where landscaping use of host plants supports a natural population of *E. atala*. Forty-four host plants located in two different sites were observed for 14 weeks. One site (Baseball Field) possessed most of the host plants (n=40) that were packed closely together in direct sunlight, while the other (Medicinal Garden) had smaller plants (n=4) that were spread out in a shaded environment. On each survey date each plant was inspected to quantify the number of larvae (caterpillars), hatched and unhatched pupae, and butterflies. Butterflies were observed on 8 of 14 observation dates. Most had recently emerged from chrysali, and some were observed laying eggs. Plants ranged in hemispheric area from 2.3 cm^2 to 2,776.5 cm^2. Larval densities ranged from 0 to 0.14 larvae cm^{-2}, and 77.3% of the plants hosted larvae at some point during the observation period. The results of this study showed notable population increase of *E. atala* in the autumn, likely skewed by heavy rainfall and a tropical storm in the latter portion of data collection. This study documents the importance of native plant landscaping at NSU to support an endangered species, and these data can be used as a baseline for future studies.

II. Introduction

Eumaeus atala (atala or coontie hairstreak) is a tropical butterfly from the family Lycadenidae (Contreras-Medina et al., 2002). It was first discovered circa 1832 by Cuban zoologist Felipe Poey, who named the species after the titular heroine of the 1801 French novella (Bartlett et al., 2004). The species is native to the Bahamas, Cuba, the Cayman Islands, and several other Caribbean islands. There is also a subspecies (*Eumaeus atala florida*) found in southeastern regions of Florida, although they can also occasionally be found in suburban or conservation areas throughout the United States (Koi, 2008). Despite challenges with maintaining populations, *E. atala* is a resilient species, having faced the threat of extinction multiple times during the past century. The rapid population decline and presumed extinction of *E. atala* from 1937 to 1959 was caused by overharvesting their favored host plant, *Zamia integrifolia* (coontie), by early Floridian settlers (Koi & Hall, 2015). This host plant serves as a food source and is crucial for the development and survival of *E. atala* during its life cycle from egg to pupa (Koi, 2008).

A typical *Eumaeus atala* begins its life as a cream-colored egg about 0.5-1.0 mm in diameter, deposited in clusters averaging 30-60 eggs on the fronds of a *Zamia integrifolia* or other Cycadaceae family plant by an adult *E. atala* (Rothschild, 2001; Koi & Hall, 2015). After this process of oviposition, the eggs remain on the frond for approximately 4 to 5 days until it hatches as a larva (caterpillar) (Culbert, n.d.).

These larvae are initially pale green (Fig. 1) and rapidly transition to a bright red hue lined with seven pairs of yellow spots on their dorsal sides (Fig 2). This distinctive coloring is a

clear indicator of aposematism, warning predators of their cycasin-rich bodies (Bowers & Larin, 1989; Rothschild, 2001). Therefore, most spiders and birds avoid *E. atala*, although Pharaoh ants, fire ants, and other ant species are among their few predators (Smith, 2002; Koi & Hall, 2015). The toxicity of *E. atala* aids them during a vulnerable stage of their development, where they must feed on their host plant for about two weeks until they reach their pupal stage (Culbert, n.d.). The quality and availability of host plants consumed during the larval stage largely determines the survival rate during the pupal stage, as well as the size of adult butterflies if they emerge. After two weeks within the chrysalis, an adult *E. atala* emerges (Culbert, n.d.).

Adult butterflies (Fig. 3) are easily recognized by their orange abdomens and dark blue wings marked with teal iridescent spots (Koi & Hall, 2015). Without careful observation, male and female atala butterflies appear indistinguishable. Both sexes have a wingspan of approximately 1.5 inches (3.81 cm) and a slow, moth-like flight pattern (Culbert, n.d.; Koi & Hall, 2015). However, male atala butterflies have consistently been observed to be slightly smaller than their female counterparts, with a differing inner wing pattern; males tend to show a greenish tint (in colder seasons) or teal (in warmer seasons) with black veins, while females are a true royal blue with a line of iridescent spots on the edge of their hindwings (Koi & Hall, 2015). Adult atala butterflies feed on flower nectar from plants such as tropical whiteweed, bluemink, coriander, and several others (Koi, 2008). They live the rest of their short lives fertilizing or laying eggs on a cycad host plant until they die roughly a month after their emergence (Koi & Hall, 2015).

Nova Southeastern University in Ft. Lauderdale, Florida, has developed a small population of *Eumaeus atala* from larval to butterfly stage in the past decade due to its settlements of ornamental cycad plants (Koi, 2008). A total of 44 plants were measured and

observed for this study. The three cycad plants studied in this survey*: Zamia integrifolia* (n=41), *Zamia polymorpha* (n=2), and *Dioon spinulosum* (Gum palm) (n=1), were compared to determine their sustainability and popularity among the *E. atala* specimen in this study. There were two location sites on Nova Southeastern University's campus where data was observed and collected. The first was NSU's Medicinal Garden, located behind the Parker building. This garden fostered a small population of *E. atala,* containing four out of the forty-four plants studied in this site. These plants were immersed with a variety of other plant and animal species, and they were somewhat concealed from direct sunlight by neighboring trees. The second site where data was collected was the outside border of NSU's baseball field. The baseball field was lined with five settlements of a total of 40 *Z. integrifolia* plants. These plants were closer packed together with less exposure to a variety of other species, and they also received direct exposure to sunlight.

Thom et al. (2015) documented that *E. atala* preferentially consumes *Zamia integrifolia* over other host plants such as *Zamia polymorpha* and *Dioon spinulosum*. Pupae often settle near the base of the plant, where they are closer to the ground and concealed by thick fronds; this provides a higher chance of survival against high wind, fire, and predators (Thom et al., 2015). Previous studies (Rothschild, 2001; Smith, 2002) documented higher populations and survival rate of larvae and pupae through the summer months. It was hypothesized that the overall population and pupae emergence rate would decrease as summer gradually turns to autumn, since *E. atala* favors tropical temperatures (Smith, 2002). The main goal of this study was to measure the growth and sustenance of the wild *E. atala florida* population at Nova Southeastern University from summer into the autumn months (from larval to butterfly stage). Monitoring the conservation efforts of Nova Southeastern University will aid in understanding how to best

provide for this threatened species in other conservation efforts throughout Florida and the United States.

III. Research Site(s) & Location

*Figures and captions in separate document

IV. Methods

Host plants (*Zamia integrifolia*) were observed at two locations on main campus, 4 plants at the Medicinal Garden in front of the Parker building and 40 plants along the walkway surrounding the baseball field. Since very little activity was observed at the Medicinal Garden (likely due to shortage of host plants and flooding from a tropical storm), data comes from the baseball field site. Two plants that died during the study were also excluded from calculations. These ornamental cycad plantings provide the resources necessary for a small *Eumaeus atala* population (Koi, 2008). Three cycad species were observed in this study: *Zamia integrifolia*, *Zamia polymorpha*, and *Dioon spinulosum* (Gum palm). However, *E. atala* populations were measured only on *Z. integrifolia*.

The activity of caterpillars and pupae on each plant were observed and recorded each Thursday morning for fourteen weeks (August 20th, 2020-November 19th, 2020) at around 8:00-11:00 AM EST because *E. atala* adult butterflies are typically more active in the morning and afternoon (Koi & Hall, 2015). The summer and autumn seasons in this study were defined by the dates of Summer (June 20th, 2020 – September 21st, 2020) and Autumn (September 22nd, 2020

– December 20th, 2020). The number of fronds, height, and perimeter of each plant were recorded twice, during the first week and last week of this study. On each of the 14 observation dates, each plant was observed for its number of live *E. atala* pupae, discarded pupae exoskeletons, caterpillars, and butterflies. Pupae that had fallen off a plant in the nearby soil were counted and attributed to the plant they were nearest to. Each plant had individual levels of growth and *E. atala* sustenance, but their data values progressed in a consistent manner throughout the 14 weeks of study.

Anomalies, such as pupae on nearby palm trees or other non-cycad plants were recorded but not counted in the data and statistical calculations of this study. There was no interference with the wild activity of the organisms except for removal of dead plants and slight shift of pupae that had fallen onto the near concrete back to the soil of the host plants.

A tape measure was used to quantify the length and perimeter of each host plant. The perimeter was used to calculate the hemisphere area of each plant for the calculations, including larval density and statistical t-tests. If a plant had died or was removed before the final week of the study, it was not included in the data figures and calculations. The number of fronds on each plant were counted by hand, and stems lacking these leaf-like fronds were not counted.

V. Data & Results

*Figures, tables, and captions in separate document

VI. Discussion

During the observation period (Summer-Fall 2020) 77.3% of the plants (*Z. integrifolia*) at the baseball field (Fig. 5) hosted larvae. Two other species of cycad were observed at the Medicinal Garden (Fig. 4): *Zamia polymorpha* (n=2) and *Dioon spinulosum* (n=1). However, *E. atala* was rarely observed on *Z. polymorpha*, only showing activity in the first few weeks of study. There was no *E. atala* activity recorded on the *D. spinulosum*. Plants (*Z. integrifolia*) at the baseball field ranged in hemispheric area from 2.3cm^2 to 2,776.5cm^2. Larval densities ranged from 0 to 0.14 larvae cm^{-2} (Fig. 6), and butterflies were observed 8 of 14 observation dates (Fig. 8). Many of these butterflies were newly emerged from their chrysali, assumed by their slow, lethargic movement and wings curled inward. The scarcity of the data collected at the Medicinal Garden was deemed negligible and was therefore excluded from data figures and calculations.

During the twelfth week of study, an unhatched pupa was seen on a palm tree near Plant 17 in Site 2. This is an uncommon but known phenomenon that occurs in nature, likely due to scarce amount of uneaten host fronds left in the settlement during this week. This pupa was not seen again for the remainder of the study, so it is likely that its exposure on the palm tree left itself susceptible to predators.

Observations suggest that *Eumaeus atala* most favored the *Zamia integrifolia* host plant, but the larger amounts of this plant observed compared to the other species may have skewed the results in its favor. The size of each plant was expressed in hemisphere area (m^2), which takes both height and width (frond density) into account. The mean hemisphere area (m^2) of the host plants was calculated for the first and last week of the study to compare their overall growth over time (Fig. 9). To better distinguish the plants' dimensions, the number of fronds on each host plant were also counted on the first and last week of study, with the taller plants generally having

wider bases that supported a higher frond count. A statistical t-test (Table 1) determined that there was also no statistical significance in the mean increase of host plant hemisphere area (t-Test, p=0.10). Plants with high frond count, regardless of height, showed the highest rates of *E. atala* activity. In accordance with Thom et al. (2015), *E. atala* larvae and pupae were consistently observed to be settled near the bottom of fronds only a few inches from the soil. This is an area for future study and analysis.

Regarding seasonality, there were higher caterpillar population (Fig. 6) and pupa emergence rate during the autumn season rather than the summer (Fig. 7). This may be due to the study beginning towards the latter portion of the summer season, where the majority of the generation born had already matured into their reproductive stage. Another factor to be considered is that the baseball field plants were settled on higher ground surrounded by concrete, protecting it from tropical storm flooding during the autumn season. This storm and subsequent rainfall produced higher levels of humidity than usual, which *E. atala* favors (Smith, 2002). The narrow location of data collection was the primary limitation of this study; recording *E. atala* activity among other cycad host plants throughout south Florida can overcome this gap in data. Moreover, it should be questioned if any difference between the Floridian and Caribbean climate in terms of volatility could have affected this study's results. Further research should be done to provide additional data to that reported in this preliminary study. Future replications of this study could feature differing regions, seasons, and other host plant species to determine and compare their population rates of *E. atala*.

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