

An assessment of the ecology, population dynamics, and threats to large yellow lady's-slipper (*Cypripedium parviflorum* var. *pubescens*) in Pennsylvania. (Project No. WRCP 17574)



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Executive Summary

There is a worldwide decline of orchid species due to a multitude of factors. This decline is likely happening in Pennsylvania as well. Large yellow lady's-slipper (*Cypripedium parviflorum* Salisb. var. *pubescens* (Willd.) Knight) is one orchid found in the state that is listed as vulnerable, but little work has been done to understand its vulnerability. The focus of this project was to further explore the life history, current population conditions, and threats associated with this species here in Pennsylvania.

A literature review was done to determine what ecological and distribution related information was already known for large yellow lady's-slipper. We discovered that Deller (2005) had completed a near exhaustive review. Information from Deller's work (2005), coupled with Pennsylvania specific information, were incorporated into a species background summary.

A combination of data sources were used to understand the distribution of large yellow lady's-slipper in Pennsylvania. The species has been documented in 15 counties within the last 25 years and one county with a record greater than 25 years. From this information, we selected 15 sites for field visits to assess population size, habitat, and potential threats. To determine population size, a low intensity monitoring approach was used at thirteen sites and included a census of vegetative and flowering stems and whether stems occurred as clusters or single stems. A high intensity monitoring approach was employed at two populations to gain a better perspective of stage structure within populations and reproductive success in terms of capsule formation. Large yellow lady's-slipper was found at 14 of the 15 sites. Population estimates ranged from six stems to over 600 stems. The stage structures of the two populations were skewed more towards the adult stages. Capsule formation varied by population and years. Given that previous population estimates from these sites were minimal or inconsistently collected, it is difficult to draw conclusions about the stability or vulnerability of these populations beyond that even the smaller populations appear to persist over time. The dataset from this project does provide an excellent baseline for future monitoring work and population-level comparisons.

The habitat at each of the 15 sites was characterized by plant community type using accepted Natural Heritage sampling protocols. Except for one site, populations were found in more continuous forest stands under mostly closed canopies. Populations were generally located on slopes ranging from gently sloping to very steep. The southeastern sites had greater boulder cover as well. Populations were found in the following terrestrial community types: Tuliptree – Beech – Maple Forests (n=9), Sugar Maple – Basswood Forests (n=2), Northern Hardwood Forest (n=2), Red Oak – Mixed Hardwood Forest (n=1), and a small forest remnant (n=1).

A combination of sources were used to conduct assessments of known and perceived threats for large yellow lady's-slipper in Pennsylvania. A list of potential threats was developed from the literature and from a public knowledge questionnaire. The species is listed as Pennsylvania Vulnerable (PV) due to drug-trade pressures and horticultural pressures so more research was done to explore the validity of these threats. Site-specific threats were also documented during visits to the 15 populations. In addition, game cameras were deployed at three populations in an attempt to capture activity at those sites. We found that all sites had some level of threat and this should be considered when planning management activities for the species.

Based on the results of this project, it is likely there are additional large yellow lady's-slipper populations in Pennsylvania. Additional survey efforts are suggested. Along with the survey efforts, we recommend using a consistent approach for estimating population size, like the low intensity one used for this project. The life history of the species, timing of site visits, and documentation of threats should all be

components of a monitoring effort. Consistent data collection would allow for more confidence when comparing multi-year population estimates and help in the determination of the actual vulnerability of the population.

Introduction

Orchids are considered one of the most charismatic plants in the plant kingdom. There is a long-standing mystique associated with orchids whether because of their aesthetic beauty, supposed medicinal values, collection value, or the thrill of discovering their elusive locations. This fascination is one factor contributing to the decline of orchids worldwide.

The trend in orchid decline is likely occurring in Pennsylvania as well. Pennsylvania is home to a variety of native orchids with many having a PA state status of rare, threatened, endangered, or vulnerable. Some Pennsylvania orchids are naturally rarer for a variety of reasons (e.g., disjunct, endemic, edge-of-range, or habitat restrictions), whereas, other species are likely declining due to external threats such as habitat degradation and loss, browsing by white-tailed deer (*Odocoileus virginianus*), competition from invasive plants, and over collecting. Anecdotal evidence from botanists' observations suggests that these are all realistic threats. However, little effort has focused on understanding the health of orchid populations in Pennsylvania and quantifying possible threats to the species. This lack of knowledge makes it difficult to develop well-informed management plans that address the actual conservation needs of a species.

In 2017, Pennsylvania's Department of Conservation and Natural Resources (PA DCNR) Wild Resource Conservation Program (WRCP) included one Pennsylvania orchid, large yellow lady's-slipper (*Cypripedium parviflorum* Salisb. var. *pubescens* (Willd.) Knight), in its request for proposals for that funding cycle. Large yellow lady's-slipper is listed as one of Pennsylvania's vulnerable orchids, but little work has been done to understand the species vulnerability in Pennsylvania. The request for proposals highlighted the need for more ecological and social information to help better inform the management of this species. This funding opportunity provided the means to begin filling the gaps in our knowledge of large yellow lady's-slipper in Pennsylvania by addressing the following questions.

- 1) What information is available about the current distribution and ecology of the species in Pennsylvania? A 2018 review of Biotics (a repository of rare species data from Pennsylvania), revealed eight known occurrences of the species in Pennsylvania, however, the extent of the species is likely much larger in Pennsylvania.
- 2) What are the current conditions of large yellow lady's-slipper populations in Pennsylvania? For documented populations of large yellow lady's-slipper, little information is known to assess the well-being of the populations and what data are available may be from just a single visit to a site and may be outdated.
- 3) What are the threats associated with populations in Pennsylvania? There are general threats associated with world-wide orchid decline, but do these threats hold true for this species as well?

Species Background

A literature review was conducted to determine what ecological and distribution related information was known for large yellow lady's-slipper. We found that a near exhaustive review of the species was completed by Deller (2005) for USDA Forest Service Region 9. Deller's review encompasses the body of knowledge compiled previously for the Finger Lakes National Forest (St. Hilaire 2003) and the Green Mountain National Forest (Allen 2002). Given this extensive review, we've provided a summary of the key information known about the species, but readers should also refer to Deller (2005) for a more thorough background on the species. In addition to the literature review, information was collected at known large yellow lady's-slipper populations and incorporated into the species review to provide a

more comprehensive understanding of the species ecology and habitat requirements as it relates to those populations found in Pennsylvania.

Distribution

Large yellow lady's-slipper (formerly *C. calceolus* Salisb. var. *pubescens* (Willd.) Corr.) is a long-lived perennial herbaceous species in the orchid family (Orchidaceae). The species occupies a wide range throughout North America, occurring in nearly every state from Georgia north to Atlantic Canada and west throughout the Midwest (Figure 1). Populations also occur as far south as the desert southwest and into Alaska.

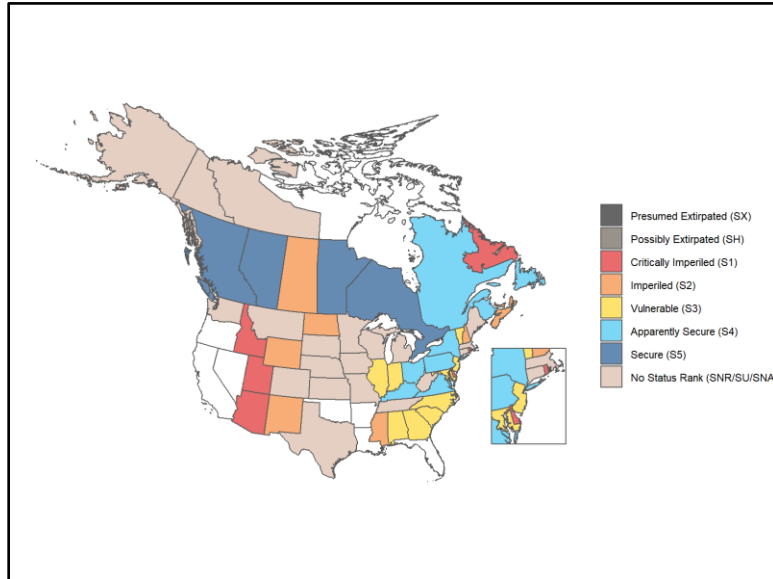


Figure 1. Map of the range of large yellow lady's-slipper in North America (NatureServe 2022).

Even though this species is wide ranging, individual populations can vary drastically from a few stems to hundreds. Large yellow lady's-slipper grows in a variety of habitats such as boggy and swampy areas, forested wetlands, rich, wooded areas with humus and decaying leaf litter, north or east facing rocky, wooded slopes, wooded loess river bluffs, moist creek sides or swales in spruce zones (NatureServe 2022). In Pennsylvania, this species is mainly found on forested slopes and rich, rocky woods, and is associated with Sugar Maple – Basswood Forests or diabase boulders (Rhoads and Block 2007). As a result of the survey work done for this project, we also found populations in Tuliptree – Beech – Maple Forests, Northern Hardwood Forests, and one population in Red Oak – Mixed Hardwood Forest.

Taxonomy

The taxonomy of the *C. parviflorum* complex has a long and complicated history. Luer (1975) cites 23 synonyms for this species, some of them within the European *C. calceolus*. *Cypripedium parviflorum* as treated in Flora of North America (Sheviak 2002) consists of three varieties: *pubescens*, *makasin*, and *parviflorum*. Variety *pubescens* is the most widespread in range and habitat preference. Variety *parviflorum* somewhat overlaps in habitat preference with var. *pubescens*, from which it is distinguished by its usually smaller pouch as well as more extensive and darker pigmentation on the sepals, however, the morphologies of varieties *pubescens* and *parviflorum* are quite variable and the two might be only distinguished as forms (Sheviak 2002). With Sheviak's opinion in mind, it is expected that there are specimens that seem to be *pubescens* or *parviflorum* but cannot safely be placed in one variety or the other. Variety *makasin* occupies a northern distribution in mostly wetland habitats. The taxonomy of this

complex is further complicated due to misidentifications in published resources, as authors traditionally included var. *makasin* in var. *parviflorum*, sometimes excluding the more southern small flowered plants (the current concept of var. *parviflorum*), which they assigned to var. *pubescens*. Large yellow lady's-slipper has a high level of genetic variation at the species level, and this likely contributes to the variability and morphological similarities between the varieties (Luer 1975, Case and Bierbaum 2013, Wallace and Case 2000, Sheviak 2000).

Species Description

Large yellow lady's-slippers are quite striking with their lime green foliage and lemon-yellow flowers (Figure 2). They can occur as a single plant or form clonal patches that give rise to multiple stems (Deller 2005). In Pennsylvania, large yellow lady's-slipper emerges in the spring before full canopy leaf-out. Each stem has one or more alternately attached leaves. From data collected during this study, we found leaf number ranged from a single leaf of a new seedling to up to six leaves. Typically, plants with less than four leaves did not flower. Of the plants that do flower, a single flower was most common, although, we did discover a few double blooms. Large yellow lady's-slipper's pouch-like flowers bloom in May and June for approximately two weeks. The sepals and petals are variable, ranging from light green to purple. The petals are sometimes spirally twisted or flat and are strongly descending. Large yellow lady's-slippers are known to be quite variable especially the coloration of the sepals and petals (Flora of North America, Rhoads and Block 2007).



Figure 2. An example of a patch of large yellow lady's-slippers.

Reproductive Biology

Large yellow lady's-slipper has the potential to reproduce both vegetatively from shoots arising from the rhizome and sexually through the production of flowers and fruit (Curtis 1943, Brackley 1985, Tremblay 1994, Light and MacConaill 1998, NH NHI 1998 all in Deller 2005). Like other members in genus *Cypripedium*, large yellow lady's-slipper depend on insects for reproductive success (Deller 2005). The unique anatomy of the flowers forces insects to fly into the front of the labellum, or pouch (Figure 3). The curved margins of the pouch prevent the insect from being able to fly back out the entrance easily,

thus potentially trapping the insect for seconds to days (Mergen 2006). If the insect is the appropriate size, it will find two exits in the back of the pouch. To exit, the insect must crawl beneath and press up against the stigma. This action causes the exit holes to widen, where the sticky, viscid pollen masses are conveniently located to brush against the insect (Deller 2005). A successful pollination event occurs when an insect correctly navigates two separate flowers (Nilsson 1979, Case and Bradford 2009). If successful pollination occurs, a capsule will form that contains dust-like seeds, with estimates of 7,000 seeds per capsule (Light and MacConaill 1998). The capsules remain upright in the winter and it is likely that seeds are wind dispersed (Mergen 2006, Arditti and Ghani 2000). Although each capsule contains thousands of seeds, seed set is low, and overall propagation of the species is assumed to be mostly vegetative (Deller 2005).



Figure 3. A close-up of the flower of a large yellow lady's-slipper.

Mycorrhizal Association

As with other orchids, large yellow lady's-slipper relies on soil mycorrhizal fungi for nutrient exchange (mycoheterotrophy). The reliance on mycorrhizae begins in the seed stage and is necessary for germination. Deller (2005) suggested that this relationship continues until the plant begins to produce adequate chloroplasts and can photosynthesize. However, Smith and Read (1998 in Light and MacConaill, 2005) reported indirect evidence that chlorophyllous orchids survive extended dormancy in part due to mycorrhizal associations. It may be that fungal associates play a role throughout the orchid's life cycle (Alexander and Hadley 1985 and Tayler et al. 2002 in Shefferson 2006).

While many different species of fungi have been found to be associated with the genus *Cypripedium*, the primary associations for *C. parviflorum* are in the family Tulasnellaceae. Previous research also suggested mycorrhizal species in the genus *Rhizoctonia*, however these were based on asexual stages comprised of many different families (Shefferson et al. 2005).

Insect Associations

As mentioned in the Reproductive Biology section, large yellow lady’s-slippers depend on insect pollination for sexual reproduction (van der Pijl and Doson 1966 and Stoutamire 1967 in Bornbusch et al. 1994 and in Deller 2005). The literature suggests that a variety of insects pollinate the species (Table 1.).

Table 1. A list of insects from the literature that have been observed visiting *Cypripedium parviflorum*. An asterisks indicates the insect successfully removed pollen.

Species	Citation
<i>Ceratina calcarata</i>	Stoutamire 1967
<i>Andrena krigiana</i> , <i>Augochlora pura</i> , <i>Lasioglossum rohweri</i>	Herring 2007
<i>Andrena perplexa</i> *, <i>A. tridens</i> *, <i>Ceratina</i> , <i>Nomada</i> , <i>Lasioglossum</i> , <i>Osmia</i>	Case and Bradford 2009
<i>Osmia proxima</i> *, <i>Osmia sp.</i> *, <i>Osmia albiventris</i> *, <i>Osmia subaustralis</i> *	Catling 2015

Dormancy

Evidence of a “cryptic stage” or below-ground dormancy has been documented for orchids in the genus *Cypripedium* (Shefferson et al. 2001, Shefferson 2006). Light and MacConaill (2005) reported dormancy in their monitored populations of large yellow lady’s-slipper. Shefferson (2006) also reported extended dormancy in his demographic study of *C. parviflorum*. Shefferson (2006) followed two individuals that remained dormant for seven years. Anecdotal evidence from individuals met during the course of this project supports a dormancy stage for this species as well (C. Elwell; personal communication).

Distribution of Large Yellow Lady’s-slipper in Pennsylvania

As previously mentioned, large yellow lady’s-slipper has a wide-ranging distribution which includes Pennsylvania (Figure 1; NatureServe 2022). However, at the start of this project, we did not have a clear understanding of the distribution of the species in the state. Two sources of information were readily available to provide some insight into the locations of known or once known populations. Rhoads and Klein (1993) produced a locational map of the species in their annotated atlas of vascular plants of Pennsylvania. Their map reflects the locations of herbarium specimens (some of them historic or not confirmed extant) and was not necessarily intended to reflect the known extant distribution of the species at the time of publication in 1993 (S. Grund; personal communication). The map may also overestimate the distribution because it was published before the taxonomic splits in the genus *Cypripedium* (S. Grund; personal communication). Given those reasons, it is probably a less reliable source for estimating the current distribution of the species in Pennsylvania.

A second source of information about distribution within the state came from records found in Biotics, a repository of rare species data from Pennsylvania. As of December 2021, there were 22 records for large yellow lady’s-slipper in Biotics with three of them categorized as historical. Some of the sites were visited under the scope of this project to verify that populations remained extant. Combined with the Biotics data, we also used population locations reported to us by reliable sources around the state to produce the following county-level distribution map (Figure 4). The distribution of the species is shown at the county level so as to protect the actual locations of populations. The map reflects populations known at the time of the project. However, the species may actually be more widespread in the state.

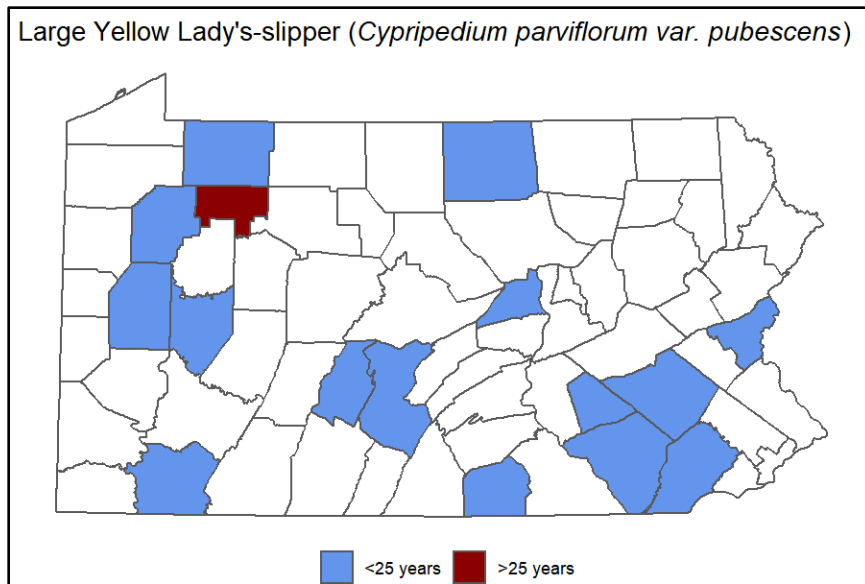


Figure 4. The county level distribution of large yellow lady's-slipper in Pennsylvania based on PNHP Biotics data.

Based on the Biotics data and our project survey efforts, large yellow lady's-slipper has been documented in 15 counties within the last 25 years and one county with a record greater than 25 years (Figure 4).

Condition of Large Yellow Lady's-slipper Populations in Pennsylvania

Field surveys for large yellow lady's-slipper were mainly planned using data from Biotics and other reliable sources. We selected 15 sites for field assessments from the list of known populations that had population information from previous surveys. The number of sites selected was based on available project funds and staff time.

For 13 of the sites, we applied a low intensity monitoring approach (or census approach) that requires a single visit to a site to determine population size and assess habitat and potential threats. This approach was similar to an EO update and could easily be done by anyone familiar with the species. For the other two sites (Site A and the Site C), we applied a high intensity monitoring approach where the fate of individual stems are followed over time. This approach provides more insight into the age/stage structure of a population and can be useful when considering management strategies for a species. The low intensity sites were visited once in May and June 2018 during peak flowering time. The high intensity sites were visited three times in 2018 and 2019.

Methods for Condition Assessments

Estimating population size

Regardless of the monitoring intensity effort, prior to visiting each site, aerial imagery was reviewed to determine the extent and overall structure of the site so that participants were familiar with the lay of the land before arrival. Each site was visited by a minimum of two people to assist with the condition assessment. Once at a site, a starting point was selected that defined the population edge and a de novo survey was conducted. Participants began a meander search through the habitat to locate large yellow lady's-slippers. Once located, plants were flagged with surveyor flags. The search continued until all participants felt that enough of the area had been searched and all plants within that area had been flagged. It is possible that some plants were overlooked, but given that we were mainly interested in a

population estimate, this method was sufficient to accomplish that goal. Depending on the habitat and the number of plants, searches lasted anywhere from 15 minutes to over an hour.

For both the low and high intensity effort, we used a combined approach to estimate population size. Stem (ramet) count was one method used. This method has been used with success in other orchid monitoring studies (see Knapp and Wiegand 2013). We did not have the time, nor did we want to disturb the sites by excavating all rhizomes to determine if stems (ramets) were arising from shared or separate rhizomes and how many plants (genets) those stems represented. Given this assumption, one can argue that our unit for estimating population size may overestimate the actual size because multiple stems can arise from a single rhizome (a single genet can have multiple ramets). To try to account for this, we also recorded the number of stem clusters and single stems in a population. This approach has also been used by others when studying this species (Shefferson et al. 2001, Light and MacConaill 2005, Shefferson 2006). As per Shefferson (2006), we used a separation distance of 20 cm to distinguish a single stem from a cluster of stems. We felt that these combined methods for estimating population size were the easiest to do and most repeatable methods for future monitoring efforts.

For the low intensity effort, once stems were flagged, the data form found in Appendix I was completed. All stems were counted, and the following information was recorded: whether a stem occurred as a single stem or in a cluster, number of vegetative stems, number of flowering stems, and if insect or deer browsing damage was observed (see Appendix I). Additional observation notes were added if something of interest was noted (Figure 5). Surveyor flags were removed once the population estimate was completed.



Figure 5. Jaci Braund, an ecologist with PNHP, flagging large yellow lady's-slipper locations at one of the monitoring sites.

The high intensity effort required a greater time investment compared to the low intensity one. Instead of only counting the total number of reproductive and non-reproductive plants in a population, data was collected on individual plants to better understand the structure of the population. Both high intensity monitoring sites had greater than 300 stems scattered across several acres. It would have been impossible to monitor all stems in those populations given limited project time so a portion of the

population was selected for monitoring instead. This subset approach in a large population still provides insight into the population structure and life cycle dynamics of the species.

To avoid drawing attention to populations by leaving flags in the field, we used a cryptic approach to tagging individuals that had been previously tested with success in a multi-year study of American ginseng (*Panax quinquefolius*) (Furedi 2004, Furedi and McGraw 2004). An aluminum nail with a unique number engraved on the nail head was placed at the base of each stem below the soil surface. If a stem went missing during the study, a metal detector was used to relocate the nail. In addition, stems with landscape identifiers were photographed, and detailed population maps drawn to aid in the relocation process.

Once an individual stem was given a unique identifier, characteristic data was collected that included whether the stem occurred as a single stem or in a cluster, number of leaves, reproductive status, and the extent of insect or deer browse damage was noted (see Appendix 2 for high intensity data sheet). The high intensity populations were visited a total of three times during the growing season to document flower production, successful capsule formation, and any browsing/insect damage or evidence of poaching.

Using the data collected for individual stems, we assigned each stem into one of four biologically meaningful stage classes based on leaf number and reproductive status. Class 1 represented the new seedling/young stem category and included all stems with two leaves. (There were no one leaf plants observed at the populations.) Class 2 was the juvenile stage and included stems with three leaves. Class 3 were the non-reproductive adults and included stems with four to six leaves that did not produce a flower. Class 4 were the reproductive adults and included stems with four to six leaves that flowered. A final class, Class SNF, was created for stems tagged in 2018 that were not relocated in 2019. The proportions of classes within each population were then calculated for 2018 and 2019 to examine the stage structure of the two populations.

Documenting habitat and threats

In addition to population estimates, we also collected vegetation data to characterize the habitat by plant community type. All vegetation data were collected following accepted Natural Heritage sampling protocols developed for the quantitative characterization of plant communities (Strakosch-Walz 2000). Since all populations were found in forest types, releve sampling plots of 20m x 20m (400m²) were used. Plots were established non-randomly to represent the plant community of interest (Mueller-Dombois and Ellenberg 1974). Vegetation plots were sampled during the same visit for population estimates, May and June 2018.

Within each plot, vegetation was visually divided into seven strata: tree canopy (variable height), tree subcanopy (>5m in height), tall shrub (2-5m), short shrub (<2m), herbaceous, non-vascular, and vines. Percent cover was estimated for each species in each stratum using modified Braun - Blanquet cover classes (Strakosch-Walz 2000). Specimens of unknown species were collected for later identification in the office and destroyed after identification. Canopy cover was estimated in the plot center and plot corners using a densiometer. These data were then used to classify plant communities using Pennsylvania's plant community classification (Fike 1999).

Abiotic data within the plot was also recorded which included location, slope, aspect, topographic position, and soil characteristics. Notes were taken on plot representativeness to the surrounding vegetation and any other significant environmental information, such as landscape context, herbivory, stand health, recent disturbance, presence of invasive plant species, or evidence of historic disturbance. Potential species threats were recorded as well. All plot centers were georeferenced using a handheld

Garmin unit. See Appendix 3 for an example of the data sheets used for collecting vegetation information.

In addition to documenting threats noted at each site, game cameras were set up at three populations in an attempt to capture “activity” at the sites.

The site and population-level data gathered for this project were entered into the PNHP rare species data base Biotics. The plant community information was entered into EcoObs, a database developed by NatureServe used by PNHP to house plant community plot data.

Results of Condition Assessments

Population Estimates

Large yellow lady’s-slipper populations were documented at 14 of the 15 sites (Table 2). The only site where the species was not relocated was Site M. This population had been previously documented in 1999. The site was revisited with the botanist who had first documented the population. The botanist led us to the original location of the population and we performed a thorough search of the area, but no large yellow lady’s-slippers were found. The botanist observed that the site was far more dominated by invasive species than when she had last visited, which is an important observation from a threats perspective.

Population estimates by stem census ranged from six stems at Site H-I to over 600 stems at Site A (Table 2). At all populations, stems were arranged as both single stems and clusters of stems (Table 2). Cluster size ranged from a few stems to almost 20 stems. It is likely that actual stem totals were greater than estimated since some populations are scattered over a large area and we may have missed stems during the de novo search. Also, given that large yellow lady’s-slipper may experience dormancy in one or more years (Shefferson et al. 2001, Shefferson 2006), there are likely more individuals in the population pool. Complete deer browsing (the entire stem is consumed and only a stump of the stem remains) prior to the census may have also reduced the number of visible stems. Since we do not have population-level estimates from previous visits for most sites, it is difficult to comment on the status of a population (increasing, decreasing, or remaining stable). However, given that we knew these populations were in existence from Biotics, we can say that the populations, even the smaller ones, are at least persisting over time. Additional future visits with comparable data collection are needed to provide a better idea of stability and vulnerability of populations. The dataset from this project does provide an excellent baseline in which to make future population-level comparisons.

In addition to recording the total number of stems at a site, we also recorded the number of flowering stems. Flowering provides some insight into plant and population age and can be linked to environmental conditions (Light and MacConaill 2005). It can take 10 to 16 years from germination for a plant to produce a flower (Curtis 1943, Kull 1995, Shefferson et al. 2001) and will continue to flower for at least six years (SVE Monocot Panel 2003 in Deller 2005). Light availability, temperature, and precipitation all play a role in flower production (Shefferson et al. 2001, Light and MacConaill 2005, Shefferson 2006). We found that flowering varied by population, ranging from two flowering stems in the smallest population at Site H-I to over 100 flowers in some of the larger populations (Table 2). The percentage of flowering stems in a population ranged from 16% at Site D to 66% at Site G (Table 2). Surprisingly, the largest population, Site A, had only 17% flowering stems. This may have been the result of a higher rate of deer browse in that year. We documented many browsed stems in 2018 at this site.

Table 2. Population estimates for 14 large yellow lady’s-slipper sites in Pennsylvania.

Population	# Total Stems	# Single Stems	# Clusters	# Flowering Stems	Comments
A	606+	52	18	100+	A subset of the population was included in this study and counted. The population is larger and likely over 800 plants in total.
B	143+	6	12	75	The population is scattered over a wide area and likely larger than estimated. Many seedlings noted.
C	118+	18	12	51+	A subset of the population was included in this study and counted. The population is larger and likely over 300 plants in total.
D	45+	12	4	7	Population scattered over wide area and likely larger than estimated.
E-1	215	37	40	135	
E-2	112	5	12	54	
F	15	7	3	9	
G	82	22	8	54	
H-1	6	4	1	2	Population scattered over wide area and likely larger than estimated.
H-2	273	192	37	123	
I	34	19	5	11	
J	12	2	3	6	
K	29	5	2	13	Plant total is the combined count from subpopulations scattered across the property.
L	63	2	7	22	
M	NA	NA	NA	NA	No large yellow lady's-slippers were found after an extensive search of the site.

Demography of Populations

Demographic studies, where plants and animals are followed over time, provide more detailed information about the age/stage structure of a population than the low intensity census approach. Demographic studies have been conducted for *Cypripedium* sp. in the past (see Shefferson et al. 2001, Light and MacConaill 2005, Shefferson 2006, and Thorpe et al. 2011). The additional leaf data and flowering status collected at the two high intensity sites (sites A and C), enabled us to take a similar demographic approach to examine the stage structure of the two populations.

As previously described in the methods section, leaf number and flowering status were used to assign individual stems found in 2018 and 2019 to one of four stage classes: Class 1 (new seedlings/young stems with two leaves), Class 2 (three leaves and non-reproductive), Class 3 (four to six leaves and non-reproductive), and Class 4 (four to six leaves and reproductive). A fifth class, Class SNF, was created for stems tagged in 2018 but not relocated in 2019. We found that all four stages were present in both populations but differed in class distribution (Table 3). The Site A population was skewed towards larger size classes in both years (greater than 90% in C3 and C4) (Table 3) with far fewer stems in classes 1 and 2. The Site C Population also had a greater number of stems in C3 and C4 in both years but had more stems represented in the smaller size classes compared to Site A (Table 3).

Table 3. Comparison of the stage structure of two populations of large yellow lady's-slipper from sampling years 2018 and 2019.

Population	Stage Classes	Number of stems/class 2018	Percent of population by class in 2018	Number of stems/class 2019	Percent of population by class in 2019
Site A	I	4	4%	1	1%

	2	4	4%	4	4%
	3	70	67%	55	52%
	4	27	25%	28	27%
	SNF	NA	NA	17	16%
Site C	1	36	30%	16	14%
	2	16	14%	21	18%
	3	16	14%	24	20%
	4	50	42%	44	37%
	SNF	NA	NA	13	11%

All of the tags used to mark individual stems were relocated in 2019, but some of the stems were not. Seventeen stems (16%) were not relocated in Site A and 13 (11%) were missing at Site C (Table 3). One of the missing stems in the Site A Population had been browsed by deer, but no other evidence of browsing or poaching was noted to explain the disappearance of the other stems. No additional investigation, like excavating the rhizome, was done to determine the fate of missing stems since we did not want to disturb the sites. Given that dormancy has been documented for the genus *Cypripedium* (Shefferson et al. 2001, Shefferson 2006) and specifically for adult large yellow lady's-slippers (Light and MacConaill 2005), some of the missing stems may have gone dormant in 2019. All of the missing stems in the Site A population and about half of those missing in the Site C Population had been recorded as adult size classes (C3 and C4) in 2018. The remaining missing stems in the Site C Population were younger stems (eight were C1 and 1 was C2) and may have died due to environmental-related stresses. Generally, new seedlings and young plants are most vulnerable to environmental stressors.

In addition to population structure, we gained more insight into reproductive success for these two populations. Reproductive success was defined as capsule formation. No seed viability studies were done under this project. Both populations were revisited later in the growing season in 2018 and 2019 to document if flowering stems were successfully pollinated and resulted in capsule formation. We found year to year variation in flowering and capsule production both within and between populations (Table 4). (Please note that a portion of the population was being monitored at both sites. Flowering rates were higher within both populations as a whole.) The number of flowering stems and subsequent capsule development was greatest for the Site C Population in both years (Table 4). In 2018, only two capsules formed in the portion of the population being monitored at Site A. The number of capsules increased to 13 in 2019. One possible explanation for low capsule formation in 2018 was that some of the flowering stems may have been browsed prior to capsule formation.

Table 4. Summary of flower and capsule formation census for two populations of large yellow lady's-slipper in Pennsylvania.

Population	2018		2019	
	Number of flowering stems	Number of capsules	Number of flowering stems	Number of capsules
Site A	27	2	28	13
Site C	50	22	44	15

The dataset for these populations is limited to only two years, yet it does provide evidence of successful pollination and subsequent capsule formation in both populations. Even if capsule formation is naturally low, a single capsule may contain an estimated 7,000 seeds (Light and MacConaill 1998) and that may be enough for new recruitment for a population, if seeds successfully germinate. Deller's review (2005) indicated that although capsules contain thousands of seeds, seed set is low, and overall propagation of the species is assumed to be mostly vegetative.

Habitats of Large Yellow Lady's-slipper in Pennsylvania

Except for Site J, the large yellow lady's-slipper populations were found in more continuous forest stands under mostly closed canopy conditions (ranging from 71% to 94% overstory cover) (Table 5). The Site J population was also shaded (47% canopy cover) but located in a small remnant successional forest patch surrounded by human-modified landscape. Populations were usually located on a forested slope ranging from gently sloping like at Site F and Site E-1 to steeply sloped like Site G and Site I. The southeastern sites had greater boulder cover as well. Site elevations ranged from 100 m in southeast Pennsylvania to 390 m in the northern tier of the state.

Table 5. Habitat characteristics of the large yellow lady's-slipper sites visited during this project. Note: habitat characteristics were recorded for Site M even though large yellow lady's-slippers were not found there.

Population	PA Region	PA Plant Community	Canopy Cover (%)	Elevation (m)	Slope (degrees)	Soil Type	Soil pH
Site A	western	Sugar Maple - Basswood Forest	80	313	25	clay loam	8
Site B	southeast	Tuliptree – Beech – Maple Forest	85	270	20	loam	7
Site C	southeast	Tuliptree – Beech – Maple Forest	85	265	20	loam	7
Site D	western	Tuliptree – Beech – Maple Forest	94	267	15	loam	7.5
Site E-1	western	Northern Hardwood Forest	71	257	4	clay loam	4.5
Site E-2	western	Red Oak - Mixed Hardwood Forest	83	330	9	clay loam	6.5
Site F	southeast	Tuliptree – Beech – Maple Forest	93	215	3	sandy loam	7
Site G	central	Sugar Maple - Basswood Forest	87	184	45	loam	7
Site H-1	southeast	Tuliptree – Beech – Maple Forest	85	225	5	loam	7
Site H-2	southeast	Tuliptree – Beech – Maple Forest	88	218	10	sandy loam	7
Site I	northern	Northern Hardwood Forest	80	390	45	loam	7
Site J	central	Remnant Forest	47	158	5	loam	7-7.5
Site K	southeast	Tuliptree – Beech – Maple Forest	85	127	25	loam	7
Site L	southeast	Tuliptree – Beech – Maple Forest	87	100	22	loam	7
Site M	southeast	Tuliptree – Beech – Maple Forest	76		10	loam	7

Eight of the populations (and the one site where large yellow lady's-slipper population was not relocated) were found in Tuliptree – Beech – Maple Forests where tuliptree (*Liriodendron tulipifera*) was the dominant tree species in the overstory canopy with other tree species, like sugar maple (*Acer saccharum*) and sweet birch (*Betula lenta*), occurring in lesser amounts. A shrub layer was usually present but variable in cover with a mix of shrubs including spicebush (*Lindera benzoin*), hophornbeam (*Carpinus caroliniana*), and witch-hazel (*Hamamelis virginiana*). The herbaceous layer at these sites was diverse with species indicative of richer sites. Soils were generally neutral loams.

Two of the populations were found in Sugar Maple – Basswood Forests where, as the community name implies, sugar maple and basswood (*Tilia americana*) were the dominant canopy species (Table 5).

Hophornbeam (*Ostrya virginia*) was the dominant shrub at both sites. These sites also had a diverse herbaceous layer with species indicative of richer sites. Soils were neutral loams.

Two of the sites were characterized as Northern Hardwood Forests (Site E-I and Site I) (Table 5), although they differed in their topographic positions. The Site E-I population was located in more of a low slope plateau where surface runoff ponded so soils were more saturated. Both sites had a canopy dominated by sugar maple. In lesser amounts were eastern hemlock (*Tsuga canadensis*) and other hardwoods. Witch-hazel was a component of the sparse tall shrub layer. The low shrub layer at Site E-I was heavily invaded by barberry (*Berberis thunbergii*). The herbaceous layers of these sites were less diverse and sparser compared to the other sites visited.

Site E-2 population was located in a Red Oak – Mixed Hardwood Forest (Table 5) where red oak (*Quercus rubra*) was the dominant canopy species. Like the Site E-I site, witch-hazel was a component of the sparse tall shrub layer and the low shrub layer was heavily invaded by barberry. The herbaceous layer was patchy.

The population found at Site J was located in a very small remnant forest patch surrounded by roadways and maintained lawns. There were only a few trees remaining and the area was too small to type to a community type.

Threats to Large Yellow Lady's-slipper

Three methods were employed to understand the threats associated with large yellow lady's-slipper across its range and within Pennsylvania. First, threats to the species across its range were compiled from a review of the literature. Second, a Pennsylvania focused survey was conducted to understand threats known or perceived in the state. Third, threats observed in the field during site visits were recorded and compiled.

Threats in the Literature

Deller's (2005) review not only provided thorough background information on large yellow lady's-slipper but also contained extensive insight into threats associated with the species. The threats listed by Deller (2005) include habitat loss, alteration, and fragmentation; herbivory by livestock and grazing by wild animals; destruction by insects and disease; competition from non-native invasive species; and collection and cultivation. See Deller (2005) for a more detailed description of these threats across the range of the species. Knapp and Wiegand (2013) documented orchid decline, including large yellow lady's-slipper in Catoctin Mountains, MD, and attributed it to intensified herbivory by white-tailed deer.

Known/Perceived Threats in Pennsylvania

In Pennsylvania, large yellow lady's-slipper has a state status of Pennsylvania Vulnerable (PV) (<http://www.naturalheritage.state.pa.us/SpeciesFeatures.aspx>; accessed 3/3/20). This means there is danger of population decline because of beauty, economic value, use as a cultivar, or other factors that indicate that persons may seek to remove these species from their native habitats. Based on notes from the Fall 2019 Pennsylvania Vascular Plant Technical Committee (VPTC), large yellow lady's-slipper was originally added to the PV list due to drug-trade pressures than horticultural collection (R. Goad; personal communication). However, recent member comments from the VPTC suggest that the drug trade is no longer a threat (R. Goad; personal communication). Large yellow lady's-slipper was traditionally used for medicinal purposes by Native Americans. However, the American Herbal Products Association agreed in the early 1990s to cease the trade of the species for medicinal purposes. The species has not been traded between root sellers across Appalachia since the late 1980s and into the early 1990s due to its overall rarity (Eric Burkhardt; personal communication). Large yellow lady's-slipper

“flower essence” is still available for sale on the internet, and upon inquiry, manufacturers follow a protocol involving collecting wild plants in the Midwest (Jaci Braund; personal communication).

Since horticultural collection (or sometimes referred to as “poaching”) may be a contributing factor to the population decline of large yellow lady’s-slipper, we pursued an informal inquiry of greenhouses in Pennsylvania in an attempt to quantify this pressure. Initially, our internet-based research focused on greenhouses only in Pennsylvania. However, “greenhouse” on the internet is broadly defined as anything from a home improvement store, to garden store, to a wholesale nursery. The internet search was then narrowed to specific stores that appeared in searches when the terms “native plants” and “orchid sellers” in Pennsylvania were used. After excluding home improvement centers and garden stores since they generally do not carry wild-collected or native plants, 18 businesses (11 native plant nurseries and 8 orchid sellers) were contacted. None of the native plant nurseries contacted were selling large yellow lady’s-slippers, and two had insufficient data. Organizations that had insufficient data either had a minimal internet presence or did not respond when contacted directly. Of the orchid sellers, four had insufficient data and only one was actively selling large yellow lady’s-slipper. When asked, the seller said they bought their plants from an unnamed distributor. Our limited research indicates that large-scale poaching to supply the horticultural industry is probably less of a threat in Pennsylvania but smaller-scale collection efforts for personal use cannot be ruled out.

Further internet research on large yellow lady’s-slipper suppliers yielded several websites from distributors around the United States. Eight distributors were identified in seven states where large yellow lady’s-slippers can be ordered from their websites and shipped to your home. One distributor obtained their supply from a larger distributor in the same state (Minnesota). Two distributors obtained their plant materials from Europe. The other five orchid sellers all propagated large yellow lady’s-slippers in-house using various tissue culture methods. Interestingly, one of the lab-propagated growers admitted to collecting wild-grown large yellow lady’s-slippers early on, however had stopped that practice in recent years and now only sells propagated plants. Based on personal correspondence with these orchid sellers, our impression was that lab propagated yellow lady’s-slippers were more widely used today for the sustainability of the market. They can continually grow seedlings from tissue in the lab with a high success rate and are then able to sell more product. Based on a 2019 report from the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), it is likely stock production of the species through artificially propagated material is a global practice (CITES 2019) (Table 6).

Table 6. Convention on International Trade in Endangered Species of Wild Fauna and Flora report generated for *Cypripedium parviflorum* international trade during the years 2013-2018. Note that in only one instance material was exported from a location in the natural range of *C. parviflorum*, Canada, and that all trade reported originated from artificially propagated material.

Year	Importer	Exporter	Origin	Importer reported quantity	Exporter reported quantity	Term	Purpose	Source
2013	Switzerland	Belgium		210	210	live	Commercial	Artificially propagated
2013	Switzerland	Germany	Belgium		50	medicine	Commercial	Artificially propagated
2013	Norway	Belgium			10	live	Commercial	Artificially propagated

2013	USA	Belgium		20	live	Commercial	Artificially propagated
2013	USA	UK		50	roots	Commercial	Artificially propagated
2014	Switzerland	Germany		80	live	Commercial	Artificially propagated
2014	Switzerland	Netherlands		8	live	Commercial	Artificially propagated
2014	Norway	Belgium		1	live	Commercial	Artificially propagated
2015	Switzerland	Germany	Netherlands	72	live	Commercial	Artificially propagated
2015	Switzerland	Netherlands		48	live	Not reported	Artificially propagated
2015	Russia	Belgium		6	live	Commercial	Artificially propagated
2015	USA	Canada		10	live	Personal	Artificially propagated
2016	Switzerland	Germany		60	live	Commercial	Artificially propagated
2017	South Korea	Belgium		20	live	Commercial	Artificially propagated
2017	USA	Belgium		50	live	Commercial	Artificially propagated

As a means of developing a better sense of additional perceived threats in Pennsylvania, a questionnaire was developed to survey public knowledge of threats to large yellow lady's-slipper in Pennsylvania. The survey included questions about knowledge of local large yellow lady's-slipper populations and what, if any, threats had been observed or were anecdotally known from these populations or other populations in the state (see Appendix 3 for survey). The survey was administered to a group of botanists and naturalists familiar with Pennsylvania plants as a written questionnaire at the 2018 Rare Plant Forum and in an email format sent to the Rare Plant Forum email list in Fall 2019. In total, the survey was given to 78 individuals. However, it is likely that some of the same individuals received both forms of the survey since the email list overlapped with attendants at the Rare Plant Forum.

The overall response rate to the survey was 15%. Although low, the survey results suggest that threats to large yellow lady's-slipper are similar to those reported in the literature as range-wide concerns. Invasive species, deer browse, and poaching were the top threats reported in the survey followed by several others (Table 7).

Table 7. A summary of threats associated with large yellow lady's-slipper from results of two surveys.

Threat	Number of times noted
Deer browsing	2
Invasive species	2
Poaching	2
Trampling	1
Habitat conversion	1
Groundhogs	1
Landslide/periodic erosional events	1

Site-specific Threats

Threat assessments were done during the 2018 and 2019 field visits to the large yellow lady's-slipper populations visited during the course of this project. The assessment included documenting and quantifying the presence of invasive plant species in the understory (shrub and herbaceous layers), if the populations were close to hiking trails or active deer trails, if plants had been trampled, signs of floral/foiar damage from deer and insects, and evidence of poaching. From the threat assessments, we found some level of threat existed for all populations visited (Table 8). The presence of invasive plant species was the most commonly noted threat across populations. Twelve of the 14 populations (86%) had invasive plants in the understory. Unfortunately, it is a common occurrence to see invasive plants like Japanese barberry, multiflora rose (*Rosa multiflora*), honeysuckle (*Lonicera* sp.), and garlic mustard (*Alliaria petiolata*) in the understory of forests in Pennsylvania. At five of the sites, the invasive cover was noted to be so great that it will likely negatively impact the populations in the near future (see Table 8; Figure 6). During the course of this study, we heard several anecdotal accounts of invasive species encroachment associated with populations disappearing.

Table 8. A summary of potential threats to large yellow lady's-slipper populations noted during site visits in 2018 and 2019. An asterisk indicates a more severe threat to the population.

Population	Invasive plants	Near trail	Deer browse	Insect damage	Trampling	Poaching
Site A	Y	Y*	Y	Y	Y	N
Site B	Y	N	Y	N	N	N
Site C	Y	Y	N	Y	N	N
Site D	Y	Y	Y	Y	Y	N
Site E-1	Y*	N	Y	Y	N	N
Site E-2	Y*	N	N	N	N	N
Site F	Y	N	N	N	N	N
Site G	Y	Y	N	N	N	N
Site H-1	N	N	N	N	N	N
Site H-2	Y	Y*	N	Y	N	N
Site I	N	Y	N	Y	N	N
Site J	Y*	Y*	Y	Y	N	N
Site K	Y*	Y*	Y	Y	N	N
Site L	Y*	Y	Y	Y	N	N



Figure 6. An example of a population of yellow lady's-slipper being invaded by Japanese barberry.

Insect damage was the second most common threat noted in the populations visited. Nine of the 14 populations (64%) had some level of foliar or floral insect damage (Table 8; Figure 7). However, insect damage was usually limited to just a few stems in the population. We did note occasional insect damage to the flower pouch, but it is unknown if this impacts pollination success and subsequent successful capsule development. Based on the two sites where we did more intensive demographic work over two years, we did not see discriminative insect damage (the same stems were not damaged year after year). It is likely that insect damage may be more of a random event and although it results in damage to individual stems, the damage is likely not a strong contributor to overall population decline.



Figure 7. Examples of insect damage to the leaves and flower of large yellow lady's-slipper.

Deer browse was reported at seven of the 14 populations (50%) (Table 9). Similar to insect damage, browsing was usually limited to a few stems in each population and resulted in variable damage to the plant (Table 9). Damage noted ranged from a single browsed leaf to browsing of almost the entire plant (Figure 8).



Figure 8. Examples of deer browse damage to the stems and leaves of yellow lady's-slippers.

An exception to the browse rate was seen at Site A in 2018. Almost 26% of the stems were browsed in the subsection of the population being monitored. However, only one stem was browsed in 2019. Since the project only allowed for two years of browse data from the Site A population, it is impossible to explain the differences in browse rates and whether deer browsing has longer-term negative effects on populations of large yellow lady's-slippers. However, numerous studies on other herbaceous species have documented the negative impacts of deer browsing at both the individual plant level and the population level. More demographic work is needed to understand this threat.

Table 9. A summary of the number of browsed stems in large yellow lady's-slipper populations where deer browse was noted.

Population	Number of plants counted	Number of plants deer browsed
Site A 2018	105	27
Site A 2019	89	1
Site D	35	2
Site E-I	37	2
Site J	13	1
Site K	29	4
Site L	44	4
SITE B	143	6

Some threats noted in the field were likely spatially correlated. Populations close to trails, whether hiking trails or deer paths, are more accessible to humans and deer and thus a greater potential for impact from deer browse, trampling, and/or poaching. We captured multiple frames of deer moving through the populations along trails and some surprise visitors as well, which included a few bears and some playful dogs (Figure 9) The trampling noted at Site A in 2018 and Site D occurred on plants (one plant at each site) located very close to the trail edge (Table 8). Although, nine of the 14 populations have some form of trail going through them, the observed incidences of deer browse and trampling were relatively low (Table 8).



Figure 9. Examples of animals (deer, dogs, and a bear) captured on game cameras that visited large yellow lady's-slipper populations during the course of this study.

Poaching is often cited as a reason for the global decline of orchids and based on our survey results, was thought to be a threat in Pennsylvania as well (Table 7). However, no evidence of poaching was noted in the 14 populations visited for this study (Table 8). Our game cameras only captured visitation by people who were working on the project. The low intensity populations were only visited once during the growing season (although site visits were when larger stems were flowering and highly visible) so it may have been possible that poaching occurred later in the season. Harvest pressures may be less for this species given that nursery stock is readily available online, but small-scale harvesting for personal collection is probably still a valid threat. Additional monitoring of easily accessible populations might shed more light on quantifying harvest pressures for this species.

Management Considerations

Based on previous occurrence data and information gathered from this project, we now have a better understanding of the distribution of large yellow lady's-slipper in Pennsylvania. As this time, large yellow lady's-slipper has been documented in 16 counties in Pennsylvania (Figure 4), but it is likely more populations exist in the state. During this project, we heard from numerous people about populations they have visited in the past or know of on private property. We found that there is a group of people in the state excited about this species and willing to show and tell you about their populations. Future survey efforts should attempt to reach out to these private landowners to document their populations.

There may be more populations than currently documented because the habitat niche of the species may be wider than originally thought. Rhodes and Block (2007) identified Sugar Maple – Basswood Forests or diabase boulders as potential habitat. However, we documented populations in a wider range of plant community types including Tuliptree – Beech – Maple Forest, Northern Hardwood Forest, Red Oak – Mixed Hardwood Forest and even in sub-optimal remnant forest patches. The construction of a species distribution model for large yellow lady's-slipper might be one way to identify potential new areas for survey effort. A model might also be helpful to identify potential habitat if there are future plans to establish new populations of this species in the state (e.g., population re-establishment and assisted migration activities).

Consistent monitoring is an important tool for gauging the health of a species. Adopting a single method of monitoring allows for consistency in data collection and more confidence when comparing multi-year population estimates. We employed two approaches in this project that were suitable for large yellow lady's-slipper and have been commonly used to monitor other plants as well. Although the high intensity approach provides more population structure and stem fate information, it is more time consuming and labor intensive than the low intensity method. The low intensity method provides basic data to evaluate population trends and can be easily taught to someone familiar with the species. We recommend this

approach and suggest that counts of both flowering and non-flowering stems be recorded along with the number of clumps and maps, if possible. The population-level data collected during the course of this project could serve as a useful baseline moving forward.

The timing of monitoring is important. Monitoring activities should coincide with timing of flowering so that counts for flowering and non-flowering stems can be done in one trip. We found that the ideal monitoring time in Pennsylvania was late May to early June. Flowers do remain visible, although withered, for some time after the peak flowering period which allows for a larger window of monitoring opportunity. Another advantage to waiting until after the peak flowering season is that you begin to see capsule formation. However, the number of capsules formed is not the same as the number of flowering stems. We recorded far more flowers than capsule formation in the two high intensity populations.

The life history of large yellow lady's-slipper should be considered when determining the time interval between monitoring events. Given that individual stems and perhaps a large proportion of the population may become dormant each year and that dormancy appears to be driven largely by climatic factors (Shefferson et al. 2001, Shefferson 2006), it may be necessary to visit some populations at a greater frequency than is currently the practice. If a population is visited and numbers are low with no obvious threats observed, we suggest revisiting this population in subsequent years to see if the decline is real or due to dormancy at the time of the initial visit.

Multiple threats to this species have been outlined in the literature and observed in the field during the course of this project. A threats assessment should be a component of a monitoring protocol. Early detection of threats, like invasive species, can lead to potential active management at a site before a threat becomes problematic. For example, active management for Japanese barberry and garlic mustard at the Site M population may have helped that population continue to persist.

If a population is declining, not all hope is lost. There is still the option to reintroduce new plants to the site. Peter Zale's group at Longwood Gardens has successfully propagated large yellow lady's-slipper in the lab and greenhouse using Pennsylvania native seed sources (P. Zale; personal communication). A partnership with Longwood Gardens would ensure the use of regionally appropriate plant material for reintroduction purposes and would help to continue the legacy of one of our beautiful orchids in Pennsylvania.

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Appendix 3. Survey form for the threats assessment

The Pennsylvania Natural Heritage Program (PNHP) will be wrapping up a two-year study on the large yellow lady's-slipper (*Cypripedium parviflorum* var. *pubescens*) in 2020. We have been studying the distribution, ecological associations, population dynamics and potential threats this species faces in PA.

At the 2018 Rare Plant Forum, we handed out a survey about the large yellow lady's-slipper and a few of you graciously took the time to fill it out. We are again asking for more information on this species in Pennsylvania in order to provide an in-depth summary of the status and current threats.

If you have seen the large yellow lady's-slipper in Pennsylvania, please consider filling out the following information and sending it to Jaci Braund (jbraund@paconserve.org) by **December 1, 2019**. Simply copy & paste the text below into the reply and add your responses. Thank you for helping to conserve and understand the large yellow lady's-slipper in PA!

- 1) Location of *Cypripedium parviflorum* var. *pubescens* population (e.g., State Game Lands, County, State Park, etc.):

- 2) If you have visited the population over multiple years, have you noticed variation in the size? Has it increased? Decreased?

- 3) Please highlight any of the following threats you have noticed in the population:
Invasive species; deer browse; habitat conversion; harvest/theft; trampling; habitat alteration (e.g., new forest canopy gaps); fire (wild or prescribed); disease: other (please specify)

- 4) Please describe what evidence of the threats highlighted above you have observed.

- 5) Do you think large yellow lady's-slipper plants are being harvested from this population?

- 6) If yes to number 5, what information has shaped your perspective? Highlight all that apply.
 - a. I have seen evidence of harvest at populations of large yellow lady's-slipper.
 - b. Someone told me they have harvested large yellow lady's-slipper.
 - c. I have second-hand knowledge of harvest of large yellow lady's-slipper (i.e. someone else observed evidence of harvest and told me).
 - d. I have heard that large yellow lady's-slipper is collected from the wild, but the information was vague.

