Exhibit A. Project Prospectus

Forecasting and monitoring "at risk" butterfly species: A case study of the Baltimore checkerspot butterfly (Euphydryas phaeton)

Introduction.

Butterfly populations are threatened by several anthropogenic factors, including habitat loss, fragmentation, and climate change.[1,2] Human population size, projected to increase from 7 to 9 billion by 2050, will exacerbate these factors. One innovative and emerging approach to butterfly conservation is to forecast "at risk" butterfly species; and monitor their population dynamics to proactively develop a management plan well-before they become classified as endangered or threatened.[3,4] The Baltimore Checkerspot (*Euphydryas phaeton*) butterfly (BCB), the state insect of Maryland, is an exemplary case of an "at-risk" butterfly species that is not yet at the brink of extinction.[5] BCB is a wetland butterfly that ranges from Canada into the eastern U.S. While not endangered, it is a "species of conservation concern" in four states (Maryland, Pennsylvania, New Jersey, and Delaware) and constitutes an important case study for this emerging approach to conservation.[5-10]

The Maryland Baltimore Checkerspot Recovery Team (BCRT), a group of citizen scientists led by Jennifer Frye of the Maryland Department of Natural Resources, is leading efforts to conserve BCBs. This effort provides a unique opportunity for me to complete research which will be useful to BCB conservation. I propose to collect baseline data on (1) BCBs abundance, (2) density of the plants that BCB lay their eggs on (i.e. their primary host plants), and (3) landscape characteristics of BCB habitats in Maryland. These data will yield the first assessment of habitat needs of the species in Maryland, and generate hypotheses for future research into their population dynamics.

Research Overview and Methods.

Overview. BCB distribution in Maryland has been roughly mapped through historic sightings and consultation with amateur lepidopterists (**Figure 1**).[5] However, neither its local abundance nor factors associated with its distribution have been analyzed systematically. I plan to analyze the data I collect to test for any associations between environmental characteristics and BCB abundance to make preliminary assessments of habitat factors important for maintaining viable populations.

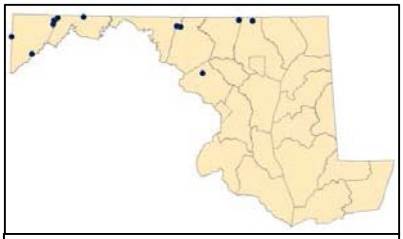

Figure 1. Distribution map of the BCB in Maryland.

Methods. The proposed research will be conducted in the Piedmont Region of Maryland, where 7 of the 11 BCB extant populations occur[A], from May 24th-June 20th. The study period represents the early part of the BCB flight season (late May-July).

Baltimore Checkerspot Butterfly Abundance

Abundance will be estimated through Pollard transect counts, a distance sampling method specific to butterflies.[12] I will walk fixed transect routes and count the number of BCBs up to 5m ahead at each site 2-3 times a week for 4 weeks.[12-17] Counts will only be made between 10.00-16.00 hours under sunny and warm (>13ºC) conditions. Transect routes and number of counts will be standardized across field sites to ensure equal counting effort. Data will be analyzed using INsect Count Analyzer (INCA) – a program which estimates an index of population size, peak emergence time, and death rate using a Zonneveld model.[18]

[A] Jennifer Frye has agreed to show me the locations of the known extant BCB populations, which will important because they are mostly located on private property.

Density of BCB Primary Host Plants

BCBs have two primary host plants: (1) *Chelone glabra* (native) and (2) P*lantago lanceolata*, which they have adopted in only some of its range.[19-24] The host plant shift has not been observed in Maryland.[5] ***Chelone***: I will count all the Chelone using a grid-like search method – the count will be treated as the actual population size. Counts will be performed twice. ***Plantago***: Using point-transect sampling, I will (1) go to randomly selected points within the field site and (2) note the number of observable Plantago and their distance from those points.[25-28] The density of English plantain will be calculated by taking the ratio of Plantago detected over the area surveyed times the probability of detecting the Plantago within the surveyed area.[24, B]

Landscape Characteristics of BCB Habitat

I will employ ArcGIS software to map and analyze the surveyed sites.[30-31] After synthesizing the population and landscape data (see **Table 1**), I will determine the field site(s) I will study. Given the limited number of extant populations, I will use correlation analysis to relate each environmental covariate to BCB abundance at each site. These associations will be used to generate testable hypotheses for future research.

Table 1. BCB Habitat Characteristics

Characteristic	**Qualitative Measurement**	**Quantitative Measurement**
Wetland	Water source (e.g. stream, river)?	Average soil water potential?
Fragmentation	Extent surrounding area has been altered by human activity (e.g. roads, buildings, farms)?	Distance between and among extant and historical BCB populations?
Human Activity	Amount of traffic (low, medium, high)?	Number of houses and people living within 10mi^2?
Edges	Types of edges surrounding the field sites?	Width of the edges?

Note on Equipment Needs. The GPSMAP®64 will be necessary to create a spatial map in ArcGIS, providing coordinates for the field sites, transects, BCBs, and BCB primary host plants. The soil water potential sensor will provide a quantitative assessment of the "wetness" of each field site.

Significance.

Dissertation Research. Ultimately, these data will be used to select suitable field site(s) in Maryland where I will collect BCB population data using more in-depth sampling methods; and as a basis for building testable hypotheses about factors associated with population viability. I will also develop predictive models of where BCB should be which integrate environmental factors; then search the sites for occupying BCB and determine why if they do or do not. I will work in tandem with the goals of BCRT. After the project is completed, Jennifer Frye and I will build the results of my analysis into conservation planning for BCB in Maryland.

Conservation. The results from this research will directly assist conservation efforts in Maryland. BCBs are rare and of conservation concern in four states and potentially form a framework for examining butterfly conservation through a pro-active perspective that identifies potential conservation risks before they occur. This will allow conservation activists and advocates to cite scientific research that can be used for public policy reforms that can prevent butterfly conservation crises.

[B] A preliminary site visit revealed Chelone is heterogeneously distributed in large clusters and thus will be countable; Plantago is primarily located on the periphery in drier sections.

References

[1] David S. Wilcove et al., "Quantifying Threats to Imperiled Species in the United States Assessing the Relative Importance of Habitat Destruction, Alien Species, Pollution, Overexploitation, and Disease," BioScience 48, no. 8 (August 1, 1998): 607–15, doi:10.2307/1313420.
[2] Owen T. Lewis and Michael J. M. Senior, "Assessing Conservation Status and Trends for the World's Butterflies: The Sampled Red List Index Approach," Journal of Insect Conservation 15, no. 1–2 (April 1, 2011): 121–28, doi:10.1007/s10841-010-9329-8.
[3] Carl Haub and Toshiko Kaneda, 2012 World Population Data Sheet (Population Reference Bureau, July 2012), http://www.prb.org/pdf12/2012-population-data-sheet_eng.pdf.
[4] Demographic Internet Staff U. S. Census Bureau, "2014 National Population Projections," accessed December 30, 2014, http://www.census.gov/population/projections/data/national/2014.html.
[5] Pat Durkin et al., Conservation and Management of the Baltimore Checkerspot (Euphydryas Phaeton Drury) in Maryland: Strategies for Statewide Monitoring and for Wetland Restoration, Captive Breeding and Release in the Piedmont Region (Maryland Department of Natural Resources), accessed December 30, 2014, http://www.dnr.state.md.us/wildlife/Plants_Wildlife/BCRT/pdfs/2013_Plan_ConservationBaltimoreCheckerspot.pdf.
[6] "Euphydryas Phaeton," NatureServe Explorer: An Online Encyclopedia of Life (Arlington, VA: NatureServe, March 2014), http://explorer.natureserve.org/servlet/NatureServe?sourceTemplate=tabular_report.wmt&loadTemplate=species_RptComprehensive.wmt&selectedReport=RptComprehensive.wmt&summaryView=tabular_report.wmt&elKey=107962&paging=home&save=true&startIndex=1&nextStartIndex=1&reset=false&offPageSelectedElKey=107962&offPageSelectedElType=species&offPageYesNo=true&post_processes=&radiobutton=radiobutton&selectedIndexes=107962&selectedIndexes=113152&selectedIndexes=110220.
[7] Maryland Wildlife and Heritage Service Natural Heritage Program, Rare, Threatened, and Endangered Animals of Maryland (Annapolis, MD: Maryland Department of Natural Resources, April 2010), http://www.dnr.state.md.us/wildlife/Plants_Wildlife/rte/pdfs/rte_Animal_List.pdf.
[8] Pennsylvania Natural Heritage Program, PNHP Species Lists (Pennsylvania Department of Conservation and Natural Resources), accessed December 30, 2014, http://www.naturalheritage.state.pa.us/species.aspx.
[9] SM Roble, Natural Heritage Resources of Virginia: Rare Animal Species. Natural Heritage Technical Report 13-05 (Richmond, VA: Virginia Department of Conservation and Recreation, Division of Natural Heritage, March 2013), http://www.dcr.virginia.gov/natural_heritage/documents/anlist2013.pdf.
[10] Wildlife Species Conservation & Research Program, Delaware's Endangered Species (Delaware Division of Fish & Wildlife, July 2013), http://www.dnrec.delaware.gov/fw/NHESP/information/Pages/Endangered.aspx.
[11] E. Pollard, "A Method for Assessing Changes in the Abundance of Butterflies," Biological Conservation 12 (1977): 115-134.
[12] Jérôme Pellet, "Seasonal Variation in Detectability of Butterflies Surveyed with Pollard Walks," Journal of Insect Conservation 12, no. 2 (2008): 155–62.
[13] Neil Collier et al., "Butterfly Communities in South Australian Urban Reserves: Estimating Abundance and Diversity Using the Pollard Walk," Austral Ecology 31, no. 2 (2006): 282–90.

[14] J. A. Thomas, "A Quick Method for Estimating Butterfly Numbers during Surveys," Biological Conservation 27, no. 3 (1983): 195–211.
[15] Astrid Caldas and Robert K. Robbins, "Modified Pollard Transects for Assessing Tropical Butterfly Abundance and Diversity," Biological Conservation 110, no. 2 (2003): 211–19.
[16] Ronald A. Royer, Jane E. Austin, and Wesley E. Newton, "Checklist and 'Pollard Walk' Butterfly Survey Methods on Public Lands," The American Midland Naturalist 140, no. 2 (1998): 358–71.
[17] Travis Longcore and Rudi Mattoni, "INsect Count Analyzer: A Tool to Assess Responses of Butterflies to Habitat Restoration," Ecological Restoration 21, no. 1 (2003): 60-61.
[18] Brian Gene Scholtens, "Prealighting Host Plant Location in the Baltimore Checkerspot Butterfly, Euphydryas Phaeton, and Its Implications for Host Range Evolution in Butterflies.," (Thesis, 1990), http://deepblue.lib.umich.edu/handle/2027.42/104172.
[19] Brian Gene Scholtens, "Host Plants and Habitats of the Baltimore Checkerspot Butterfly, Euphydryas Phaeton (Lepidoptera: Nymphalidae), in the Great Lakes Region," Great Lakes Entomologist 24, no. 4 (1991): 207–17.
[20] M. Deane Bowers, Nancy E. Stamp, and Sharon K. Collinge, "Early Stage of Host Range Expansion by a Specialist Herbivore, Euphydryas Phaeton (Nymphalidae)," Ecology 73, no. 2 (April 1, 1992): 526–36, doi:10.2307/1940758.
[21] M. Deane Bowers and Leif Richardson, "USE OF TWO OVIPOSITION PLANTS IN POPULATIONS OF EUPHYDRYAS PHAETON DRURY (NYMPHALIDAE)," Journal of the Lepidopterists' Society 67, no. 4 (2013): 1–3.
[22] Scholtens, "Host Plants and Habitats of the Baltimore Checkerspot Butterfly, Euphydryas Phaeton (Lepidoptera: Nymphalidae), in the Great Lakes Region," Great Lakes Entomologist 24, no. 4 (1991): 207-217.
[23] NE Stamp, "New Oviposition Plant for Euphydryas Phaeton (Nymphalidae)," Journal of the Lepidopterists' Society 33, no. 3 (1979): 203–4.
[24] Len Thomas et al., "Distance Sampling," Encyclopedia of Environmetrics, vol. 1 (2002): 544–552.
[25] Len Thomas et al., "Distance Software: Design and Analysis of Distance Sampling Surveys for Estimating Population Size," Journal of Applied Ecology 47, no. 1 (2010): 5–14.
[26] Stephen T. Buckland et al., "Line Transect Methods for Plant Surveys," Biometrics 63, no. 4 (2007): 989–98.
[27] T. A. Marques et al., "Point Transect Sampling along Linear Features," Biometrics 66, no. 4 (2010): 1247–55.
[28] Lucio Barabesi and Marzia Marcheselli, "Species Abundance Estimation Using Point-to-Plant Sampling in a Design-Based Setting," Environmental and Ecological Statistics 9, no. 4 (2002): 393–403.
[29] Lauren M. Scott and Mark V. Janikas, "Spatial Statistics in ArcGIS," in Handbook of Applied Spatial Analysis (Springer, 2010), 27–41.
[30] Michael S. Rosenberg and Corey Devin Anderson, "PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis. Version 2," Methods in Ecology and Evolution 2, no. 3 (2011): 229–32.

Exhibit B. Project Budget

Project Budget

Total Estimated Budget	**$ 1,060**
Total Requested Amount	**$ 1,000**
Amount to be Provided From Other Resources[1]	**$ 60**

Maryland Field Research Expenses

May 24th to June 20th, 2015 Field Research in Maryland: $ 730

Car Trip to Maryland from Boston, MA[2]: $70 (gas)[3] = $70

Housing in Maryland: $500/month x 1 month = $500

Local Travel to Collect Baseline Population Data for Selection of Field Sites

Car Trip to and from Field Site to Field Site[4]:

$8/trip * 5 days/week * 4 weeks = $160

Equipment and Software: $ 330

Soil Water Potential Sensor[5]: $80

GPSMAP® 64[6]: $250

[1] Though the total budget exceeds the total award amount for the Tufts Graduate Student Research Competition, I plan to complete all research necessary for the completion of the research project using funds from my current fellowship.

[2] Distance between Boston, MA and Maryland: 441 miles.

[3] All gas estimates are calculated using the "My Tripe Calculator" available on www.fueleconomy.gov (http://www.fueleconomy.gov/trip/#?), which uses current gas prices and the make and model of your vehicle to estimate the fuel cost.

[4] Estimated mileage to and between different field sites in Maryland: 100 miles.

[5] http://www.irrometer.com/sensors.html

[6] https://buy.garmin.com/en-US/US/on-the-trail/handhelds/gpsmap-64/prod140020.html