From:	Marshall, Michael E
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Subject:	HENA draft
Date:	Tuesday, January 23, 2018 9:59:44 AM
Attachments:	HENA_Intro_Species Biology_Species Needs_01232018.docx
Importance:	High

Hello Rebekah,

Welcome back, and hope you are feeling better! I've pulled together a draft for the HENA SSA--Biology and Species Needs sections. Although the elicitation questions I sent out to the technical team may result in edits to this, I figured I'd let you have plenty of time a look at the draft before the technical team gets a review. I'd be curious to get your sense of the direction the report is going....some of the images, maps, tables, etc...are placeholders (i.e. will make them look much better for a final draft).

I'm envisioning the next section (Current Conditions) starting with some type of resilience ranking rules...for example...we might use EO Viability scores directly to characterize populations as low, moderate, or highly resilient....or perhaps we use population abundance estimates and growth rates where we have them.....the model could play in to this as well.

Then, current condition would report the number and resilience category of each population, probably in map and tabular form. Redundancy would be a report of the number and distribution of these pops across the range, and perhaps within representative units....Representation will hopefully be informed by the technical team input.

Finally....we should reschedule our Core Team call....how does the rest of folks week look?

Thanks!

Mike

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Work Schedule 1st Week
Monday-Thursday --> In Office 7:30-4:30 CST

Work Schedule 2nd Week
Monday-Thursday --> In Office 7:30-4:30 CST

INTRODUCTION

The dwarf-flowered heartleaf (*Hexastylis naniflora*) is a species of plant endemic to the upper Piedmont region of western North Carolina and upstate South Carolina. The dwarf-flowered heartleaf has been listed as threatened under the Endangered Species Act of 1973, as amended (Act), since 1989 (FR 54 14964-14967). The Species Status Assessment (SSA) framework (USFWS 2016, entire) is intended to support an in-depth review of the species' biology and threats, an evaluation of its biological status, and an assessment of the resources and conditions needed to maintain long-term viability. The intent is for the SSA Report to be easily updated as new information becomes available and to support all functions of the Endangered Species Program from Candidate Assessment to Listing to Consultations to Recovery.

A Recovery Plan for the species was never completed. However, over the last 29 years, the U.S. Fish and Wildlife Service (Service) has worked closely with partners to make significant progress toward recovery of the species. The Service is initiating a Species Status Assessment (SSA) to aid in determining the appropriateness of reclassifying the species. In the event that the SSA does not support reclassification, the SSA would be used to inform the development of a Recovery Plan. Importantly, the SSA Report does not result in a decision by the Service on whether this species should be proposed for reclassification under the Act. Instead, this SSA Report provides a review of the available information strictly related to the biological status of the dwarf-flowered heartleaf. The reclassification decision will be made by the Service after reviewing this document and all relevant laws, regulations, and policies, and the results of a proposed decision will be announced in the *Federal Register,* with appropriate opportunities for public input.

For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in its natural systems over time. Using the SSA framework (Figure 1), we consider what the species needs to maintain viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation (Wolf *et al.* 2015, entire).

- **Resiliency** describes the ability of populations to withstand stochastic events (arising from random factors). We can measure resiliency based on metrics of population health; for example, birth versus death rates and population size. Highly resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of anthropogenic activities.

- **Representation** describes the ability of a species to adapt to changing environmental conditions. Representation can be measured by the breadth of genetic or environmental diversity within and among populations and gauges the probability that a species is capable of adapting to environmental changes. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or human caused) in its environment. In the absence of species-specific genetic and ecological diversity information, we evaluate representation based on the extent and variability of habitat characteristics across the geographical range.
- **Redundancy** describes the ability of a species to withstand catastrophic events. Measured by the number of populations, their resiliency, and their distribution (and connectivity), redundancy gauges the probability that the species has a margin of safety to withstand or can bounce back from catastrophic events (such as a rare destructive natural event or episode involving many populations).

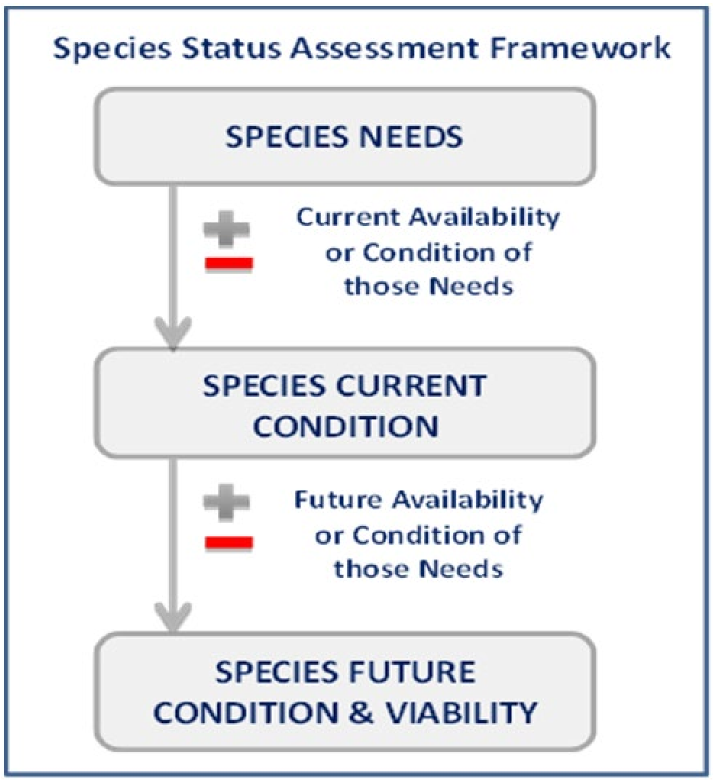

Figure 1- Species Status Assessment Framework

To evaluate the biological status of the dwarf-flowered heartleaf, both currently and into the future, we assessed a range of conditions to allow us to consider the species' resiliency, redundancy, and representation (together, the 3Rs). This SSA Report provides a thorough assessment of biology and natural history and assesses demographic risks, stressors, and limiting factors in the context of determining the viability and risks of extinction for the species.

The format for this SSA Report includes: (1) Biology and Species Needs (2) Current Conditions (3) Influences on Viability and (4) Future Conditions. This document is a compilation of the best available scientific and commercial information and a description of past, present, and likely future risk factors to the dwarf-flowered heartleaf.

Biology and Species Needs:

In this chapter we provide basic biological information about the dwarf-flowered heartleaf, including its taxonomic history, species description, distribution, life history traits, and habitat characteristics. We then use this information to outline the resource needs within various life stages of Nashville Crayfish. Here we report those aspects of the life history of the dwarf-flowered heartleaf that are important to our analysis.

Taxonomy and species description

Dwarf-flowered heartleaf is a rare low growing herbaceous plant in the birthwort family (Aristolochiaceae). The species was described by Blomquist (1957) in his revision of the North American members of the genus Hexastylis. The dwarf-flowered heartleaf has been recognized as part of the Virginica Group, and this group was further subdivided into three Subgroups: Virginica, Shuttleworthii, and Heterophylla (Blomquist 1957; Whittemore and Gaddy 1997). Three species have been recognized in the Heterophylla complex, *Hexastylis naniflora, H. heterophylla* and *H. minor*, and field biologist have generally recognized that considerable morphological overlap occurs (Murrell et al. 2007). One of the main concerns regarding this complex was the inability to distinguish between species without access to fresh flowers. Even with fresh flowers, Blomquist (1957) and Gaddy (1987) still recognized considerable overlap in flower morphology making species delineation difficult.

Murell et al. 2007, conducted a comparative genetic analysis using Inter Simple Sequence Repeats, and were unable to find banding patterns that could be used to separate *H. naniflora* from the other members within the complex. However, based on biogeographical, ecological, molecular, morphological, as well as micromorphological work, their results show that *H. naniflora* is a well-defined species.

Although there has been considerable disagreement on the generic distinctiveness of *Hexastylis* and *Asarum* (Barringer 1993 and Kelly 1997, 1998, 2001), a recent phylogeny estimate using chloroplast genes supports that *Hexastylis* is a monophyletic clade and therefore should be recognized as a genus (Niedenberger 2010). Additionally, most North American publications recognize *Hexastylis* at the generic level (Flora of North America 1997, Weakley 2015).

The Service is not aware of any proposed changes in taxonomy that would affect the continued legal status of *H. naniflora* under the Act. However, within the range of *H. naniflora* there are populations which fall outside of the range of published values for key floral characteristics, overlapping with values described for *H. heterophylla* or *H. minor* (Figure 2; Weakley, 2010; Murrell et al. 2007; Gaddy 1987). These geographic areas of overlap in key characters have been the focus of recent genetic analyses (Murrell et al. 2007; Renninger, 2010; Murrell, 2015).

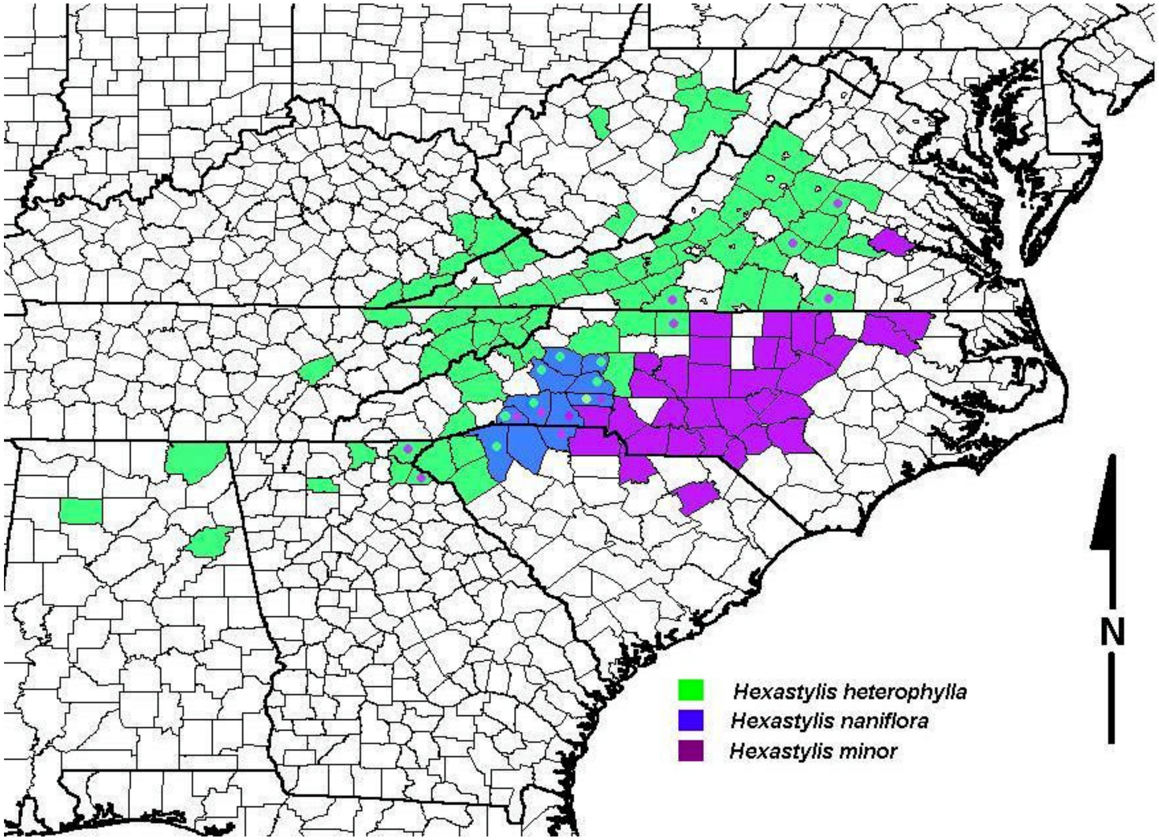

Figure 2: *From Murrell et al. 2007.* Distribution map showing county records for the three species in the *H. heterophylla* complex. Data was gathered from herbarium specimens, Element Of Occurrence (EOC) sheets and field studies. Dots within *H. heterophylla* counties indicates co-occurrence with *H. minor*. Light dot within *H. naniflora* counties indicates co-occurrence with *H. heterophylla*, dark dot indicates co-occurrence with *H. minor*.

The most outstanding characteristic of this species is the small flowers, which are one of the smallest of any species in North America (Blomquist 1957). The plant's heart shaped leaves are dark green in color, evergreen, and leathery, and are supported by long thin petioles from a subsurface rhizome. The shape of the leaf blades, their pattern of variegation, and the ridged reticulation inside the calyx-tube, place this species inside the Virginica group. It differs from all the other members of this group, aside from the small flowers, in having no flare in the calyx-tube. Maximum height rarely exceeds 15 centimeters (cm). The jug shaped flowers are usually beige to dark brown in color and appear from mid-March to early June. The flowers are small and inconspicuous and are found near the base of the petioles. The fruit matures from mid-May to early July (Blomquist 1957, Gaddy 1980, 1981). Characteristics that distinguish it from other *Hexastylis* species are found in floral structures and pollen characters (Gaddy 1987, Padgett 2004, Niedenberger 2010). *H. naniflora* has a smaller calyx tube orifice, which is typically 5mm or less (sometimes up to 7mm) and the ovary is half-inferior, rather than superior (Blomquist 1957, Gaddy 1987, Padgett 2004, HDR 2005). Pollen surface features have also been shown to be an effective character to identify *H. naniflora*, as it has a microporate surface and, unlike any other *Hexastylis* species, lacks gemmae entirely (Padgett 2004, Niedenberger 2010).

Distribution

Although dwarf-flowered heartleaf is restricted in range, it is not as rare as once thought (USFWS 2010, NCNHP 2016). The species occurs in the upper piedmont of North and South Carolina (Figure 3). In North Carolina, it is found in Alexander, Burke, Caldwell, Catawba, Cleveland, Gaston, Iredell, Lincoln, Polk, and Rutherford Counties. In South Carolina, it is in Cherokee, Greenville, and Spartanburg Counties. When dwarf-flowered heartleaf was federally listed in 1989, the listing rule described 24 extant "populations" (and 1 extirpated population) distributed across eight counties in North and South Carolina. As of 2010, the distribution of this species consisted of 108 populations distributed across 12 counties in these two states. Since 1989, the county distribution has expanded to include the following North Carolina counties: Alexander, Caldwell, Iredell, and Polk. The species has not been discovered in any additional counties in South Carolina.

The species appears to be restricted to Pacolet sandy loam, Madison gravelly sandy loam, and Musella fine sandy loam soils (Gaddy 1981,1987). These soils are restricted to an area from near Charlotte, North Carolina west to the foot of the mountains near Rutherfordton, North Carolina, and from Hickory, North Carolina southward to just south of Spartanburg, South Carolina (Murrell et al. 2007).

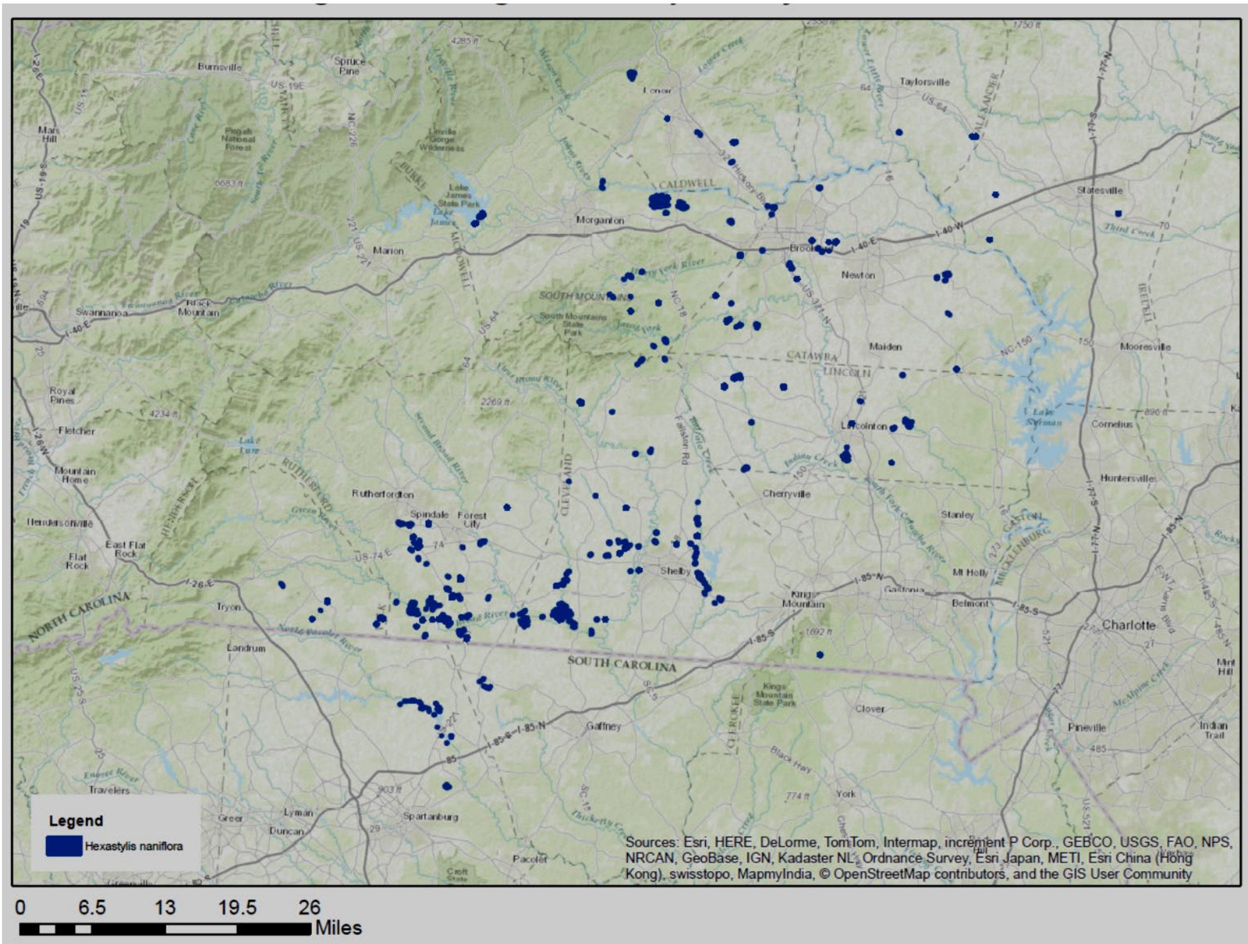

Figure 3--this is a placeholder…we will generate a new map in Arc GIS.

Many of those working with *Hexastylis naniflora* have used the terms “sub site”, “site”, “location”, “occurrence” (often, but not always, in reference to Natural Heritage Program Element Occurrence (EO) Records), “subpopulation” and “population” interchangeably. Others have aggregated smaller sites into populations according to subjective criteria which have never been explicitly defined. This generates discrepancies among sources with respect to the abundance and distribution of the species, with the net result being that available data are usually not comparable from one source to the next. We describe how the numerous small, site-specific locations containing *H. naniflora* have been aggregated into proxies for 113 biological populations for purposes of this review, using mapping standards devised by NatureServe and its network of Natural Heritage Programs, in the Species Needs section of this report.

Life History

Demographics

The Service is aware of a single effort to collect demographic-level data (survivorship and recruitment of tagged individuals) for dwarf-flowered heartleaf. This effort was conducted during the 1990-1991 field seasons, within a portion of the Peters Creek population in Spartanburg County, SC (Newberry, 1993). This study demonstrated a 96.1% survival rate over these two consecutive seasons, with 50% of the mortality occurring in plants located at the highest position on the forested slope (away from the adjacent floodplain). Mortality was highest in small plants bearing fewer than four leaves. Plant size was variable, with the largest plant bearing 45 leaves and 33 flowers, and situated in the floodplain. In general, plants located in the floodplain were larger than plants located on adjacent slopes. The percentage of flowering plants averaged 70%, with the highest frequency of flowering occurring among plants situated in the floodplain (USFWS 2010).

Pollination and Dispersal

The pollination of Hexastylis has not been well studied but the genus was thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal by ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (H. heterophylla, H. naniflora, and H. minor), which employ myrmecochory as a method for seed dispersal.

Ants appear to be a primary dispersal agent for the dwarf-flowered heartleaf (Gaddy 1986; Jones et al. 2014). All diaspores of *Hexastylis naniflora* presented to ants (*Aphaenogaster rudis*) were quickly removed (Gaddy, 1986). This is not to say that they are not occasionally, or even frequently, dispersed and/or pollinated by other means. Jones et al. (2014) suggests that the pollination mechanism is facultative, benefiting from more than one method of pollination/fertilization. Ants were the pollinators in a controlled experiment, and their data supports that when outside molesting forces/pollinators (biotic and abiotic) were limited by their caging procedure, the efficiency of pollination decreased by almost 50%, however, caged flowers did produce seeds, indicating pollinization occurred via some alternative method.

Habitat

Dwarf-flowered heartleaf appears to have a restricted range due to its habitat requirements. The habitat where dwarf-flowered heartleaf exists is limited in size and scope due to a multitude of factors including soil type, moisture availability, and slope aspect. This unique combination of factors limits not only the range of dwarf-flowered heartleaf, but also the size of a given population. With the limited range and size in populations, questions arise regarding gene flow among populations. How much is occurring and how often does it occur? It is due, in part, to narrow habitat requirements that conservation measures have been implemented for the protection of the species. Any efforts made to protect this species must consider giving protection to the available habitat.

Dwarf-flowered heartleaf occurs on piedmont uplands on acidic sandy-loam soils that are very deep and moderately permeable (Gaddy 1981, 1987). Typical habitats for this species include mesic to dry bluffs, slopes, or ravines in deciduous forests that are frequently associated with *Kalmia latifolia* (Padgett 2004, Weakley 2015, USFWS 2015), or in moist soils adjacent to creeks, streamheads, or along lakes and rivers. Plants have been observed to grow larger and have more frequent flowering in floodplains along rivers, lakes, and streams (Newberry 1993). Wagner (2013) conducted a habitat suitability study to quantify the habitat requirements for dwarf-flowered heartleaf, which may be used for helping identify the species when not in flower (relative to other *Hexastylis* species habitat preferences), find new populations, or identify suitable sites for transplants. A model was created to predict habitat suitability to determine how many EOs are found within habitat considered fair to excellent (Figure 4; Wagner 2015). Based on the model, the strongest habitat correlations were that the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Wagner 2015). Populations found in areas with a percent slope of greater than 28, soil codes other than 6, 7, or 11, or elevation less than 199 m or greater than 415 m are unlikely to be *H. naniflora.* The model accurately predicts habitat suitability at a local scale 81% of the time.

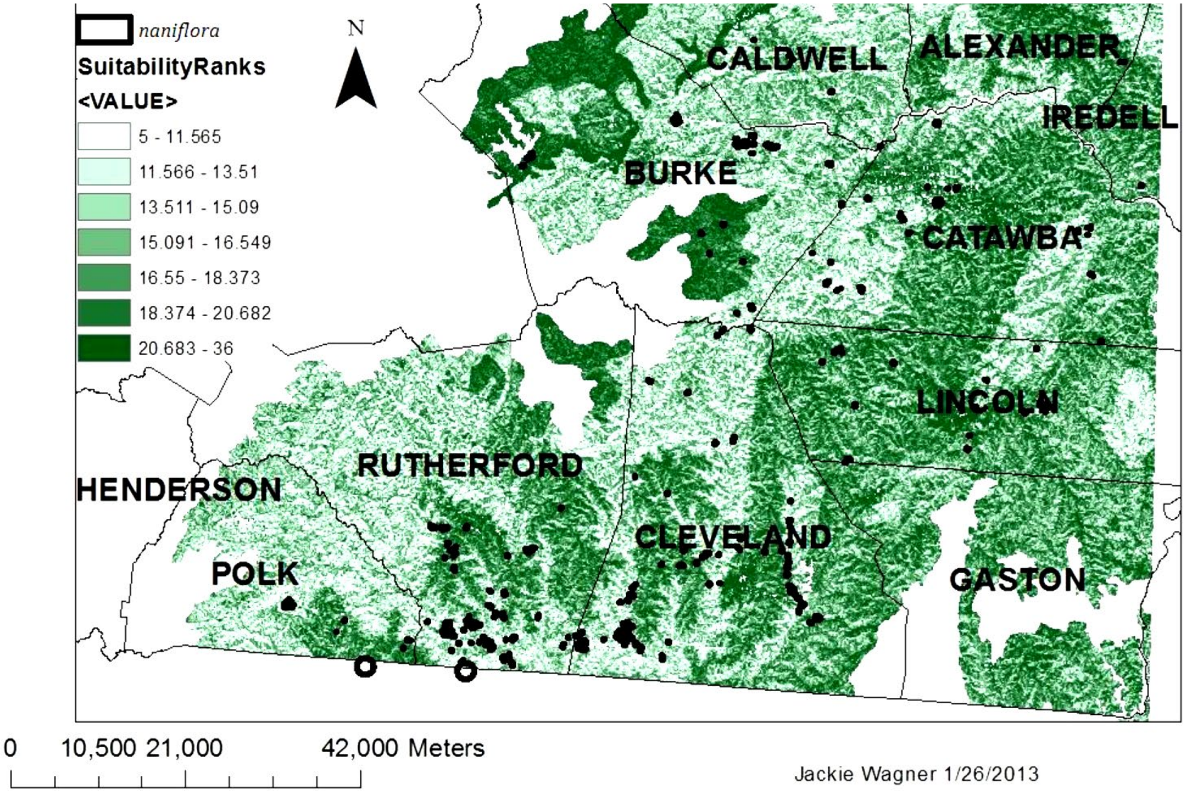

Figure 4: The site suitability ranks based on five habitat variables across the counties where *H. naniflora* is known to exist in NC. The smaller numbers indicate a higher rank and lighter green areas denote more suitable habitat. Known populations of *H. naniflora* are outlined in black.

Soils

The species grows in acidic soils along bluffs and adjacent slopes, in boggy areas next to streams and creekheads, and along the slopes of nearby hillsides and ravines (Gaddy 1980, 1981). The plant is primarily found inhabiting north- to northwest-facing slopes, bluffs, and ravines in close proximity to creeks or streams. Within these areas exists the soil type required for H. naniflora to grow. It grows primarily on well-drained, sandy, acidic soils, and will not grow in heavy clay (Gaddy 1981).

The results of soil samples analyzed at the Clemson Soil Lab suggest that major differences in soil chemistry exist between the species in the *H. heterophylla* complex. Statistical analysis of the soil samples showed that many of the basic elements were significantly different among the three species. Those significant differences occurred in Phosphorous (P), Potassium (K), Magnesium (Mg), Zinc (Zn), Manganese (Mn), (Na), Sodium, and Cation Exchange Capacity (CEC). Slightly significant differences were seen in Buffer pH (Bu pH), and Acidity (Table 8).

Soil chemistry showed marked differences between the species in the complex. The results indicated that soil chemistry is very different between *H. naniflora* and *H. minor* localities. The results also show that *H. heterophylla* and *H. naniflora* are found in soils where the chemistry is more similar, but still showed significant differences. It would appear that differentiation in soil types could be used as a proxy for species delineation. The soil analysis also indicates that soils must be considered when trying to select sites for relocation of imperiled populations of *H. naniflora*.

The analysis did not show statistically significant differences among the three species in the *H. heterophylla* complex. However, *H. naniflora* appears to have an association with three oak species that is lacking in the other two species in the complex. There are a number of oak species (*Q. coccinea*, *Q. prinus* (*Q. Montana*), and *Q. velutina*), that tend to co-occur with only *H. naniflora*, but are not present with the other two species in the complex. This may be the result of some microbial need or specific soil nutrient required for those species to occur in the same habitat.

Fire

There are little data on the response to fire by *Hexastylis naniflora*, however, prescribed burns have been conducted within the population at Cowpens National Battlefield in Cherokee County SC. Preliminary data at this site and recent annual monitoring data of this population support the theory that moderate controlled burns do not negatively affect this population (Walker et al. 2009). Additionally, a dormant season wildfire in Caldwell County did not show evidence of negative impacts to a population in Caldwell County (USFWS 2010). Fire suppression could be a hazard to *H. naniflora* by allowing fire intolerant, nonnative and invasive plants to thrive, as well as the accumulation of thick duff or leaf litter that may shade low growing species (Wagner 2013).

Genetics

Analyses on ecology, morphology, soil chemistry, pollen, and molecular genetics have been evaluated for *Hexastylis naniflora* to determine the boundaries within the *Hexastylis heterophylla* complex, which consists of *H. heterophylla*, *H. minor*, and *H. naniflora* (Murrell 2015, Wagner 2013, Niedenburger 2010, USFWS 2010, Murrell et al. 2007, Padgett 2004). These analyses support the continued recognition of these taxa as well-defined, discrete species. Scanning electron microscopy (SEM) consistently distinguished *H. naniflora* from other members of the *H. heterophylla* complex based on pollen microscopy. Principal Components Analysis of floral characters and soil chemistry also consistently distinguished *H. naniflora* from *H. minor* and *H. heterophylla*. However, efforts to obtain consistently distinct banding patterns using Inter Simple Sequence Repeats (ISSRs) were unsuccessful at distinguishing *H. naniflora* from other members of this group (Murrell et al., 2007). These results were based upon an extremely small sample size (n=10 *H. naniflora* individuals), and therefore warrant further investigation.

Field observations demonstrate that there are some populations of dwarf-flowered heartleaf with morphological characteristics that do not fit within the range of published values for key traits, overlapping with values for *H. heterophylla* or *H. minor* (Gaddy 1987, Murrell et al. 2007, USFWS 2010, Weakley 2015). These populations with overlap in key characteristics were the focus of a genetic analysis conducted at Appalachian State University (ASU) through funding provided by NCDOT (Murrell 2015). In some populations, floral characteristics are highly variable, which suggests the potential for hybridization or individuals with highly variable flower size and shape (Murrell 2015). Additionally, no vegetative characters were previously known to consistently distinguish *Hexastylis naniflora* from other close relatives. Given the difficulties with field identification of the species, particularly when not in flower, this study sought to develop a microsatellite library of molecular markers to resolve variation in populations of dwarf-flowered heartleaf and apply the markers to populations with highly variable characters, as identified by NCDOT biologists. The morphological and micromorphological information from those variable populations were compared to molecular results with morphological, micromophological, and distributional data to determine genetic structure, biological boundaries, and placement of putative hybrids or intermediate populations of *H. naniflora* (Murrell 2015).

The preliminary findings of this study suggest that populations in the southern range of dwarf-flowered heartleaf exhibit a more uniform genetic pattern, with some possible hybridization with *H. minor*. Populations in the northern part of the range appear to have hybridized with both *H. heterophylla* and *H. minor*, although there are still individuals with “pure” *H. naniflora* genotypes in the northern range (Murrell 2015). It is critical to note that although these data provide anecdotal evidence of hybridization with the *Hexastylis heterophylla* group, intraspecific variation may be caused by forces other than hybridization, such as convergent morphological evolution (Dobzhansky 1937), or the species is in the process of speciation and this study shows a case of incomplete speciation (Murrell 2015), and/or other environmental factors are at play (Wagner 2013). On May 11, 2016 a meeting was held with USFWS, NCNHP, NCDOT, and ASU to discuss the status of *H. naniflora* and the current work being conducted among the agencies (Amoroso 2016). Based on discussions during this meeting, the results of this study reported by ASU to NCDOT in 2015 are preliminary. Dr. Matt Estep (ASU) provided additional preliminary results to NCNHP in May 2016, showing which populations were sampled, sample size, and percent of samples that show evidence of hybridization, and hybridizing with which species. ASU continues to work towards a more definitive explanation of the variation in the *H. heterophylla* complex (Murrell 2015, Amoroso 2016).

SPECIES NEEDS

For the purpose of this report, we define viability as the ability of the species to sustain populations in the wild over time. Species with greater numbers (redundancy) of healthy populations (resiliency), encompassing a broad array of ecological and genetic diversity in a spatial arrangement that maintains adequate gene flow (representation), are more likely to be viable. Using the Species Status Assessment framework, we describe the species' viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation.

Delineating Populations

As stated in the USFWS five-year review, "many of those working with dwarf-flowered heartleaf have used the terms "sub site," "site," "location," "occurrence" (often, but not always, in reference to Natural Heritage Program Element Occurrence Records), and "population" interchangeably, while others have aggregated sites into populations according to subjective criteria which have never been explicitly defined. This has generated considerable discrepancies among sources with respect to the number of known populations within a given area (or across the species' range), to the extent that numbers are not comparable from one source to the next. The tendency to treat each location as a separate population also artificially inflates the actual number of populations known.

In recent years, NatureServe and its member Natural Heritage Programs have devised mapping standards to balance the need for fine-scale, highly site-specific EO records (required for monitoring and management) with the need to aggregate these records in meaningful units of conservation interest that may approximate biological populations (NatureServe 2002). "Since USFWS does not maintain its own database of known locations of *Hexastylis naniflora*, it regards the NHP databases as the best repository for this information" (USFWS 2010).

We delineate populations for the purposes of this SSA according to the NatureServe (2002) convention. Separation distances are a key component to delineating populations from EO records. For the dwarf-flowered heartleaf, we used the EO Data Standard which provides a Default Separation Distance of 1 km (~0.62 miles) for plant and animal elements that lack EO specs, noting that situations involving dispersal barriers could involve even shorter distances. While gene flow declines over distance at different rates for different elements, the minimum default EO separation distance of 1 km has been accepted by the Network as the most suitable round-number metric-system approximation broadly applicable to many (but not all) situations. This results in several dwarf-flowered heartleaf populations being stand-alone EOs, as well as many populations being aggregates of several EOs.

Population Resiliency

For the dwarf-flowered heartleaf to maintain viability, its populations or some portion thereof must be resilient. Stochastic factors that have the potential to affect dwarf-flowered heartleaf include impacts to its habitat, particularly human development pressures, but also climate change and presence of invasive species. Other factors that influence the resiliency of Nashville crayfish populations include abundance within populations, and habitat factors such as elevation, slope, aspect, and soil type. Influencing those factors are elements of dwarf-flowered heartleaf ecology that determine whether populations can grow to maximize habitat occupancy, thereby increasing resiliency of populations. These factors and habitat elements are discussed below (Figure 5).

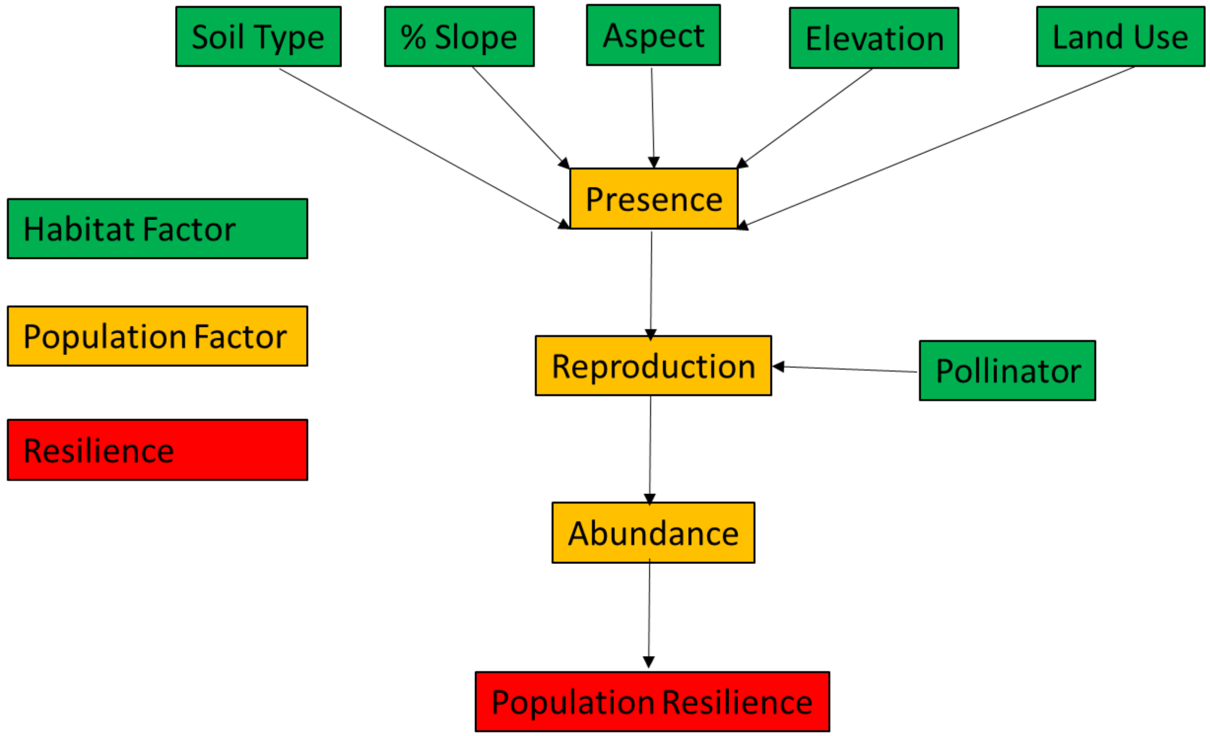

Figure 5--Conceptual diagram describing population and habitat factors influencing population resilience for dwarf-flowered heartleaf.

Habitat Factors: Slope, Aspect, Elevation, Soil, and Forest Type

In a site suitability model generated with habitat rankings from 5 (the most suitable) to 36 (least suitable habitat) across 7 rank classes, the most common classification included the following microhabitat elements: the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Table 1; Wagner 2015). More importantly, populations found in areas with a percent slope greater than 28, or elevation less than 199 m or greater than 415 m, are unlikely to be dwarf-flowered heartleaf. Slope aspect analysis shows that *H. naniflora* has already adapted to the cooler, wetter conditions of north facing slopes suggesting that this species would fare poorly under climate change scenarios predicting warmer and drier environments (mimicking south facing slopes) throughout their range, supporting a similar claim from Warren (2008).

Table 1. Frequency of element occurrence records (EORs) for each habitat variable. For continuous variables (slope and elevation) data were grouped into classes with the value shown being the top end of the range. * indicates most common classification for that habitat variable.

slope		land use (LU)		soil		elevation		aspect	
% slope	EORs	LU type	EORs	soil code	EORs	Elev. (m)	EORs	aspect	EOR
5.5	20	open water	1	6*	123	<199	0	N*	57
8	50	Low intensity residential	14	7	59	229.8571	34	NE	32
10.5*	54	High intensity residential	3	10	1	260.7143*	65	NW	32
13	35	Commercial/ Industrial	1	11	8	291.5714	29	E	13
15.5	22	Deciduous Forest*	153	other	0	322.4286	34	W	10
18	10	Evergreen Forest	13			353.2857	24	SE	22
20.5	3	Mixed Forest	1			384.1429	10	SW	13
23	3	Grassland	3			415	4	S	20
25.5	0	Pasture	11			More	0		
28	2								
>28	0								

Reproduction and Presence of Pollinators

The pollination of Hexastylis has not been well studied but the genus is thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal via ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (H. heterophylla, H. naniflora, and H. minor), which employ myrmecochory as a method for seed dispersal.

Because the flower for this species is oftentimes partially or completely covered with soil and leaf litter, possibly inhibiting pollinator activity (Gonzalez 1972), there is still uncertainty in the pollination mechanism for dwarf-flowered heartleaf. Otte (1977) suggests that a variety of possible pollinators reside in this leaf litter, however, the calyx opening is considered to be far too small for efficient pollinating (Gaddy 1981). There are, however, invertebrates within this proposed size limit that could theoretically act as pollinators. It is possible that the species employs self-pollination, with or without a vector, or that cross-pollination occurs by a number of invertebrates. Jones et al. 2014 investigated pollination of dwarf-flowered heartleaf under a manipulative experimental design, and found that while insects may play a significant role in pollination, even without them, flowers managed to produce a partial seed set. However, two points need to be considered here. First, although flowers managed to produce seeds in the absence of insect pollinators, the efficiency of pollination decreased by almost 50%. Also, even if successful pollination occurs in the absence of insect vectors, the dispersal of plants amongst populations would be limited, and could result in decreased resilience due to genetic concerns such as limited gene flow and issues associated with potential inbreeding depression.

Abundance

The influence of stochastic variation in demographic (reproductive and mortality) rates is much higher for small populations than large ones. Stochastic variation in demographic rates causes small populations to fluctuate randomly in size. In general, the smaller the population, the greater the probability that fluctuations will lead to extinction. There are also genetic concerns with small populations, including reduced availability of compatible mates, genetic drift, and inbreeding depression. Small populations of dwarf-flowered heartleaf have low resilience, leaving them particularly vulnerable to stochastic events.

As of 2016, the combined databases of the NCNHP and SCNDNR contain 239 Element Occurrence Records (EORs) for *H. naniflora* (NCNHP2016, SCDNR 2016). These EORs depict roughly 113 locations which are sufficiently geographically distinct to be regarded as proxies for populations of the species (See *Delineating Populations*). Thus, the total number of populations has increased more than four-fold (from 24 to 113) since the species was listed in 1989.

At this time, the largest known populations have been monitored by NCNHP and NCDOT. The estimates for entire populations are based on a consistent monitoring methodology developed by NCDOT, USFWS, and NCNHP with monitoring plots representing roughly 10% of a population. Populations were delineated to get a more accurate boundary and size of the area occupied. All rosettes are counted annually in each monitoring plot to estimate an extrapolated population size, based on the number and density in the plots. As a result of these efforts, better estimates of population sizes for the largest known populations are available, compared to when the last five-year review was completed in 2010 (Robinson and Padgett 2016).

The 113 EORs have been estimated to contain anywhere from a single rosette to over 100,000 rosettes. Table A1 (Appendix A) was created by NCNHP to replicate the same format and population data as Table B2 of the most recent USFWS five-year review of *H. naniflora* (USFWS 2010), for comparison of changes since 2010. Table A1 summarizes the largest occurrences of *H. naniflora*, with the size of the population based on the number of rosettes it was last estimated to contain. The number of populations estimated to contain over 1,000 rosettes is 26. This is approximately 23% of the total known populations and many of these populations contain well over 1,000 individuals.

There are, however, 13 populations (12% of all known) that are simply known to be extant, with no available estimate of population size (NCNHP 2016, SCDNR 2016). If the most recent population estimates for each EOR are compiled across years of observation, the 113 populations could conservatively be estimated to contain a collective total of more than 300,000 rosettes (NCNHP 2016, SCDNR 2016).

Appendix A

Table A1: Populations of *Hexastylis naniflora* estimated to contain over 1,000 rosettes in 2010, with updated information in 2016[1], and protection measures for these populations.

Populations known to be afforded some protection are indicated in **bold**.[2]

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
1	NC	Burke	029	A	Island Creek Heath Bluff (Lovelady Sites)	50,000+ (2016)	0	Not protected.
2	NC	Burke	178.159-178.161 178.254-178.256 178.280-178.282	A	Catawba River: Love Lady (Hoyle and Micol Creek)	~6,738 (2008-2011)	Awaiting data from NCDOT.	Possible NCDOT protected R-O-W, protection unconfirmed. In early 2016, the Town of Valdese applied to CWMTF to acquire part of this population.
3	**NC**	**Caldwell**	**227**	**A**	**Peaked Top Rare Plant Site (Foothills Landfill)**	**16,463 (2014)**	**16,463 (2014)**	**Conservation Easement (Foothills Conservancy of NC).**
4	**NC**	**Caldwell**	**044**	**AB**	**Little Gunpowder Creek Rare Plant Site 1**	**2,707 (2015)**	**2,424 (2015)**	**DOT mitigation site.**
5	NC	Catawba	158	D	South Fork Catawba River: Jacob Fork, Camp Creek	123 (2016)	27 (2015)	Some plants occur within an undetermined acreage within NCDOT R-O-W (Bassette 2016). Protection unconfirmed.
6	**NC**	**Catawba**	**243.012,** 243.242 243.269, **243.270**	**A**	**Catawba Wildlife Club**	**1,000-1300 (1995-2007)**	**>1,000 (1995-2007)**	**Catawba County Wildlife Club Registered Heritage Area (EO 12) The Nature Conservancy Conservation Easement (EO 270).**
7	NC	Catawba	031	C?	US 321, southeast Hickory	1,202 (2010)	161 (2010)	161 plants within NCDOT R-O-W (Bassette 2016).
8	**NC**	**Catawba**	**96.184,** 96.038 96.039	**A**	**Murrays Mill/Upper Balls Creek**	**~9,150 (2005)**	**11,860 (2013)**	**Fee title by NCDOT (~34 acres).**

9	**NC**	**Cleveland**	100.014, **100.049, 100.050, 100.051, 100.073, 100.074 100.149, 100.233**	**A**	**Broad River/Sandy Run Natural Areas (aka "Broad River Tract")**	**~33,500 (2001-2015)**	**32,702 (2015)**	**Fee title by Broad River Greenway (1,000 acres).**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
			100.236, 100.237 **100.238, 100.239 100.240, 100.241,** 100.246					
10	NC	Cleveland	208	A?	Buffalo Creek: Potts Creek	3,572 (2007)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
11	NC	Cleveland	211.072, 211.194		First Broad River: Hop-Hornbeam Natural Area	1,965 (2000)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
12	NC	Cleveland	214.201, **214.202, 214.203,** 214.204, **214.205, 214.209,** 214.212, **214.213,**	A	Buffalo Creek: tributaries north and south of SR2047	6,447 (200-2007)		Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 202, 203, 205, 209, 213).
13	NC	Cleveland	216.028, 216.196, 216.197, 216.198, **216.199, 216.200**	A	Buffalo Creek: Kings Mountain Reservoir	3,310 (200-2016)	~500? (2000-2007) (awaiting updates)	Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 199, 200).
14	NC	Cleveland	157	AB	First Broad River: Crooked Run Creek	330 (2010)		None of the population is protected within NCDOT R-O-W, determined too far away from roadside, DOT stopped monitoring in 2010 (Bassette 2016).
15	**NC**	**Cleveland/ Rutherford**	**276**	**A**	**Cliffside Steam Station**	**39,545 (2016)**	**~39,545 (2016)**	**Voluntary agreement with Duke Energy; protected acreage unknown.**
16	NC	Lincoln	261.252, 261.259, 261.263	B	Lincoln County Airport and Leepers Creek	7,032 (2005-2006)		Not protected.
17	NC	Polk	023	A	Mills Creek Forest and Seep	1,459 (2016)		Not protected.

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
18	**NC**	**Polk**	**125**	**A**	**Broad River: New Hope Springhead Swamp**	**12,235 (2016)**	**1,000-3,000 estimated (2016)**	**New Hope Springhead Swamp Registered Heritage Area (voluntary conservation agreement).**
19	**NC**	**Rutherford**	**154**	**C**	**Second Broad River (Forest City Industrial Complex)**	**2,576 (2016)**	**2478 (2008)**	**Deed restrictions (12 acres). Significant threats from industrial development, invasive exotics, erosion, sedimentation in a fragmented landscape.**
20	**NC**	**Rutherford**	99.009, 99.010, 99.037, 99.052, 99.053, 99.055, 99.061, 99.063, 99.075, **99.076,** 99.079, 99.090, 99.121, **99.172,** 99.181, 99.182, 99.249-.251, 99.267, 99.268	**A**	**Broad River: Hensons Creek Ravine, Brice Rare Plant Site, Sandy Mush Outcrop**	**106,940 (2016)**	**~200-250 (2007-2012)**	**Parris Heartleaf Registered Heritage Area (voluntary conservation agreement on subEO 076 and 172).**
21	NC	Rutherford	176.167-176.170	A	Broad River: Cleghorn Creek Tributary/US221	6,750 (2016)		Not protected.
22	NC	Rutherford	177.016, 177.107, 177.122, 177.063, 177.164, 177.165, 177.166	A	Broad River: Floyds Creek, Long Branch	12,687 (2008-2016)		Not protected.
23	**NC**	**Rutherford**	247.013, 247.056, 247.080, **247.106,** 247.114, 247.115, 247.173, 247.174, **247.286**	**A**	**Broad River: Floyds Creek (aka New Bethel Rare Plant Site)**	**~5,480 (2003-2015)**	**4,873 (2015)**	**Tate Conservation Easement (8 acres) (SubEOs 106 and 286).**
24	**SC**	**Cherokee**	**016, 017, 018**	**BC**	**Cowpens Battlefield**	**2,823 (2016)**	**2,823? (2016)**	**National Park Service, National Battlefield.**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on- site protection (year)	Protection comments
25	SC	Greenville /Spartanburg	002, 004, 024, 032, 033, 036, 038, 039, 040, 041, 042, 049, 050, 054	Various AB-BD	South Pacolet River and tributaries	5,205 (1991-1998)		Not protected.
26	**SC**	**Spartanburg**	**011, 014, 026--028, 047, 048, 057**	**Various AB-BD**	**Peters Creek Heritage Preserve**	**3,306 (2016)**	**3,306 (2016)**	**State Heritage Preserve (194 acres).**
27	**SC**	**Spartanburg**	**007, 029**	**BD, H?**	**Blalock Reservoir**	**3,505 (2016)**	**3,505? (2016)**	**Restrictive covenants (protected acreage unknown); significant threats to remaining population.**
28[6]	**NC**	**Lincoln**	302.046, **302.262**	**A**	**Rhyne Preserve**	**19,880 (2016)**	**19,873 (2016)**	**Rhyne Preserve (fee title by Catawba Lands Conservancy).**

[1]This table was replicated in the same format and populations as Table B.2 from the most recent USFWS five-year Review of *Hexastylis naniflora* (USFWS 2010) for comparison of changes since the time of the five-year review. Populations that are not believed to contain over 1,000 rosettes but were included in the original Table B.2 are included here, but the "NHP EO rank" and in the "Latest estimate of population size" reflects the smaller size.

[2] In the case of partially protected populations, the column "NHP EO numbers" indicates the specific portions of the population afforded protection in bold font.

[3]NHP EO numbers use the following format: Principal EO.SubEO. Only one number is listed if it is a stand-alone principal EO.

[4]NHP EO rank specifications are defined in Appendix B2. In cases involving principal/sub EOs, only the rank for the principal EO is given.

[5] EO ranks with two letters indicate a degree of uncertainty within the range provided (e.g. a rank of "AB" indicates possibly excellent to good viability).

[6]Population that is currently estimated to have over 1,000 rosettes that was not known to be large populations in 2010 have been added to this table.

From:	Reid, Rebekah N
To:	Marshall, Michael E
Cc:	Stephanie DeMay; Becker, Drew N; Endries, Mark
Subject:	Re: HENA draft
Date:	Tuesday, January 23, 2018 11:07:01 AM
Importance:	High

Thanks, Mike. I am back in the office this week and I'll dive into the intro section shortly.

I am available for a call anytime Wed. or Thurs. I also have a window of time on Fri. from about 10:00 - 1:00.

Rebekah Reid

US Fish and Wildlife Service
Asheville Ecological Services Field Office
160 Zillicoa St.
Asheville, NC 28801
phone: 828-258-3939 x238
cell: 828-782-0090

On Tue, Jan 23, 2018 at 9:59 AM, Marshall, Michael <michael_marshall@fws.gov> wrote:

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Alternate email: mmarshall@ag.tamu.edu

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To:	Reid, Rebekah N
Cc:	Stephanie DeMay; Becker, Drew N; Endries, Mark
Subject:	Re: HENA draft
Date:	Tuesday, January 23, 2018 11:17:42 AM
Importance:	High

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From:	Endries, Mark
To:	Marshall, Michael E
Cc:	Reid, Rebekah N; Stephanie DeMay; Becker, Drew N
Subject:	Re: HENA draft
Date:	Tuesday, January 23, 2018 1:24:22 PM
Importance:	High

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Mark

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USFWS
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To:	Endries, Mark
Cc:	Reid, Rebekah N; Stephanie DeMay; Becker, Drew N
Subject:	Re: HENA draft
Date:	Tuesday, January 23, 2018 1:28:07 PM
Importance:	High

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To: Marshall, Michael E
Cc: Reid, Rebekah N; Stephanie DeMay; Becker, Drew N
Subject: Re: HENA draft
Date: Tuesday, January 23, 2018 1:34:47 PM
Importance: High

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This email correspondence and any attachments to and from this sender is subject to the Freedom of Information Act and may be disclosed to third parties.

--
Mike Marshall
SSA Program Specialist
U.S. Fish and Wildlife Service Region 4
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From:	Reid, Rebekah N
To:	Endries, Mark
Cc:	Marshall, Michael E; Stephanie DeMay; Becker, Drew N
Subject:	Re: HENA draft
Date:	Thursday, January 25, 2018 11:23:26 AM
Attachments:	HENA_Intro_Species Biology_Species Needs_01232018 RNR Comments.docx

Mike,

Attached are some comments on the draft. I wanted to get this to you before our call. I realize it is a little last minute so we can discuss at a different time if needed.

Thanks.

Rebekah Reid

US Fish and Wildlife Service
Asheville Ecological Services Field Office
160 Zillicoa St.
Asheville, NC 28801
phone: 828-258-3939 x238
cell: 828-782-0090

On Tue, Jan 23, 2018 at 1:34 PM, Endries, Mark <mark_endries@fws.gov> wrote:

> Works great for me.
>
> Mark
>
> **Mark Endries**
> USFWS
> 160 Zillicoa St
> Asheville, NC 28801
> Office: 828.258.3939 ext. 231
> Mobile: 828.215.1740
>
> On Tue, Jan 23, 2018 at 1:28 PM, Marshall, Michael <michael_marshall@fws.gov> wrote:
>
>> Sure thing....does 1:00 pm est work better?
>>
>> On Tue, Jan 23, 2018 at 1:24 PM, Endries, Mark <mark_endries@fws.gov> wrote:
>>
>>> I need to leave at 2:30 on Thursdays. Could we move it earlier?
>>>
>>> Mark
>>>
>>> **Mark Endries**
>>> USFWS
>>> 160 Zillicoa St
>>> Asheville, NC 28801
>>> Office: 828.258.3939 ext. 231
>>> Mobile: 828.215.1740

On Tue, Jan 23, 2018 at 11:17 AM, Marshall, Michael <michael_marshall@fws.gov> wrote:

I'm going to suggest Thursday (1/25) at 2:00 est for our Core Team call....please let me know if this does not work for anyone.

Thanks!

Mike

On Tue, Jan 23, 2018 at 11:07 AM, Reid, Rebekah <rebekah_reid@fws.gov> wrote:

Thanks, Mike. I am back in the office this week and I'll dive into the intro section shortly.

I am available for a call anytime Wed. or Thurs. I also have a window of time on Fri. from about 10:00 - 1:00.

Rebekah Reid

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160 Zillicoa St.
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On Tue, Jan 23, 2018 at 9:59 AM, Marshall, Michael <michael_marshall@fws.gov> wrote:

Hello Rebekah,

Welcome back, and hope you are feeling better! I've pulled together a draft for the HENA SSA--Biology and Species Needs sections. Although the elicitation questions I sent out to the technical team may result in edits to this, I figured I'd let you have plenty of time a look at the draft before the technical team gets a review. I'd be curious to get your sense of the direction the report is going....some of the images, maps, tables, etc...are placeholders (i.e. will make them look much better for a final draft).

I'm envisioning the next section (Current Conditions) starting with some type of resilience ranking rules...for example...we might use EO Viability scores directly to characterize populations as low, moderate, or highly resilient....or perhaps we use population abundance estimates and growth rates where we have them.....the model could play in to this as well.

Then, current condition would report the number and resilience category of each population, probably in map and tabular form. Redundancy would be a report of the number and distribution of these pops across the range, and perhaps within representative units....Representation will hopefully be informed by the technical team input.

Finally....we should reschedule our Core Team call....how does the rest of folks week look?

Thanks!

Mike

--
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INTRODUCTION

The dwarf-flowered heartleaf (*Hexastylis naniflora*) is a species of plant endemic to the upper Piedmont region of western North Carolina and upstate South Carolina. The dwarf-flowered heartleaf has been listed as threatened under the Endangered Species Act of 1973, as amended (Act), since 1989 (FR 54 14964-14967). The Species Status Assessment (SSA) framework (USFWS 2016, entire) summarizes the information complied and reviewed by the US Fish and Wildlife Service (Service), incorporating the best available scientific and commercial data, to conduct an in-depth review of the species' biology and threats, evaluate its biological status, and assess the resources and conditions needed to maintain long-term viability. The intent is for the SSA to be easily updated as new information becomes available and to support all functions of the Endangered Species Program from Listing to Consultations to Recovery.

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A Recovery Plan for the species was never completed. However, over the last 29 years, the Service has worked closely with partners to make significant progress toward recovery of the species. The Service is initiating this SSA to aid in determining the appropriateness of reclassifying the species. In the event that the SSA does not support reclassification, the SSA would be used to inform the development of a Recovery Plan. Importantly, the SSA does not result in a decision by the Service on whether this species should be proposed for reclassification under the Act. Instead, this SSA provides a review of the available information strictly related to the biological status of the dwarf-flowered heartleaf. The reclassification decision will be made by the Service after reviewing this document and all relevant laws, regulations, and policies. The results of a proposed decision will be announced in the *Federal Register,* with appropriate opportunities for public input.

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For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in its natural systems over time. Using the SSA framework (Figure 1), we consider what the species needs to maintain viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation (Wolf *et al.* 2015, entire).

- **Resiliency** describes the ability of populations to withstand stochastic events (arising from random factors). We can measure resiliency based on metrics of population health;

for example, birth versus death rates and population size. Highly resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of anthropogenic activities.

- **Redundancy** describes the ability of a species to withstand catastrophic events. Measured by the number of populations, their resiliency, and their distribution (and connectivity), redundancy gauges the probability that the species has a margin of safety to withstand or can bounce back from catastrophic events (such as a rare destructive natural event or episode involving many populations).
- **Representation** describes the ability of a species to adapt to changing environmental conditions. Representation can be measured by the breadth of genetic or environmental diversity within and among populations and gauges the probability that a species is capable of adapting to environmental changes. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or human caused) in its environment. In the absence of species-specific genetic and ecological diversity information, we evaluate representation based on the extent and variability of habitat characteristics across the geographical range.

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Deleted: <#>**Redundancy** describes the ability of a species to withstand catastrophic events. Measured by the number of populations, their resiliency, and their distribution (and connectivity), redundancy gauges the probability that the species has a margin of safety to withstand or can bounce back from catastrophic events (such as a rare destructive natural event or episode involving many populations).¶

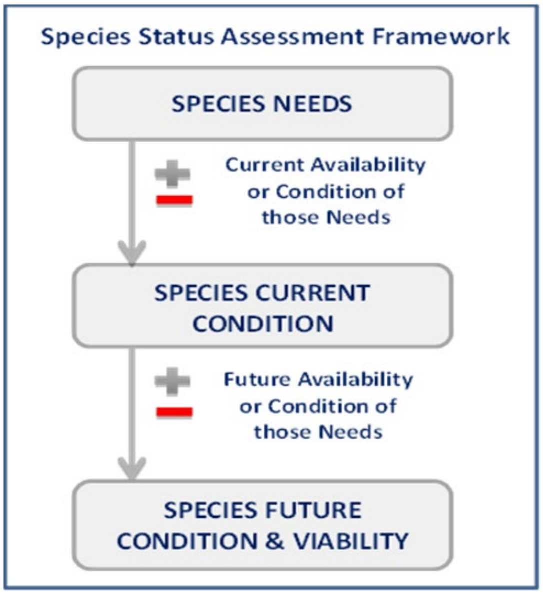

Figure 1- Species Status Assessment Framework

To evaluate the biological status of the dwarf-flowered heartleaf, both currently and into the future, we assessed a range of conditions to allow us to consider the species' resiliency, redundancy, and representation (together, the 3Rs). This SSA provides a thorough assessment of biology and natural history, and assesses demographic risks, stressors, and limiting factors in the context of determining the viability and risks of extinction for the species.

The format for this SSA includes: (1) Biology and Species Needs (2) Current Conditions (3) Influences on Viability and (4) Future Conditions. This document is a compilation of the best available scientific and commercial information, and a description of past, present, and likely future risk factors to the dwarf-flowered heartleaf.

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Biology and Species Needs:

In this chapter, we provide basic biological information about the dwarf-flowered heartleaf, including its taxonomic history, species description, distribution, life history traits, and habitat characteristics. We then use this information to outline the resource needs within various life stages of dwarf-flowered heartleaf. Here we report those aspects of the life history of the dwarf-flowered heartleaf that are important to our analysis.

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Taxonomy and species description

Commented [RNR1]: Should genetics (at the end) be moved to this section? I'm imagining this in three subsection just to clearly delineate the information – Species Description (morphology), Taxonomy, and Genetics. NHP's range-wide report is set up this way and it flows well.

Dwarf-flowered heartleaf is a rare, low growing herbaceous plant in the birthwort family (Aristolochiaceae). The species was described by Blomquist (1957) in his revision of the North American members of the genus *Hexastylis*. The dwarf-flowered heartleaf has been recognized as part of the Virginica Group, and this group was further subdivided into three Subgroups: Virginica, Shuttleworthii, and Heterophylla (Blomquist 1957; Whittemore and Gaddy 1997). Three species have been recognized in the Heterophylla complex, *Hexastylis naniflora, H. heterophylla* and *H. minor*, and field biologist have generally recognized that considerable morphological overlap occurs (Murrell et al. 2007). One of the main concerns regarding this complex was the inability to distinguish between species without access to fresh flowers. Even with fresh flowers, Blomquist (1957) and Gaddy (1987) still recognized considerable overlap in flower morphology making species delineation difficult.

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Murell et al. 2007, conducted a comparative genetic analysis using Inter Simple Sequence Repeats, and were unable to find banding patterns that could be used to separate *H. naniflora* from the other members within the complex. However, based on biogeographical, ecological, molecular, morphological, as well as micromorphological work, their results show that *H. naniflora* is a well-defined species.

Although there has been considerable disagreement on the generic distinctiveness of *Hexastylis* and *Asarum* (Barringer 1993 and Kelly 1997, 1998, 2001), a recent phylogeny estimate using chloroplast genes supports that *Hexastylis* is a monophyletic clade and therefore should be recognized as a genus (Niedenberger 2010). Additionally, most North American publications recognize *Hexastylis* at the generic level (Flora of North America 1997, Weakley 2015).

The Service is not aware of any proposed changes in taxonomy that would affect the continued legal status of *H. naniflora* under the Act. However, within the range of *H. naniflora* there are populations which fall outside of the range of published values for key floral characteristics, overlapping with values described for *H. heterophylla* or *H. minor* (Figure 2; Weakley, 2010; Murrell et al. 2007; Gaddy 1987). These geographic areas of overlap in key characters have been the focus of recent genetic analyses (Murrell et al. 2007; Renninger, 2010; Murrell, 2015).

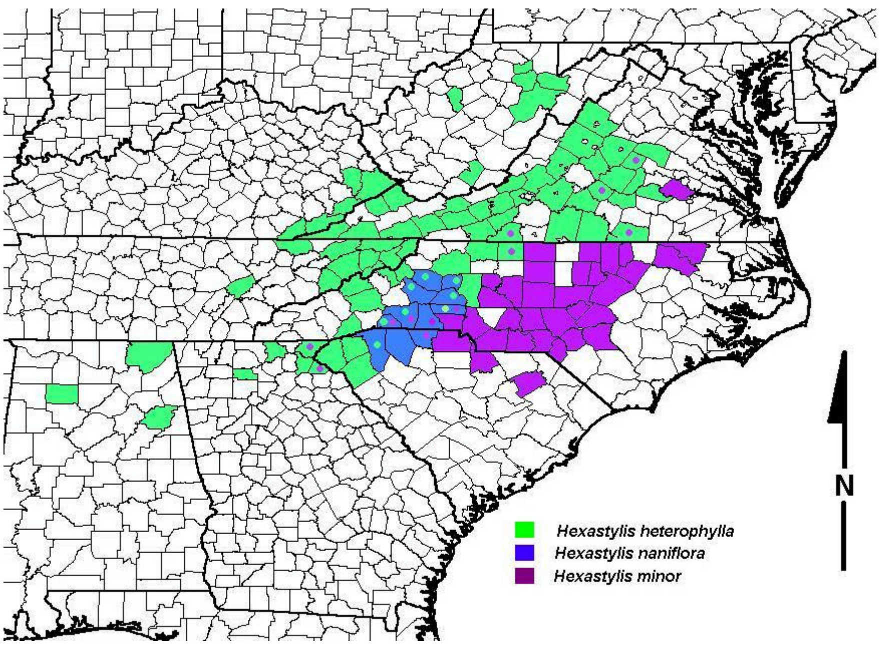

Figure 2: *From Murrell et al. 2007*. Distribution map showing county records for the three species in the *H. heterophylla* complex. Data was gathered from herbarium specimens, Element Of Occurrence (EOC) sheets and field studies. Dots within *H. heterophylla* counties indicates co-occurrence with *H. minor*. Light dot within *H. naniflora* counties indicates co-occurrence with *H. heterophylla*, dark dot indicates co-occurrence with *H. minor*.

Commented [RNR2]: Iredell is missing from HENA range on this map.

The most outstanding characteristic of this species is the small flowers, which are one of the smallest of any species in North America (Blomquist 1957). The plant's heart shaped leaves are dark green in color, evergreen, and leathery, and are supported by long thin petioles from a subsurface rhizome. The shape of the leaf blades, their pattern of variegation, and the ridged reticulation inside the calyx-tube, place this species inside the Virginica group. It differs from all the other members of this group, aside from the small flowers, in having no flare in the calyx-tube. Maximum height rarely exceeds 15 centimeters (cm). The jug shaped flowers are usually beige to dark brown in color and appear from mid-March to early June. The flowers are small and inconspicuous and are found near the base of the petioles. The fruit matures from mid-May to early July (Blomquist 1957, Gaddy 1980, 1981). Characteristics that distinguish it from other *Hexastylis* species are found in floral structures and pollen characters (Gaddy 1987, Padgett 2004, Niedenberger 2010). *H. naniflora* has a smaller calyx tube orifice, which is typically 5mm or less (sometimes up to 7mm) and the ovary is half-inferior, rather than superior (Blomquist 1957, Gaddy 1987, Padgett 2004, HDR 2005). Pollen surface features have also been shown to be an effective character to identify *H. naniflora*, as it has a microporate surface and, unlike any other *Hexastylis* species, lacks gemmae entirely (Padgett 2004, Niedenberger 2010).

Distribution

Although dwarf-flowered heartleaf is restricted in range, it is not as rare as once thought (USFWS 2010, NCNHP 2016). When dwarf-flowered heartleaf was federally listed in 1989, the listing rule described 24 extant "populations" (and one extirpated population) distributed across eight counties in the upper Piedmont of North and South Carolina. Since 1989, the range has expanded to include four additional counties in North Carolina. In North Carolina, it is found in Alexander, Burke, Caldwell, Catawba, Cleveland, Gaston, Iredell, Lincoln, Polk, and Rutherford Counties. In South Carolina, it is in Cherokee, Greenville, and Spartanburg Counties. As of 2016, the distribution of this species consisted of 113 populations distributed across 12 counties in these two states (Figure 3).

The species appears to be restricted to Pacolet sandy loam, Madison gravelly sandy loam, and Musella fine sandy loam soils (Gaddy 1981,1987). These soils are restricted to an area from near

Deleted: The species occurs in the upper piedmont of North and South Carolina (Figure 3)…

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Deleted: Since 1989, the county distribution has expanded to include the following North Carolina counties: Alexander, Caldwell, Iredell, and Polk. The species has not been discovered in any additional counties in South Carolina.…

Charlotte, North Carolina west to the foot of the mountains near Rutherfordton, North Carolina, and from Hickory, North Carolina southward to just south of Spartanburg, South Carolina (Murrell et al. 2007).

Commented [RNR3]: Would this be better suited in the habitat section? A soils map to accompany this description might be a good addition to the report…especially if soil is discussed further.

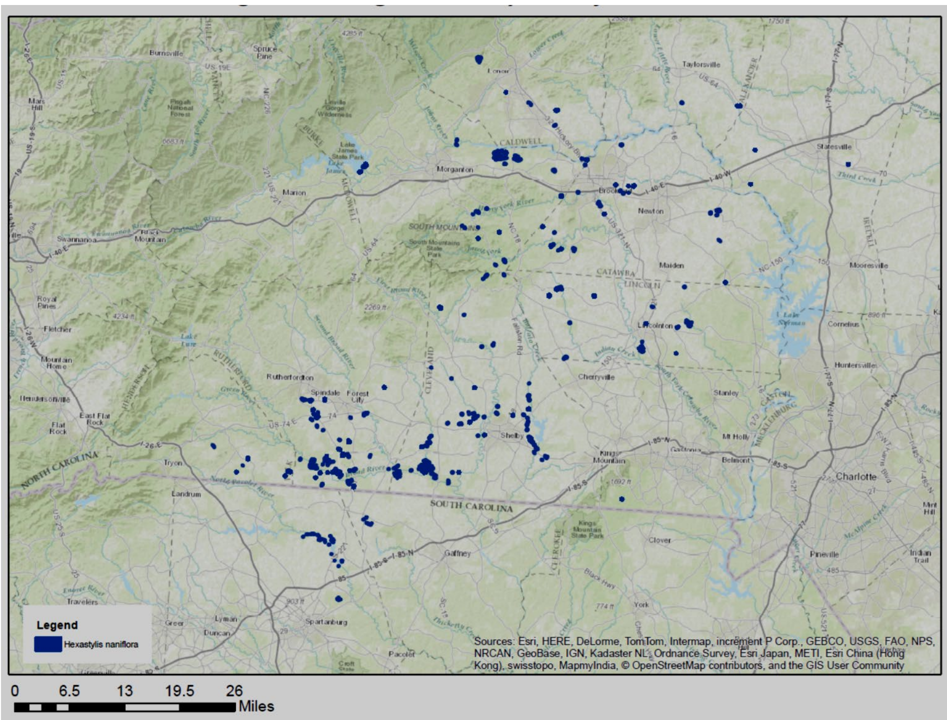

Figure 3--this is a placeholder…we will generate a new map in Arc GIS.

Many of those working with *Hexastylis naniflora* have used the terms "sub site", "site", "location", "occurrence" (often, but not always, in reference to Natural Heritage Program Element Occurrence (EO) Records), "subpopulation" and "population" interchangeably. Others have aggregated smaller sites into populations according to subjective criteria which have never been explicitly defined. This generates discrepancies among sources with respect to the abundance and distribution of the species, with the net result being that available data are usually not comparable from one source to the next. We describe how the numerous small, site-specific locations containing *H. naniflora* have been aggregated into proxies for 113 biological populations for purposes of this review, using mapping standards devised by NatureServe and its network of Natural Heritage Programs, in the Species Needs section of this report.

Life History

Demographics

The Service is aware of a single effort to collect demographic-level data (survivorship and recruitment of tagged individuals) for dwarf-flowered heartleaf. This effort was conducted during the 1990-1991 field seasons, within a portion of the Peters Creek population in Spartanburg County, SC (Newberry, 1993). This study demonstrated a 96.1% survival rate over these two consecutive seasons, with 50% of the mortality occurring in plants located at the highest position on the forested slope (away from the adjacent floodplain). Mortality was highest in small plants bearing fewer than four leaves. Plant size was variable, with the largest plant bearing 45 leaves and 33 flowers, and situated in the floodplain. In general, plants located in the floodplain were larger than plants located on adjacent slopes. The percentage of flowering plants averaged 70%, with the highest frequency of flowering occurring among plants situated in the floodplain (USFWS 2010).

Pollination and Dispersal

The pollination of Hexastylis has not been well studied but the genus was thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal by ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (*H. heterophylla, H. naniflora, and H. minor*), which employ myrmecochory as a method for seed dispersal.

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Ants appear to be a primary dispersal agent for the dwarf-flowered heartleaf (Gaddy 1986; Jones et al. 2014). All diaspores of *Hexastylis naniflora* presented to ants (*Aphaenogaster rudis*) were quickly removed (Gaddy, 1986). This is not to say that they are not occasionally, or even frequently, dispersed and/or pollinated by other means. Jones et al. (2014) suggests that the pollination mechanism is facultative, benefiting from more than one method of pollination/fertilization. Ants were the pollinators in a controlled experiment, and their data supports that when outside molesting forces/pollinators (biotic and abiotic) were limited by their caging procedure, the efficiency of pollination decreased by almost 50%, however, caged flowers did produce seeds, indicating pollinization occurred via some alternative method.

Habitat

Dwarf-flowered heartleaf appears to have a restricted range due to its habitat requirements. The habitat where dwarf-flowered heartleaf exists is limited in size and scope due to a multitude of factors including soil type, moisture availability, and slope aspect. This unique combination of factors limits not only the range of dwarf-flowered heartleaf, but also the size of a given population. With the limited range and size in populations, questions arise regarding gene flow among populations. How much is occurring and how often does it occur? It is due, in part, to narrow habitat requirements that conservation measures have been implemented for the protection of the species. Any efforts made to protect this species must consider giving protection to the available habitat.

Dwarf-flowered heartleaf occurs on piedmont uplands on acidic sandy-loam soils that are very deep and moderately permeable (Gaddy 1981, 1987). Typical habitats for this species include mesic to dry bluffs, slopes, or ravines in deciduous forests that are frequently associated with *Kalmia latifolia* (Padgett 2004, Weakley 2015, USFWS 2015), or in moist soils adjacent to creeks, streamheads, or along lakes and rivers. Plants have been observed to grow larger and have more frequent flowering in floodplains along rivers, lakes, and streams (Newberry 1993). Wagner (2013) conducted a habitat suitability study to quantify the habitat requirements for dwarf-flowered heartleaf, which may be used for helping identify the species when not in flower (relative to other *Hexastylis* species habitat preferences), find new populations, or identify suitable sites for transplants. A model was created to predict habitat suitability to determine how many EOs are found within habitat considered fair to excellent (Figure 4; Wagner 2015). Based on the model, the strongest habitat correlations were that the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Wagner 2015). Populations found in areas with a percent slope of greater than 28, soil codes other than 6, 7, or 11, or elevation less than 199 m or greater than 415 m are unlikely to be *H. naniflora.* The model accurately predicts habitat suitability at a local scale 81% of the time.

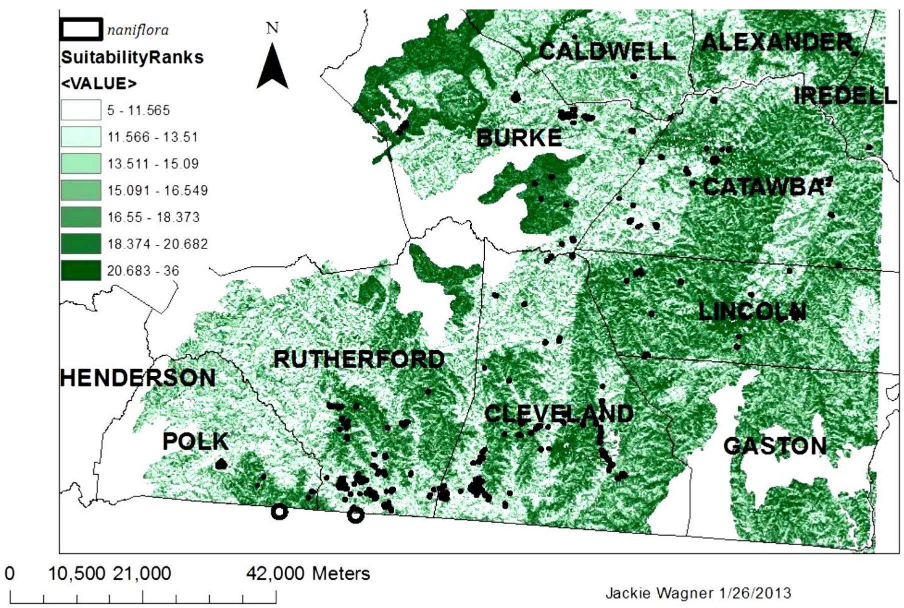

Figure 4: The site suitability ranks based on five habitat variables across the counties where *H. naniflora* is known to exist in NC. The smaller numbers indicate a higher rank and lighter green areas denote more suitable habitat. Known populations of *H. naniflora* are outlined in black.

Soils

The species grows in acidic soils along bluffs and adjacent slopes, in boggy areas next to streams and creekheads, and along the slopes of nearby hillsides and ravines (Gaddy 1980, 1981). The plant is primarily found inhabiting north- to northwest-facing slopes, bluffs, and ravines in close proximity to creeks or streams. Within these areas exists the soil type required for *H. naniflora*

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to grow. It grows primarily on well-drained, sandy, acidic soils, and will not grow in heavy clay (Gaddy 1981).

The results of soil samples analyzed at the Clemson Soil Lab suggest that major differences in soil chemistry exist between the species in the *H. heterophylla* complex. Statistical analysis of the soil samples showed that many of the basic elements were significantly different among the three species. Those significant differences occurred in Phosphorous (P), Potassium (K), Magnesium (Mg), Zinc (Zn), Manganese (Mn), (Na), Sodium, and Cation Exchange Capacity (CEC). Slightly significant differences were seen in Buffer pH (Bu pH), and Acidity.

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Soil chemistry showed marked differences between the species in the complex. The results indicated that soil chemistry is very different between *H. naniflora* and *H. minor* localities. The results also show that *H. heterophylla* and *H. naniflora* are found in soils where the chemistry is more similar, but still showed significant differences. It would appear that differentiation in soil types could be used as a proxy for species delineation. The soil analysis also indicates that soils must be considered when trying to select sites for relocation of imperiled populations of *H. naniflora*.

Thirteen population sites in North Carolina and South Carolina were examined using the Carolina Vegetation Survey (CVS) method to compare species richness between the three species of the *Hexastylis heterophylla* complex. The analysis did not show statistically significant differences among the three species in the *H. heterophylla* complex. However, *H. naniflora* appears to have an association with three oak species that is lacking in the other two species in the complex. There are a number of oak species (*Q. coccinea*, *Q. prinus* (*Q. montana*), and *Q. velutina*), that tend to co-occur with only *H. naniflora*, but are not present with the other two species in the complex. This may be the result of some microbial need or specific soil nutrient required for those species to occur in the same habitat.

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Fire

There are little data on the response to fire by *Hexastylis naniflora*, however, prescribed burns have been conducted within the population at Cowpens National Battlefield in Cherokee County, SC. Preliminary data at this site and recent annual monitoring data of this population support the theory that moderate controlled burns do not negatively affect this population (Walker et al. 2009). Additionally, a dormant season wildfire in Caldwell County did not show evidence of negative impacts to a population in Caldwell County (USFWS 2010). Fire suppression could be a hazard to *H. naniflora* by allowing fire intolerant, nonnative and invasive plants to thrive, as well as the accumulation of thick duff or leaf litter that may shade low growing species (Wagner 2013).

Genetics

Analyses on ecology, morphology, soil chemistry, pollen, and molecular genetics have been evaluated for *Hexastylis naniflora* to determine the boundaries within the *Hexastylis heterophylla* complex, which consists of *H. heterophylla*, *H. minor*, and *H. naniflora* (Murrell 2015, Wagner 2013, Niedenburger 2010, USFWS 2010, Murrell et al. 2007, Padgett 2004). These analyses support the continued recognition of these taxa as well-defined, discrete species. Scanning electron microscopy (SEM) consistently distinguished *H. naniflora* from other members of the *H. heterophylla* complex based on pollen microscopy. Principal Components Analysis of floral characters and soil chemistry also consistently distinguished *H. naniflora* from *H. minor* and *H. heterophylla*. However, efforts to obtain consistently distinct banding patterns using Inter Simple Sequence Repeats (ISSRs) were unsuccessful at distinguishing *H. naniflora* from other members of this group (Murrell et al., 2007). These results were based upon an extremely small sample size (n=10 *H. naniflora* individuals), and therefore warrant further investigation.

Field observations demonstrate that there are some populations of dwarf-flowered heartleaf with morphological characteristics that do not fit within the range of published values for key traits, overlapping with values for *H. heterophylla* or *H. minor* (Gaddy 1987, Murrell et al. 2007, USFWS 2010, Weakley 2015). These populations with overlap in key characteristics were the focus of a genetic analysis conducted at Appalachian State University (ASU) through funding provided by NCDOT (Murrell 2015). In some populations, floral characteristics are highly variable, which suggests the potential for hybridization or individuals with highly variable flower size and shape (Murrell 2015). Additionally, no vegetative characters were previously known to consistently distinguish *Hexastylis naniflora* from other close relatives. Given the difficulties with field identification of the species, particularly when not in flower, this study sought to develop a microsatellite library of molecular markers to resolve variation in populations of dwarf-flowered heartleaf and apply the markers to populations with highly variable characters, as identified by NCDOT biologists. The morphological and micromorphological information from those variable populations were compared to molecular results with morphological, micromophological, and distributional data to determine genetic structure, biological boundaries, and placement of putative hybrids or intermediate populations of *H. naniflora* (Murrell 2015).

The preliminary findings of this study suggest that populations in the southern range of dwarf-flowered heartleaf exhibit a more uniform genetic pattern, with some possible hybridization with *H. minor*. Populations in the northern part of the range appear to have hybridized with both *H. heterophylla* and *H. minor*, although there are still individuals with "pure" *H. naniflora* genotypes in the northern range (Murrell 2015). It is critical to note that although these data provide anecdotal evidence of hybridization with the *Hexastylis heterophylla* group, intraspecific variation may be caused by forces other than hybridization, such as convergent morphological evolution (Dobzhansky 1937), or the species is in the process of speciation and this study shows a case of incomplete speciation (Murrell 2015), and/or other environmental factors are at play (Wagner 2013). On May 11, 2016 a meeting was held with USFWS, NCNHP, NCDOT, and ASU to discuss the status of *H. naniflora* and the current work being conducted among the agencies (Amoroso 2016). Based on discussions during this meeting, the results of this study reported by ASU to NCDOT in 2015 are preliminary. Dr. Matt Estep (ASU) provided additional preliminary results to NCNHP in May 2016, showing which populations were sampled, sample size, and percent of samples that show evidence of hybridization, and hybridizing with which species. ASU continues to work towards a more definitive explanation of the variation in the *H. heterophylla* complex (Murrell 2015, Amoroso 2016).

SPECIES NEEDS

Commented [RNR4]: The guidance documents and several examples I have seen discuss needs on an Individual, Population, and Species level. Do we need to be more explicit earlier in the SSA about what is an individual need vs. population need before jumping to species needs?

For the purpose of this report, we define viability as the ability of the species to sustain populations in the wild over time. Species with greater numbers (redundancy) of healthy populations (resiliency), encompassing a broad array of ecological and genetic diversity in a spatial arrangement that maintains adequate gene flow (representation), are more likely to be viable. Using the Species Status Assessment framework, we describe the species' viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation.

Delineating Populations

As stated in the USFWS five-year review, many of those working with dwarf-flowered heartleaf have used the terms "sub site," "site," "location," "occurrence" (often, but not always, in reference to Natural Heritage Program Element Occurrence Records), and "population" interchangeably, while others have aggregated sites into populations according to subjective criteria which have never been explicitly defined. This has generated considerable discrepancies among sources with respect to the number of known populations within a given area (or across the species' range), to the extent that numbers are not comparable from one source to the next. The tendency, in the past, to treat each location as a separate population also artificially inflated the actual number of populations known.

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In recent years, NatureServe and its member Natural Heritage Programs have devised mapping standards to balance the need for fine-scale, highly site-specific EO records (required for monitoring and management) with the need to aggregate these records in meaningful units of conservation interest that may approximate biological populations (NatureServe 2002). "Since USFWS does not maintain its own database of known locations of *Hexastylis naniflora*, it regards the NHP databases as the best repository for this information" (USFWS 2010).

We delineate populations for the purposes of this SSA according to the NatureServe (2002) convention. Separation distances are a key component to delineating populations from EO records. For the dwarf-flowered heartleaf, we used the EO Data Standard which provides a Default Separation Distance of 1 km (~0.62 miles) for plant and animal elements that lack EO specs, noting that situations involving dispersal barriers could involve even shorter distances. While gene flow declines over distance at different rates for different elements, the minimum default EO separation distance of 1 km has been accepted by the Network as the most suitable round-number metric-system approximation broadly applicable to many (but not all) situations. This results in several dwarf-flowered heartleaf populations being stand-alone EOs, as well as many populations being aggregates of several EOs.

Population Resiliency

Commented [RNR5]: Isn't resilience discussed on a population level? This document has it under the species level.

For the dwarf-flowered heartleaf to maintain viability, its populations or some portion thereof must be resilient. Stochastic factors that have the potential to affect dwarf-flowered heartleaf include impacts to its habitat, particularly human development pressures, but also climate change and presence of invasive species. Other factors that influence the resiliency of dwarf-flowered heartleaf populations include abundance within populations, and habitat factors such as elevation, slope, aspect, and soil type. Influencing those factors are elements of dwarf-flowered heartleaf ecology that determine whether populations can grow to maximize habitat occupancy, thereby increasing resiliency of populations. These factors and habitat elements are discussed below (Figure 5).

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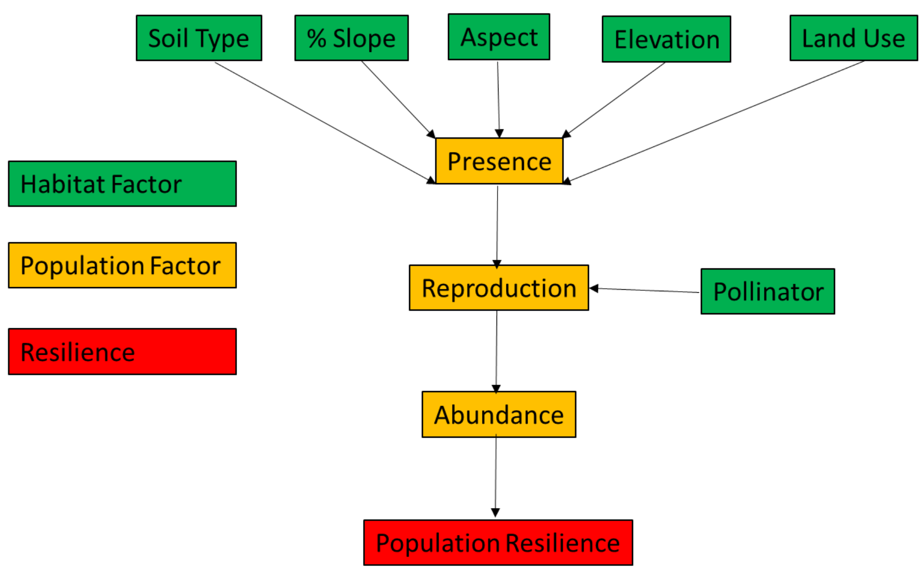

Figure 5--Conceptual diagram describing population and habitat factors influencing population resilience for dwarf-flowered heartleaf.

Habitat Factors: Slope, Aspect, Elevation, Soil, and Forest Type

Commented [RNR6]: How is this different from habitat discussion earlier on in the SSA? Same question with the pollinator section below. I'm struggling to see how they are different.

In a site suitability model generated with habitat rankings from 5 (the most suitable) to 36 (least suitable habitat) across 7 rank classes, the most common classification included the following microhabitat elements: the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Table 1; Wagner 2015). More importantly, populations found in areas with a percent slope greater than 28, or elevation less than 199 m or greater than 415 m, are unlikely to be dwarf-flowered heartleaf. Slope aspect analysis shows that *H. naniflora* has already adapted to the cooler, wetter conditions of north facing slopes suggesting that this species would fare poorly under climate change scenarios predicting warmer and drier environments (mimicking south facing slopes) throughout their range, supporting a similar claim from Warren (2008).

Table 1. Frequency of element occurrence records (EORs) for each habitat variable. For continuous variables (slope and elevation) data were grouped into classes with the value shown being the top end of the range. * indicates most common classification for that habitat variable.

slope		land use (LU)		soil		elevation		aspect	
% slope	EORs	LU type	EORs	soil code	EORs	Elev. (m)	EORs	aspect	EOR
5.5	20	open water	1	6*	123	<199	0	N*	57
8	50	Low intensity residential	14	7	59	229.8571	34	NE	32
10.5*	54	High intensity residential	3	10	1	260.7143*	65	NW	32
13	35	Commercial/ Industrial	1	11	8	291.5714	29	E	13
15.5	22	Deciduous Forest*	153	other	0	322.4286	34	W	10
18	10	Evergreen Forest	13			353.2857	24	SE	22
20.5	3	Mixed Forest	1			384.1429	10	SW	13
23	3	Grassland	3			415	4	S	20
25.5	0	Pasture	11			More	0		
28	2								
>28	0								

Reproduction and Presence of Pollinators

The pollination of Hexastylis has not been well studied but the genus is thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal via ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (*H. heterophylla, H. naniflora, and H. minor*), which employ myrmecochory as a method for seed dispersal.

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Because the flower for this species is oftentimes partially or completely covered with soil and leaf litter, possibly inhibiting pollinator activity (Gonzalez 1972), there is still uncertainty in the pollination mechanism for dwarf-flowered heartleaf. Otte (1977) suggests that a variety of possible pollinators reside in this leaf litter, however, the calyx opening is considered to be far too small for efficient pollinating (Gaddy 1981). There are, however, invertebrates within this proposed size limit that could theoretically act as pollinators. It is possible that the species employs self-pollination, with or without a vector, or that cross-pollination occurs by a number of invertebrates. Jones et al. 2014 investigated pollination of dwarf-flowered heartleaf under a manipulative experimental design, and found that while insects may play a significant role in pollination, even without them, flowers managed to produce a partial seed set. However, two points need to be considered here. First, although flowers managed to produce seeds in the absence of insect pollinators, the efficiency of pollination decreased by almost 50%. Also, even if successful pollination occurs in the absence of insect vectors, the dispersal of plants amongst populations would be limited, and could result in decreased resilience due to genetic concerns such as limited gene flow and issues associated with potential inbreeding depression.

Abundance

Commented [RNR7]: Should abundance be discussed with distribution or vice versa?

The influence of stochastic variation in demographic (reproductive and mortality) rates is much higher for small populations than large ones. Stochastic variation in demographic rates causes small populations to fluctuate randomly in size. In general, the smaller the population, the greater the probability that fluctuations will lead to extinction. There are also genetic concerns with small populations, including reduced availability of compatible mates, genetic drift, and inbreeding depression. Small populations of dwarf-flowered heartleaf have low resilience, leaving them particularly vulnerable to stochastic events.

As of 2016, the combined databases of the NCNHP and SCNDNR contain 239 Element Occurrence Records (EORs) for *H. naniflora* (NCNHP2016, SCDNR 2016). These EORs depict roughly 113 locations which are sufficiently geographically distinct to be regarded as proxies for populations of the species (See *Delineating Populations*). Thus, the total number of populations has increased more than four-fold (from 24 to 113) since the species was listed in 1989.

Commented [RNR8]: I've seen 239 and 293 referenced. We just need to verify with NHP. They may check it in response to elicitation questions.

At this time, the largest known populations have been monitored by NCNHP and NCDOT. The estimates for entire populations are based on a consistent monitoring methodology developed by NCDOT, USFWS, and NCNHP with monitoring plots representing roughly 10% of a population. Populations were delineated to get a more accurate boundary and size of the area occupied. All rosettes are counted annually in each monitoring plot to estimate an extrapolated population size, based on the number and density in the plots. As a result of these efforts, better estimates of population sizes for the largest known populations are available, compared to when the last five-year review was completed in 2010 (Robinson and Padgett 2016).

Commented [RNR9]: Would it be helpful to include some of the population trend analysis here? Or, would that come later? I think long-term population stability would equate or relate to population resiliency.

The 113 EORs have been estimated to contain anywhere from a single rosette to over 100,000 rosettes. Table A1 (Appendix A) was created by NCNHP to replicate the same format and population data as Table B2 of the most recent USFWS five-year review of *H. naniflora* (USFWS 2010), for comparison of changes since 2010. Table A1 summarizes the largest occurrences of *H. naniflora*, with the size of the population based on the number of rosettes it was last estimated to contain. The number of populations estimated to contain over 1,000 rosettes is 26. This is approximately 23% of the total known populations and many of these populations contain well over 1,000 individuals.

There are, however, 13 populations (12% of all known) that are simply known to be extant, with no available estimate of population size (NCNHP 2016, SCDNR 2016). If the most recent population estimates for each EOR are compiled across years of observation, the 113 populations could conservatively be estimated to contain a collective total of more than 300,000 rosettes (NCNHP 2016, SCDNR 2016).

Appendix A

Table A1: Populations of *Hexastylis naniflora* estimated to contain over 1,000 rosettes in 2010, with updated information in 2016[1], and protection measures for these populations.

Populations known to be afforded some protection are indicated in **bold**.[2]

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
1	NC	Burke	029	A	Island Creek Heath Bluff (Lovelady Sites)	50,000+ (2016)	0	Not protected.
2	NC	Burke	178.159-178.161 178.254-178.256 178.280-178.282	A	Catawba River: Love Lady (Hoyle and Micol Creek)	~6,738 (2008-2011)	Awaiting data from NCDOT.	Possible NCDOT protected R-O-W, protection unconfirmed. In early 2016, the Town of Valdese applied to CWMTF to acquire part of this population.
3	**NC**	**Caldwell**	**227**	**A**	**Peaked Top Rare Plant Site (Foothills Landfill)**	**16,463 (2014)**	**16,463 (2014)**	**Conservation Easement (Foothills Conservancy of NC).**
4	**NC**	**Caldwell**	**044**	**AB**	**Little Gunpowder Creek Rare Plant Site 1**	**2,707 (2015)**	**2,424 (2015)**	**DOT mitigation site.**
5	NC	Catawba	158	D	South Fork Catawba River: Jacob Fork, Camp Creek	123 (2016)	27 (2015)	Some plants occur within an undetermined acreage within NCDOT R-O-W (Bassette 2016). Protection unconfirmed.
6	**NC**	**Catawba**	**243.012,** 243.242 243.269, **243.270**	**A**	**Catawba Wildlife Club**	**1,000-1300 (1995-2007)**	**>1,000 (1995-2007)**	**Catawba County Wildlife Club Registered Heritage Area (EO 12) The Nature Conservancy Conservation Easement (EO 270).**
7	NC	Catawba	031	C?	US 321, southeast Hickory	1,202 (2010)	161 (2010)	161 plants within NCDOT R-O-W (Bassette 2016).
8	**NC**	**Catawba**	**96.184,** 96.038 96.039	**A**	**Murrays Mill/Upper Balls Creek**	**~9,150 (2005)**	**11,860 (2013)**	**Fee title by NCDOT (~34 acres).**
9	**NC**	**Cleveland**	100.014, **100.049, 100.050, 100.051, 100.073, 100.074 100.149, 100.233**	**A**	**Broad River/Sandy Run Natural Areas (aka "Broad River Tract")**	**~33,500 (2001-2015)**	**32,702 (2015)**	**Fee title by Broad River Greenway (1,000 acres).**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
			100.236, 100.237 **100.238, 100.239 100.240, 100.241,** 100.246					
10	NC	Cleveland	208	A?	Buffalo Creek: Potts Creek	3,572 (2007)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
11	NC	Cleveland	211.072, 211.194		First Broad River: Hop-Hornbeam Natural Area	1,965 (2000)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
12	NC	Cleveland	214.201, **214.202, 214.203,** 214.204, **214.205, 214.209,** 214.212, **214.213,**	A	Buffalo Creek: tributaries north and south of SR2047	6,447 (200-2007)		Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 202, 203, 205, 209, 213).
13	NC	Cleveland	216.028, 216.196, 216.197, 216.198, **216.199, 216.200**	A	Buffalo Creek: Kings Mountain Reservoir	3,310 (200-2016)	~500? (2000-2007) (awaiting updates)	Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 199, 200).
14	NC	Cleveland	157	AB	First Broad River: Crooked Run Creek	330 (2010)		None of the population is protected within NCDOT R-O-W, determined too far away from roadside, DOT stopped monitoring in 2010 (Bassette 2016).
15	**NC**	**Cleveland/ Rutherford**	**276**	**A**	**Cliffside Steam Station**	**39,545 (2016)**	**~39,545 (2016)**	**Voluntary agreement with Duke Energy; protected acreage unknown.**
16	NC	Lincoln	261.252, 261.259, 261.263	B	Lincoln County Airport and Leepers Creek	7,032 (2005-2006)		Not protected.
17	NC	Polk	023	A	Mills Creek Forest and Seep	1,459 (2016)		Not protected.

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
18	**NC**	**Polk**	**125**	**A**	**Broad River: New Hope Springhead Swamp**	**12,235 (2016)**	**1,000-3,000 estimated (2016)**	**New Hope Springhead Swamp Registered Heritage Area (voluntary conservation agreement).**
19	**NC**	**Rutherford**	**154**	**C**	**Second Broad River (Forest City Industrial Complex)**	**2,576 (2016)**	**2478 (2008)**	**Deed restrictions (12 acres). Significant threats from industrial development, invasive exotics, erosion, sedimentation in a fragmented landscape.**
20	**NC**	**Rutherford**	99.009, 99.010, 99.037, 99.052, 99.053, 99.055, 99.061, 99.063, 99.075, **99.076,** 99.079, 99.090, 99.121, **99.172,** 99.181, 99.182, 99.249-.251, 99.267, 99.268	**A**	**Broad River: Hensons Creek Ravine, Brice Rare Plant Site, Sandy Mush Outcrop**	**106,940 (2016)**	**~200-250 (2007-2012)**	**Parris Heartleaf Registered Heritage Area (voluntary conservation agreement on subEO 076 and 172).**
21	NC	Rutherford	176.167-176.170	A	Broad River: Cleghorn Creek Tributary/US221	6,750 (2016)		Not protected.
22	NC	Rutherford	177.016, 177.107, 177.122, 177.063, 177.164, 177.165, 177.166	A	Broad River: Floyds Creek, Long Branch	12,687 (2008-2016)		Not protected.
23	**NC**	**Rutherford**	247.013, 247.056, 247.080, **247.106,** 247.114, 247.115, 247.173, 247.174, **247.286**	**A**	**Broad River: Floyds Creek (aka New Bethel Rare Plant Site)**	**~5,480 (2003-2015)**	**4,873 (2015)**	**Tate Conservation Easement (8 acres) (SubEOs 106 and 286).**
24	**SC**	**Cherokee**	**016, 017, 018**	**BC**	**Cowpens Battlefield**	**2,823 (2016)**	**2,823? (2016)**	**National Park Service, National Battlefield.**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
25	SC	Greenville /Spartanburg	002, 004, 024, 032, 033, 036, 038, 039, 040, 041, 042, 049, 050, 054	Various AB-BD	South Pacolet River and tributaries	5,205 (1991-1998)		Not protected.
26	**SC**	**Spartanburg**	**011, 014, 026--028, 047, 048, 057**	**Various AB-BD**	**Peters Creek Heritage Preserve**	**3,306 (2016)**	**3,306 (2016)**	**State Heritage Preserve (194 acres).**
27	**SC**	**Spartanburg**	**007, 029**	**BD, H?**	**Blalock Reservoir**	**3,505 (2016)**	**3,505? (2016)**	**Restrictive covenants (protected acreage unknown); significant threats to remaining population.**
28[6]	**NC**	**Lincoln**	302.046, **302.262**	**A**	**Rhyne Preserve**	**19,880 (2016)**	**19,873 (2016)**	**Rhyne Preserve (fee title by Catawba Lands Conservancy).**

[1]This table was replicated in the same format and populations as Table B.2 from the most recent USFWS five-year Review of *Hexastylis naniflora* (USFWS 2010) for comparison of changes since the time of the five-year review. Populations that are not believed to contain over 1,000 rosettes but were included in the original Table B.2 are included here, but the "NHP EO rank" and in the "Latest estimate of population size" reflects the smaller size.

[2] In the case of partially protected populations, the column "NHP EO numbers" indicates the specific portions of the population afforded protection in bold font.

[3]NHP EO numbers use the following format: Principal EO.SubEO. Only one number is listed if it is a stand-alone principal EO.

[4]NHP EO rank specifications are defined in Appendix B2. In cases involving principal/sub EOs, only the rank for the principal EO is given.

[5] EO ranks with two letters indicate a degree of uncertainty within the range provided (e.g. a rank of "AB" indicates possibly excellent to good viability).

[6]Population that is currently estimated to have over 1,000 rosettes that was not known to be large populations in 2010 have been added to this table.

From:	Marshall, Michael E
To:	Reid, Rebekah N; Endries, Mark
Subject:	HENA draft
Date:	Thursday, April 19, 2018 11:13:15 AM
Importance:	High

Hey guys,

I'm real close to being ready to send out a draft....would you prefer to review only the Future Conditions, or should I send the entire SSA? As for an entire SSA, I'm still working on merging all of the chapters together and organizing the appendices...also, don't have an executive summary or table of contents yet....

Let me know.

Thanks,

Mike

--
Mike Marshall
SSA Program Specialist
U.S. Fish and Wildlife Service Region 4
Cell: 512-461-6217
Alternate email: mmarshall@ag.tamu.edu

Work Schedule 1st Week
Monday-Thursday --> In Office 7:30-4:30 CST

Work Schedule 2nd Week
Monday-Thursday --> In Office 7:30-4:30 CST

From:	Endries, Mark
To:	Marshall, Michael E
Cc:	Reid, Rebekah N
Subject:	Re: HENA draft
Date:	Thursday, April 19, 2018 11:20:29 AM
Importance:	High

I'd like to see the whole SSA.

Thanks!

Mark

Mark Endries
USFWS
160 Zillicoa St
Asheville, NC 28801
Office: 828.258.3939 ext. 231
Mobile: 828.215.1740

On Thu, Apr 19, 2018 at 11:13 AM, Marshall, Michael <michael_marshall@fws.gov> wrote:

> Hey guys,
>
> I'm real close to being ready to send out a draft....would you prefer to review only the Future Conditions, or should I send the entire SSA? As for an entire SSA, I'm still working on merging all of the chapters together and organizing the appendices...also, don't have an executive summary or table of contents yet....
>
> Let me know.
>
> Thanks,
>
> Mike
>
> --
> Mike Marshall
> SSA Program Specialist
> U.S. Fish and Wildlife Service Region 4
> Cell: 512-461-6217
> Alternate email: mmarshall@ag.tamu.edu
>
> **Work Schedule 1st Week**
> Monday-Thursday --> In Office 7:30-4:30 CST
>
> **Work Schedule 2nd Week**
> Monday-Thursday --> In Office 7:30-4:30 CST
>
>

From:	Reid, Rebekah N
To:	Endries, Mark
Cc:	Marshall, Michael E
Subject:	Re: HENA draft
Date:	Thursday, April 19, 2018 11:23:44 AM
Importance:	High

Me, too.

Rebekah Reid

US Fish and Wildlife Service
Asheville Ecological Services Field Office
160 Zillicoa St.
Asheville, NC 28801
phone: 828-258-3939 x238
cell: 828-782-0090

On Thu, Apr 19, 2018 at 11:20 AM, Endries, Mark <mark_endries@fws.gov> wrote:

> I'd like to see the whole SSA.
>
> Thanks!
>
> Mark
>
> **Mark Endries**
> USFWS
> 160 Zillicoa St
> Asheville, NC 28801
> Office: 828.258.3939 ext. 231
> Mobile: 828.215.1740
>
> On Thu, Apr 19, 2018 at 11:13 AM, Marshall, Michael <michael_marshall@fws.gov> wrote:
>
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> >
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> > Thanks,
> >
> > Mike
> >
> > --
> > Mike Marshall

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Work Schedule 2nd Week
Monday-Thursday --> In Office 7:30-4:30 CST

From:	Marshall, Michael E
To:	Reid, Rebekah N
Cc:	Endries, Mark
Subject:	Re: HENA draft
Date:	Thursday, April 19, 2018 11:26:36 AM
Importance:	High

Sounds good! I may need to send without an executive summary if that's ok....lemme keep chipping away. Coming soon!

Mike

On Thu, Apr 19, 2018 at 11:23 AM, Reid, Rebekah <rebekah_reid@fws.gov> wrote:

> Me, too.
>
> Rebekah Reid
>
> US Fish and Wildlife Service
> Asheville Ecological Services Field Office
> 160 Zillicoa St.
> Asheville, NC 28801
> phone: 828-258-3939 x238
> cell: 828-782-0090
>
>
>
> On Thu, Apr 19, 2018 at 11:20 AM, Endries, Mark <mark_endries@fws.gov> wrote:
>
>> I'd like to see the whole SSA.
>>
>> Thanks!
>>
>> Mark
>>
>> **Mark Endries**
>> USFWS
>> 160 Zillicoa St
>> Asheville, NC 28801
>> Office: 828.258.3939 ext. 231
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>>
>> On Thu, Apr 19, 2018 at 11:13 AM, Marshall, Michael <michael_marshall@fws.gov> wrote:
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> > **Work Schedule 2nd Week**
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> >
> > *This email correspondence and any attachments to and from this sender is subject to the Freedom of Information Act and may be disclosed to third parties.*

--
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INTRODUCTION

The dwarf-flowered heartleaf (*Hexastylis naniflora*) is a species of plant endemic to the upper Piedmont region of western North Carolina and upstate South Carolina. The dwarf-flowered heartleaf has been listed as threatened under the Endangered Species Act of 1973, as amended (Act), since 1989 (FR 54 14964-14967). The Species Status Assessment (SSA) framework (USFWS 2016, entire) summarizes the information complied and reviewed by the US Fish and Wildlife Service (Service), incorporating the best available scientific and commercial data, to conduct an in-depth review of the species' biology and threats, evaluate its biological status, and assess the resources and conditions needed to maintain long-term viability. The intent is for the SSA to be easily updated as new information becomes available and to support all functions of the Endangered Species Program from Listing to Consultations to Recovery.

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A Recovery Plan for the species was never completed. However, over the last 29 years, the Service has worked closely with partners to make significant progress toward recovery of the species. The Service is initiating this SSA to aid in determining the appropriateness of reclassifying the species. In the event that the SSA does not support reclassification, the SSA would be used to inform the development of a Recovery Plan. Importantly, the SSA does not result in a decision by the Service on whether this species should be proposed for reclassification under the Act. Instead, this SSA provides a review of the available information strictly related to the biological status of the dwarf-flowered heartleaf. The reclassification decision will be made by the Service after reviewing this document and all relevant laws, regulations, and policies. The results of a proposed decision will be announced in the *Federal Register,* with appropriate opportunities for public input.

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For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in its natural systems over time. Using the SSA framework (Figure 1), we consider what the species needs to maintain viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation (Wolf *et al.* 2015, entire).

- **Resiliency** describes the ability of populations to withstand stochastic events (arising from random factors). We can measure resiliency based on metrics of population health;

for example, birth versus death rates and population size. Highly resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of anthropogenic activities.

- **Redundancy** describes the ability of a species to withstand catastrophic events. Measured by the number of populations, their resiliency, and their distribution (and connectivity), redundancy gauges the probability that the species has a margin of safety to withstand or can bounce back from catastrophic events (such as a rare destructive natural event or episode involving many populations).
- **Representation** describes the ability of a species to adapt to changing environmental conditions. Representation can be measured by the breadth of genetic or environmental diversity within and among populations and gauges the probability that a species is capable of adapting to environmental changes. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or human caused) in its environment. In the absence of species-specific genetic and ecological diversity information, we evaluate representation based on the extent and variability of habitat characteristics across the geographical range.

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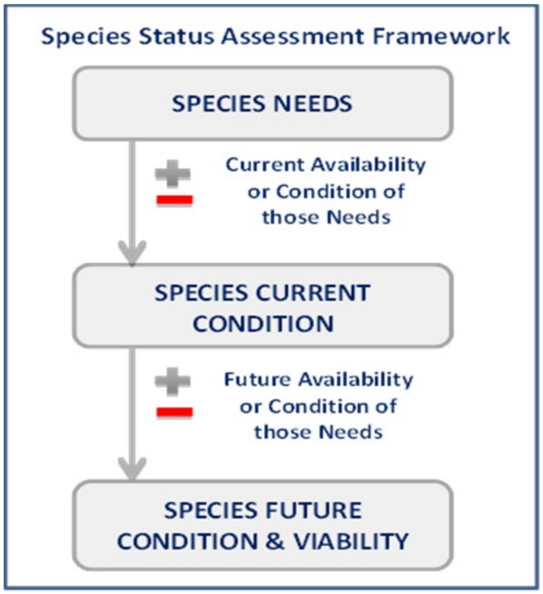

Figure 1- Species Status Assessment Framework

To evaluate the biological status of the dwarf-flowered heartleaf, both currently and into the future, we assessed a range of conditions to allow us to consider the species' resiliency, redundancy, and representation (together, the 3Rs). This SSA provides a thorough assessment of biology and natural history, and assesses demographic risks, stressors, and limiting factors in the context of determining the viability and risks of extinction for the species.

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The format for this SSA includes: (1) Biology and Species Needs (2) Current Conditions (3) Influences on Viability and (4) Future Conditions. This document is a compilation of the best available scientific and commercial information, and a description of past, present, and likely future risk factors to the dwarf-flowered heartleaf.

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Biology and Species Needs:

In this chapter, we provide basic biological information about the dwarf-flowered heartleaf, including its taxonomic history, species description, distribution, life history traits, and habitat characteristics. We then use this information to outline the resource needs within various life stages of dwarf-flowered heartleaf. Here we report those aspects of the life history of the dwarf-flowered heartleaf that are important to our analysis.

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Taxonomy and species description

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Dwarf-flowered heartleaf is a rare, low growing herbaceous plant in the birthwort family (Aristolochiaceae). The species was described by Blomquist (1957) in his revision of the North American members of the genus *Hexastylis*. The dwarf-flowered heartleaf has been recognized as part of the Virginica Group, and this group was further subdivided into three Subgroups: Virginica, Shuttleworthii, and Heterophylla (Blomquist 1957; Whittemore and Gaddy 1997). Three species have been recognized in the Heterophylla complex, *Hexastylis naniflora, H. heterophylla* and *H. minor*, and field biologist have generally recognized that considerable morphological overlap occurs (Murrell et al. 2007). One of the main concerns regarding this complex was the inability to distinguish between species without access to fresh flowers. Even with fresh flowers, Blomquist (1957) and Gaddy (1987) still recognized considerable overlap in flower morphology making species delineation difficult.

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Murell et al. 2007, conducted a comparative genetic analysis using Inter Simple Sequence Repeats, and were unable to find banding patterns that could be used to separate *H. naniflora* from the other members within the complex. However, based on biogeographical, ecological, molecular, morphological, as well as micromorphological work, their results show that *H. naniflora* is a well-defined species.

Although there has been considerable disagreement on the generic distinctiveness of *Hexastylis* and *Asarum* (Barringer 1993 and Kelly 1997, 1998, 2001), a recent phylogeny estimate using chloroplast genes supports that *Hexastylis* is a monophyletic clade and therefore should be recognized as a genus (Niedenberger 2010). Additionally, most North American publications recognize *Hexastylis* at the generic level (Flora of North America 1997, Weakley 2015).

The Service is not aware of any proposed changes in taxonomy that would affect the continued legal status of *H. naniflora* under the Act. However, within the range of *H. naniflora* there are populations which fall outside of the range of published values for key floral characteristics, overlapping with values described for *H. heterophylla* or *H. minor* (Figure 2; Weakley, 2010; Murrell et al. 2007; Gaddy 1987). These geographic areas of overlap in key characters have been the focus of recent genetic analyses (Murrell et al. 2007; Renninger, 2010; Murrell, 2015).

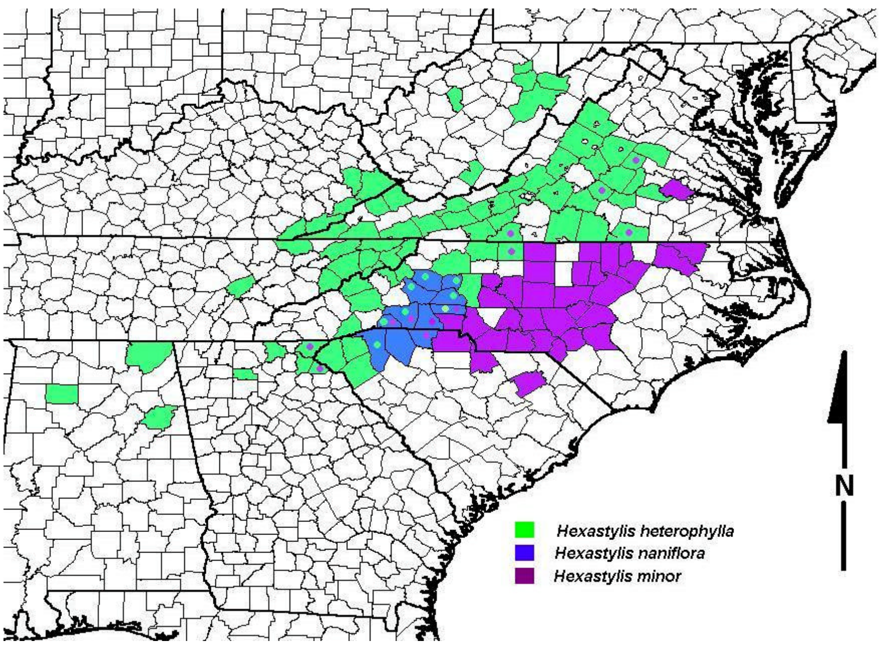

Figure 2: *From Murrell et al. 2007*. Distribution map showing county records for the three species in the *H. heterophylla* complex. Data was gathered from herbarium specimens, Element Of Occurrence (EOC) sheets and field studies. Dots within *H. heterophylla* counties indicates co-occurrence with *H. minor*. Light dot within *H. naniflora* counties indicates co-occurrence with *H. heterophylla*, dark dot indicates co-occurrence with *H. minor*.

Commented [RNR2]: Iredell is missing from HENA range on this map.

The most outstanding characteristic of this species is the small flowers, which are one of the smallest of any species in North America (Blomquist 1957). The plant's heart shaped leaves are dark green in color, evergreen, and leathery, and are supported by long thin petioles from a subsurface rhizome. The shape of the leaf blades, their pattern of variegation, and the ridged reticulation inside the calyx-tube, place this species inside the Virginica group. It differs from all the other members of this group, aside from the small flowers, in having no flare in the calyx-tube. Maximum height rarely exceeds 15 centimeters (cm). The jug shaped flowers are usually beige to dark brown in color and appear from mid-March to early June. The flowers are small and inconspicuous and are found near the base of the petioles. The fruit matures from mid-May to early July (Blomquist 1957, Gaddy 1980, 1981). Characteristics that distinguish it from other *Hexastylis* species are found in floral structures and pollen characters (Gaddy 1987, Padgett 2004, Niedenberger 2010). *H. naniflora* has a smaller calyx tube orifice, which is typically 5mm or less (sometimes up to 7mm) and the ovary is half-inferior, rather than superior (Blomquist 1957, Gaddy 1987, Padgett 2004, HDR 2005). Pollen surface features have also been shown to be an effective character to identify *H. naniflora*, as it has a microporate surface and, unlike any other *Hexastylis* species, lacks gemmae entirely (Padgett 2004, Niedenberger 2010).

Distribution

Although dwarf-flowered heartleaf is restricted in range, it is not as rare as once thought (USFWS 2010, NCNHP 2016). When dwarf-flowered heartleaf was federally listed in 1989, the listing rule described 24 extant "populations" (and one extirpated population) distributed across eight counties in the upper Piedmont of North and South Carolina. Since 1989, the range has expanded to include four additional counties in North Carolina. In North Carolina, it is found in Alexander, Burke, Caldwell, Catawba, Cleveland, Gaston, Iredell, Lincoln, Polk, and Rutherford Counties. In South Carolina, it is in Cherokee, Greenville, and Spartanburg Counties. As of 2016, the distribution of this species consisted of 113 populations distributed across 12 counties in these two states (Figure 3).

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Deleted: Since 1989, the county distribution has expanded to include the following North Carolina counties: Alexander, Caldwell, Iredell, and Polk. The species has not been discovered in any additional counties in South Carolina.…

The species appears to be restricted to Pacolet sandy loam, Madison gravelly sandy loam, and Musella fine sandy loam soils (Gaddy 1981,1987). These soils are restricted to an area from near

Charlotte, North Carolina west to the foot of the mountains near Rutherfordton, North Carolina, and from Hickory, North Carolina southward to just south of Spartanburg, South Carolina (Murrell et al. 2007).

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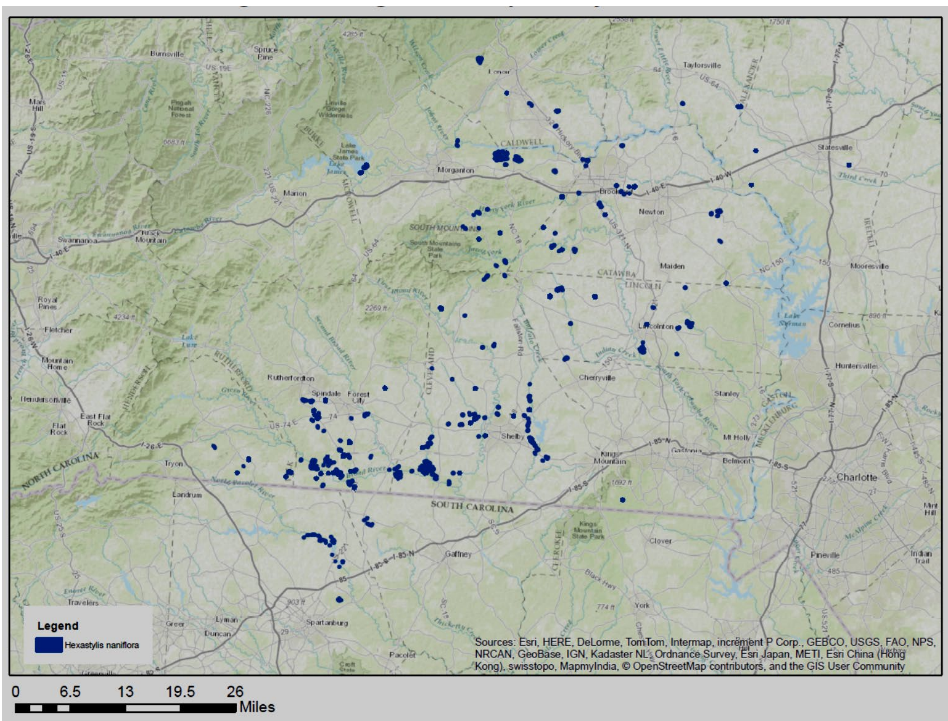

Figure 3--this is a placeholder…we will generate a new map in Arc GIS.

Many of those working with *Hexastylis naniflora* have used the terms “sub site”, “site”, “location”, “occurrence” (often, but not always, in reference to Natural Heritage Program Element Occurrence (EO) Records), “subpopulation” and “population” interchangeably. Others have aggregated smaller sites into populations according to subjective criteria which have never been explicitly defined. This generates discrepancies among sources with respect to the abundance and distribution of the species, with the net result being that available data are usually not comparable from one source to the next. We describe how the numerous small, site-specific locations containing *H. naniflora* have been aggregated into proxies for 113 biological populations for purposes of this review, using mapping standards devised by NatureServe and its network of Natural Heritage Programs, in the Species Needs section of this report.

Life History

Demographics

The Service is aware of a single effort to collect demographic-level data (survivorship and recruitment of tagged individuals) for dwarf-flowered heartleaf. This effort was conducted during the 1990-1991 field seasons, within a portion of the Peters Creek population in Spartanburg County, SC (Newberry, 1993). This study demonstrated a 96.1% survival rate over these two consecutive seasons, with 50% of the mortality occurring in plants located at the highest position on the forested slope (away from the adjacent floodplain). Mortality was highest in small plants bearing fewer than four leaves. Plant size was variable, with the largest plant bearing 45 leaves and 33 flowers, and situated in the floodplain. In general, plants located in the floodplain were larger than plants located on adjacent slopes. The percentage of flowering plants averaged 70%, with the highest frequency of flowering occurring among plants situated in the floodplain (USFWS 2010).

Pollination and Dispersal

The pollination of Hexastylis has not been well studied but the genus was thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal by ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (*H. heterophylla, H. naniflora, and H. minor*), which employ myrmecochory as a method for seed dispersal.

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Ants appear to be a primary dispersal agent for the dwarf-flowered heartleaf (Gaddy 1986; Jones et al. 2014). All diaspores of *Hexastylis naniflora* presented to ants (*Aphaenogaster rudis*) were quickly removed (Gaddy, 1986). This is not to say that they are not occasionally, or even frequently, dispersed and/or pollinated by other means. Jones et al. (2014) suggests that the pollination mechanism is facultative, benefiting from more than one method of pollination/fertilization. Ants were the pollinators in a controlled experiment, and their data supports that when outside molesting forces/pollinators (biotic and abiotic) were limited by their caging procedure, the efficiency of pollination decreased by almost 50%, however, caged flowers did produce seeds, indicating pollinization occurred via some alternative method.

Habitat

Dwarf-flowered heartleaf appears to have a restricted range due to its habitat requirements. The habitat where dwarf-flowered heartleaf exists is limited in size and scope due to a multitude of factors including soil type, moisture availability, and slope aspect. This unique combination of factors limits not only the range of dwarf-flowered heartleaf, but also the size of a given population. With the limited range and size in populations, questions arise regarding gene flow among populations. How much is occurring and how often does it occur? It is due, in part, to narrow habitat requirements that conservation measures have been implemented for the protection of the species. Any efforts made to protect this species must consider giving protection to the available habitat.

Dwarf-flowered heartleaf occurs on piedmont uplands on acidic sandy-loam soils that are very deep and moderately permeable (Gaddy 1981, 1987). Typical habitats for this species include mesic to dry bluffs, slopes, or ravines in deciduous forests that are frequently associated with *Kalmia latifolia* (Padgett 2004, Weakley 2015, USFWS 2015), or in moist soils adjacent to creeks, streamheads, or along lakes and rivers. Plants have been observed to grow larger and have more frequent flowering in floodplains along rivers, lakes, and streams (Newberry 1993). Wagner (2013) conducted a habitat suitability study to quantify the habitat requirements for dwarf-flowered heartleaf, which may be used for helping identify the species when not in flower (relative to other *Hexastylis* species habitat preferences), find new populations, or identify suitable sites for transplants. A model was created to predict habitat suitability to determine how many EOs are found within habitat considered fair to excellent (Figure 4; Wagner 2015). Based on the model, the strongest habitat correlations were that the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Wagner 2015). Populations found in areas with a percent slope of greater than 28, soil codes other than 6, 7, or 11, or elevation less than 199 m or greater than 415 m are unlikely to be *H. naniflora.* The model accurately predicts habitat suitability at a local scale 81% of the time.

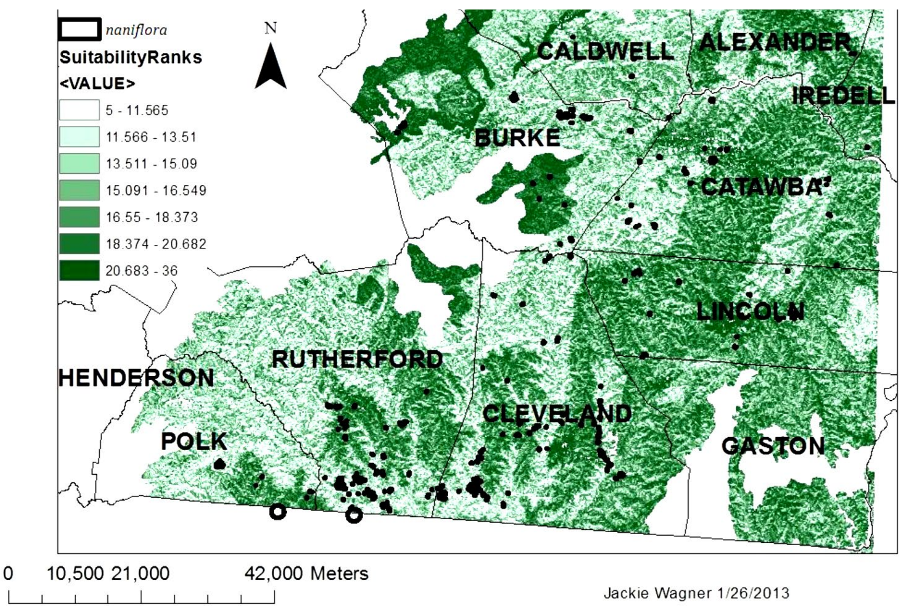

Figure 4: The site suitability ranks based on five habitat variables across the counties where *H. naniflora* is known to exist in NC. The smaller numbers indicate a higher rank and lighter green areas denote more suitable habitat. Known populations of *H. naniflora* are outlined in black.

Soils

The species grows in acidic soils along bluffs and adjacent slopes, in boggy areas next to streams and creekheads, and along the slopes of nearby hillsides and ravines (Gaddy 1980, 1981). The plant is primarily found inhabiting north- to northwest-facing slopes, bluffs, and ravines in close proximity to creeks or streams. Within these areas exists the soil type required for *H. naniflora*

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to grow. It grows primarily on well-drained, sandy, acidic soils, and will not grow in heavy clay (Gaddy 1981).

The results of soil samples analyzed at the Clemson Soil Lab suggest that major differences in soil chemistry exist between the species in the *H. heterophylla* complex. Statistical analysis of the soil samples showed that many of the basic elements were significantly different among the three species. Those significant differences occurred in Phosphorous (P), Potassium (K), Magnesium (Mg), Zinc (Zn), Manganese (Mn), (Na), Sodium, and Cation Exchange Capacity (CEC). Slightly significant differences were seen in Buffer pH (Bu pH), and Acidity.

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Soil chemistry showed marked differences between the species in the complex. The results indicated that soil chemistry is very different between *H. naniflora* and *H. minor* localities. The results also show that *H. heterophylla* and *H. naniflora* are found in soils where the chemistry is more similar, but still showed significant differences. It would appear that differentiation in soil types could be used as a proxy for species delineation. The soil analysis also indicates that soils must be considered when trying to select sites for relocation of imperiled populations of *H. naniflora*.

Thirteen population sites in North Carolina and South Carolina were examined using the Carolina Vegetation Survey (CVS) method to compare species richness between the three species of the *Hexastylis heterophylla* complex. The analysis did not show statistically significant differences among the three species in the *H. heterophylla* complex. However, *H. naniflora* appears to have an association with three oak species that is lacking in the other two species in the complex. There are a number of oak species (*Q. coccinea*, *Q. prinus* (*Q. montana*), and *Q. velutina*), that tend to co-occur with only *H. naniflora*, but are not present with the other two species in the complex. This may be the result of some microbial need or specific soil nutrient required for those species to occur in the same habitat.

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Fire

There are little data on the response to fire by *Hexastylis naniflora*, however, prescribed burns have been conducted within the population at Cowpens National Battlefield in Cherokee County, SC. Preliminary data at this site and recent annual monitoring data of this population support the theory that moderate controlled burns do not negatively affect this population (Walker et al. 2009). Additionally, a dormant season wildfire in Caldwell County did not show evidence of negative impacts to a population in Caldwell County (USFWS 2010). Fire suppression could be a hazard to *H. naniflora* by allowing fire intolerant, nonnative and invasive plants to thrive, as well as the accumulation of thick duff or leaf litter that may shade low growing species (Wagner 2013).

Genetics

Analyses on ecology, morphology, soil chemistry, pollen, and molecular genetics have been evaluated for *Hexastylis naniflora* to determine the boundaries within the *Hexastylis heterophylla* complex, which consists of *H. heterophylla*, *H. minor*, and *H. naniflora* (Murrell 2015, Wagner 2013, Niedenburger 2010, USFWS 2010, Murrell et al. 2007, Padgett 2004). These analyses support the continued recognition of these taxa as well-defined, discrete species. Scanning electron microscopy (SEM) consistently distinguished *H. naniflora* from other members of the *H. heterophylla* complex based on pollen microscopy. Principal Components Analysis of floral characters and soil chemistry also consistently distinguished *H. naniflora* from *H. minor* and *H. heterophylla*. However, efforts to obtain consistently distinct banding patterns using Inter Simple Sequence Repeats (ISSRs) were unsuccessful at distinguishing *H. naniflora* from other members of this group (Murrell et al., 2007). These results were based upon an extremely small sample size (n=10 *H. naniflora* individuals), and therefore warrant further investigation.

Field observations demonstrate that there are some populations of dwarf-flowered heartleaf with morphological characteristics that do not fit within the range of published values for key traits, overlapping with values for *H. heterophylla* or *H. minor* (Gaddy 1987, Murrell et al. 2007, USFWS 2010, Weakley 2015). These populations with overlap in key characteristics were the focus of a genetic analysis conducted at Appalachian State University (ASU) through funding provided by NCDOT (Murrell 2015). In some populations, floral characteristics are highly variable, which suggests the potential for hybridization or individuals with highly variable flower size and shape (Murrell 2015). Additionally, no vegetative characters were previously known to consistently distinguish *Hexastylis naniflora* from other close relatives. Given the difficulties with field identification of the species, particularly when not in flower, this study sought to develop a microsatellite library of molecular markers to resolve variation in populations of dwarf-flowered heartleaf and apply the markers to populations with highly variable characters, as identified by NCDOT biologists. The morphological and micromorphological information from those variable populations were compared to molecular results with morphological, micromophological, and distributional data to determine genetic structure, biological boundaries, and placement of putative hybrids or intermediate populations of *H. naniflora* (Murrell 2015).

The preliminary findings of this study suggest that populations in the southern range of dwarf-flowered heartleaf exhibit a more uniform genetic pattern, with some possible hybridization with *H. minor*. Populations in the northern part of the range appear to have hybridized with both *H. heterophylla* and *H. minor*, although there are still individuals with "pure" *H. naniflora* genotypes in the northern range (Murrell 2015). It is critical to note that although these data provide anecdotal evidence of hybridization with the *Hexastylis heterophylla* group, intraspecific variation may be caused by forces other than hybridization, such as convergent morphological evolution (Dobzhansky 1937), or the species is in the process of speciation and this study shows a case of incomplete speciation (Murrell 2015), and/or other environmental factors are at play (Wagner 2013). On May 11, 2016 a meeting was held with USFWS, NCNHP, NCDOT, and ASU to discuss the status of *H. naniflora* and the current work being conducted among the agencies (Amoroso 2016). Based on discussions during this meeting, the results of this study reported by ASU to NCDOT in 2015 are preliminary. Dr. Matt Estep (ASU) provided additional preliminary results to NCNHP in May 2016, showing which populations were sampled, sample size, and percent of samples that show evidence of hybridization, and hybridizing with which species. ASU continues to work towards a more definitive explanation of the variation in the *H. heterophylla* complex (Murrell 2015, Amoroso 2016).

SPECIES NEEDS

Commented [RNR4]: The guidance documents and several examples I have seen discuss needs on an Individual, Population, and Species level. Do we need to be more explicit earlier in the SSA about what is an individual need vs. population need before jumping to species needs?

For the purpose of this report, we define viability as the ability of the species to sustain populations in the wild over time. Species with greater numbers (redundancy) of healthy populations (resiliency), encompassing a broad array of ecological and genetic diversity in a spatial arrangement that maintains adequate gene flow (representation), are more likely to be viable. Using the Species Status Assessment framework, we describe the species' viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation.

Delineating Populations

As stated in the USFWS five-year review, many of those working with dwarf-flowered heartleaf have used the terms "sub site," "site," "location," "occurrence" (often, but not always, in reference to Natural Heritage Program Element Occurrence Records), and "population" interchangeably, while others have aggregated sites into populations according to subjective criteria which have never been explicitly defined. This has generated considerable discrepancies among sources with respect to the number of known populations within a given area (or across the species' range), to the extent that numbers are not comparable from one source to the next. The tendency, in the past, to treat each location as a separate population also artificially inflated the actual number of populations known.

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In recent years, NatureServe and its member Natural Heritage Programs have devised mapping standards to balance the need for fine-scale, highly site-specific EO records (required for monitoring and management) with the need to aggregate these records in meaningful units of conservation interest that may approximate biological populations (NatureServe 2002). "Since USFWS does not maintain its own database of known locations of *Hexastylis naniflora*, it regards the NHP databases as the best repository for this information" (USFWS 2010).

We delineate populations for the purposes of this SSA according to the NatureServe (2002) convention. Separation distances are a key component to delineating populations from EO records. For the dwarf-flowered heartleaf, we used the EO Data Standard which provides a Default Separation Distance of 1 km (~0.62 miles) for plant and animal elements that lack EO specs, noting that situations involving dispersal barriers could involve even shorter distances. While gene flow declines over distance at different rates for different elements, the minimum default EO separation distance of 1 km has been accepted by the Network as the most suitable round-number metric-system approximation broadly applicable to many (but not all) situations. This results in several dwarf-flowered heartleaf populations being stand-alone EOs, as well as many populations being aggregates of several EOs.

Population Resiliency

Commented [RNR5]: Isn't resilience discussed on a population level? This document has it under the species level.

For the dwarf-flowered heartleaf to maintain viability, its populations or some portion thereof must be resilient. Stochastic factors that have the potential to affect dwarf-flowered heartleaf include impacts to its habitat, particularly human development pressures, but also climate change and presence of invasive species. Other factors that influence the resiliency of dwarf-flowered heartleaf populations include abundance within populations, and habitat factors such as elevation, slope, aspect, and soil type. Influencing those factors are elements of dwarf-flowered heartleaf ecology that determine whether populations can grow to maximize habitat occupancy, thereby increasing resiliency of populations. These factors and habitat elements are discussed below (Figure 5).

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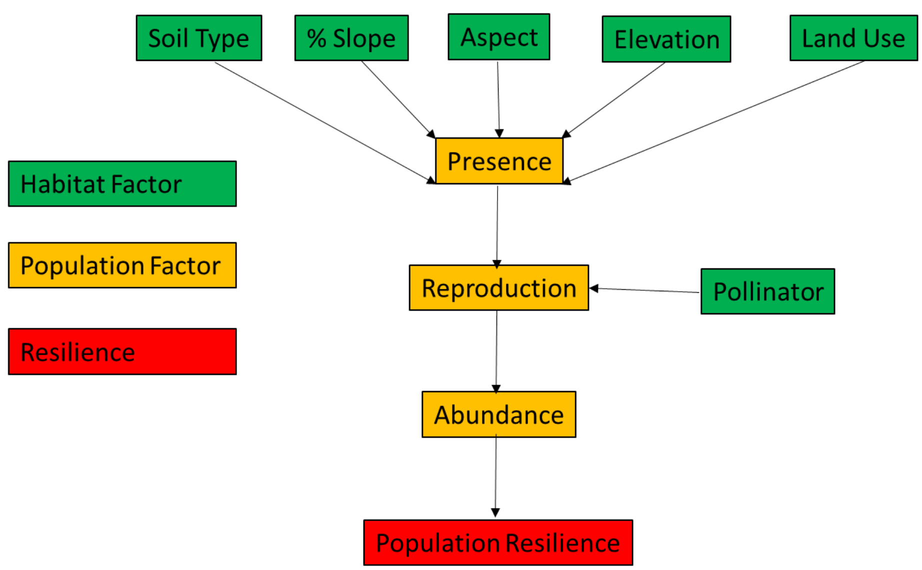

Figure 5--Conceptual diagram describing population and habitat factors influencing population resilience for dwarf-flowered heartleaf.

Habitat Factors: Slope, Aspect, Elevation, Soil, and Forest Type

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In a site suitability model generated with habitat rankings from 5 (the most suitable) to 36 (least suitable habitat) across 7 rank classes, the most common classification included the following microhabitat elements: the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Table 1; Wagner 2015). More importantly, populations found in areas with a percent slope greater than 28, or elevation less than 199 m or greater than 415 m, are unlikely to be dwarf-flowered heartleaf. Slope aspect analysis shows that *H. naniflora* has already adapted to the cooler, wetter conditions of north facing slopes suggesting that this species would fare poorly under climate change scenarios predicting warmer and drier environments (mimicking south facing slopes) throughout their range, supporting a similar claim from Warren (2008).

Table 1. Frequency of element occurrence records (EORs) for each habitat variable. For continuous variables (slope and elevation) data were grouped into classes with the value shown being the top end of the range. * indicates most common classification for that habitat variable.

slope		land use (LU)		soil		elevation		aspect	
% slope	EORs	LU type	EORs	soil code	EORs	Elev. (m)	EORs	aspect	EOR
5.5	20	open water	1	6*	123	<199	0	N*	57
8	50	Low intensity residential	14	7	59	229.8571	34	NE	32
10.5*	54	High intensity residential	3	10	1	260.7143*	65	NW	32
13	35	Commercial/ Industrial	1	11	8	291.5714	29	E	13
15.5	22	Deciduous Forest*	153	other	0	322.4286	34	W	10
18	10	Evergreen Forest	13			353.2857	24	SE	22
20.5	3	Mixed Forest	1			384.1429	10	SW	13
23	3	Grassland	3			415	4	S	20
25.5	0	Pasture	11			More	0		
28	2								
>28	0								

Reproduction and Presence of Pollinators

The pollination of Hexastylis has not been well studied but the genus is thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal via ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (*H. heterophylla, H. naniflora, and H. minor*), which employ myrmecochory as a method for seed dispersal.

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Because the flower for this species is oftentimes partially or completely covered with soil and leaf litter, possibly inhibiting pollinator activity (Gonzalez 1972), there is still uncertainty in the pollination mechanism for dwarf-flowered heartleaf. Otte (1977) suggests that a variety of possible pollinators reside in this leaf litter, however, the calyx opening is considered to be far too small for efficient pollinating (Gaddy 1981). There are, however, invertebrates within this proposed size limit that could theoretically act as pollinators. It is possible that the species employs self-pollination, with or without a vector, or that cross-pollination occurs by a number of invertebrates. Jones et al. 2014 investigated pollination of dwarf-flowered heartleaf under a manipulative experimental design, and found that while insects may play a significant role in pollination, even without them, flowers managed to produce a partial seed set. However, two points need to be considered here. First, although flowers managed to produce seeds in the absence of insect pollinators, the efficiency of pollination decreased by almost 50%. Also, even if successful pollination occurs in the absence of insect vectors, the dispersal of plants amongst populations would be limited, and could result in decreased resilience due to genetic concerns such as limited gene flow and issues associated with potential inbreeding depression.

Abundance

Commented [RNR7]: Should abundance be discussed with distribution or vice versa?

The influence of stochastic variation in demographic (reproductive and mortality) rates is much higher for small populations than large ones. Stochastic variation in demographic rates causes small populations to fluctuate randomly in size. In general, the smaller the population, the greater the probability that fluctuations will lead to extinction. There are also genetic concerns with small populations, including reduced availability of compatible mates, genetic drift, and inbreeding depression. Small populations of dwarf-flowered heartleaf have low resilience, leaving them particularly vulnerable to stochastic events.

As of 2016, the combined databases of the NCNHP and SCNDNR contain 239 Element Occurrence Records (EORs) for *H. naniflora* (NCNHP2016, SCDNR 2016). These EORs depict roughly 113 locations which are sufficiently geographically distinct to be regarded as proxies for populations of the species (See *Delineating Populations*). Thus, the total number of populations has increased more than four-fold (from 24 to 113) since the species was listed in 1989.

Commented [RNR8]: I've seen 239 and 293 referenced. We just need to verify with NHP. They may check it in response to elicitation questions.

At this time, the largest known populations have been monitored by NCNHP and NCDOT. The estimates for entire populations are based on a consistent monitoring methodology developed by NCDOT, USFWS, and NCNHP with monitoring plots representing roughly 10% of a population. Populations were delineated to get a more accurate boundary and size of the area occupied. All rosettes are counted annually in each monitoring plot to estimate an extrapolated population size, based on the number and density in the plots. As a result of these efforts, better estimates of population sizes for the largest known populations are available, compared to when the last five-year review was completed in 2010 (Robinson and Padgett 2016).

Commented [RNR9]: Would it be helpful to include some of the population trend analysis here? Or, would that come later? I think long-term population stability would equate or relate to population resiliency.

The 113 EORs have been estimated to contain anywhere from a single rosette to over 100,000 rosettes. Table A1 (Appendix A) was created by NCNHP to replicate the same format and population data as Table B2 of the most recent USFWS five-year review of *H. naniflora* (USFWS 2010), for comparison of changes since 2010. Table A1 summarizes the largest occurrences of *H. naniflora*, with the size of the population based on the number of rosettes it was last estimated to contain. The number of populations estimated to contain over 1,000 rosettes is 26. This is approximately 23% of the total known populations and many of these populations contain well over 1,000 individuals.

There are, however, 13 populations (12% of all known) that are simply known to be extant, with no available estimate of population size (NCNHP 2016, SCDNR 2016). If the most recent population estimates for each EOR are compiled across years of observation, the 113 populations could conservatively be estimated to contain a collective total of more than 300,000 rosettes (NCNHP 2016, SCDNR 2016).

Appendix A

Table A1: Populations of *Hexastylis naniflora* estimated to contain over 1,000 rosettes in 2010, with updated information in 2016[1], and protection measures for these populations.

Populations known to be afforded some protection are indicated in **bold**.[2]

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
1	NC	Burke	029	A	Island Creek Heath Bluff (Lovelady Sites)	50,000+ (2016)	0	Not protected.
2	NC	Burke	178.159-178.161 178.254-178.256 178.280-178.282	A	Catawba River: Love Lady (Hoyle and Micol Creek)	~6,738 (2008-2011)	Awaiting data from NCDOT.	Possible NCDOT protected R-O-W, protection unconfirmed. In early 2016, the Town of Valdese applied to CWMTF to acquire part of this population.
3	**NC**	**Caldwell**	**227**	**A**	**Peaked Top Rare Plant Site (Foothills Landfill)**	**16,463 (2014)**	**16,463 (2014)**	**Conservation Easement (Foothills Conservancy of NC).**
4	**NC**	**Caldwell**	**044**	**AB**	**Little Gunpowder Creek Rare Plant Site 1**	**2,707 (2015)**	**2,424 (2015)**	**DOT mitigation site.**
5	NC	Catawba	158	D	South Fork Catawba River: Jacob Fork, Camp Creek	123 (2016)	27 (2015)	Some plants occur within an undetermined acreage within NCDOT R-O-W (Bassette 2016). Protection unconfirmed.
6	**NC**	**Catawba**	**243.012,** 243.242 243.269, **243.270**	**A**	**Catawba Wildlife Club**	**1,000-1300 (1995-2007)**	**>1,000 (1995-2007)**	**Catawba County Wildlife Club Registered Heritage Area (EO 12) The Nature Conservancy Conservation Easement (EO 270).**
7	NC	Catawba	031	C?	US 321, southeast Hickory	1,202 (2010)	161 (2010)	161 plants within NCDOT R-O-W (Bassette 2016).
8	**NC**	**Catawba**	**96.184,** 96.038 96.039	**A**	**Murrays Mill/Upper Balls Creek**	**~9,150 (2005)**	**11,860 (2013)**	**Fee title by NCDOT (~34 acres).**
9	**NC**	**Cleveland**	100.014, **100.049, 100.050, 100.051, 100.073, 100.074 100.149, 100.233**	**A**	**Broad River/Sandy Run Natural Areas (aka "Broad River Tract")**	**~33,500 (2001-2015)**	**32,702 (2015)**	**Fee title by Broad River Greenway (1,000 acres).**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
			100.236, 100.237 **100.238, 100.239 100.240, 100.241,** 100.246					
10	NC	Cleveland	208	A?	Buffalo Creek: Potts Creek	3,572 (2007)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
11	NC	Cleveland	211.072, 211.194		First Broad River: Hop-Hornbeam Natural Area	1,965 (2000)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
12	NC	Cleveland	214.201, **214.202, 214.203,** 214.204, **214.205, 214.209,** 214.212, **214.213,**	A	Buffalo Creek: tributaries north and south of SR2047	6,447 (200-2007)		Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 202, 203, 205, 209, 213).
13	NC	Cleveland	216.028, 216.196, 216.197, 216.198, **216.199, 216.200**	A	Buffalo Creek: Kings Mountain Reservoir	3,310 (200-2016)	~500? (2000-2007) (awaiting updates)	Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 199, 200).
14	NC	Cleveland	157	AB	First Broad River: Crooked Run Creek	330 (2010)		None of the population is protected within NCDOT R-O-W, determined too far away from roadside, DOT stopped monitoring in 2010 (Bassette 2016).
15	**NC**	**Cleveland/ Rutherford**	**276**	**A**	**Cliffside Steam Station**	**39,545 (2016)**	**~39,545 (2016)**	**Voluntary agreement with Duke Energy; protected acreage unknown.**
16	NC	Lincoln	261.252, 261.259, 261.263	B	Lincoln County Airport and Leepers Creek	7,032 (2005-2006)		Not protected.
17	NC	Polk	023	A	Mills Creek Forest and Seep	1,459 (2016)		Not protected.

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
18	**NC**	**Polk**	**125**	**A**	**Broad River: New Hope Springhead Swamp**	**12,235 (2016)**	**1,000-3,000 estimated (2016)**	**New Hope Springhead Swamp Registered Heritage Area (voluntary conservation agreement).**
19	**NC**	**Rutherford**	**154**	**C**	**Second Broad River (Forest City Industrial Complex)**	**2,576 (2016)**	**2478 (2008)**	**Deed restrictions (12 acres). Significant threats from industrial development, invasive exotics, erosion, sedimentation in a fragmented landscape.**
20	**NC**	**Rutherford**	99.009, 99.010, 99.037, 99.052, 99.053, 99.055, 99.061, 99.063, 99.075, **99.076,** 99.079, 99.090, 99.121, **99.172,** 99.181, 99.182, 99.249-.251, 99.267, 99.268	**A**	**Broad River: Hensons Creek Ravine, Brice Rare Plant Site, Sandy Mush Outcrop**	**106,940 (2016)**	**~200-250 (2007-2012)**	**Parris Heartleaf Registered Heritage Area (voluntary conservation agreement on subEO 076 and 172).**
21	NC	Rutherford	176.167-176.170	A	Broad River: Cleghorn Creek Tributary/US221	6,750 (2016)		Not protected.
22	NC	Rutherford	177.016, 177.107, 177.122, 177.063, 177.164, 177.165, 177.166	A	Broad River: Floyds Creek, Long Branch	12,687 (2008-2016)		Not protected.
23	**NC**	**Rutherford**	247.013, 247.056, 247.080, **247.106,** 247.114, 247.115, 247.173, 247.174, **247.286**	**A**	**Broad River: Floyds Creek (aka New Bethel Rare Plant Site)**	**~5,480 (2003-2015)**	**4,873 (2015)**	**Tate Conservation Easement (8 acres) (SubEOs 106 and 286).**
24	**SC**	**Cherokee**	**016, 017, 018**	**BC**	**Cowpens Battlefield**	**2,823 (2016)**	**2,823? (2016)**	**National Park Service, National Battlefield.**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
25	SC	Greenville /Spartanburg	002, 004, 024, 032, 033, 036, 038, 039, 040, 041, 042, 049, 050, 054	Various AB-BD	South Pacolet River and tributaries	5,205 (1991-1998)		Not protected.
26	**SC**	**Spartanburg**	**011, 014, 026--028, 047, 048, 057**	**Various AB-BD**	**Peters Creek Heritage Preserve**	**3,306 (2016)**	**3,306 (2016)**	**State Heritage Preserve (194 acres).**
27	**SC**	**Spartanburg**	**007, 029**	**BD, H?**	**Blalock Reservoir**	**3,505 (2016)**	**3,505? (2016)**	**Restrictive covenants (protected acreage unknown); significant threats to remaining population.**
28[6]	**NC**	**Lincoln**	302.046, **302.262**	**A**	**Rhyne Preserve**	**19,880 (2016)**	**19,873 (2016)**	**Rhyne Preserve (fee title by Catawba Lands Conservancy).**

[1]This table was replicated in the same format and populations as Table B.2 from the most recent USFWS five-year Review of *Hexastylis naniflora* (USFWS 2010) for comparison of changes since the time of the five-year review. Populations that are not believed to contain over 1,000 rosettes but were included in the original Table B.2 are included here, but the "NHP EO rank" and in the "Latest estimate of population size" reflects the smaller size.

[2] In the case of partially protected populations, the column "NHP EO numbers" indicates the specific portions of the population afforded protection in bold font.

[3]NHP EO numbers use the following format: Principal EO.SubEO. Only one number is listed if it is a stand-alone principal EO.

[4]NHP EO rank specifications are defined in Appendix B2. In cases involving principal/sub EOs, only the rank for the principal EO is given.

[5] EO ranks with two letters indicate a degree of uncertainty within the range provided (e.g. a rank of "AB" indicates possibly excellent to good viability).

[6]Population that is currently estimated to have over 1,000 rosettes that was not known to be large populations in 2010 have been added to this table.

INTRODUCTION

The dwarf-flowered heartleaf (*Hexastylis naniflora*) is a species of plant endemic to the upper Piedmont region of western North Carolina and upstate South Carolina. The dwarf-flowered heartleaf has been listed as threatened under the Endangered Species Act of 1973, as amended (Act), since 1989 (FR 54 14964-14967). The Species Status Assessment (SSA) framework (USFWS 2016, entire) is intended to support an in-depth review of the species' biology and threats, an evaluation of its biological status, and an assessment of the resources and conditions needed to maintain long-term viability. The intent is for the SSA Report to be easily updated as new information becomes available and to support all functions of the Endangered Species Program from Candidate Assessment to Listing to Consultations to Recovery.

A Recovery Plan for the species was never completed. However, over the last 29 years, the U.S. Fish and Wildlife Service (Service) has worked closely with partners to make significant progress toward recovery of the species. The Service is initiating a Species Status Assessment (SSA) to aid in determining the appropriateness of reclassifying the species. In the event that the SSA does not support reclassification, the SSA would be used to inform the development of a Recovery Plan. Importantly, the SSA Report does not result in a decision by the Service on whether this species should be proposed for reclassification under the Act. Instead, this SSA Report provides a review of the available information strictly related to the biological status of the dwarf-flowered heartleaf. The reclassification decision will be made by the Service after reviewing this document and all relevant laws, regulations, and policies, and the results of a proposed decision will be announced in the *Federal Register,* with appropriate opportunities for public input.

For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in its natural systems over time. Using the SSA framework (Figure 1), we consider what the species needs to maintain viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation (Wolf *et al.* 2015, entire).

- **Resiliency** describes the ability of populations to withstand stochastic events (arising from random factors). We can measure resiliency based on metrics of population health; for example, birth versus death rates and population size. Highly resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of anthropogenic activities.

- **Representation** describes the ability of a species to adapt to changing environmental conditions. Representation can be measured by the breadth of genetic or environmental diversity within and among populations and gauges the probability that a species is capable of adapting to environmental changes. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or human caused) in its environment. In the absence of species-specific genetic and ecological diversity information, we evaluate representation based on the extent and variability of habitat characteristics across the geographical range.
- **Redundancy** describes the ability of a species to withstand catastrophic events. Measured by the number of populations, their resiliency, and their distribution (and connectivity), redundancy gauges the probability that the species has a margin of safety to withstand or can bounce back from catastrophic events (such as a rare destructive natural event or episode involving many populations).

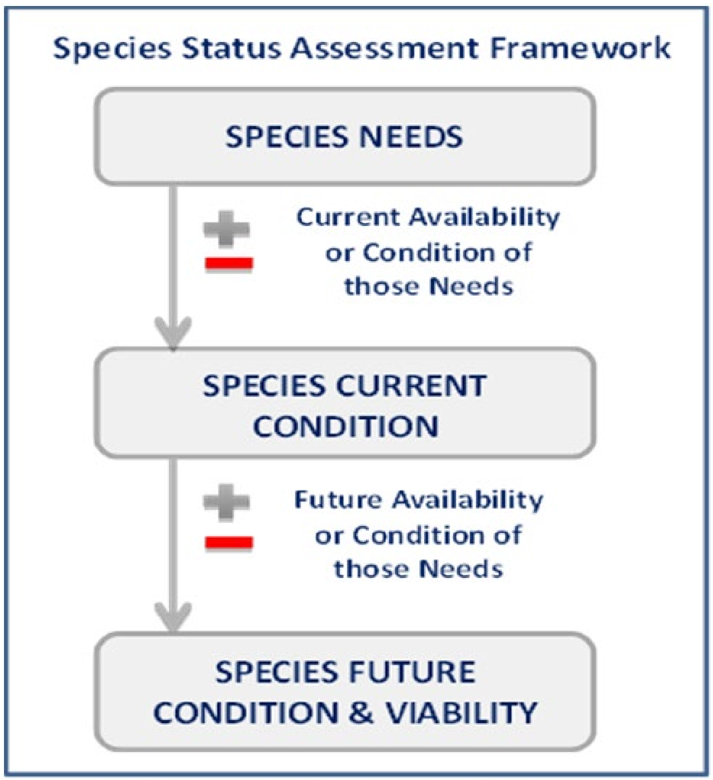

Figure 1- Species Status Assessment Framework

To evaluate the biological status of the dwarf-flowered heartleaf, both currently and into the future, we assessed a range of conditions to allow us to consider the species' resiliency, redundancy, and representation (together, the 3Rs). This SSA Report provides a thorough assessment of biology and natural history and assesses demographic risks, stressors, and limiting factors in the context of determining the viability and risks of extinction for the species.

The format for this SSA Report includes: (1) Biology and Species Needs (2) Current Conditions (3) Influences on Viability and (4) Future Conditions. This document is a compilation of the best available scientific and commercial information and a description of past, present, and likely future risk factors to the dwarf-flowered heartleaf.

Biology and Species Needs:

In this chapter we provide basic biological information about the dwarf-flowered heartleaf, including its taxonomic history, species description, distribution, life history traits, and habitat characteristics. We then use this information to outline the resource needs within various life stages of Nashville Crayfish. Here we report those aspects of the life history of the dwarf-flowered heartleaf that are important to our analysis.

Taxonomy and species description

Dwarf-flowered heartleaf is a rare low growing herbaceous plant in the birthwort family (Aristolochiaceae). The species was described by Blomquist (1957) in his revision of the North American members of the genus Hexastylis. The dwarf-flowered heartleaf has been recognized as part of the Virginica Group, and this group was further subdivided into three Subgroups: Virginica, Shuttleworthii, and Heterophylla (Blomquist 1957; Whittemore and Gaddy 1997). Three species have been recognized in the Heterophylla complex, *Hexastylis naniflora, H. heterophylla* and *H. minor*, and field biologist have generally recognized that considerable morphological overlap occurs (Murrell et al. 2007). One of the main concerns regarding this complex was the inability to distinguish between species without access to fresh flowers. Even with fresh flowers, Blomquist (1957) and Gaddy (1987) still recognized considerable overlap in flower morphology making species delineation difficult.

Murell et al. 2007, conducted a comparative genetic analysis using Inter Simple Sequence Repeats, and were unable to find banding patterns that could be used to separate *H. naniflora* from the other members within the complex. However, based on biogeographical, ecological, molecular, morphological, as well as micromorphological work, their results show that *H. naniflora* is a well-defined species.

Although there has been considerable disagreement on the generic distinctiveness of *Hexastylis* and *Asarum* (Barringer 1993 and Kelly 1997, 1998, 2001), a recent phylogeny estimate using chloroplast genes supports that *Hexastylis* is a monophyletic clade and therefore should be recognized as a genus (Niedenberger 2010). Additionally, most North American publications recognize *Hexastylis* at the generic level (Flora of North America 1997, Weakley 2015).

The Service is not aware of any proposed changes in taxonomy that would affect the continued legal status of *H. naniflora* under the Act. However, within the range of *H. naniflora* there are populations which fall outside of the range of published values for key floral characteristics, overlapping with values described for *H. heterophylla* or *H. minor* (Figure 2; Weakley, 2010; Murrell et al. 2007; Gaddy 1987). These geographic areas of overlap in key characters have been the focus of recent genetic analyses (Murrell et al. 2007; Renninger, 2010; Murrell, 2015).

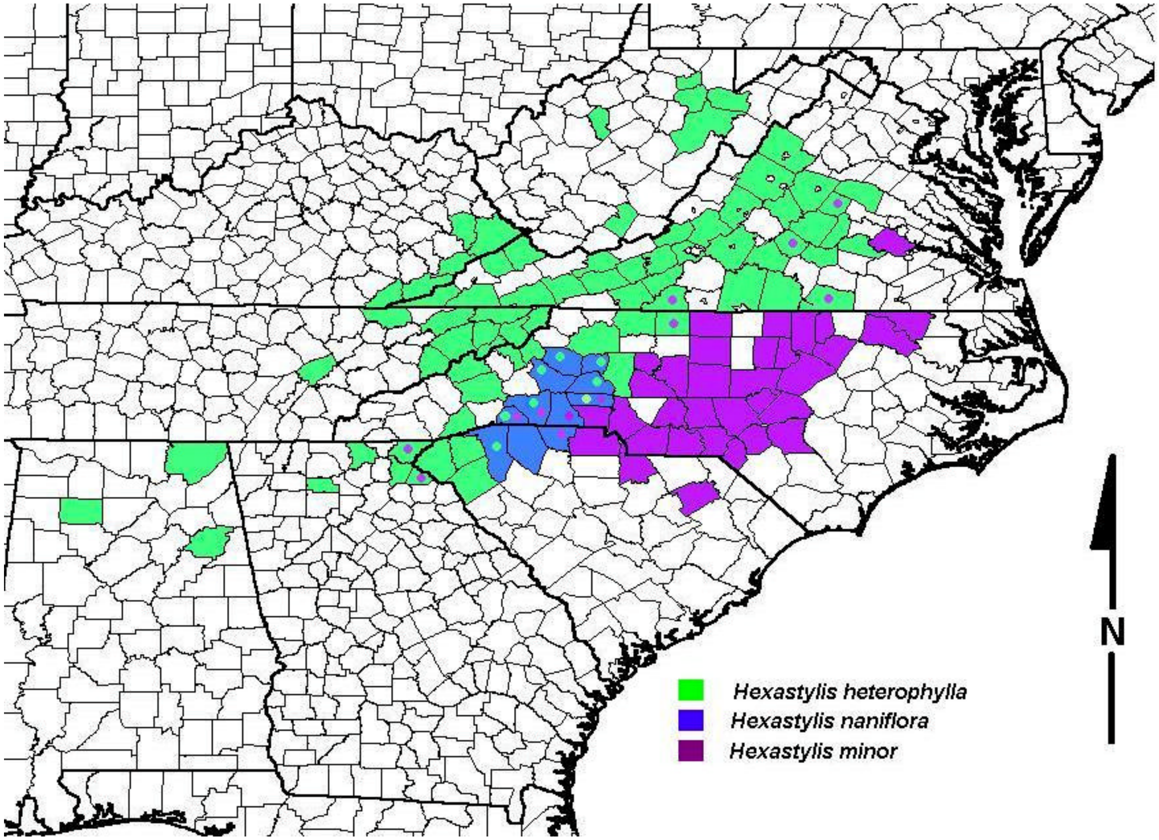

Figure 2: *From Murrell et al. 2007.* Distribution map showing county records for the three species in the *H. heterophylla* complex. Data was gathered from herbarium specimens, Element Of Occurrence (EOC) sheets and field studies. Dots within *H. heterophylla* counties indicates co-occurrence with *H. minor*. Light dot within *H. naniflora* counties indicates co-occurrence with *H. heterophylla*, dark dot indicates co-occurrence with *H. minor*.

The most outstanding characteristic of this species is the small flowers, which are one of the smallest of any species in North America (Blomquist 1957). The plant's heart shaped leaves are dark green in color, evergreen, and leathery, and are supported by long thin petioles from a subsurface rhizome. The shape of the leaf blades, their pattern of variegation, and the ridged reticulation inside the calyx-tube, place this species inside the Virginica group. It differs from all the other members of this group, aside from the small flowers, in having no flare in the calyx-tube. Maximum height rarely exceeds 15 centimeters (cm). The jug shaped flowers are usually beige to dark brown in color and appear from mid-March to early June. The flowers are small and inconspicuous and are found near the base of the petioles. The fruit matures from mid-May to early July (Blomquist 1957, Gaddy 1980, 1981). Characteristics that distinguish it from other *Hexastylis* species are found in floral structures and pollen characters (Gaddy 1987, Padgett 2004, Niedenberger 2010). *H. naniflora* has a smaller calyx tube orifice, which is typically 5mm or less (sometimes up to 7mm) and the ovary is half-inferior, rather than superior (Blomquist 1957, Gaddy 1987, Padgett 2004, HDR 2005). Pollen surface features have also been shown to be an effective character to identify *H. naniflora*, as it has a microporate surface and, unlike any other *Hexastylis* species, lacks gemmae entirely (Padgett 2004, Niedenberger 2010).

Distribution

Although dwarf-flowered heartleaf is restricted in range, it is not as rare as once thought (USFWS 2010, NCNHP 2016). The species occurs in the upper piedmont of North and South Carolina (Figure 3). In North Carolina, it is found in Alexander, Burke, Caldwell, Catawba, Cleveland, Gaston, Iredell, Lincoln, Polk, and Rutherford Counties. In South Carolina, it is in Cherokee, Greenville, and Spartanburg Counties. When dwarf-flowered heartleaf was federally listed in 1989, the listing rule described 24 extant "populations" (and 1 extirpated population) distributed across eight counties in North and South Carolina. As of 2010, the distribution of this species consisted of 108 populations distributed across 12 counties in these two states. Since 1989, the county distribution has expanded to include the following North Carolina counties: Alexander, Caldwell, Iredell, and Polk. The species has not been discovered in any additional counties in South Carolina.

The species appears to be restricted to Pacolet sandy loam, Madison gravelly sandy loam, and Musella fine sandy loam soils (Gaddy 1981,1987). These soils are restricted to an area from near Charlotte, North Carolina west to the foot of the mountains near Rutherfordton, North Carolina, and from Hickory, North Carolina southward to just south of Spartanburg, South Carolina (Murrell et al. 2007).

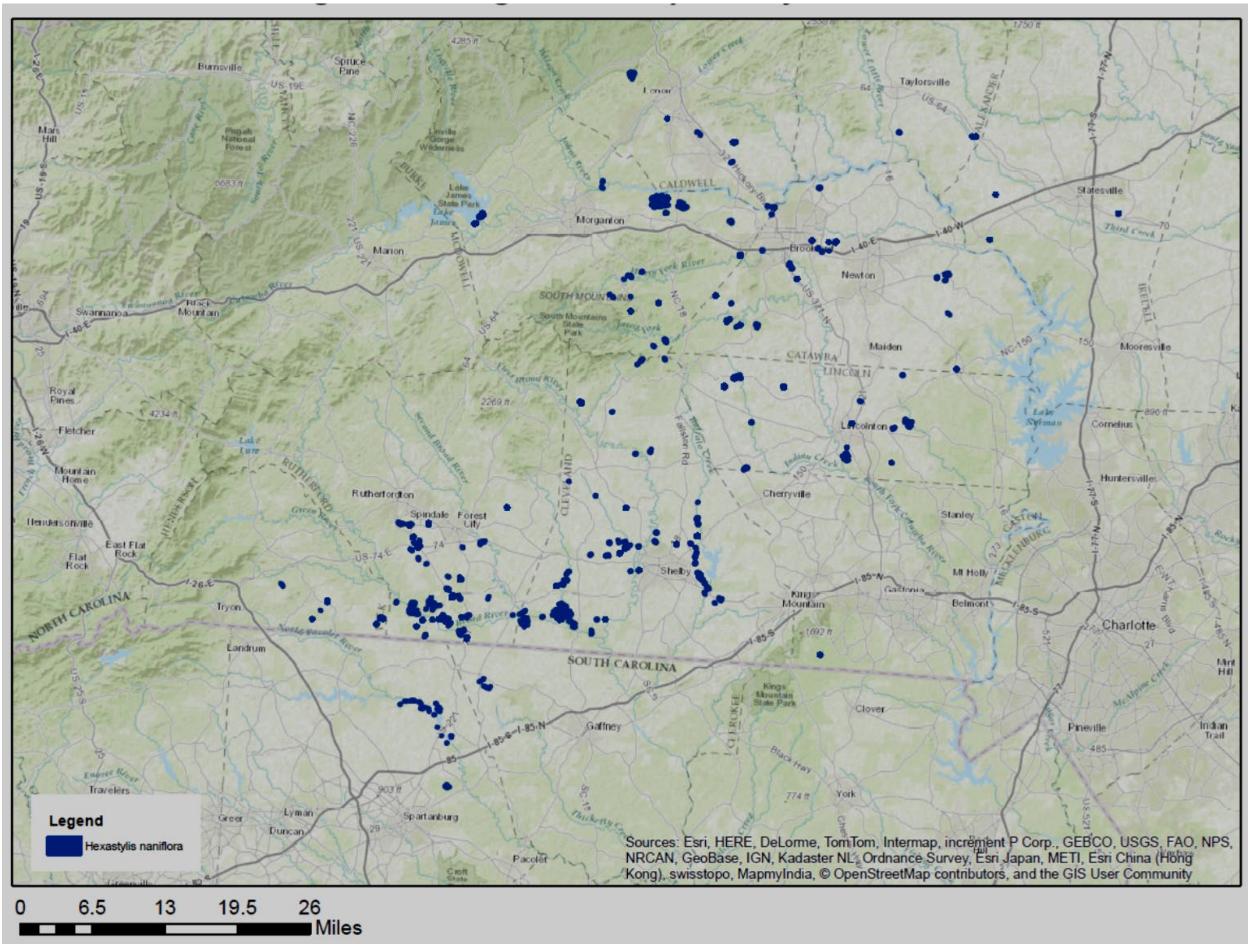

Figure 3--this is a placeholder…we will generate a new map in Arc GIS.

Many of those working with *Hexastylis naniflora* have used the terms "sub site", "site", "location", "occurrence" (often, but not always, in reference to Natural Heritage Program Element Occurrence (EO) Records), "subpopulation" and "population" interchangeably. Others have aggregated smaller sites into populations according to subjective criteria which have never been explicitly defined. This generates discrepancies among sources with respect to the abundance and distribution of the species, with the net result being that available data are usually not comparable from one source to the next. We describe how the numerous small, site-specific locations containing *H. naniflora* have been aggregated into proxies for 113 biological populations for purposes of this review, using mapping standards devised by NatureServe and its network of Natural Heritage Programs, in the Species Needs section of this report.

Life History

Demographics

The Service is aware of a single effort to collect demographic-level data (survivorship and recruitment of tagged individuals) for dwarf-flowered heartleaf. This effort was conducted during the 1990-1991 field seasons, within a portion of the Peters Creek population in Spartanburg County, SC (Newberry, 1993). This study demonstrated a 96.1% survival rate over these two consecutive seasons, with 50% of the mortality occurring in plants located at the highest position on the forested slope (away from the adjacent floodplain). Mortality was highest in small plants bearing fewer than four leaves. Plant size was variable, with the largest plant bearing 45 leaves and 33 flowers, and situated in the floodplain. In general, plants located in the floodplain were larger than plants located on adjacent slopes. The percentage of flowering plants averaged 70%, with the highest frequency of flowering occurring among plants situated in the floodplain (USFWS 2010).

Pollination and Dispersal

The pollination of Hexastylis has not been well studied but the genus was thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal by ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (H. heterophylla, H. naniflora, and H. minor), which employ myrmecochory as a method for seed dispersal.

Ants appear to be a primary dispersal agent for the dwarf-flowered heartleaf (Gaddy 1986; Jones et al. 2014). All diaspores of *Hexastylis naniflora* presented to ants (*Aphaenogaster rudis*) were quickly removed (Gaddy, 1986). This is not to say that they are not occasionally, or even frequently, dispersed and/or pollinated by other means. Jones et al. (2014) suggests that the pollination mechanism is facultative, benefiting from more than one method of pollination/fertilization. Ants were the pollinators in a controlled experiment, and their data supports that when outside molesting forces/pollinators (biotic and abiotic) were limited by their caging procedure, the efficiency of pollination decreased by almost 50%, however, caged flowers did produce seeds, indicating pollinization occurred via some alternative method.

Habitat

Dwarf-flowered heartleaf appears to have a restricted range due to its habitat requirements. The habitat where dwarf-flowered heartleaf exists is limited in size and scope due to a multitude of factors including soil type, moisture availability, and slope aspect. This unique combination of factors limits not only the range of dwarf-flowered heartleaf, but also the size of a given population. With the limited range and size in populations, questions arise regarding gene flow among populations. How much is occurring and how often does it occur? It is due, in part, to narrow habitat requirements that conservation measures have been implemented for the protection of the species. Any efforts made to protect this species must consider giving protection to the available habitat.

Dwarf-flowered heartleaf occurs on piedmont uplands on acidic sandy-loam soils that are very deep and moderately permeable (Gaddy 1981, 1987). Typical habitats for this species include mesic to dry bluffs, slopes, or ravines in deciduous forests that are frequently associated with *Kalmia latifolia* (Padgett 2004, Weakley 2015, USFWS 2015), or in moist soils adjacent to creeks, streamheads, or along lakes and rivers. Plants have been observed to grow larger and have more frequent flowering in floodplains along rivers, lakes, and streams (Newberry 1993). Wagner (2013) conducted a habitat suitability study to quantify the habitat requirements for dwarf-flowered heartleaf, which may be used for helping identify the species when not in flower (relative to other *Hexastylis* species habitat preferences), find new populations, or identify suitable sites for transplants. A model was created to predict habitat suitability to determine how many EOs are found within habitat considered fair to excellent (Figure 4; Wagner 2015). Based on the model, the strongest habitat correlations were that the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Wagner 2015). Populations found in areas with a percent slope of greater than 28, soil codes other than 6, 7, or 11, or elevation less than 199 m or greater than 415 m are unlikely to be *H. naniflora.* The model accurately predicts habitat suitability at a local scale 81% of the time.

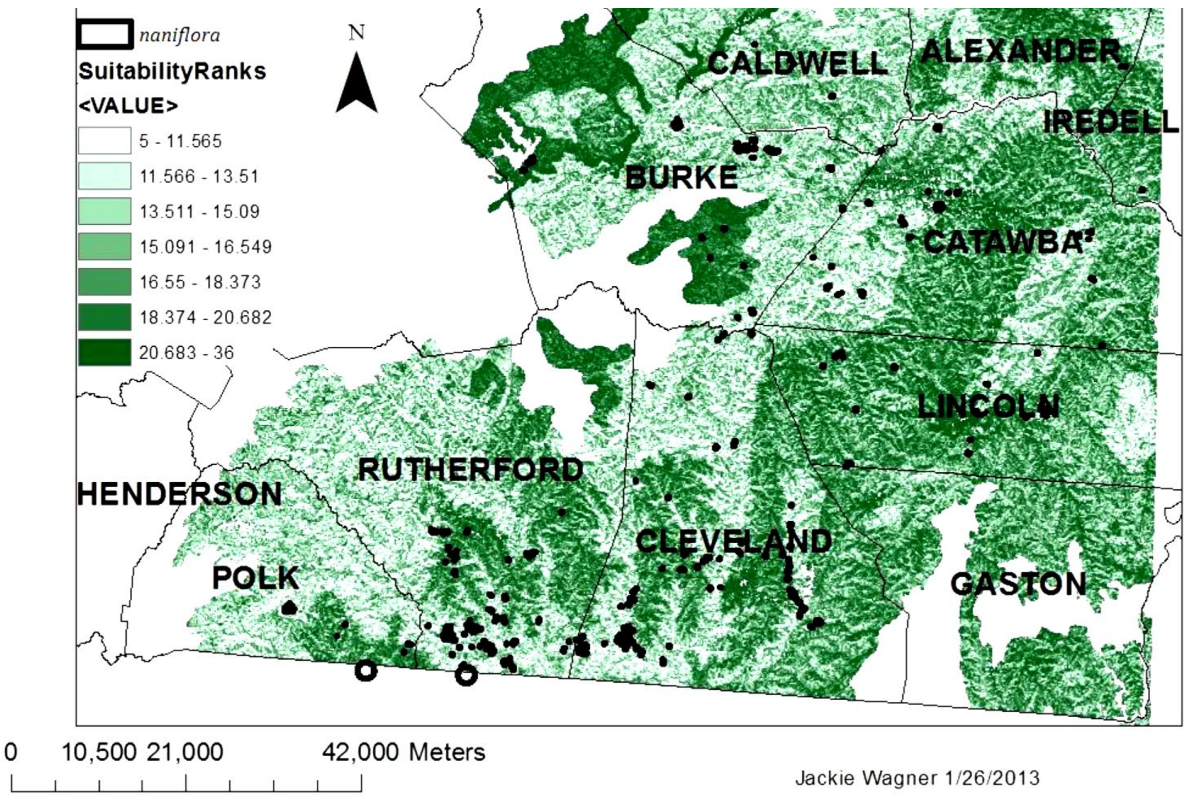

Figure 4: The site suitability ranks based on five habitat variables across the counties where *H. naniflora* is known to exist in NC. The smaller numbers indicate a higher rank and lighter green areas denote more suitable habitat. Known populations of *H. naniflora* are outlined in black.

Soils

The species grows in acidic soils along bluffs and adjacent slopes, in boggy areas next to streams and creekheads, and along the slopes of nearby hillsides and ravines (Gaddy 1980, 1981). The plant is primarily found inhabiting north- to northwest-facing slopes, bluffs, and ravines in close proximity to creeks or streams. Within these areas exists the soil type required for H. naniflora to grow. It grows primarily on well-drained, sandy, acidic soils, and will not grow in heavy clay (Gaddy 1981).

The results of soil samples analyzed at the Clemson Soil Lab suggest that major differences in soil chemistry exist between the species in the *H. heterophylla* complex. Statistical analysis of the soil samples showed that many of the basic elements were significantly different among the three species. Those significant differences occurred in Phosphorous (P), Potassium (K), Magnesium (Mg), Zinc (Zn), Manganese (Mn), (Na), Sodium, and Cation Exchange Capacity (CEC). Slightly significant differences were seen in Buffer pH (Bu pH), and Acidity (Table 8).

Soil chemistry showed marked differences between the species in the complex. The results indicated that soil chemistry is very different between *H. naniflora* and *H. minor* localities. The results also show that *H. heterophylla* and *H. naniflora* are found in soils where the chemistry is more similar, but still showed significant differences. It would appear that differentiation in soil types could be used as a proxy for species delineation. The soil analysis also indicates that soils must be considered when trying to select sites for relocation of imperiled populations of *H. naniflora*.

The analysis did not show statistically significant differences among the three species in the *H. heterophylla* complex. However, *H. naniflora* appears to have an association with three oak species that is lacking in the other two species in the complex. There are a number of oak species (*Q. coccinea*, *Q. prinus* (*Q. Montana*), and *Q. velutina*), that tend to co-occur with only *H. naniflora*, but are not present with the other two species in the complex. This may be the result of some microbial need or specific soil nutrient required for those species to occur in the same habitat.

Fire

There are little data on the response to fire by *Hexastylis naniflora*, however, prescribed burns have been conducted within the population at Cowpens National Battlefield in Cherokee County SC. Preliminary data at this site and recent annual monitoring data of this population support the theory that moderate controlled burns do not negatively affect this population (Walker et al. 2009). Additionally, a dormant season wildfire in Caldwell County did not show evidence of negative impacts to a population in Caldwell County (USFWS 2010). Fire suppression could be a hazard to *H. naniflora* by allowing fire intolerant, nonnative and invasive plants to thrive, as well as the accumulation of thick duff or leaf litter that may shade low growing species (Wagner 2013).

Genetics

Analyses on ecology, morphology, soil chemistry, pollen, and molecular genetics have been evaluated for *Hexastylis naniflora* to determine the boundaries within the *Hexastylis heterophylla* complex, which consists of *H. heterophylla*, *H. minor*, and *H. naniflora* (Murrell 2015, Wagner 2013, Niedenburger 2010, USFWS 2010, Murrell et al. 2007, Padgett 2004). These analyses support the continued recognition of these taxa as well-defined, discrete species. Scanning electron microscopy (SEM) consistently distinguished *H. naniflora* from other members of the *H. heterophylla* complex based on pollen microscopy. Principal Components Analysis of floral characters and soil chemistry also consistently distinguished *H. naniflora* from *H. minor* and *H. heterophylla*. However, efforts to obtain consistently distinct banding patterns using Inter Simple Sequence Repeats (ISSRs) were unsuccessful at distinguishing *H. naniflora* from other members of this group (Murrell et al., 2007). These results were based upon an extremely small sample size (n=10 *H. naniflora* individuals), and therefore warrant further investigation.

Field observations demonstrate that there are some populations of dwarf-flowered heartleaf with morphological characteristics that do not fit within the range of published values for key traits, overlapping with values for *H. heterophylla* or *H. minor* (Gaddy 1987, Murrell et al. 2007, USFWS 2010, Weakley 2015). These populations with overlap in key characteristics were the focus of a genetic analysis conducted at Appalachian State University (ASU) through funding provided by NCDOT (Murrell 2015). In some populations, floral characteristics are highly variable, which suggests the potential for hybridization or individuals with highly variable flower size and shape (Murrell 2015). Additionally, no vegetative characters were previously known to consistently distinguish *Hexastylis naniflora* from other close relatives. Given the difficulties with field identification of the species, particularly when not in flower, this study sought to develop a microsatellite library of molecular markers to resolve variation in populations of dwarf-flowered heartleaf and apply the markers to populations with highly variable characters, as identified by NCDOT biologists. The morphological and micromorphological information from those variable populations were compared to molecular results with morphological, micromophological, and distributional data to determine genetic structure, biological boundaries, and placement of putative hybrids or intermediate populations of *H. naniflora* (Murrell 2015).

The preliminary findings of this study suggest that populations in the southern range of dwarf-flowered heartleaf exhibit a more uniform genetic pattern, with some possible hybridization with *H. minor*. Populations in the northern part of the range appear to have hybridized with both *H. heterophylla* and *H. minor*, although there are still individuals with “pure” *H. naniflora* genotypes in the northern range (Murrell 2015). It is critical to note that although these data provide anecdotal evidence of hybridization with the *Hexastylis heterophylla* group, intraspecific variation may be caused by forces other than hybridization, such as convergent morphological evolution (Dobzhansky 1937), or the species is in the process of speciation and this study shows a case of incomplete speciation (Murrell 2015), and/or other environmental factors are at play (Wagner 2013). On May 11, 2016 a meeting was held with USFWS, NCNHP, NCDOT, and ASU to discuss the status of *H. naniflora* and the current work being conducted among the agencies (Amoroso 2016). Based on discussions during this meeting, the results of this study reported by ASU to NCDOT in 2015 are preliminary. Dr. Matt Estep (ASU) provided additional preliminary results to NCNHP in May 2016, showing which populations were sampled, sample size, and percent of samples that show evidence of hybridization, and hybridizing with which species. ASU continues to work towards a more definitive explanation of the variation in the *H. heterophylla* complex (Murrell 2015, Amoroso 2016).

SPECIES NEEDS

For the purpose of this report, we define viability as the ability of the species to sustain populations in the wild over time. Species with greater numbers (redundancy) of healthy populations (resiliency), encompassing a broad array of ecological and genetic diversity in a spatial arrangement that maintains adequate gene flow (representation), are more likely to be viable. Using the Species Status Assessment framework, we describe the species' viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation.

Delineating Populations

As stated in the USFWS five-year review, "many of those working with dwarf-flowered heartleaf have used the terms "sub site," "site," "location," "occurrence" (often, but not always, in reference to Natural Heritage Program Element Occurrence Records), and "population" interchangeably, while others have aggregated sites into populations according to subjective criteria which have never been explicitly defined. This has generated considerable discrepancies among sources with respect to the number of known populations within a given area (or across the species' range), to the extent that numbers are not comparable from one source to the next. The tendency to treat each location as a separate population also artificially inflates the actual number of populations known.

In recent years, NatureServe and its member Natural Heritage Programs have devised mapping standards to balance the need for fine-scale, highly site-specific EO records (required for monitoring and management) with the need to aggregate these records in meaningful units of conservation interest that may approximate biological populations (NatureServe 2002). “Since USFWS does not maintain its own database of known locations of *Hexastylis naniflora*, it regards the NHP databases as the best repository for this information” (USFWS 2010).

We delineate populations for the purposes of this SSA according to the NatureServe (2002) convention. Separation distances are a key component to delineating populations from EO records. For the dwarf-flowered heartleaf, we used the EO Data Standard which provides a Default Separation Distance of 1 km (~0.62 miles) for plant and animal elements that lack EO specs, noting that situations involving dispersal barriers could involve even shorter distances. While gene flow declines over distance at different rates for different elements, the minimum default EO separation distance of 1 km has been accepted by the Network as the most suitable round-number metric-system approximation broadly applicable to many (but not all) situations. This results in several dwarf-flowered heartleaf populations being stand-alone EOs, as well as many populations being aggregates of several EOs.

Population Resiliency

For the dwarf-flowered heartleaf to maintain viability, its populations or some portion thereof must be resilient. Stochastic factors that have the potential to affect dwarf-flowered heartleaf include impacts to its habitat, particularly human development pressures, but also climate change and presence of invasive species. Other factors that influence the resiliency of Nashville crayfish populations include abundance within populations, and habitat factors such as elevation, slope, aspect, and soil type. Influencing those factors are elements of dwarf-flowered heartleaf ecology that determine whether populations can grow to maximize habitat occupancy, thereby increasing resiliency of populations. These factors and habitat elements are discussed below (Figure 5).

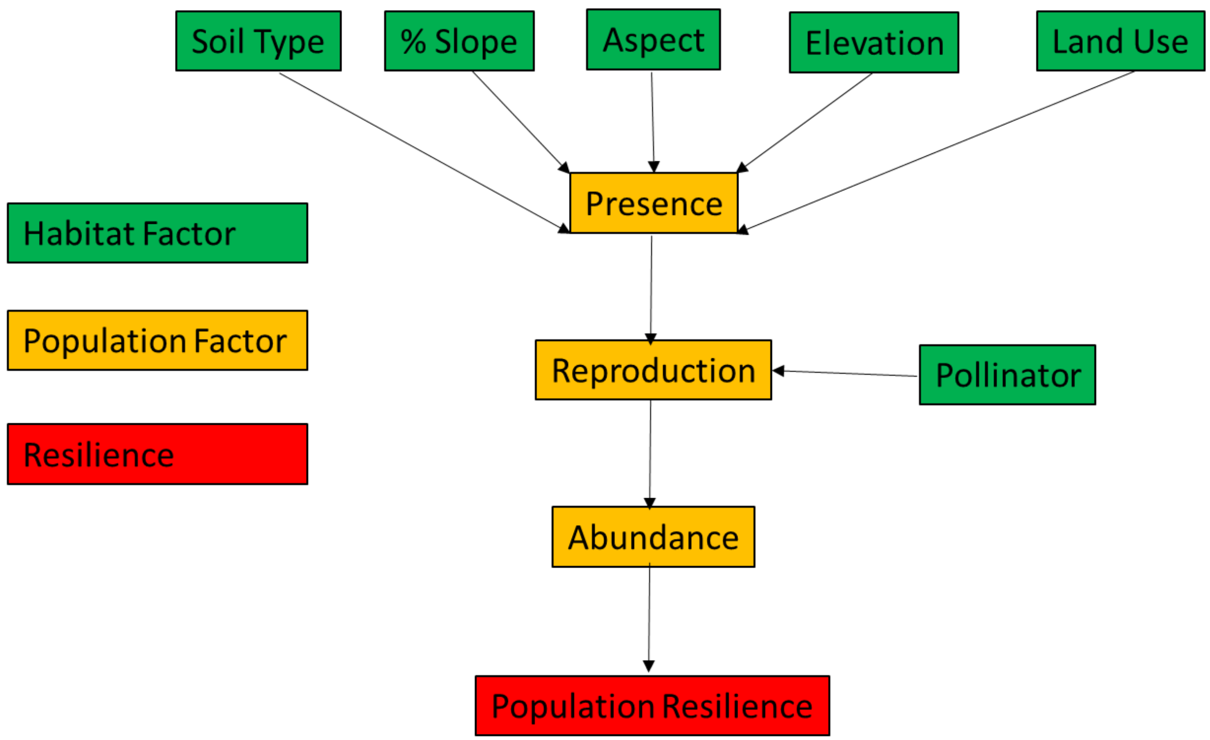

Figure 5--Conceptual diagram describing population and habitat factors influencing population resilience for dwarf-flowered heartleaf.

Habitat Factors: Slope, Aspect, Elevation, Soil, and Forest Type

In a site suitability model generated with habitat rankings from 5 (the most suitable) to 36 (least suitable habitat) across 7 rank classes, the most common classification included the following microhabitat elements: the slope aspect was north-facing, soil type is Pacolet sandy loam, elevation was between 230-260m, percent slope is 8-10%, and land use is deciduous forest (Table 1; Wagner 2015). More importantly, populations found in areas with a percent slope greater than 28, or elevation less than 199 m or greater than 415 m, are unlikely to be dwarf-flowered heartleaf. Slope aspect analysis shows that *H. naniflora* has already adapted to the cooler, wetter conditions of north facing slopes suggesting that this species would fare poorly under climate change scenarios predicting warmer and drier environments (mimicking south facing slopes) throughout their range, supporting a similar claim from Warren (2008).

Table 1. Frequency of element occurrence records (EORs) for each habitat variable. For continuous variables (slope and elevation) data were grouped into classes with the value shown being the top end of the range. * indicates most common classification for that habitat variable.

slope		land use (LU)		soil		elevation		aspect	
% slope	EORs	LU type	EORs	soil code	EORs	Elev. (m)	EORs	aspect	EOR
5.5	20	open water	1	6*	123	<199	0	N*	57
8	50	Low intensity residential	14	7	59	229.8571	34	NE	32
10.5*	54	High intensity residential	3	10	1	260.7143*	65	NW	32
13	35	Commercial/ Industrial	1	11	8	291.5714	29	E	13
15.5	22	Deciduous Forest*	153	other	0	322.4286	34	W	10
18	10	Evergreen Forest	13			353.2857	24	SE	22
20.5	3	Mixed Forest	1			384.1429	10	SW	13
23	3	Grassland	3			415	4	S	20
25.5	0	Pasture	11			More	0		
28	2								
>28	0								

Reproduction and Presence of Pollinators

The pollination of Hexastylis has not been well studied but the genus is thought to be pollinated by insects including flies, wasps, and thrips (Otte 1977). Additionally, Lengyel et al. (2010) described how, within the family Aristolochiaeae, more than 50% of the plant lineage is myrmecochorous (seed dispersal via ants). This supports Gaddy's work (1986), which found three species within the Hexastylis Heterophylla subgroup (H. heterophylla, H. naniflora, and H. minor), which employ myrmecochory as a method for seed dispersal.

Because the flower for this species is oftentimes partially or completely covered with soil and leaf litter, possibly inhibiting pollinator activity (Gonzalez 1972), there is still uncertainty in the pollination mechanism for dwarf-flowered heartleaf. Otte (1977) suggests that a variety of possible pollinators reside in this leaf litter, however, the calyx opening is considered to be far too small for efficient pollinating (Gaddy 1981). There are, however, invertebrates within this proposed size limit that could theoretically act as pollinators. It is possible that the species employs self-pollination, with or without a vector, or that cross-pollination occurs by a number of invertebrates. Jones et al. 2014 investigated pollination of dwarf-flowered heartleaf under a manipulative experimental design, and found that while insects may play a significant role in pollination, even without them, flowers managed to produce a partial seed set. However, two points need to be considered here. First, although flowers managed to produce seeds in the absence of insect pollinators, the efficiency of pollination decreased by almost 50%. Also, even if successful pollination occurs in the absence of insect vectors, the dispersal of plants amongst populations would be limited, and could result in decreased resilience due to genetic concerns such as limited gene flow and issues associated with potential inbreeding depression.

Abundance

The influence of stochastic variation in demographic (reproductive and mortality) rates is much higher for small populations than large ones. Stochastic variation in demographic rates causes small populations to fluctuate randomly in size. In general, the smaller the population, the greater the probability that fluctuations will lead to extinction. There are also genetic concerns with small populations, including reduced availability of compatible mates, genetic drift, and inbreeding depression. Small populations of dwarf-flowered heartleaf have low resilience, leaving them particularly vulnerable to stochastic events.

As of 2016, the combined databases of the NCNHP and SCNDNR contain 239 Element Occurrence Records (EORs) for *H. naniflora* (NCNHP2016, SCDNR 2016). These EORs depict roughly 113 locations which are sufficiently geographically distinct to be regarded as proxies for populations of the species (See *Delineating Populations*). Thus, the total number of populations has increased more than four-fold (from 24 to 113) since the species was listed in 1989.

At this time, the largest known populations have been monitored by NCNHP and NCDOT. The estimates for entire populations are based on a consistent monitoring methodology developed by NCDOT, USFWS, and NCNHP with monitoring plots representing roughly 10% of a population. Populations were delineated to get a more accurate boundary and size of the area occupied. All rosettes are counted annually in each monitoring plot to estimate an extrapolated population size, based on the number and density in the plots. As a result of these efforts, better estimates of population sizes for the largest known populations are available, compared to when the last five-year review was completed in 2010 (Robinson and Padgett 2016).

The 113 EORs have been estimated to contain anywhere from a single rosette to over 100,000 rosettes. Table A1 (Appendix A) was created by NCNHP to replicate the same format and population data as Table B2 of the most recent USFWS five-year review of *H. naniflora* (USFWS 2010), for comparison of changes since 2010. Table A1 summarizes the largest occurrences of *H. naniflora*, with the size of the population based on the number of rosettes it was last estimated to contain. The number of populations estimated to contain over 1,000 rosettes is 26. This is approximately 23% of the total known populations and many of these populations contain well over 1,000 individuals.

There are, however, 13 populations (12% of all known) that are simply known to be extant, with no available estimate of population size (NCNHP 2016, SCDNR 2016). If the most recent population estimates for each EOR are compiled across years of observation, the 113 populations could conservatively be estimated to contain a collective total of more than 300,000 rosettes (NCNHP 2016, SCDNR 2016).

Appendix A

Table A1: Populations of *Hexastylis naniflora* estimated to contain over 1,000 rosettes in 2010, with updated information in 2016[1], and protection measures for these populations.

Populations known to be afforded some protection are indicated in **bold**.[2]

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
1	NC	Burke	029	A	Island Creek Heath Bluff (Lovelady Sites)	50,000+ (2016)	0	Not protected.
2	NC	Burke	178.159-178.161 178.254-178.256 178.280-178.282	A	Catawba River: Love Lady (Hoyle and Micol Creek)	~6,738 (2008-2011)	Awaiting data from NCDOT.	Possible NCDOT protected R-O-W, protection unconfirmed. In early 2016, the Town of Valdese applied to CWMTF to acquire part of this population.
3	**NC**	**Caldwell**	**227**	**A**	**Peaked Top Rare Plant Site (Foothills Landfill)**	**16,463 (2014)**	**16,463 (2014)**	**Conservation Easement (Foothills Conservancy of NC).**
4	**NC**	**Caldwell**	**044**	**AB**	**Little Gunpowder Creek Rare Plant Site 1**	**2,707 (2015)**	**2,424 (2015)**	**DOT mitigation site.**
5	NC	Catawba	158	D	South Fork Catawba River: Jacob Fork, Camp Creek	123 (2016)	27 (2015)	Some plants occur within an undetermined acreage within NCDOT R-O-W (Bassette 2016). Protection unconfirmed.
6	**NC**	**Catawba**	**243.012,** 243.242 243.269, **243.270**	**A**	**Catawba Wildlife Club**	**1,000-1300 (1995-2007)**	**>1,000 (1995-2007)**	**Catawba County Wildlife Club Registered Heritage Area (EO 12) The Nature Conservancy Conservation Easement (EO 270).**
7	NC	Catawba	031	C?	US 321, southeast Hickory	1,202 (2010)	161 (2010)	161 plants within NCDOT R-O-W (Bassette 2016).
8	**NC**	**Catawba**	**96.184,** 96.038 96.039	**A**	**Murrays Mill/Upper Balls Creek**	**~9,150 (2005)**	**11,860 (2013)**	**Fee title by NCDOT (~34 acres).**

9	**NC**	**Cleveland**	100.014, **100.049, 100.050, 100.051, 100.073, 100.074 100.149, 100.233**	**A**	**Broad River/Sandy Run Natural Areas (aka "Broad River Tract")**	**~33,500 (2001-2015)**	**32,702 (2015)**	**Fee title by Broad River Greenway (1,000 acres).**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
			100.236, 100.237 **100.238, 100.239 100.240, 100.241,** 100.246					
10	NC	Cleveland	208	A?	Buffalo Creek: Potts Creek	3,572 (2007)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
11	NC	Cleveland	211.072, 211.194		First Broad River: Hop-Hornbeam Natural Area	1,965 (2000)		Awaiting verification that plants are protected in NCDOT R-O-W. Protection unconfirmed.
12	NC	Cleveland	214.201, **214.202, 214.203,** 214.204, **214.205, 214.209,** 214.212, **214.213,**	A	Buffalo Creek: tributaries north and south of SR2047	6,447 (200-2007)		Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 202, 203, 205, 209, 213).
13	NC	Cleveland	216.028, 216.196, 216.197, 216.198, **216.199, 216.200**	A	Buffalo Creek: Kings Mountain Reservoir	3,310 (200-2016)	~500? (2000-2007) (awaiting updates)	Awaiting verification that plants are protected in NCDOT in R-O-W, bold in EO column. Protection unconfirmed (EO 199, 200).
14	NC	Cleveland	157	AB	First Broad River: Crooked Run Creek	330 (2010)		None of the population is protected within NCDOT R-O-W, determined too far away from roadside, DOT stopped monitoring in 2010 (Bassette 2016).
15	**NC**	**Cleveland/ Rutherford**	**276**	**A**	**Cliffside Steam Station**	**39,545 (2016)**	**~39,545 (2016)**	**Voluntary agreement with Duke Energy; protected acreage unknown.**
16	NC	Lincoln	261.252, 261.259, 261.263	B	Lincoln County Airport and Leepers Creek	7,032 (2005-2006)		Not protected.
17	NC	Polk	023	A	Mills Creek Forest and Seep	1,459 (2016)		Not protected.

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on-site protection (year)	Protection comments
18	**NC**	**Polk**	**125**	**A**	**Broad River: New Hope Springhead Swamp**	**12,235 (2016)**	**1,000-3,000 estimated (2016)**	**New Hope Springhead Swamp Registered Heritage Area (voluntary conservation agreement).**
19	**NC**	**Rutherford**	**154**	**C**	**Second Broad River (Forest City Industrial Complex)**	**2,576 (2016)**	**2478 (2008)**	**Deed restrictions (12 acres). Significant threats from industrial development, invasive exotics, erosion, sedimentation in a fragmented landscape.**
20	**NC**	**Rutherford**	99.009, 99.010, 99.037, 99.052, 99.053, 99.055, 99.061, 99.063, 99.075, **99.076,** 99.079, 99.090, 99.121, **99.172,** 99.181, 99.182, 99.249-.251, 99.267, 99.268	**A**	**Broad River: Hensons Creek Ravine, Brice Rare Plant Site, Sandy Mush Outcrop**	**106,940 (2016)**	**~200-250 (2007-2012)**	**Parris Heartleaf Registered Heritage Area (voluntary conservation agreement on subEO 076 and 172).**
21	NC	Rutherford	176.167-176.170	A	Broad River: Cleghorn Creek Tributary/US221	6,750 (2016)		Not protected.
22	NC	Rutherford	177.016, 177.107, 177.122, 177.063, 177.164, 177.165, 177.166	A	Broad River: Floyds Creek, Long Branch	12,687 (2008-2016)		Not protected.
23	**NC**	**Rutherford**	247.013, 247.056, 247.080, **247.106,** 247.114, 247.115, 247.173, 247.174, **247.286**	**A**	**Broad River: Floyds Creek (aka New Bethel Rare Plant Site)**	**~5,480 (2003-2015)**	**4,873 (2015)**	**Tate Conservation Easement (8 acres) (SubEOs 106 and 286).**
24	**SC**	**Cherokee**	**016, 017, 018**	**BC**	**Cowpens Battlefield**	**2,823 (2016)**	**2,823? (2016)**	**National Park Service, National Battlefield.**

USFWS population number	State	County	NHP EO numbers (2016)[3]	NHP EO rank (2016)[4,5]	Site	Latest estimate of population size (year)	Estimate of plants on- site protection (year)	Protection comments
25	SC	Greenville /Spartanburg	002, 004, 024, 032, 033, 036, 038, 039, 040, 041, 042, 049, 050, 054	Various AB-BD	South Pacolet River and tributaries	5,205 (1991-1998)		Not protected.
26	**SC**	**Spartanburg**	**011, 014, 026--028, 047, 048, 057**	**Various AB-BD**	**Peters Creek Heritage Preserve**	**3,306 (2016)**	**3,306 (2016)**	**State Heritage Preserve (194 acres).**
27	**SC**	**Spartanburg**	**007, 029**	**BD, H?**	**Blalock Reservoir**	**3,505 (2016)**	**3,505? (2016)**	**Restrictive covenants (protected acreage unknown); significant threats to remaining population.**
28[6]	**NC**	**Lincoln**	302.046, **302.262**	**A**	**Rhyne Preserve**	**19,880 (2016)**	**19,873 (2016)**	**Rhyne Preserve (fee title by Catawba Lands Conservancy).**

[1]This table was replicated in the same format and populations as Table B.2 from the most recent USFWS five-year Review of *Hexastylis naniflora* (USFWS 2010) for comparison of changes since the time of the five-year review. Populations that are not believed to contain over 1,000 rosettes but were included in the original Table B.2 are included here, but the "NHP EO rank" and in the "Latest estimate of population size" reflects the smaller size.

[2] In the case of partially protected populations, the column "NHP EO numbers" indicates the specific portions of the population afforded protection in bold font.

[3]NHP EO numbers use the following format: Principal EO.SubEO. Only one number is listed if it is a stand-alone principal EO.

[4]NHP EO rank specifications are defined in Appendix B2. In cases involving principal/sub EOs, only the rank for the principal EO is given.

[5] EO ranks with two letters indicate a degree of uncertainty within the range provided (e.g. a rank of "AB" indicates possibly excellent to good viability).

[6]Population that is currently estimated to have over 1,000 rosettes that was not known to be large populations in 2010 have been added to this table.