8-2015

HABITAT CHARACTERISTICS AND DEMOGRAPHY OF CALYPSO BULBOSA AND CYPRIPEDIUM ARIETINUM IN THE GRAND SABLE DUNES, MICHIGAN

Adrienne L. Bozic
Northern Michigan University, adrienne_bozic@yahoo.com

Recommended Citation

Bozic, Adrienne L., "HABITAT CHARACTERISTICS AND DEMOGRAPHY OF CALYPSO BULBOSA AND CYPRIPEDIUM ARIETINUM IN THE GRAND SABLE DUNES, MICHIGAN" (2015). *All NMU Master's Theses*. 67.
https://commons.nmu.edu/theses/67

HABITAT CHARACTERISTICS AND DEMOGRAPHY OF *CALYPSO BULBOSA* AND *CYPRIPEDIUM ARIETINUM* IN THE GRAND SABLE DUNES, MICHIGAN

By

Adrienne L. Bozic

THESIS

Submitted to
Northern Michigan University
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE

Office of Graduate Education and Research

August 2015

SIGNATURE APPROVAL FORM

HABITAT CHARACTERISTICS AND DEMOGRAPHY OF *CALYPSO BULBOSA* AND *CYPRIPEDIUM ARIETINUM* IN THE GRAND SABLE DUNES, MICHIGAN

This thesis by Adrienne L. Bozic is recommended for approval by the student's Thesis Committee and Department Head in the Department of Biology and by the Assistant Provost of Graduate Education and Research.

Dr. Alan J. Rebertus: Date

Dr. Donna M. Becker: Date

Dr. Jill B.K. Leonard: Date

Dr. John Rebers: Date

Dr. Brian D. Cherry Date
Assistant Provost of Graduate Education and Research

ABSTRACT

HABITAT CHARACTERISTICS AND DEMOGRAPHY OF *CALYPSO BULBOSA* AND *CYPRIPEDIUM ARIETINUM* IN THE GRAND SABLE DUNES, MICHIGAN

By

Adrienne Lynn Bozic

I studied the distribution, habitat characteristics, and reproductive demography of *Calypso bulbosa* and *Cypripedium arietinum* in the Grand Sable Dunes, Pictured Rocks National Lakeshore, Michigan, USA. Data for *Cypripedium arietinum* was collected from 345, 1-m radius plots in a stratified (by island) random sampling design. The total number of *C. arietinum* in 2009 was 3,596,000 (95% CI= 2,645,000-4,547,000). *C. arietinum* has several close vegetative associates, including *Chimaphila umbellata* and the moss *Pleurozium schreberi*. The population appears to be stable to expanding. Nearly 100 *Calypso bulbosa* were monitored in eight, 4 x 4-m permanent plots from 2008 to 2014. The minimum number of *Calypso bulbosa* in the Grand Sable Dunes was 321 plants in 2008; however, over the 6-year period (2008-2014) that number declined 40.5% with all plots pooled (mean=41.4%, SD=28.4, n=8 plots). The habitat models of *Calypso* show that it is strongly associated with several other orchids, and occurs primarily in moist swales. Climate change may have a tremendous impact on the Grand Sable Dunes and its plant communities. The long-term effects of climate change on these orchids and their associated mycorrhizae are unknown.

Keywords. Grand Sable Dunes, *Calypso bulbosa*, *Cypripedium arietinum*, Pictured Rocks National Lakeshore.

ACKNOWLEDGEMENTS

I thank my advisor, Dr. Alan Rebertus, and committee members Drs. Jill Leonard and Donna Becker for their indispensable guidance. LuAnne Crupi provided enormous help with document formatting. I also thank Mike Rotter, Katie Grezsiak, and Laura Garrison for their assistance in the field and other worthy contributions to this project. The rest know who you are, especially my Dad, and Wilfred.

Deepest gratitude to Dr. John Anderton for his kindness, guidance, and support.

Special thanks to Pictured Rocks National Lakeshore and the National Park Service, who provided funding for this project, generous staff and logistical support, and a phenomenal study site. This project would not have been possible or nearly as enjoyable without Chief of Science, Bruce Leutscher. I would also like to thank Sigma Xi and South Florida Orchid Society for their generous financial assistance and support of student scientific research.

This thesis follows the format prescribed by the *American Midland Naturalist*, to which Chapter One (*Habitat Characteristics and Demography of Calypso bulbosa in the Grand Sable Dunes, Michigan)* of this manuscript has been submitted.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: HABITAT CHARACTERISTICS AND DEMOGRAPHY OF *CALYPSO BULBOSA* IN THE GRAND SABLE DUNES, MICHIGAN

INTRODUCTION

Calypso bulbosa (L.) Oakes has a wide, circumboreal distribution, but is considered rare throughout most of its range. It exhibits a narrow range of habitat requirements and is vulnerable to even slight disruptions in its environment; it is classified as threatened or endangered in several U.S. states and in Sweden and Finland (Currah *et al.*, 1986). Many populations at the southern edge of its range have been extirpated or are declining (Reddoch and Reddoch, 1997; Kartesz, 2013) so its viability in the Great Lakes region is of concern. Orchids are especially vulnerable to climate change due in large part to their small population sizes, narrow habitat requirements, and intricate life histories (Liu *et al.*, 2010).

In eastern North America, *Calypso bulbosa* is mainly found in forested peatlands or in old-growth forest stands dominated by conifers such as eastern white cedar (*Thuja occidentalis*), balsam fir (*Abies balsamea*), or white spruce (*Picea glauca*) (Case, 1987; Higman and Penskar, 1996; Reddoch and Reddoch, 1997). In western North America it typically occurs in mid-montane, conifer-dominated communities (Klinkenburg, 2010). *Calypso bulbosa* occurs in all stages of succession, but is more prevalent in mature or "preclimax" forests (Reeves, 2005).

In Michigan, *C. bulbosa* appears to occur in a wider range of habitats than elsewhere in the Great Lakes region (Schmidt, 2003). Habitats in Michigan include old, undisturbed, heavily wooded *Picea glauca-Abies balsamea-Thuja occidentalis* swamps (Case, 1987), coniferous woods (*Thuja occidentalis-Abies balsamea*, *Picea glauca-Abies balsamea*, *Tsuga canadensis*) (Schmidt, 2003), mixed damp woods of conifers and hardwoods (Voss, 1972), and dry *Thuja* and *Abies* woods over limestone bedrock along Upper Great Lakes shores (Case, 1987). This study describes populations located in isolated jack pine (*Pinus banksiana*) forests in a perched dune system along Lake Superior's south shore.

Key habitat requirements for *Calypso* that are most often cited include undisturbed, mature coniferous forests with cool, moist soils and moderate to heavy shade (Caljouw, 1981; Case, 1987; Higman and Penskar, 1996). Old-growth stands with numerous light gaps and coarse woody debris are also important (Mousley, 1924; Case, 1987; Reddoch and Reddoch, 1997; Morissette *et al*., 2009). Coarse woody debris provides a good substrate for mycorrhizal fungi (Sippola and Renvall, 1999; Desponts *et al.*, 2004), which are essential for seed germination and seedling development of orchids (Currah *et al.*, 1988; Zettler, 1998). Moist, well-rotted logs covered in moss also provide a good substrate for *Calypso* colonization (Case, 1987).

In Michigan, *Calypso* is among the first plants to bloom in spring, typically appearing in mid-May to early June (Case, 1987). Each ramet typically produces a single leaf and a solitary flower (Proctor and Harder, 1995). The flower provides neither nectar nor usable pollen, instead relying on bright color, anther-like hairs, and sweet smell to deceive naive pollinators, mainly *Bombus* and *Psithyrus* bumblebees (Mosquin, 1970; Ackerman, 1981; Boyden, 1982; Proctor and Harder, 1995). In Minnesota, Smith (2012) reports that the flowering period of *Calypso* is synchronized with the emergence of bumblebee queens who forage close to the ground and encounter the flowers head-on. They tend to investigate the showy flowers at first, inadvertently pollinating many of them, but quickly learn to avoid the nectarless flowers. Although selfing is possible (Alexandersson and Ågren, 2000), the mechanical removal of pollinia by a bumblebee is usually required to initiate pollination (Mosquin, 1970). Seed capsules are rarely produced in the Great Lakes region (Mousley, 1924; Case, 1987), but are more common elsewhere throughout its range. Despite the paucity of seed capsule formation, continual reseeding is vital to the continuation of a colony (Case, 1987). Each seed pod may contain 10,000-20,000 seeds (Kershaw *et al*., 1998). After pollination the flower quickly senesces, the single leaf disappears, and the underground corm produces one or two new shoot buds (Higman and Penskar, 1996; Alexandersson and Ågren, 2000). These new shoot buds will become the new corm, while the previous year's corms remain in sequence, attached to the younger corms for 2 to 4 years (Currah *et al*., 1988).

Few studies have focused on demographic aspects of *Calypso*. Case (1987) monitored a population in a cedar swamp in Michigan for twenty years but did not present any quantitative data. He did note that most plants only thrived for two to three seasons followed by a decline that seemed to coincide with an increase in herbaceous groundcover and *Abies* seedlings, or with the deterioration of a “nurse” log or stump. Plants do not persist long due to the transitory nature of their substrate, usually rotting logs, moss, or leaf litter (Case, 1987). Dormancy of 1-2 years has also been reported in *Calypso* (Schmidt, 2003), possibly complicating interpretation of demographic trends, especially for short-term studies (Kéry and Gregg, 2004).

The initial goal of my study was to determine the population status of *Calypso* in the Grand Sable Dunes (GSD). This was partially motivated by heavy visitation to the area by orchid enthusiasts, and a prosecuted case of illegal collection of another orchid species. I set up permanent plots that included about one-third of the total *Calypso* population at GSD, and conducted monitoring over a six-year period that focused on survivorship, flowering, dormancy, and herbivory. The second goal of this project was to describe the abiotic and biotic variables associated with *Calypso* habitat. I used multivariate techniques to define the vascular, non-vascular, and lichen communities that were closely associated with this rare orchid; and to identify any physical variables that were good predictors of *Calypso* habitat. Although many important features of *Calypso* habitat have been identified in the literature, the majority of the information is anecdotal and non-quantitative.

METHODS

STUDY AREA

The Grand Sable Dunes (GSD) are an 890-ha perched dune system sitting atop glacial moraines 30-100 m above Lake Superior in Pictured Rocks National Lakeshore, Upper Peninsula of Michigan (46° 39' N, and 86° 03' W) (Fig. 1.1). Most of the dune soils are classified as Shelldrake-Duneland complex, derived from sandy aeolian deposits that are excessively drained and have low water-holding capacity (USDA, 2003). Climatically, Lake Superior highly moderates coastal temperature extremes and increases winter snowfall. Average temperatures range from 23.8 C in summer to -12.2 C in winter. Average annual precipitation is 81-86 cm, with annual snowfalls as high as 4.6 m on the uplands near Lake Superior (Albert, 1995). Plant communities include open dunes, sand barrens, patches of jack pine forest, and a variety of transitional habitats. The GSD are considered a biodiversity "hot spot" occupied by a suite of unusual, rare, and narrowly-restricted plant species.

FIELD METHODS

In May 2008 I located three *Calypso* populations in two coniferous forest "islands" in the dune complex. A crew of eight persons delimited population boundaries and attempted to locate and map every orchid with a GPS. Next, I established eight, 4-m by 4-m permanent plots, subjectively located in the core areas of the populations. After a baseline condition was established in 2008, plots were re-visited in 2009, 2010, 2012, and 2104 to examine demographic trends. Only six of the eight plots were visited in 2010. Surveys were usually timed to coincide with peak flowering or slightly later. In 2009 and 2012, however, plots were visited twice about a week apart to insure better flowering data. Initially, 110 orchids were monitored, including some just outside the plots, but later this was reduced to 79 orchids found only within the plot boundaries. I also collected data on leaf size (length and width), % of plants flowering, % of plants pollinated, and evidence of leaf and flower herbivory.

To study the characteristics of the physical and biotic environment of *Calypso* habitat, I used a separate sampling scheme. Because of the orchid's rarity and clumped populations, I subjectively placed twenty 5-m radius plots that collectively included most of the plants in the three populations. The *Calypso* plots were then compared to 62 randomly located plots that were more widely distributed on the jack pine islands. None of the random plots contained *Calypso*. My goal was to identify more subtle community and microsite differences that were presumably associated with "prime" *Calypso* habitat.

In each 3-m radius plot, all vascular plant species, lichens, bryophytes, litter, rock, and bare ground were inventoried, and cover was visually estimated using a Braun-Blanquet scale. Woody cover was estimated by height strata: <15 cm, 15-100 cm, 1-5 m, 5-15 m, and >15 m. Coarse woody debris was determined by measuring the length and midpoint diameter of log portions ≥10 cm diameter within the plot, and the volume was then calculated assuming a cylindrical shape. A decay class ranging from 1 (least decayed) to 5 (most decayed) was assigned to each log portion (Maser *et al*., 1979). Sapling-size trees (4-9 cm DBH) were counted and DBH measured within the 3-m radius plots; trees ≥10-cm DBH were sampled within a 5-m radius plot. Finally, all *Calypso* plants were counted in the 5-m plot. I additionally used eight

HOBO® data loggers to monitor temperatures near ground level in the valley bottoms containing *Calypso*; three additional loggers were set up in the surrounding uplands for comparison.

DATA ANALYSIS

Binary logistic regression (IBM SPSS Statistics ver. 21) was used to model the probability of flowering as a function of current and previous year's leaf area. Leaf area was computed as the area of an ellipse from length and width measurements. Discriminant analysis was used to determine which environmental variables best separated *Calypso* from random (non-*Calypso*) plots. This analysis was appropriate because the two groups were known *a priori*, the sampling of the rarer group was non-random, and prior probabilities for each group were unknown (Norusis, 2006). I used nonmetric multidimensional scaling (NMS) to explore compositional patterns on the forested dune islands and identify species most strongly associated with *Calypso* (PCORD ver. 6.0; McCune and Mefford, 2011). Separate ordinations were used for vascular plants and mosses. NMS is an ordination method based on ranked distances and avoids the assumption of linear relationships among variables. I used the program's "auto pilot" setting on "slow and thorough" and Sorensen's distance. Monte Carlo tests, with 250 random permutations of the data set, were used to assess the significance of ordination axes.

RESULTS

POPULATION SIZE, DISTRIBUTION, AND DEMOGRAPHIC TRENDS

Surveys in 2008 revealed 321 orchids in three populations located on two jack pine islands. The "east" population had 51 orchids within 420-m^2; the "west," 245 orchids in 0.34-ha; and the "northwest," 23 orchids concentrated in 15-m^2, plus two isolated plants 60-m away.

Many vegetative plants could have been missed in the extensive “west population,” but the other two were probably complete censuses.

A smaller subset of orchids, 79 in eight permanent plots, were followed from 2008-2014. The average mortality of marked orchids was around 15% per year, except for 2010 when about one-third of the orchids disappeared (Table 1.1). A total of 42 new orchids were recorded between 2009 and 2014. New orchids were recorded every year, but peaked in 2009 and declined steeply through 2014. Because plots were only revisited every other year after 2010, some new plants may have come and gone in between surveys, so the data only reflect minimum recruitment. It was unknown whether the new plants were from seedling or asexual (corm splitting) origin. Roughly 10% of the plants each year were classified as “reactivated,” meaning they were recorded absent during a survey and then reappeared in a later census (Table 1.1). The longest hiatus was three years. Some “reactivated” plants may have been dormant or recovered from herbivory (see Herbivory).

Over the six-year period (2008-2014) the population had a net decline of 40.5% with all orchids pooled (plot mean=41.4%, SD=28.4, n=8 plots). The estimated λ, finite rate of change, was 0.85 (15% annual decline). An extinction time was calculated from the equation $N_t = N_0 \lambda^t$, where N_t is the population size at time t, in years, and N_0 is the current population size. Assuming λ does not change, the population would face extirpation in about 20 years.

Following the fates of marked individual orchids revealed a surprisingly dynamic population. Of the 79 plants that were followed throughout the study, only 39% remained in 2014 (mean 37%, SD=21.9, n=8 plots) (Fig. 1.2). “Reactivated” plants were considered alive in the survivorship curve even during years when they were not recorded. The final year, 2014, does not take into account future reactivation for that year, so the ending survivorship may be slightly higher than 39%. It should be emphasized that the original cohort was not comprised of seedlings, so these data cannot be used to compute an expected lifespan of *Calypso*.

FLOWERING AND POLLINATION

The average flowering rate for the first three years was around 20%, but dropped to 11% in 2012 and 4% in 2014 (Table 1.2). Pollination rates were more variable and may reflect differences in the year-to-year timing of surveys. The 2009 survey was probably most accurate because we were able to visit all plots twice. Flowering was strongly related to current year's leaf area (Table 1.3, Fig. 1.3) In 2009, both current and previous year's leaf area performed equally well in the model, but in 2010 the previous year's leaf area was a better predictor of flowering than the current year's leaf area. The logistic models were much more effective in predicting the non-flowering state compared to flowering (Table 1.3).

HERBIVORY AND OTHER STRESS FACTORS

Herbivory was negligible in 2008 and 2009, increased substantially in 2010 and 2012, and was again negligible in 2014 (Table 1.2). In 2010 I suspect that herbivory was actually >31% because nearly half the plants surveyed could not be located and a few were found nipped off at the base. Clipped off flower heads accounted for nearly half the herbivory; the rest was leaf herbivory. Pooling all years and plants together, the herbivory rate on flowering plants was 32%, but only 7% for vegetative plants (Chi-square with continuity correction=33.14, df=1, P<0.001). Based on odds ratios, flowering plants were 6.3 times more likely to be eaten than vegetative plants.

Besides herbivory, another potential source of plant mortality or reduced vigor was the smothering effect of hardwood leaf litter, especially *Acer* species. At some point in the study 8% (n=79) of the *Calypso* were found trapped beneath compacted hardwood leaf litter. Such plants were often chlorotic and/or malformed. Heavy, late snow packs in 2013 and 2014 may have compounded this problem. The small northwest population was the only site where hardwoods were a significant component of the overstory.

TRANSITION STATES

In 2008-09 flowering and vegetative plants were equally likely to disappear the following year (Table 1.4). Sample sizes were smaller for 2009-2010, but vegetative plants were more likely to disappear. The most surprising result was that plants that flowered were 3.6 times more likely to flower again the subsequent year as opposed to becoming vegetative. New plants were overwhelmingly vegetative. Two of the 11 new plants in 2010 were almost certainly re-activated (i.e. possibly dormant in 2009), and there was at least one instance in 2009 of a second, new orchid coming from the same corm. Several other new plants had very large leaves, suggesting they were coming from well-established corms.

CHARACTERISTICS OF *CALYPSO* HABITAT

Physiographically, all three populations of *Calypso* were located in moist swales or valleys dominated by balsam fir intermixed with 50-70 year-old jack pine, although the latter tended to be more dominant on the surrounding slopes. The “east” population was on the protected, leeward side of an active dune; the other populations were in valleys between long-stabilized, heavily-wooded dunes of 15-20 m relief. Most orchids occurred on slopes between 5-9%, and none occurred on slopes >20%.

On average, *Calypso* microsites were 1.6^{o} C cooler in June than random sites. The most noticeable difference was nighttime low temperatures, when *Calypso* sites averaged 2.3^{o} C cooler. During the hottest part of the day, *Calypso* sites averaged 1.8^{o} C cooler than random sites.

The NMS ordination converged on stable, 2-dimensional solution with final stress of 15.7 after 50 iterations, which is considered a fairly useable representation (McCune and Grace, 2002). Both axes had stress values significantly lower than random (P=0.004 for both). The first axis explained most of the variation (r^2=0.63), and corresponded to a moisture gradient: *Calypso* and other species found in the interdunal valleys were at the mesic end, while jack pine forest and dune transition communities clustered at the xeric end (Fig. 1.4). The second axis was more difficult to interpret (r^2=0.20), but partly segregated hardwood (bottom) from more acidic, coniferous understories (top). The main vascular plant species associated with *Calypso* were *Abies balsamea, Picea glauca, Polygala paucifolia, Plantanthera* spp. (*P. hookeri and P. orbciulata*), *Neottia convallariodes, Viola blanda, Goodyera oblongifolia, Vaccinium membranaceum, V. angustifolium,* and *Cynoglossum boreale* (Fig. 1.4).

The moss and lichen ordination converged on a 2-dimensional solution with final stress of 10.49 after 30 iterations. Both axes had stress values significantly lower than random (both P=0.02). The first axis accounted for the most variation (r^2=0.63) and corresponded with the same moisture gradient found with vascular plants. On the left side of the diagram, *Polytrichum* spp. were indicators of xeric jack pine forests. On the right side, *Ptilium crista-castrensis, Plagiomnium ciliare, Hylocomium splendens, Thuidium recognitum,* and *Peltigera* lichens were associated with *Calypso* in cooler, more shaded habitats. *Pleurozium schreberi* and *Dicranum* spp. were common in jack pine forests but also closely associated with *Calypso* in the interdunal valleys. Average cover of moss and lichens in plots with *Calypso* was 31 ± 25 % (n=20, mean and SD).

For the discriminant analysis comparing characteristics of *Calypso* and non-*Calypso* habitat, the only variable included in the final model was class 4-5 (fairly rotten) coarse woody debris (CWD), which was more than twice as high in *Calypso* plots compared to non-*Calypso* (12.5 m^3/ha vs 5.2 m^3/ha; F=17.699; df=1,80; P<0.001). Average cover of coarse woody debris was 14 ± 9% (n=20). Average *Abies balsamea* cover was considerably higher in *Calypso* plots (46.5 % vs 27.52 % in random) but significance was marginal (F=3.058; df=1, 80; P=0.084). The discriminant function (score = -1.029+0.148*CWD) resulted in significantly different average scores for each group (Wilk's Lambda=0.819, Chi-Square= 15.885, df=1, P<0.001), but the eigenvalue was only 0.221, indicating high within-group variation. The discriminant function correctly classified 77% of the plots lacking *Calypso* and 57% of the plots with *Calypso*; however, the classification rate for the rarer group tends to be overinflated (Norusis, 2006).

DISCUSSION

HABITAT

The habitat for *Calypso* at Grand Sable Dune (GSD) is fairly unique in that it occupies rather isolated, low pockets in jack pine islands that are not far from heavily disturbed, active dunes. Most of the jack pine stands are located on older dunes 2 km from Lake Superior, or in protected valleys between active dunes. Dunes exhibit much varied microtopography, and in the jack pine forests, which represent some of the most stabilized areas of the dunes, this topography becomes a long-term feature—unlike the constantly shifting sands of the open dunes.

Calypso inhabits deep valleys in the GSD, where cold air sinks and moisture collects. These areas also receive finer aeolian sands and silt that form a cap over coarser sands (Walter Loope, USGS, pers.comm.) and provide better moisture retention and nutrients than more wind-exposed areas.

The cooling effect in the valleys was most noticeable in nighttime low temperatures, which were more than 2 C cooler than nearby upper slopes; but daytime temperatures were also cooler, which probably was related to heavier shading. According to Caljouw (1981), *Calypso* is intolerant of soil temperatures >15 C. Based on the ordination, and to a lesser extent the discriminant analysis, balsam fir seedlings and saplings provide an important shading component for *Calypso* habitat. A strong association between fir and *Calypso* was also found by Morissette et al. (2009) on Anticosti Island. Very heavy shading, however, is known to reduce flowering and fruiting success in *Calypso* (Abelli *et al*., 2013). Likewise, Case (1987) observed that *Calypso* declined when fir seedlings became too dense. Treefall gaps may be important in maintaining sufficient light levels for *Calypso* in older stands with fir (Case, 1987; Reddoch and Reddoch, 1997; Morissette *et al*., 2009). The abundance of dead woody material in my *Calypso* plots was indicative of frequent small gaps. More extensive canopy disturbance (logging, blowdowns, and budworm damage) is probably harmful to *Calypso* (Case, 1987; Higman and Penskar, 1996).

The understory vegetation associated with *Calypso* included species that are typical of shaded coniferous forest, but a few species more characteristic of mixed and deciduous forest were also important (*e.g*., *Acer rubrum, Lonicera canadensis*), particularly around the small "northwest" population. Another obvious trend was the number of other orchid species associated with *Calypso* (*e.g*., *Plantanthera* spp., *Neottia convallariodes*, and *Goodyera oblongifolia*), which probably indicates a similar need for cool, moist, shaded conditions by these orchids or their mycorrhizal partners. Mosses are another of the most important components of *Calypso* habitat in the GSD, providing a moist, cool microhabitat that may be equally important for *Calypso* and its associated mycorrhizal fungi. Rasmussen *et al.* (2006) found that successful seed germination and seedling development in the orchid *Dactylorhiza* was very sensitive to temperature, primarily due to the growing requirements of its mycorrhizal fungi. Mosses are known to be important habitat for many fungi, including many mycorrhizal species (Zhang and Guo, 2007). Ironically, on Anticosti Island in Lake Huron, Morissette *et al*. (2009) found that *Calypso* was negatively associated with moss and other vegetative groundcover. Moss may be more important in the sandy soils of GSD, where its protective cover outweighs its competitive effects.

The occurrence of *Polygala paucifolia* near *Calypso* is intriguing, because their flowers are superficially similar and their phenologies overlap. *Calypso* provides no nectar reward for pollinators, but inexperienced pollinators may mistake it for *P. paucifolia*. Such floral mimicry systems have previously been shown in orchids (Gigord *et al.*, 2002), so further study of these species may be worthwhile. However, this has occurred comparatively rarely in the evolution of the Orchidaceae (Dafni, 1984; Johnson, 2000).

DEMOGRAPHY

Overall, *Calypso* at GSD declined over the 6-year period, but this trend was spatially quite variable: two populations, including the largest and most extensive (also the most visited), declined 25-38%, while one small, isolated population seemed to be flourishing. Survivorship of marked orchids was only about 40% for the 6-year span of this study. While it could be argued that some missing orchids were dormant and may reappear later, Shefferson *et al.* (2005) found that dormancy in *Cypripedium calceolus* was mainly the result of stress, and plants that resprouted had much poorer chances of future survival than other stages. The high rate of population turnover in *Calypso* at GSD agrees with Case's (1987) observations. Some of the trends should be interpreted cautiously given the high rates of herbivory in 2010, which may have impacted survival, flowering, and fruit set. Primack and Stacy (1998) recommended four years as a minimum for studying most demographic and reproductive trends in orchids, but given the highly variable rates of herbivory in this study, longer term studies may provide more clues into *Calypso*'s natural history and demographic vulnerabilities.

FLOWERING AND POLLINATION

The percentage of flowering *Calypso* was relatively low from 2008-2014, ranging from 4-20%, similar to the rate reported by Morissette *et al*. (2009). Low rates of flowering may be a consequence of *Calypso* being a rewardless orchid. The orchid family is renowned for its unusually high occurrence of non-rewarding flowers compared to other plant families, with roughly one-third of all orchid species thought to be rewardless (van der Pijl and Dodson, 1966; Gill, 1989). This deceptive pollination strategy often results in high levels of pollen limitation (Walsh *et al*., 2014), with pollination success being positively correlated with the spatial distribution of flowering plants (Internicola *et al.*, 2006; Sun *et al*., 2009). Isolated plants have greater reproductive success than clusters of flowering plants, presumably because pollinators quickly leave flower patches after finding no rewards (Internicola *et al.*, 2006). Pollination of rewardless orchids appears to be carried out primarily by newly emergent, inexperienced insects that sample a small number of nectarless flowers before learning to seek out more profitable food sources (Heinrich, 1975; Nilsson, 1992; Smithson and Gigord, 2003). Orchid pollen is usually not itself a reward because in the Orchidaceae, pollen is clumped into larger pollinia that are frequently affixed to the pollinator (Johnson and Edwards, 2000). The evolution of a rewardless pollination strategy seems paradoxical because many rewardless orchids exhibit extremely low fruit set compared to species that offer nectar or usable pollen (Gill, 1989; Neiland and Wilcock, 1998). Some have hypothesized that deception reduces geitonogamous pollination (pollination of a flower by an agent using pollen from the same plant) by incentivizing pollinators to leave non-rewarding flowers after receiving the pollinia (Smithson, 2002; Jersáková *et al*., 2006; Johnson *et al.*, 2004; Sun *et al*., 2009), referred to as the “outcrossing hypothesis” (Jersáková *et al.*, 2006).

Flowering was strongly related to current year’s leaf area for the three years tested. Previous year’s leaf area was equal or better at predicting flowering, which suggests that corm reserves set the stage for future flowering success. The importance of leaf area and flowering agrees with unpublished data on *Calypso* from the White Mountains of Arizona (Tom Gibson, University of Wisconsin, pers.comm.), and is widely reported for orchids (Gill, 1989; Primack and Stacy, 1998; Shefferson *et al*., 2005; Gregg, 2004; Shefferson, 2006). The evidence is circumstantial, but the very low flowering rate in 2014 followed multiple years of heavy herbivory, during which flowering and leaf size generally declined.

Our study also found that *Calypso* that flowered the previous year were more likely to flower again. Primack and Stacy (1998) found that *Cypripedium acaule* could sustain repeated flowering for about four consecutive years, but the number of years depended mainly on loss of leaf area. The ability to flower consecutively also seems to depend on whether fruit is set or not. Fruit production depletes reserves and leads to diminished leaf area in the following year (Snow and Whigham, 1989; Primack and Stacy, 1998). However, Primack and Stacy (1998) found that flowering alone (without fruit set) incurred no costs to *Cypripedium acaule*. Shefferson *et al*. (2005) found that flowering in *Cypripedium calceolus* was associated with higher survival and lower rates of dormancy in future years. Only one seed pod was observed during my study at GSD, which may explain the high rate of repeat flowering. Case (1987) also noted that capsule production is rare in the Great Lakes region, but "much more common" in mountainous regions of the West.

FUTURE PROSPECTS FOR *CALYPSO*

Given survivorship trends and low fruit set reported for the Great Lakes region, *Calypso* may be vulnerable to local extinction. High rates of herbivory in 2010, and to a lesser degree 2012, are cause for immediate concern. Plants damaged by herbivores frequently do not appear above ground the following year (Ostlie, 1990; Bender, 1989), which has ramifications for population stability. Moreover, Shefferson *et al.* (2005) concluded that such dormancy may result in costs to adult survival because wild plants experiencing dormancy have higher mortality rates. Flower herbivory, which accounted for about half the instances, may have been perpetrated by snowshoe hares (*Lepus americana*) although the only evidence is the presence of hare pellets in some of the plots that were hardest hit. Some leaf herbivory was consistent with damage patterns caused by slugs. Only a single slug species appeared to be active in my study sites in May, which was subsequently identified by DNA analysis as a dusky slug, *Arion fuscus* (Rachel Sines, Northern Michigan University, pers.comm.). *Arion fuscus* is an invasive European species only recently confirmed in the USA from specimens collected in New England (Barr *et al*., 2009). Its activity so early in spring may be important given *Calypso*'s phenology. According to Correll (1950) the whole plant is frequently attacked by rodents, slugs, and fungi, particularly in the eastern United States. Traps were very effective in capturing slugs when a specimen was needed for identification and documentation, and could be considered as a management tool if necessary.

Trampling of orchids also poses a serious threat to *Calypso* at GSD, but supporting data are lacking. The greatest risk by far is the inadvertent damage of dormant or vegetative plants, which superficially resemble *Maianthemum canadense*, and are likely unnoticed while searching for or photographing this sought-after rarity (see Case, 1987 for examples). Even with detailed maps that included orchids outside of the plots, we accidently damaged at least four orchids throughout the study, two of which failed to reappear the following year. *Calypso* is highly susceptible to even slight disturbances in its environment (Currah *et al*., 1988). Trampling and picking are the primary reasons for its rapid decline in some locations (Pojar, 1994). A decline in the frequency of *C. bulbosa* due largely to a growing illegal international trade, caused the International Union for the Conservation of Nature and Natural Resources to list it as a species vulnerable to extinction on a global scale (Currah *et al*., 1988). Transplanting or cultivating wild orchids is rarely successful due to their need for specific soil fungi that are not usually present at transplant sites or in controlled environments (Currah *et al*., 1988). Although *Calypso* is widespread in its distribution, population extermination is conceivable if plants are not considered within a management plan.

Climate change and its effects on *Calypso* and other orchids at GSD is fairly complex because of the interplay of lake temperature, water levels, and dune dynamics. Plant communities within the dune complex are extremely isolated and strongly influenced by Superior's coastal climate. Lake Superior's summer water temperature rose 3.4 C during the past 100 years (mostly in the past 30 years), and is warming at a rate roughly twice that of regional air temperatures (Austin and Colmon, 2008). The lake's temperature is predicted to rise by another 6 C by 2070-2100 (Trumpickas *et al*., 2009), which does not bode well for temperature-sensitive plants that are blocked from northward migration by Superior's south shore. Orchids may be especially sensitive because their flowering time is not influenced by temperature (Willis *et al*., 2008). Flowering times for *Calypso* may become further removed from the influence of snowmelt, and thus occur under drier conditions. In 2012, for example, snowmelt occurred about four weeks earlier than the long-term normal (Blunden and Arndt, 2013), continuing a trend of earlier snow melt that has accelerated over the past 40 years (Brown and Robinson, 2011).

Ironically, potential orchid habitat may be expanding within the GSD because of prolonged low water levels in Lake Superior. In perched dune systems, active dune building is associated with high lake levels that carve into shoreline banks and make sand available for redistribution and dune building. Low lake levels, in contrast, lead to dune quiescence (Loope *et al*., 2004). These lake-level variations drive expansion and contraction of the forested islands across the dunes. Aerial photographs taken over the past 50 years show a fivefold increase in forest cover across the dunes (Loope and McEachern, 1998) and show that afforestation began at least 125 years ago at the landward edge of the dunes. The presence of buried forests and soil types indicate that such changes are not new; however, the current period of lower lake levels is projected to continue due to declining ice cover and increased evaporation (Assel *et al*., 2004). Thus, as jack pine continues to expand lakeward, it may increase potential *Calypso* habitat over the short term.

REFERENCES

ABELLI, T., A. JÄKÄLÄNIEUM, L. WANNAS, P. MUTIKAINEN AND J. TUOMI. 2013. Pollen limitation and fruiting failure related to canopy closure in *Calypso bulbosa* (Orchidaceae), a northern food-deceptive orchid with a single flower. *J Linn. Soc.*, 171:744-750.

ACKERMAN, J. D. 1981. Pollination biology of *Calypso bulbosa* var. *occidentalis* (Orchidaceae): a food-deception system. *Madrona*, 3:101-110.

ALBERT, D. A. 1995. Regional landscape ecosystems of Michigan, Minnesota, and Wisconsin: a working map and classification. U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station, Gen. Tech. Rep. NC-178. 250 p.

ALEXANDERSSON, R. AND J. ÅGREN. 2000. Genetic structure in the nonrewarding, bumblebee-pollinated orchid, *Calypso bulbosa*. *Heredity*, 85:401-409.

ANDERTON, J.B. AND W.L.LOOPE. 1995. Buried soils in a perched dune-field as indicators of late-Holocene lake-level change in the Lake Superior Basin. Quat. Res. 44:190-199.

ASSEL, R. A., F. H. QUINN AND C. E. SELLINGER. 2004. Hydrological factors of the recent record drop in Laurentian Great Lakes water levels. *Bull. Am. Meteorol. Soc.*, 85:1143-1151.

AUSTIN, J. AND S. COLMON. 2008. A century of temperature variability in Lake Superior. *Limnol. Oceanog.,* 53:2724-2730.

BARR, N.B., A. COOK, P. ELDER, J. MOLONGOKI, D. PRASHER, AND D.G. ROBINSON. 2009. Application of a DNA barcode using the 16S rRNA gene to diagnose pest *Arion* species in the USA. Journal of Molluscan Studies 75: 187-191.

BENDER, J. 1989. Progress report on the ram's-head lady's slipper project at The Ridges Sanctuary, Bailey's Harbor, Wisconsin: year three. Unpublished report to the Ridges Sanctuary, Bailey's Harbor, WI.

BLUNDER, J. AND D. S. ARNDT. 2013. State of the climate. *Bull Am. Metero. Soc.*, 94 (special supplement). 240 p.

BOYDEN, T. C. 1982. The pollination biology of *Calypso bulbosa* var. *Americana* (Orchidaceae). Initial deception of bumblebee visitors. *Oecologia,* 55:178-184.

BROWN, R. D. AND D. A. ROBINSON. 2011. Northern Hemisphere spring snow cover variability and change over 1922-2010 including an assessment of uncertainty. *Cryosphere*, 5:219-229.

CALJOUW, C. 1981. Life history and management recommendations for *Calypso bulbosa* in Scraggly Lake Public Lot, T7R8WELS. Report to Bureau of Public Lands, Maine.

CASE, F. W. 1987. Orchids of the Western Great Lakes Region. Cranbrook Institute of Science Bulletin 48. Bloomfield Hills, MI. 251 p.

CORRELL, D.S. 1950. Native orchids of North America. Chronica Botanica. Waltham, MA.

CURRAH, R. S., S. HAMBLETON, AND A. SMRECIU. 1986. Orchids of Alberta: Studies of their microhabitat, mycotrophy, and micropropagation. University of Alberta, Edmonton. 108 p.

CURRAH, R. S., S. HAMBLETON, AND A. SMRECIU. 1988. Mycorrhizae and mycorrhizal fungi of *Calypso bulbosa*. *Am. J. Bot.*, 75:739-752.

DAFNI, A. 1984. Mimicry and deception in pollination. Annual Review of Ecology and Systematics 15: 259–278.

DAVIS, R.W. 1986. The pollination biology of *Cypripedium acaule* (Orchidaceae). Rhodora 88: 445–450.

DESPONTS, M., G. BRUNET, L. BELANGER, AND M. BOUCHARD. 2004. The eastern boreal old-growth balsam fir forest: a distinct ecosystem. *Can. J. Bot.*, 82:830–849.

GILL, D. 1989. Fruiting failure, pollinator inefficiency, and speciation in orchids, p. 458-481 *In*: D. Otte and J.A. Endler (eds.). Speciation and its consequences. Sinauer, Sunderland, MA. 679 p.

GIGORD, L. D. B., M. R. MACNAIR, M. STRITESKY, AND A. SMITHSON. 2002. The potential for floral mimicry in rewardless orchids: an experimental study. *Proc. R. Soc. London Ser. B, 262:1389-1395.*

GREGG, K. B. 2004. Recovery of showy lady's slippers (*Cypripedium reginae* Walter) from moderate and severe herbivory by white-tailed deer (*Odocoileus virginianus* Zimmerman). *Nat. Areas J.*, 24:232-241.

HEINRICH, B. 1975. Bee flowers: a hypothesis on flower variety and blooming times. Evolution 29: 325–334.

HIGMAN, P. J. AND M. R. PENSKAR. 1996. Special plant abstract for *Calypso bulbosa* (*Calypso* orchid). Michigan Natural Features Inventory, Lansing, MI. 3 p.

INTERNICOLA, A. I, N. JUILLET, A. SMITHSON AND L. D. B. GIGORD. 2006. Experimental investigation of the effect of spatial aggregation on reproductive success in a rewardless orchid. *Oecologia*, 150:435-441.

JERSÁKOVÁ, J., S.D. JOHNSON, AND P. KINDLMANN. 2006. Mechanisms and evolution of deceptive pollination in orchids. Biol Rev Camb Philos Soc. 81(2): 219–235.

JOHNSON S.D., AND T.J. EDWARDS. 2000. The structure and function of orchid pollinaria. *Plant Systematics and Evolution* 222: 243-269.

JOHNSON S.D., C.I. PETER, AND J. ÅGREN. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. Ecology 84: 2919–2927.

JOHNSON S.D., C.I. PETER, AND J. ÅGREN. 2004. The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. Proceedings of the Royal Society of London Biological Sciences 271: 803–809.

KARTESZ, J.T., 2013. The Biota of North America Program (BONAP). 2013. *North American Plant Atlas.* (http://bonap.net/napa). Chapel Hill, N.C. [maps generated from Kartesz, J.T. 2013. Floristic Synthesis of North America, Version 1.0. Biota of North America Program (BONAP).

KERSHAW, L., A. MACKINNON, AND J. POJAR. 1998. Plants of the Rocky Mountains. Edmonton, AB: Lone Pine Publishing. 384 p.

KÉRY, M., AND K. B. GREGG. 2004. Demographic analysis of dormancy and survival in the terrestrial orchid *Cypripedium reginae*. *J. Ecol.*, 92:686-695.

KLINKENBERG, B. (ED). 2010. *Calypso bulbosa* L. *In*: E-Flora BC: Electronic Atlas of the Plants of British Columbia. Lab for Advanced Spatial Analysis, Department of Geography, University of British Columbia. Vancouver, BC.

LIU, H., C. FENG, Y. B. LUO, B. S. CHEN, Z. S. WANG AND H.Y. GU. 2010. Potential challenges of climate change to orchid conservation in a wild orchid hotspot in southwestern China. *Bot. Rev*., 76: 174-192.

LOOPE, W. L., T. G. FISCHER, H. M. JOL, R. J. GOBLE, J. B. ANDERTON, AND W. L. BLEWETT. 2004. A Holocene history of dune-mediated landscape change along the southeastern shore of Lake Superior. *Geomorphology*, 61:303-322.

LOOPE, W. L. AND A. K. MCEACHERN. 1998. Habitat change in a perched dune system along Lake Superior: Pitcher's thistle and lake level change, p. 227-229. *In*: M.J. Mac, P. A. Opler, C. E. Puckett Haecker, and P. D. Doran (eds.) Status and trends of the Nation's biological resources, Vol. 1. U.S. Geological Survey, Reston, VA. 436 p.

MASER, C., R.G. ANDERSON, K. CROMACK JR., J.T. WILLIAMS, AND R.E. MARTIN. 1979. Dead and down woody material. In J.W. Thomas (technical editor). Wildlife habitats in managed forests: the Blue Mountains of Oregon and Washington. USDA Forest Service Agricultural Handbook No. 553. pp.78-95.

MCCUNE, B. AND J. B. GRACE. 2002. Analysis of ecological communities. Mjm Software Design. Gleneden Beach, OR, 300 p.

MCCUNE, B. AND M. MEDFORD. 2011. PC-ORD: Multivariate analysis of ecological data, Version 6. Mjm Software, Gleneden Beach, OR, 28 p.

MORISSETTE, È. M., C. LAVOIE, AND J. HUOT. 2009. Fairy slipper (*Calypso bulbosa*) on Anticosti Island: The occurrence of a rare plant in an environment strongly modified by white-tailed deer. *Botany*, 87:1223-1231.

MOSQUIN, T. 1970. The reproductive biology of *Calypso bulbosa* (Orchidaceae). *Can. Field-Nat.,* 84:291-296.

MOUSLEY, H. 1924. *Calypso*. J. N.Y. Bot. Gard. 25 (290):1-30.

NEILAND, M.R.M., AND C.C. WILCOCK. 1998. Fruit set, nectar reward, and rarity in the Orchidaceae. American Journal of Botany 85: 1657–1671.

NILSSON, L.A. 1992. Orchid pollination biology. Trends in Ecology and Evolution 7: 255–259

NORUSIS, M. J. 2006. SPSS 14.0 Guide to data analysis. Prentice-Hall, Upper Saddle River, NJ. 368 p.

OSTLIE, W. 1990. The Nature Conservancy Element stewardship abstracts. Contract to U.S. Forest Service, Eastern Region.

POJAR, J., A. MACKINNON, EDS. 1994. Plants of the Pacific Northwest Coast: Washington, Oregon, British Columbia and Alaska. Lone Pine Publishing. Redmond, WA.

PRIMACK, R. AND E. STACY. 1998. Cost of reproduction in the pink lady's slipper orchid (*Cypripedium acaule*, Orchidaceae): an eleven-year experimental study of three populations. *Am. J. Bot.,* 85:1672–1679.

PROCTOR, H. C. AND L. D. HARDER. 1994. Pollen load, capsule weight, and seed production in three orchid species. Canadian Journal of Botany. 72(2): 249-255.

PROCTOR, H. C. AND L. D. HARDER. 1995. Effect of pollination success on floral longevity in the orchid *Calypso bulbosa* (Orchidaceae). *Am. J. Bot.,* 82:1131–1136.

RASMUSSEN, H., T. F. ANDERSON, AND B. JOHANSEN. 2006. Temperature sensitivity of *in vitro* germination and seedling development of *Dactylorhiza majalis* (Orchidaceae) with and without a mycorrhizal fungus. *Plant Cell Envir.,* 13:171-177.

REDDOCH, J. M. AND A. H. REDDOCH. 1997. The orchids in the Ottawa district: floristics, phytogeography, population studies and historical review. *Can. Field Nat*., 111:1–185.

REEVES, S. L. 2005. *Calypso bulbosa*. *In:* Fire effects information system. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. <http://www.fs.fed.us/database/feis/> (accessed 1 Dec. 2007).

SCHMIDT, L. 2003. Conservation assessment for *Calypso bulbosa,* fairy slipper. Conservation Assessment for the Eastern Region, U.S.D.A. Forest Service.

SHEFFERSON, R. P. 2006. Survival costs of adult dormancy and the confounding influence of size in lady's slipper orchids, genus *Cypripedium*. *Oikos*, 115:253-262.

SHEFFERSON, R. P., T. KULL, AND K. TALI. 2005. Adult whole-plant dormancy induced by stress in long-lived orchids. *Ecology*, 86:3099-3104.

SIPPOLA, A. L., AND P. RENVALL. 1999. Wood-decomposing fungi and seed-tree cutting: a 40-year perspective. *For. Ecol. Manage*., 115:183–201.

SMITH, W.R. 2012. Native Orchids of Minnesota. University Of MN Press, Minneapolis, MN.

SMITHSON, A. 2002. The consequences of rewardlessness in orchids: reward supplementation experiments with *Anacamptis morio* (Orchidaceae). American Journal of Botany 89: 1579–1587.

SMITHSON, A. AND L.D. GIGORD. 2003. The evolution of empty flowers revisited. American Naturalist 161: 537–552.

SNOW, A.A., AND WHIGHAM, D.F. 1989. Costs of flower and fruit production in *Tipularia discolor*. *Ecology*, 70:1286-1293.

SUN, H-Q, J. CHENG, F-M ZHANG, Y-B LUO. 2009. Reproductive success of non-rewarding *Cypripedium japonicum* benefits from low spatial dispersion pattern and asynchronous flowering. Annals of Botany 103:1227 –1237.

TRUMPICKAS, J., B. J. SHUTER, AND C. K. MINNS. 2009. Forecasting impacts of climate change on Great Lakes surface water temperature. *J. Great Lakes Res*., 35: 454-463.

USDA, NRCS. 2003. Soil Survey of Alger County, Interim Report.

VAN DER PIJL, L. AND C.H. DODSON. 1966. Orchid flowers: their pollination and evolution. University Miami Press. Coral Glabes, FL.

VOSS, E.G. 1972. Michigan flora – Part I Gymnosperms and monocots. Cranbrook Institute of Science Bulletin 55, Bloomfield Hills, MI. 488 p.

WALSH, R.P., P.M. ARNOLD, AND H.J. MICHAELS. 2014. Effects of pollination limitation and seed predation on female reproductive success of a deceptive orchid. AoB PLANTS 6: plu031; doi:10.1093/aobpla/plu031

WILLIS, C.G. ET AL. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proc. Natl. Acad. Sci. U.S.A*., 105: 17029+.

ZETTLER, L. W. 1998. Orchid conservation in the 21st century: The value of including mycorrhizal fungi to preserve endangered species. *N. Am. Native Orchid J*., 4:261-269.

ZHANG, Y., AND D. GUO. 2007. Arbuscular mycorrhizal structure and fungi associated with mosses. *Mycorrhiza*, 17:319-324.

Table 1.1. Demographic statistics for *Calypso* based on orchids in permanent plot. Averages (± STD) are based on 8 permanent plots with 2-18 orchids. For plots that were not censused every year, averages were used to compute annual rates. Two plots were not surveyed in 2010, so the 2010-2012 values include two plots where the census period was 2009-2012. Pooled data is for all orchids combined. Reactivated plants were orchids that disappeared from 1-3 years but reappeared during a later census, presumably after being dormant or recovering from herbivory.

	Annual mortality (%)			New plants/year (%)			Reactivated plants/year (%)		
Year	average	pooled	n	average	pooled	n	average	pooled	n
2008-09	11.18 ±10.95	14.47	76	18.82 ±15.90	22.99	87	---	----	---
2009-10	33.72 ±21.22	31.34	67	9.17 ±14.90	18.19	44	9.72 ±13.92	6.82	44
2010-12	15.84 ±11.91	13.63	76	5.80 ±9.68	11.32	46	12.57 ±16.32	13.85	65
2012-14	14.91 ±17.05	16.43	69	0.95 ±2.52	2.71	46	7.14 ±17.50	2.08	48

Table 1.2 Flowering, pollination, and herbivory rates for *Calypso* 2008-2014 in permanent plots, Grand Sable Dunes. Pollination was not assessed in 2012 or 2014.

Year	n	% flowering	% pollinated	% herbivory
2008	110	18	50	3
2009	100	27	26	12
2010	45	22	10	31
2012	79	11	---	23
2014	54	4	---	4

Table 1.3. Logistic regression models and statistics of flowering probability as a function of leaf area. For 2009 and 2010, models for both current and previous year's leaf area are given. Flowering probability= $1/(1+e^{(-z)})$, where "z" is the logit given below. For % correctly classified, "0" is for non-flowering plants, and "1" is for flowering plants. ** = $P<0.001$. No leaf area data was collected after 2010.

Year	Model logit	n	Omnibus Chi-square	Nagelkerke R^2	% Correctly Classified		
					=0	=1	overall
2008	-4.64+0.52*(area08)	103	31.6**	0.45	96.5	35.3	86.4
2009	-3.64+0.49*(area08)	87	27.7**	0.40	90.9	38.1	78.2
2009	-4.49+0.62*(area09)	95	32.8**	0.44	91.8	31.8	77.9
2010	-6.29+0.98*(area09)	31	18.3**	0.65	95.7	75.0	90.3
2010	-3.07+0.40*(area10)	32	8.4**	0.34	91.7	62.5	84.4

Table 1.4. Transition probabilities between life history stages of *Calypso*, 2008-2009 and 2009-2010 in 8 permanent plots in the Grand Sable Dunes, Michigan. The sample included some orchids just outside the plot boundaries that were followed in 2008 and 2009. Two permanent plots were not visited in 2010.

To:	Vegetative		Flowering		Absent		N	
From:	08-09	09-10	08-09	09-10	08-09	09-10	08-09	09-10
Vegetative	0.56	0.44	0.20	0.00	0.24	0.56	89	52
Flowering	0.26	0.07	0.47	0.71	0.26	0.21	19	14

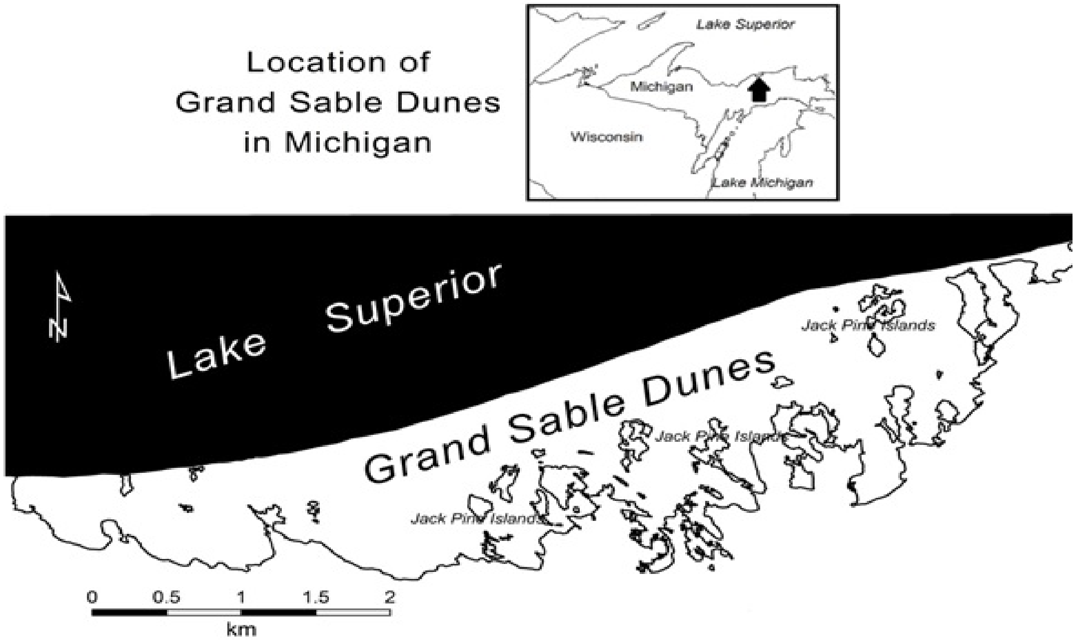

Figure 1.1. Location of the Grand Sable Dunes in the eastern Upper Peninsula, MI.

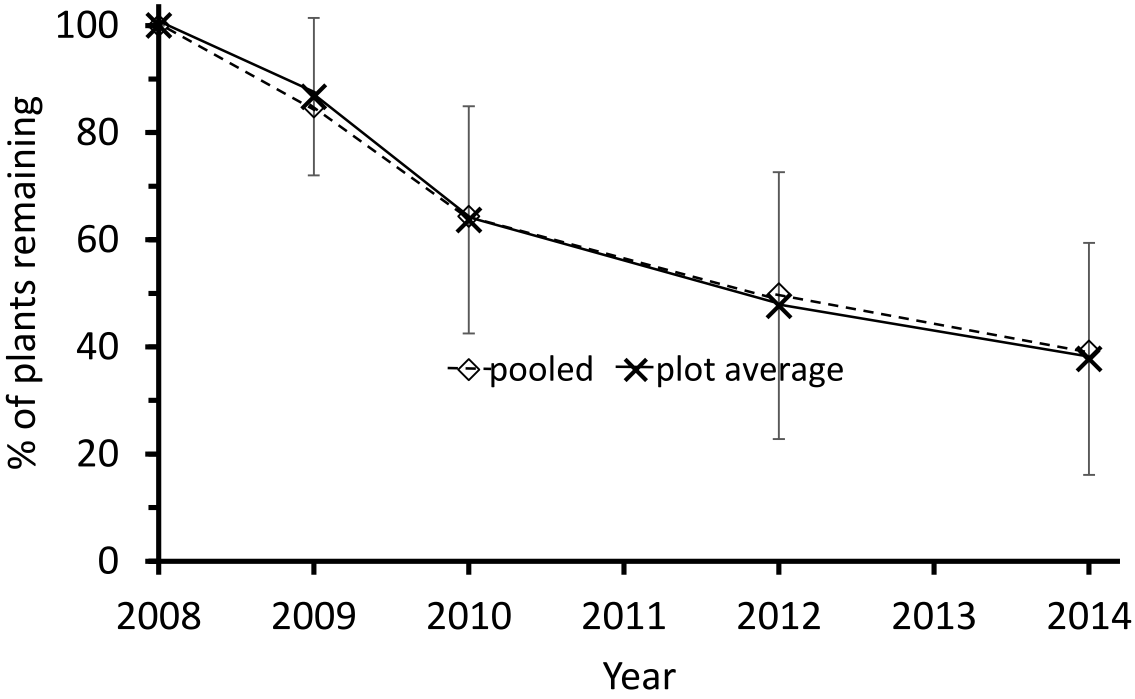

Figure 1.2. Survivorship curves for 79 *Calypso* plants 2008-2014. The solid line is based on plot averages ± SD (n=8 plots). The dotted line is for all 79 orchids pooled together.

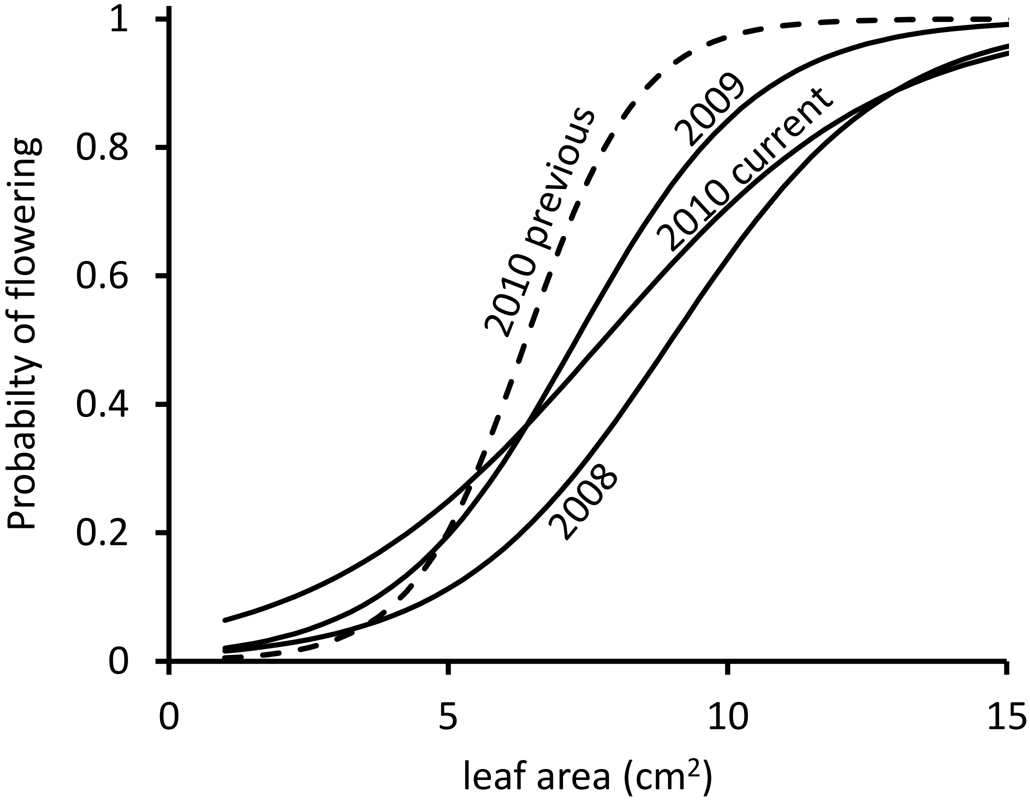

Figure 1.3. Logistic regression models of *Calypso* flowering probabilities as a function of leaf area, 2008-2010, Grand Sable Dunes, Michigan. The best model in 2010 (dashed line) was based on the previous year's leaf area; all other models are based on the current year's leaf area.

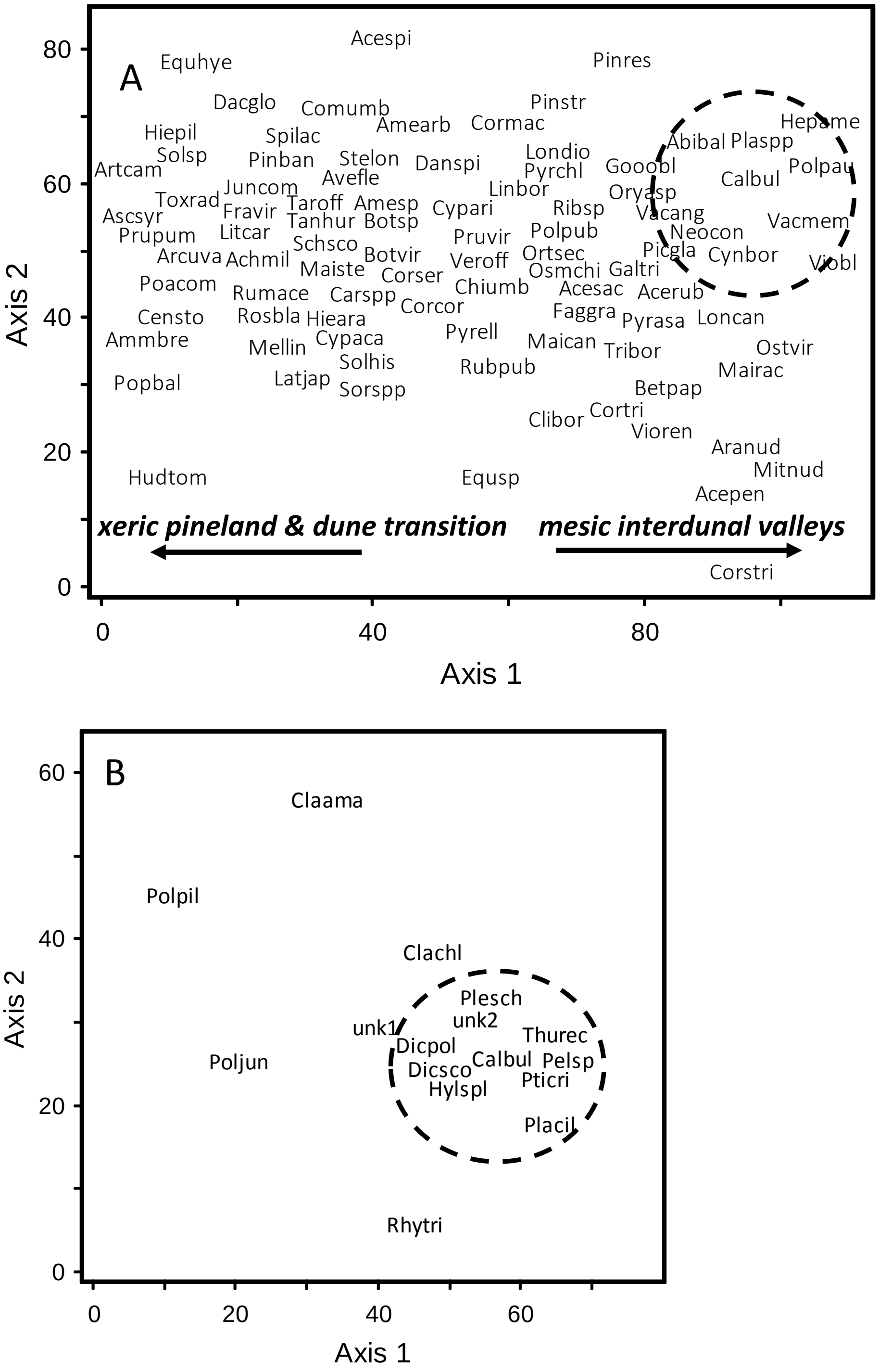

Figure 1.4 NMS ordination diagrams of vascular (A) and moss-lichen (B) communities on forest islands in the Grand Sable Dunes (n=82 and 51, respectively). The dashed circle shows the position of *Calypso* (Calbul) and closely associated species. Species codes are the first three letters of the genus and specific epithet (Appendix A and B below).

APPENDIX A

Vascular plant species codes used in NMS ordination diagrams.

code	**scientific name**	**common name**
ABIBAL	Abies balsamea	BALSAM FIR
ACEPEN	Acer pensylvanicum	STRIPED MAPLE
ACERUB	Acer rubrum	RED MAPLE
ACESAC	Acer saccharum	SUGAR MAPLE
ACESPI	Acer spicatum	MOUNTAIN MAPLE
ACHMIL	Achillea millefolium	YARROW
AMEARB	Amelanchier arborea	JUNEBERRY
AMMBRE	Ammophila breviligulata	MARRAM GRASS
ARANUD	Aralia nudicaulis	WILD SARSAPARILLA
ARCUVA	Arctostaphylos uva-ursi	BEARBERRY
ARTCAM	Artemisia campestris	WORMWOOD
ASCSYR	Asclepias syriaca	COMMON MILKWEED
AVEFLE	Avenella flexuosa	HAIR GRASS
BETPAP	Betula papyrifera	PAPER BIRCH
BOTSP	Botrychium sp.	MOONWORT
BOTVIR	Botrypus virginianus	RATTLESNAKE FERN
CALBUL	Calypso bulbosa	CALYPSO
CARSPP	mostly Carex pensylvanica	SEDGE
CENSTO	Centaurea stoebe	SPOTTED KNAPWEED
CHIUMB	Chimaphila umbellata	PIPSISSEWA
CLIBOR	Clintonia borealis	BLUEBEAD LILY; CORN LILY
COMUMB	Comandra umbellata	BASTARD TOADFLAX
CORCOR	Corylus cornuta	BEAKED HAZELNUT
CORMAC	Coralorrhiza maculata	SPOTTED CORAL-ROOT
CORSER	Cornus sericea	RED OSIER DOGWOOD
CORSTR	Corallorhiza striata	STRIPED CORAL-ROOT
CORTRI	Corallorhiza trifida	EARLY CORAL-ROOT
CYNBOR	Cynoglossum boreale	WILD COMFREY
CYPACA	Cypripedium acaule	PINK LADY'S SLIPPER
CYPARI	Cypripedium arietinum	RAM'S HEAD LADY'S SLIPPER
DACGLO	Dactylis glomerata	ORCHARD GRASS
DANSPI	Danthonia spicata	POVERTY GRASS; OATGRASS
EQUHYE	Equisetum hyemale	SCOURING RUSH
FAGGRA	Fagus grandifolia	AMERICAN BEECH
FRAVIR	Fragaria virginiana	WILD STRAWBERRY
GALTRI	Galium triflorum	FRAGRANT BEDSTRAW
GOOOBL	Goodyera oblongifolia	MENZIES' RATTLESNAKE PLANTAIN
HEPAME	Hepatica americana	ROUND-LOBED HEPATICA
HIEARA	Hieracium aurantiacum	ORANGE HAWKWEED, DEVIL'S-PAINTBRUSH
HIEPIL	Hieracium piloselloides	KING DEVIL, YELLOW HAWKWEED
HUDTOM	Hudsonia tomentosa	BEACH HEATH
JUNCOM	Juniperus communis	COMMON or GROUND JUNIPER

LATJAP	Lathyrus japonicus	BEACH PEA
LINBOR	Linnaea borealis	TWINFLOWER
LITCAR	Lithospermum caroliniense	PLAINS PUCCOON
LONCAN	Lonicera canadensis	CANADIAN FLY HONEYSUCKLE
LONDIO	Lonicera dioica	GLAUCOUS HONEYSUCKLE
MAICAN	Maianthemum canadense	CANADA MAYFLOWER
MAIRAC	Maianthemum racemosum	FALSE SPIKENARD
MAISTE	Maianthemum stellata	STARRY FALSE SOLOMON SEAL
MELLIN	Melampyrum lineare	COW WHEAT
MITNUD	Mitella nuda	BISHOP'S CAP
NEOCON	Neottia convallarioides	BROAD LEAVED TWAYBLADE
ORTSEC	Orthilia secunda	ONE SIDED PYROLA
ORYASP	Oryzopsis asperifolia	ROUGH-LEAVED RICE-GRASS
OSMCHI	Osmorhiza chilensis	SWEET CICELY
OSTVIR	Ostrya virginiana	IRONWOOD; HOP HORNBEAM
PICGLA	Picea glauca	WHITE SPRUCE
PINBAN	Pinus banksiana	JACK PINE
PINRES	Pinus resinosa	RED PINE
PINSTR	Pinus strobus	WHITE PINE
PLASPP	Platanthera spp.	ORCHID
POACOM	Poa compressa	CANADA BLUEGRASS
POLPAU	Polygala paucifolia	GAY WINGS
POLPUB	Polygonatum pubescens	DOWNY SOLOMON SEAL
POPBAL	Populus balsamifera	BALSAM POPLAR
PRUPUM	Prunus pumila	SAND CHERRY
PRUVIR	Prunus virginiana	CHOKE CHERRY
PYRASA	Pyrola asarifolia	PINK PYROLA
PYRCHL	Pyrola chlorantha	SHINLEAF
PYRELL	Pyrola elliptica	LARGE LEAVED SHINLEAF
RIBSP	Ribes sp.	GOOSEBERRY
ROSBLA	Rosa blanda	WILD ROSE
RUBPUB	Rubus pubescens	DWARF RASPBERRY
RUMACE	Rumex acetosella	SHEEP SORREL
SCHSCO	Schizachyrium scoparium	LITTLE BLUESTEM
SOLHIS	Solidago hispida	HAIRY GOLDENROD
SOLSP	Solidago sp.	GOLDENROD
SORSPP	Sorbus sp.	MOUNTAIN ASH
SPILAC	Spiranthes lacera	SLENDER LADIES'-TRESSES
STELON	Stellaria longipes	STARWORT
TANHUR	Tanacetum huronense	LAKE HURON TANSY
TAROFF	Taraxacum officinae	DANDELION
TOXRAD	Toxicodendron radicans	POISON IVY
TRIBOR	Trientalis borealis	STARFLOWER
VACANG	Vaccinium angustifolium	BLUEBERRY
VACMEM	Vaccinium membranaceum	TALL BILBERRY
VEROFF	Veronica officinalis	COMMON SPEEDWELL
VIOBLA	Viola blanda	SWEET WHITE VIOLET
VIOREN	Viola renifolia	KIDNEY-LEAVED VIOLET

APPENDIX B

Moss species codes used in NMS ordination diagrams.

species code	**scientific name**	**common name**
CLAAMA	Cladonia sp.	GOBLET LICHEN
CLACHL	Cladonia chlorophaea	PIXIE GOBLET LICHEN
DICPOL	Dicranum polysetum	WAVY BROOM MOSS
DICSCO	Dicranum scoparium	BROOM MOSS
HYLSPL	Hylocomium splendens	STAIR STEP MOSS
PELSP	Peltigera canina	DOG TOOTH LICHEN
PLACIL	Plagiomnium ciliare	LEAF MOSS/ THREAD MOSS
PLESCH	Pleurozium schreberi	RED-STEMMED MOSS
POLJUN	Polytrichum juniperinum	HAIRY CAP MOSS
POLPIL	Polytrichum piliferum	AWNED HAIRY CAP MOSS
PTICRI	Ptilium crista-castrensis	OSTRICH PLUME MOSS
RHYTRI	Rhytidiadephus triquetris	SHAGGY MOSS
THUREC	Thuidium recognitum	FERN MOSS
UNK1	unknown sp.	UNKNOWN SP.
UNK2	unknown sp.	UNKNOWN SP.

CHAPTER 2: HABITAT CHARACTERISTICS AND DEMOGRAPHY OF *CYPRIPEDIUM ARIETINUM* IN THE GRAND SABLE DUNES, MICHIGAN

INTRODUCTION

Pictured Rocks National Lakeshore (PIRO) boasts a flora comprised of over 700 vascular plant species (Chadde, 1996), including numerous state- and federally-listed species. This high species richness results in part from a diversity of glacial landforms, which give rise to a wide variety of wetland and terrestrial habitats. Among the rare species are (1) endemics to restricted habitats; and (2) arctic, western, and coastal plain disjuncts associated with microclimates along the coast of Lake Superior.

The most significant biodiversity “hot spot” in PIRO is the 890-ha Grand Sable Dunes. This Critical Dune Area (MDNR, 1989) is described as one of the best examples of a perched dune system in the world (Dorr and Eschman, 1972). The dunes are occupied by a suite of unusual, rare, and narrowly-restricted plant species, including one federally-threatened plant species and one endangered, six threatened, and four species of concern listed by the state of Michigan. This study will focus on ram’s head lady’s slipper (*Cypripedium arietinum*), which is extremely rare across most of its range, but thrives in this “atypical” dune habitat.

Cypripedium arietinum W.T. Aiton (Orchidaceae), the ram’s head lady’s slipper, is threatened or endangered in every state in which it is found, including Connecticut, Maine, Massachusetts, Minnesota, New Hampshire, New York, Vermont, and Wisconsin (USDA, 2013). The only exception is Michigan, where it is currently listed as ‘special concern’ (MNFI, 2013). Only about 300 extant occurrences of this unique orchid are known from throughout its entire range, which spans northeastern North America and the Great Lakes region west to Manitoba and Saskatchewan. It is believed to be declining in most parts of its range; however, little is known about this species’ population biology or specific habitat requirements.

Cypripedium arietinum is a spring-flowering perennial that typically emerges in May. It is readily identifiable by its conical, pointed, hairy lip, and fully divided, linear lateral sepals (Cribb, 1997). It is also the smallest native *Cypripedium* in the Great Lakes region (Case, 1987). Clusters of up to twelve arise from a single knotty rhizome (Brzeskiewicz, 2000). The plants develop rapidly after appearing above-ground and can flower within a week (Case, 1987). Plants will remain in bloom for a week if weather is cool and the flower is not pollinated (Case, 1987). If the bloom is pollinated, hormones in the plant cause the upper sepal to drop down within an hour or two, sealing the entry to the lip and excluding additional visitors (Case, 1987). Seed formation then begins and seed capsules become apparent one to two weeks after pollination has occurred (Fleming, 2000). Orchid seeds are almost microscopic and extremely lightweight, containing 96% of their volume as air (Smith, 1993). This allows them to float and enables long-distance transport, though Brower (1977) suggests that *C. arietinum* may not disperse any great distance by wind due to the density of trees in its habitat. Nonetheless, the potential for long-distance seed dispersal in this wind-dispersed family may facilitate geographic isolation among populations (Gill, 1989). Isolated populations such as those found at the Grand Sable Dunes were likely founded by one or a few individuals, further limiting potential genetic variability through founder effects. The geographic range of *C. arietinum* is entirely north of the southernmost limit of the last Wisconsin ice sheet, and neither Case (1994) nor Bornbusch *et al.* (1994) found variation at any allozyme locus surveyed. Case (1994) suggested that the lack of genetic variation in *C. arietinum* was caused by a genetic bottleneck that occurred prior to the species' spread into previously glaciated territory.

Three general habitat preferences have been described (Case, 1987; Brzeskiewicz, 2000; Blaney and Mazerolle, 2007): 1) Cool, dense, white cedar-black spruce-balsam fir (*Thuja* *occidentalis-Picea mariana-Abies balsamea*) forested swamps and bogs, 2) Mixed hardwood/ conifer forests over moist soil of sandy loam or clay, or over limestone exposures, and 3) Nearly pure sand over limestone, mulched with pine or cedar needles; especially under cover of jack pine (*Pinus banksiana*), juniper (*Juniperus communis*, *J. horizontalis*), red pine (*Pinus resinosa*), or cedar (*Thuja occidentalis*). A common feature of most sites appears to be an open, uncrowded understory with little competition from other plants, and partial canopy cover (Alverson and Solheim, 1981; Brzeskiewicz, 2000).

A primary objective of this research was the development of a predictive habitat model to represent the relationship between *C. arietinum* and the biotic and abiotic factors that determine its distribution at the GSD. In addition, estimates of population size, distribution, herbivory, flowering, and pollination were collected to better understand the status and basic biology of *C. arietinum* in the Grand Sable Dunes.

METHODS

STUDY AREA

The 890-ha Grand Sable Dunes (GSD) are expansive, wind-blown sand dunes perched atop glacial moraines. They cover roughly eight kilometers between the Sable River and Au Sable Point in Pictured Rocks National Lakeshore, Alger County, Upper Peninsula of Michigan. Present day soil cover at the GSD is predominantly sand of the Shelldrake-Duneland complex, consisting largely of quartz (87-94%) with lesser amounts of feldspar (10-18%), magnetite (1-3%), and traces of other minerals (Kost *et al.*, 2007). Because the sand contains and overlies calcareous materials, it is neutral to slightly alkaline. This soil complex is derived from sandy aeolian (windblown) deposits that are excessively drained and have low water-holding capacity, with less than one inch of moderately decomposed organic matter at the surface (USDA, 2003).

Occupying the dunes are numerous forested islands, treed in primarily jack pine (*Pinus banksiana*) with occasional pockets of balsam poplar (*Populus balsamifera*) and aspen (*Populus tremuloides* and *P. balsamifera*). The orchids are found primarily in these forested stands.

FIELD METHODS

A series of random points was generated across the Grand Sable Dunes using a random number generator and Arc GIS 9.3 software (ESRI). Through analysis of aerial photos, plots that did not occur within forested areas were eliminated. After an initial field visit plots that did not occur under a jack pine canopy were also eliminated. Sampling was performed using a nested plot design of 1-m, 3-m, and 5-m radius circles. At plot center, light availability, slope, and aspect were measured. Within a 1-m radius, all *C. arietinum* stems were counted, and reproductive status (flowering vs. vegetative, evidence of pollination) was determined. Herbivory was assessed in a subset of 80 plots, where an herbivory index of 0 - 3 was assigned to indicate the damage level over the entire plot: 0 = none, 1 = light, 2 = moderate, and 3 = heavy. Within a 3-m radius circle, soil pH and moisture, and depth of duff and A horizon were measured. Additionally, all plant species, lichens and bryophytes in the 3-m plot were inventoried, and coverage values for species and strata were assigned using a modified Braun-Blanquet scale. Cover of woody understory vegetation was estimated for three height strata: <1 m, 1-5 m, and >5 m. Cover percentages for the following ground variables were also estimated in the 3-m radius plots: bare ground, rock, litter, tree boles, live vegetation, and coarse woody debris. Coarse woody debris volume was computed by measuring the length and midpoint diameter of logs and assuming a cylindrical shape. Only portions of logs $\geq$10 cm diameter located within the plot were considered in the volume computations. Finally, sapling-size trees (4-9 cm dbh) were counted and dbh measured within the 3-m radius plots. In the 5-m plot, trees ≥10-cm dbh were sampled.

DATA ANALYSIS

Population sizes for *C. arietinum* were estimated using stratified random sampling, with each jack pine island considered a stratum. A total of 345, 1-m radius plots were used in the analyses. Final estimates for mean densities, total population size and 95% confidence limits were computed using stratum weights proportional to island size, as described in Krebs (1989, p. 213-216). For the smallest islands (<0.2 ha), and islands where plot samples failed to detect any orchids, I scoured the island and noted whether orchids were present or not. This data was not used in the total population estimate, but it did provide a clearer picture of the distributional limits of the *C. arietinum*.

I used Nonmetric Multidimensional Scaling (NMS) in the program PC-ORD version 6.0 (McCune and Mefford, 2011) to explore relationships in species composition that might be used to predict *C. arietinum* habitat. Vascular species were included in an ordination that included 307 plots. Moss and lichen species were identified in a subset of 157 plots, and these were included in a separate analysis with *C. arietinum* cover. NMS was originally run in the "autopilot" mode under the "slow and thorough method" to determine the best distance measure, appropriate number of axes, and to assess final stress and stability of the solution. Sorensen's and Jaccard's distance measures were used for the vascular plants and moss-lichen datasets, respectively. Significance of axes were tested with Monte Carlo randomization tests based on 250 iterations.

Nonparametric multiplicative regression (NPMR), performed with Hyperniche version 2.2 (McCune and Mefford, 2009), was used to model orchid presence/absence or abundance as a function of environmental variables described in field methods. NPMR was used over traditional regression because it makes no assumptions about the shape of the response curve. Local variation in *C. arietinum* densities may reflect the history of vegetative spread, so binary models of orchid presence/absence were considered more reliable for identifying important environmental predictors. I used a Gaussian weighting function with a local mean estimator in a forward stepwise regression. Best models were selected based on two criteria: (1) diminishing returns in log B plotted against the number of variables, and (2) significance of the final model based on Monte Carlo tests with 100 random permutations of the data.

RESULTS

POPULATION SIZE

Cypripedium arietinum was found on practically every jack pine island in the eastern end of the dunes. The estimated total population size (rounded to 1000s) for the Grand Sable Dunes was 3,596,000 with a 95% confidence interval of 2,645,000 – 4,547,000 orchids. The broad confidence limits reflect the patchiness of orchid distribution, especially within islands.

REPRODUCTIVE SUCCESS

In the summer of 2008, 272 plots were visited to collect demographic data on reproductive status. Each plot was visited once during a one-week span. During that time 79% of *C. arietinum* were vegetative (non-flowering) and 19% were flowering (n=272). Of the plants that were flowering, 30% of them had been pollinated. Due to the short blooming season for *C. arietinum* and number of plants checked, no follow up was conducted to see if flowering plants were eventually pollinated. Plants may have achieved these reproductive benchmarks after my single visit, so the results represent a snapshot in time of the reproductive status of *C. arietinum* during the blooming season.

HERBIVORY

Snowshoe hares and slugs are common in the dunes, and patterns of leaf damage observed throughout the study suggest both are feeding on *C. arietinum*. Of the 80 plots that were assessed for herbivory, 72.5% showed no browse damage, 12.5 % showed light damage, 2.5% showed moderate damage, and 12.5% showed heavy damage.

COMMUNITY AND HABITAT RELATIONSHIPS

NMS converged on a stable 3-dimensional solution with final stress of 15.05 after 30 iterations (Fig. 2.1). The final stress value would be considered "fair" and typical for ecological datasets with large sample sizes (Clarke, 1993). The proportions of variance (r^2) represented by the three axes, in order, were 0.467, 0.231, and 0.163 (cumulative r^2=0.861). All three ordination axes had stress values lower than random (all P=0.02) based on 250 Monte Carlo permutations of the data.

Based on NMS analysis of 307 plots, the major vascular plant species associated with *C. arietinum* were *Chimaphila umbellata, Spiranthes lacera, Goodyera oblongifolia, Pyrola chlorantha, Orthilia secunda, Amelanchier laevis, Deschampsia flexuosa,* and *Pinus banksiana* (Fig. 2.1A). Plants in the lower right of Fig. 2.1A represent open jack pine forests bordering exposed dunes. At the opposite end, dry pine forest quickly transitions to mesic back dunes and sheltered valleys that support mature beech-maple forest. Finally, vegetation near the top-right of the ordination corresponds to young *Populus* spp. stands (especially *P. balsamifera*) that occur nearest the lake. The relative position of *C. arietinum* along the axes indicates its relatively neutral position with respect to environmental gradients: it reaches highest densities where jack pine is dominant in the island interiors, and is less abundant or absent from the transitional communities along dune edges and more mesic, sheltered valleys and back dunes.

A separate ordination analysis was conducted on a subset of 133 plots with moss and lichen data (Fig. 2.1B). Again, a 3-dimensional solution was recommended, and a stable final stress of 14.3 was achieved after 45 iterations. All three axes had stress values that were significantly lower than random (all P=0.004). The proportions of variance (r^2) represented by the three axes, in order, were 0.172, 0.174, and 0.168 (cumulative r^2=0.514). The species most closely associated with *C. arietinum* included *Pleurozium schreberi*, *Dicranum polysetum* and *Peltigera* spp.

NPMR response curves for *C. arietinum* were constructed using two methods, one based on presence/absence of *C. arietinum* and the other based on abundance/density of the orchid. The best explanatory model of *C. arietinum* orchid presence/absence included *Chimaphila umbellata* cover (sensitivity 0.881, Fig. 2.2A) and cover of the woody mid-understory (1-5 m tall) stratum (sensitivity 0.286, Fig. 2.2B). LogB for the 2-variable model was 8.59 and P=0.001 based on 100 random permutations. The probability of *C. arietinum* occurrence increased to nearly 0.9 when *Chimaphila* cover exceeded 6% (Fig. 2.2A), confirming the close association between these species revealed in the ordination. *C. arietinum* was less likely to be found under heavy cover of woody understory species, in this case mainly *Abies balsamea* (Fig. 2.2B). In a dataset that included only mosses and lichens, the best model of orchid occurrence included *Pleurozium schreberi* (sensitivity 1.16, Fig. 2.2C) and *Dicranum polysetum* (sensitivity=0.154). The model was relatively weak (LogB=2.92), but still significant P=0.029 (100 random permutations).

Some variables not chosen by the NPMR selection algorithm were examined non-statistically using histograms to demonstrate differences in orchid density across physical and biotic gradients. *Cypripedium arietinum* reaches its highest densities in areas that are flat or have modest slope (Fig. 2.3A). This may not represent a preference *per se*; rather it probably reflects the modest relief characteristic of most of the jack pine islands in the Grand Sable Dunes, which tend to form in wind/sand sheltered valleys. The slope position histogram (Fig. 2.3B) tells a similar story to the gradient data: orchid density is highest in flat areas and lower slopes, which again reflects the pattern of jack pine invasion into more sheltered valleys in the otherwise very rugged, exposed dune topography.

Cypripedium arietinum reached highest densities under partial shade (20-40% canopy cover) (Fig. 2.3C). The spherical densiometer mostly captured larger openings in the canopy and not the finer penetration of light through the needle canopy. In addition, densiometer calculations do not take into account understory shrubs and small trees. Orchid densities were several times higher in areas lacking woody understory vegetation (Fig. 2.3D). Most of the understory is composed of balsam fir seedlings and saplings that cast heavy shade.

The jack pine islands are composed of predominantly first-generation trees, so coarse woody debris (CWD) is generally minimal. However, orchid density was highest in areas with some CWD and snags (figs. 2.3E and 2.3F) indicating a trend for orchids to be more common in older jack pine forests. The highest CWD, however, was found in the most mature stands, where some canopy turnover to more shade-tolerant spruce and fir has occurred. In these areas *C. arietinum* was less common.

DISCUSSION

POPULATION STATUS

With an estimate of 3.5 million *C. arietinum* for the Grand Sable Dunes, this is the largest population ever reported. The population is also poised to increase in the short term:

investigation of aerial photographs taken over the last fifty years demonstrates a fivefold increase in forest cover (Loope and McEachern, 1998). Dune-building events are tied to geologically high lake levels while periods of dune stabilization and forest expansion are correlated to low lake phases (Anderton and Loope, 1995). The Grand Sable Dunes have experienced relative stability for the past 150 years, allowing jack pine forests to increase and move into areas of the central and eastern dunes. The occurrence of *C. arietinum* on remote islands that are only three or four decades old suggests that long-distance dispersal events at the GSD are fairly common, and that it is only a matter of time before all the islands are occupied. The huge population size on some of the larger islands no doubt improves the odds of long-distance dispersal. Countering this expansion will be the succession of jack pine to fir and spruce, which will make these stands less suitable for *C. arietinum.*

Perhaps the most intriguing biological question concerning *C. arietinum* at the GSD is what supports this mega-population of such an endangered plant? The extremely high density of orchids in the sheltered valleys of the Grand Sable Dunes may be associated with a wind-blown silt cap that settles on the hardwood dominated back dunes and sheltered jack pine valleys on the interior side of main dunes (Walter Loope, USGS, pers. comm.). The silt cap holds moisture and nutrients, while the underlying coarser sands provide favorable drainage. Another large part of this answer may lie in the paleoecology of this orchid and its sister taxon. *Cypripedium arietinum* was once more widely distributed in loess-dominated habitats associated with former glaciations. The strong clonal habits of *C. arietinum* may have evolved for rapid spread in dynamic, wind-disturbed forest stands. The Grand Sable Dunes represent a last vestige of a habitat that was once widespread in the Northern Hemisphere. It is thought that *C. arietinum* derived from an ancestor whose range was continuous across northern North America and eastern Asia. Evidence for this can be found in a closely related species occurring in Asia, *Cypripedium plectrochilon* (Sing-Chi, 1983). While the two species have been found to be distinct, they share so many morphological and ecological characteristics as to have once been considered conspecific (Franchet, 1894; Correll, 1950). The primitive section of *Cypripedium* that includes *C. arietinum* and *C. plectrochilon* likely migrated to Asia from North America via the Beringian link, probably along coastal areas and continental islands (Sing-Chi, 1983). This migration route can be still found in other orchid species (Sing-Chi, 1983). Today, *C. arietinum* continues to persist in small populations within suboptimal habitat where competition is rare.

HABITAT RELATIONSHIPS

It was surprisingly difficult to model the habitat preferences of *C. arietinum*, probably because many of these populations are young and still expanding within islands and colonizing new ones. Habitats may be suitable, but unoccupied. The other issue is that at the scale of my plots (1-m radius), local abundance may be heavily influenced by clonal spread. Thus, the number of orchids in a plot could be more a function of time than environmental conditions. I consider the models based on presence-absence to be more reliable than those based on orchid abundance, although the general trends were usually similar.

Chimaphila umbellata was the most important forecaster of *C. arietinum* presence, but was not an important predictor of abundance. A positive relationship was demonstrated between *C. arietinum* and *C. umbellata*, with increases in *C. umbellata* resulting in increasing probabilities of occurrence for *C. arietinum*. In general, *C. arietinum* shared habitat with a suite of other ericads and orchids, such as *Pyrola chlorantha, Orthilia secunda, Spiranthes lacera, Cypripedium acaule,* and *Goodyera oblongifolia.* All these species are common in acidic, mossy, coniferous forests.

The association with heavy *Pleurozium* moss and some coarse woody debris could favor mycorrhizal association in the otherwise unhospitable dune habitat. Mosses are known to be important habitat for many fungi, including many mycorrhizal species (Zhang and Guo, 2007). Colonization by a compatible fungus is essential for germination and early seedling development in most terrestrial orchids (Smith and Read, 1997). Because their seeds are so small they contain few stored food reserves. Upon infection by mycorrhizal fungus, the seed germinates and develops into a seedling that consumes the fungal sugars (Rasmussen, 1995). Without this infusion of nutrients, the seed cannot survive.

The picture emerging from the forest stand structure data suggests that *C. arietinum* favors somewhat older (40-50 yrs) jack pine with open, mossy understories, and modest quantities of coarse woody debris. *Cypripedium arietinum* showed a negative relationship with shrub and understory tree cover in both models, which agrees with numerous publications that highlight the need for open, uncrowded understory with little competition from other plants (Alverson and Solheim, 1981; Bender, 1989; Ostlie, 1990; Sabourin *et al.*,1999; Brzeskiewicz, 2000; Fleming, 2000; Blaney and Mazerolle, 2007).

The Grand Sable Dunes is an important stronghold for *Cypripedium arietinum*; its population may rival the combined total for all other sites in its range. This provides an opportunity to better understand the demographic forces that lead to population changes, and a benchmark against which to compare the genetic issues associated with smaller, isolated populations that are so pervasive across its range. Smaller populations are too easily affected by stochastic events, such as local disturbances, that can radically affect their status. Effects of climate change, for example, may be easier to assess in a large population where long-term trends can be monitored in a series of permanent plots. Other opportunities exist to study poorly understood aspects of orchid biology, such as mycorrhizal associations, seed dispersal, and vegetative spread. Finally, this population may be suitable as a source of propagules for reintroduction of the orchid in former or potential new habitats.

REFERENCES

ALVERSON, W. S., AND S. SOLHEIM. 1981. Field Survey Report on Endangered, Threatened and Rare Vascular Plants of the Chequamegon National Forest, Wisc. Contr. USFS.

ANDERTON, J.B. AND W.L. LOOPE. 1995. Buried soils in a perched dune-field as indicators of late- Holocene lake-level change in the Lake Superior Basin. Quat. Res. 44:190-199.

BENDER, J. 1989. Progress report on the ram's-head lady's slipper project at The Ridges Sanctuary, Bailey's Harbor, Wisconsin: year three. Unpublished report to the Ridges Sanctuary, Bailey's Harbor, WI.

BLANEY, S., AND D. MAZEROLLE. 2007. Nova Scotia Provincial Status Report on Ram's-head Lady Slipper (*Cypripedium arietinum* R.Br.). Prepared for Nova Scotia Species at Risk Working Group. 31 p.

BORNBUSCH A.H., L.A. SWENDER AND D.L. HOOGERWERT. 1994. Genetic variation in Massachusetts populations of *Cypripedium arietinum* R. Brown in Ait. and *C. acaule* Ait. (Orchidaceae). Rhodora 96: 354–369

BROWER, A.E. 1977. Ram's-head lady's-slipper (*Cypripedium arietinum* R. Br.) in Maine and its relevance to the Critical Areas Program. Planning Report 25. State Planning Office, Augusta, Maine. 14 pp.

BRZESKIEWICZ, M. 2000. Conservation Assessment for Ram's Head Lady Slipper (*Cypripedium arietinum*). USDA Forest Service, Eastern Region, Minneapolis, Minnesota, USA.

CASE, F.W. 1987. Orchids of the Western Great Lakes Region (revised edition). Cranbrook Institute of Science, Bloomfield Hills, MI. 251p.

CASE, M.A. 1994. Extensive variation in the levels of genetic diversity and degree of relatedness among five species of *Cypripedium* (Orchidaceae). Am. J. Bot. 8:175-184.

CHADDE, STEVE W. 1996. Plants of Pictured Rocks National Lakeshore. Pocket Flora Press. 52 pp.

CLARKE, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18:117-143.

CORRELL, D. S. 1950. Native orchids of North America. Chronica Botanica. Waltham, MA.

CRIBB, P. 1997. The genus *Cypripedium*. Timber Press, Inc. Portland, Oregon. 301 p.

DORR, J. A. AND D. F. ESCHMAN. 1972. Geology of Michigan. University of Michigan Press, Ann Arbor, Michigan, USA. 476 pp.

FLEMING, T. 2000. The ram's head lady's slipper, *Cypripedium arietinum*: a study of species biology and conservation management. Undergraduate thesis, Marlboro College, Vermont. 129 pp.

FRANCHET, A. 1894. Les *Cypripedium*, de l'Asie central et de l'Asie orientale. J. Bot. 8:225-233, 249-256.

GILL, D. E. 1989. Fruiting failure, pollinator inefficiency, and speciation in orchids. In D. Otte and J. A. Endler [eds.], Speciation and its consequences, 458-481. Sinauer, Sunderland, MA.

KOST, M.A., D.A. ALBERT, J.G. COHEN, B.S. SLAUGHTER, R.K. SCHILLO, C.R. WEBER, AND K.A. CHAPMAN. 2007. Natural Communities of Michigan: Classification and Description. Michigan Natural Features Inventory, Report No. 2007-21, Lansing, MI.

KREBS, C.J. 1989. Ecological methods. Harper Collins, New York. 654 pp.

LOOPE, W.L. AND A.K. MCEACHERN. 1998. Habitat change in a perched dune system along Lake Superior: Pitcher's thistle and lake level change. Pp. 227-229 in Status and trends of the Nation's biological resources, Vol. 1. United States Department of Interior, U.S. Geological Survey, Biological Resources Division.

MCCUNE, B., AND M. MEDFORD. 2009. Hyperniche, Version 2.2. MJM Software, Gleneden Beach, OR.

MCCUNE, B., AND M. MEDFORD. 2011. PC-ORD: Multivariate Analysis of Ecological Data, Version 6. MJM Software, Gleneden Beach, OR.

[MDNR]. Michigan Department of Natural Resources. 1989. Atlas of critical dune areas in Michigan. MDNR, Lansing, Michigan, USA.

[MNFI]. Michigan Natural Features Inventory. 2013. Rare Species Explorer (Web Application). Available online at http://web4.msue.msu.edu/mnfi/explorer.

OSTLIE, W. 1990. The Nature Conservancy Element stewardship abstracts. Contract to U.S. Forest Service, Eastern Region.

RASMUSSEN, H. N. 1995. Terrestrial orchids: from seed to mycotrophic plant. Cambridge Univ. Press, Cambridge, UK. 238 p.

SABOURIN, A., D. PAQUETTE AND L. COUILLARD. 1999. La situation du cypripède tête-de-bélier (*Cypripedium arietinum*) au Québec. Gouvernement du Québec, Ministère del'Environnement, Direction de la Conservation et du Patrimoine Écologique, Québec, 35pp.

SING-CHI, C. 1983. Two pairs of vicarious species of *Cypripedium* (Orchidaceae) from eastern Asia and eastern North America. Brittonia 35: 159-163.

SMITH, W. R. 1993. Orchids of Minnesota. University of Minnesota Press, Minneapolis, MN. 172 pp.

SMITH, S. E. AND D. J. READ. 1997. Mycorrhizal symbiosis. Academic Press, Cambridge, MA.

USDA, NRCS. 2003. Soil Survey of Alger County, Interim Report.

USDA, NRCS. 2013. The PLANTS Database (http://plants.usda.gov, 16 March 2013). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

ZHANG, Y., AND D. GUO. 2007. Arbuscular mycorrhizal structure and fungi associated with mosses. *Mycorrhiza*, 17:319-324.

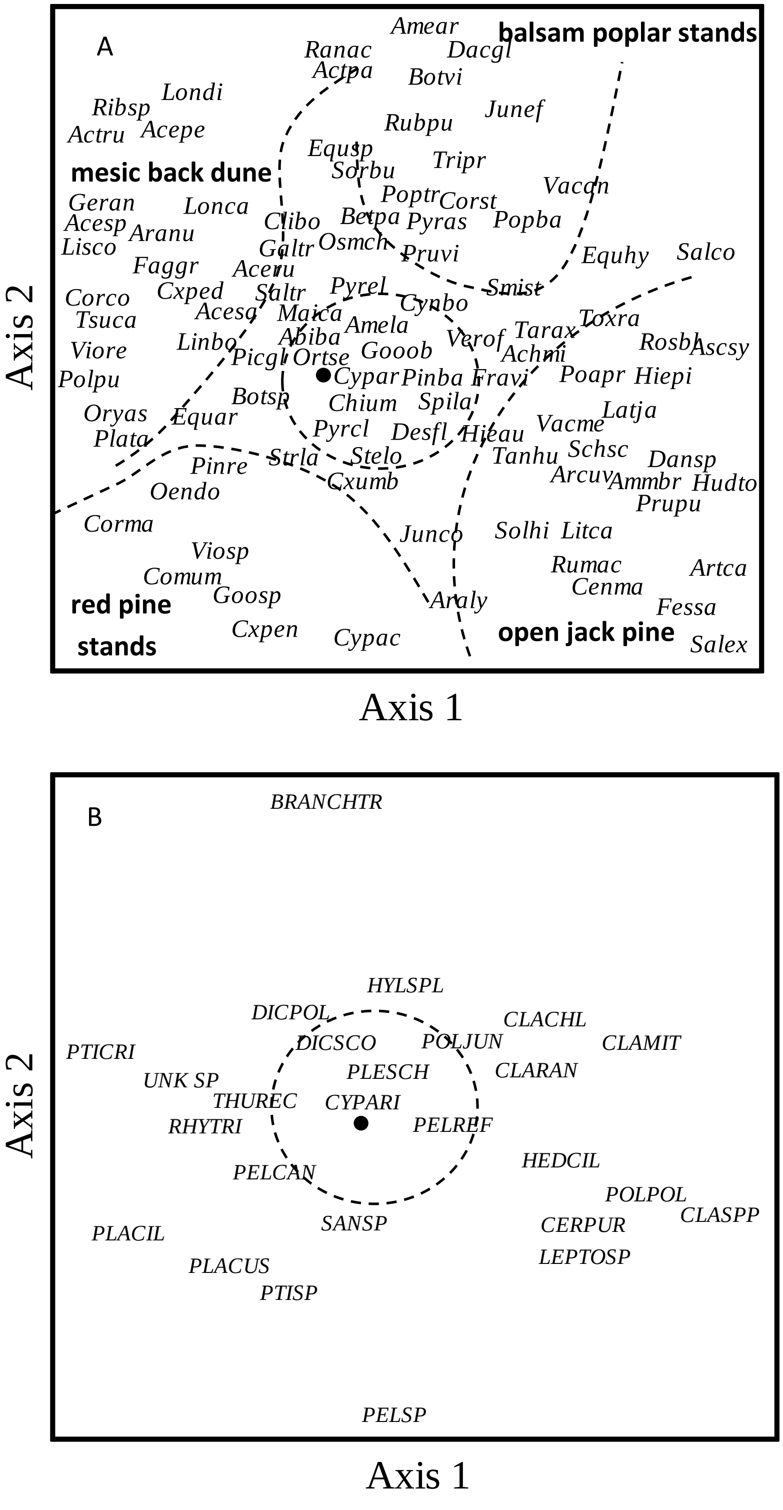

Fig. 2.1. NMS ordination of vascular plant (A) and moss and lichen (B) communities of forested islands in the GSD. The dashed circle (size arbitrary) in the center of each panel shows the position of *C. arietinum* (●*CYPARI*) and closely associated species. Other dashed lines show the approximate boundaries of different communities on the islands. In both ordinations, the third axis (not shown) did not aid in the interpretation. See Appendix for species acronyms.

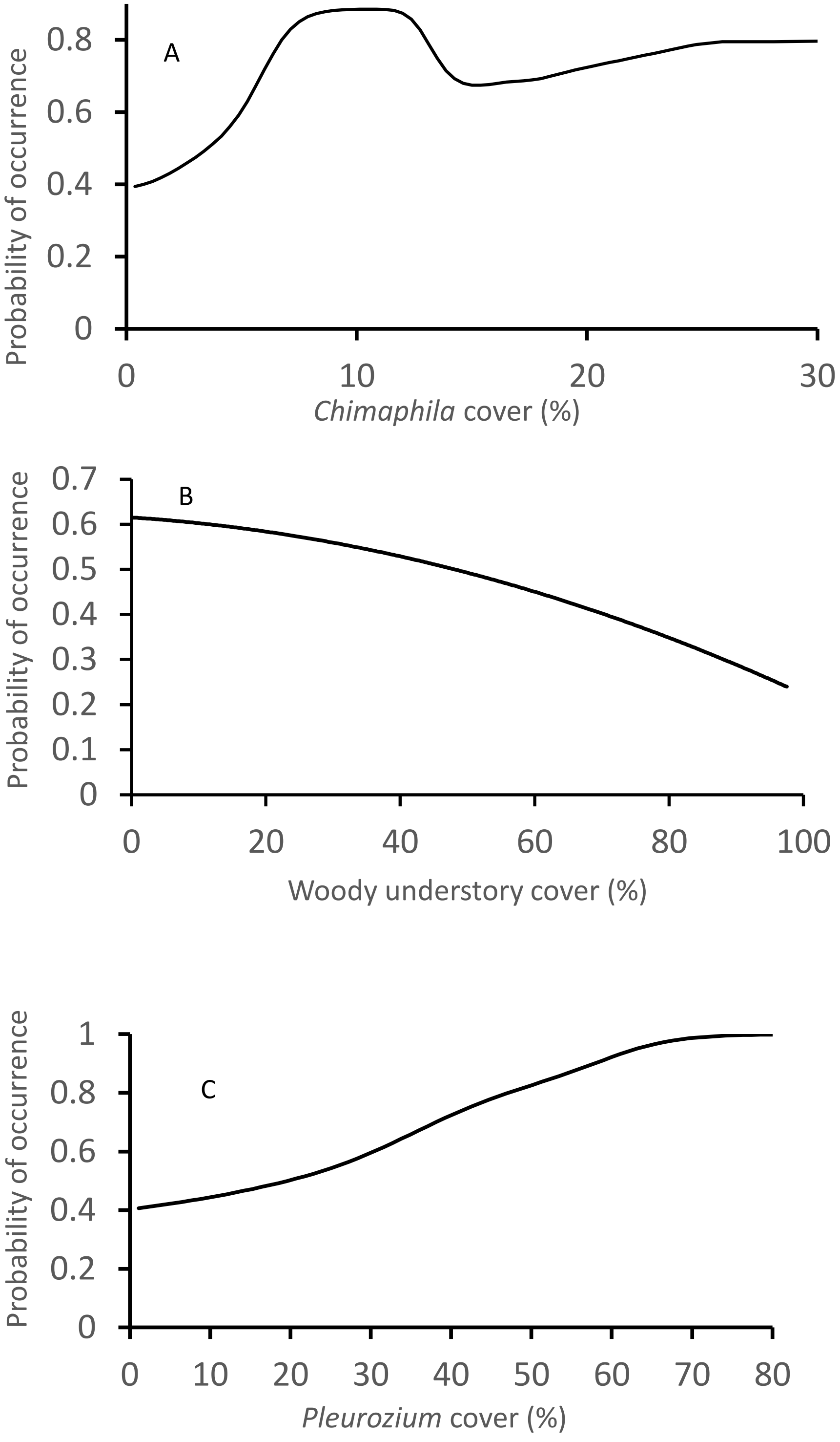

Fig. 2.2 NMPR model of *C. arietinum* abundance as a function of (A) *Chimaphila umbellata* cover, (B) *Pleurozium schreberi* moss cover, and (C) woody understory cover in the 1-5 m height range.

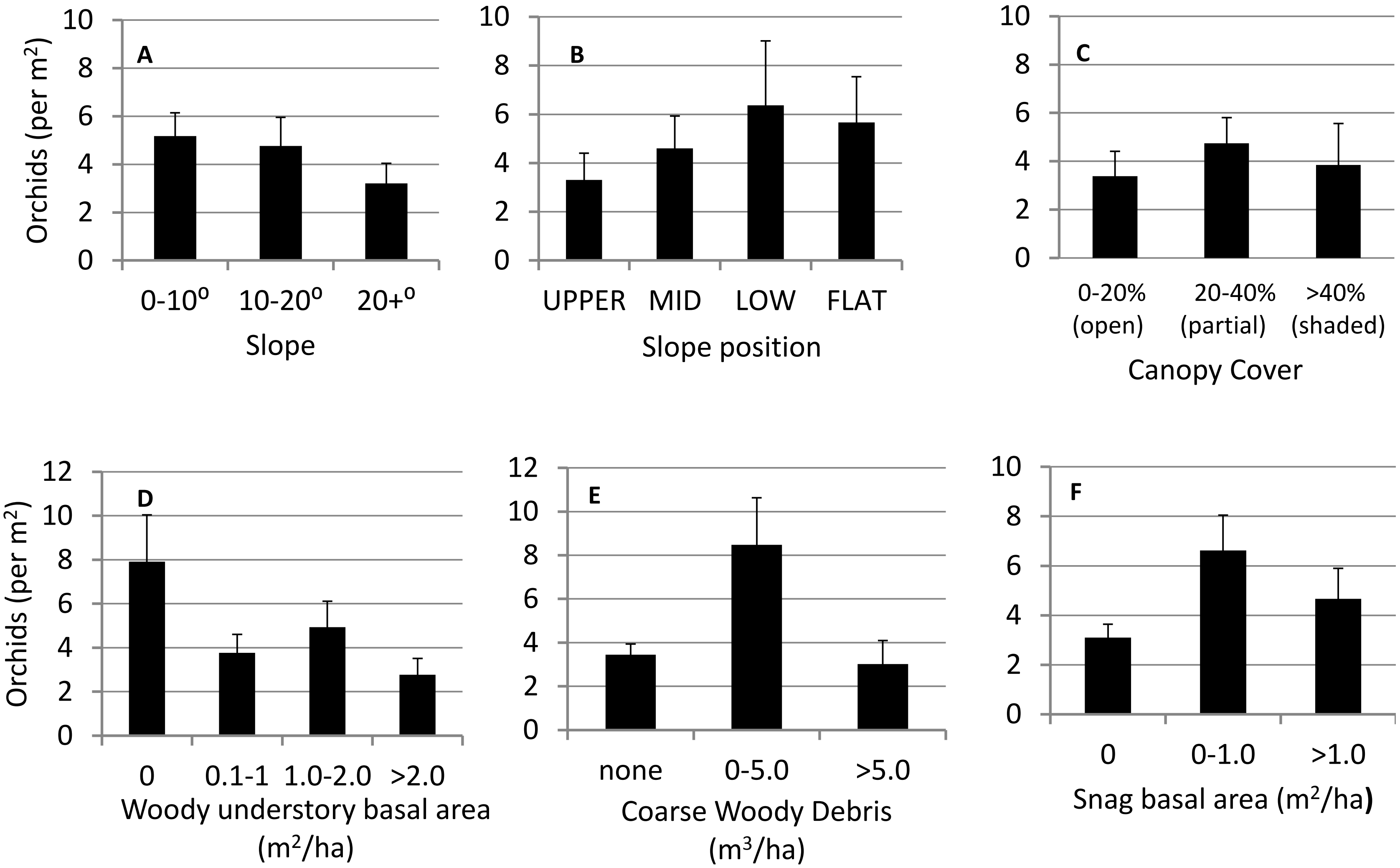

Fig. 2.3 Histograms of *C. arietinum* densities (mean ±SE) by (A) slope, (B) slope position, (C) canopy cover, (D) basal area of woody understory vegetation (5-15 cm dbh), (E) volume of coarse woody debris, and (F) snags (>10 cm dbh) basal area.

APPENDIX A

Vascular plant species codes used in NMS ordination diagrams.

code	**scientific name**	**common name**
ABIBA	Abies balsamea	BALSAM FIR
ACEPE	Acer pensylvanicum	STRIPED MAPLE
ACERU	Acer rubrum	RED MAPLE
ACESA	Acer saccharum	SUGAR MAPLE
ACESP	Acer spicatum	MOUNTAIN MAPLE
ACHMI	Achillea millefolium	YARROW
ACTPA	Actaea pachypoda	WHITE BANEBERRY, DOLL'S-EYES
ACTRU	Actaea rubra	RED BANEBERRY
AMEAR	Amelanchier arborea	JUNEBERRY
AMELA	Amelanchier sp.	JUNEBERRY
AMMBR	Ammophila breviligulata	MARRAM GRASS
ARALY	Arabidopsis lyrata	SAND CRESS
ARANU	Aralia nudicaulis	WILD SARSAPARILLA
ARCUV	Arctostaphylos uva-ursi	BEARBERRY
ARTCA	Artemisia campestris	WORMWOOD
ASCSY	Asclepias syriaca	COMMON MILKWEED
BETPA	Betula papyrifera	PAPER BIRCH
BOTSP	Botrychium sp.	MOONWORT
BOTVI	Botrypus virginianus	RATTLESNAKE FERN
CENMA	Centaurea stoebe	SPOTTED KNAPWEED
CHIUM	Chimaphila umbellata	PIPSISSEWA
CLIBO	Clintonia borealis	BLUEBEAD LILY; CORN LILY
COMUM	Comandra umbellata	BASTARD TOADFLAX
CORCO	Corylus cornuta	BEAKED HAZELNUT
CORMA	Coralorrhiza maculata	SPOTTED CORAL-ROOT
CORST	Cornus sericea	RED OSIER DOGWOOD
CXPED	Carex pedunculata	SEDGE
CXPEN	Carex pensylvanica	SEDGE
CXUMB	Carex umbellata	SEDGE
CYNBO	Cynoglossum boreale	WILD COMFREY
CYPAC	Cypripedium acaule	PINK LADY'S SLIPPER
CYPAR	Cypripedium arietinum	RAM'S HEAD LADY'S SLIPPER
DACGL	Dactylis glomerata	ORCHARD GRASS
DANSPI	Danthonia spicata	POVERTY GRASS; OATGRASS
DESFLE	Avenella flexuosa	HAIR GRASS
EQUAR	Equisetum arvense	SCOURING RUSH
EQUHY	Equisetum hyemale	SCOURING RUSH
EQUSP	Equisetum sp.	SCOURING RUSH
FAGGR	Fagus grandifolia	AMERICAN BEECH
FESSA	Festuca saximontana	FESCUE
FRAVI	Fragaria virginiana	WILD STRAWBERRY
GALTRI	Galium triflorum	FRAGRANT BEDSTRAW

GOOOB	Goodyera oblongifolia	MENZIES' RATTLESNAKE PLANTAIN
GOOSP	Goodyera sp.	RATTLESNAKE PLANTAIN
HIEAR	Hieracium aurantiacum	ORANGE HAWKWEED, DEVIL'S-PAINTBRUSH
HIEPI	Hieracium piloselloides	KING DEVIL, YELLOW HAWKWEED
HUDTO	Hudsonia tomentosa	BEACH HEATH
JUNCO	Juniperus communis	COMMON or GROUND JUNIPER
JUNEF	Juncus effusus	SOFT-STEMMED RUSH
LATJA	Lathyrus japonicus	BEACH PEA
LINBO	Linnaea borealis	TWINFLOWER
LISCON	Listera convallarioides	BROAD LEAVED TWAYBLADE
LITCA	Lithospermum caroliniense	PLAINS PUCCOON
LONCA	Lonicera canadensis	CANADIAN FLY HONEYSUCKLE
LONDI	Lonicera dioica	GLAUCOUS HONEYSUCKLE, RED HONEYSUCKLE
MAICAN	Maianthemum canadense	CANADA MAYFLOWER
OENDO	Oenothera oakesiana	EVENING-PRIMROSE
ORTSE	Orthilia secunda	ONE SIDED PYROLA
ORYAS	Oryzopsis asperifolia	ROUGH-LEAVED RICE-GRASS
OSMCH	Osmorhiza chilensis	SWEET CICELY
PICGL	Picea glauca	WHITE SPRUCE
PINBA	Pinus banksiana	JACK PINE
PINRES	Pinus resinosa	RED PINE
PLATA	Platanthera spp.	ORCHID
POAPR	Poa compressa	CANADA BLUEGRASS
POLPU	Polygonatum pubescens	DOWNY SOLOMON SEAL
POPBA	Populus balsamifera	BALSAM POPLAR
POPTR	Populus tremuloides	TREMBLING ASPEN
PRUPUM	Prunus pumila	SAND CHERRY
PRUVI	Prunus virginiana	CHOKE CHERRY
PYRAS	Pyrola asarifolia	PINK PYROLA
PYRCH	Pyrola chlorantha	SHINLEAF
PYREL	Pyrola elliptica	LARGE LEAVED SHINLEAF
RIBSP	Ribes sp.	GOOSEBERRY
ROSBL	Rosa blanda	WILD ROSE
RUBPU	Rubus pubescens	DWARF RASPBERRY
RUMAC	Rumex acetosella	SHEEP SORREL
SALCO	Salix cordata	SAND-DUNE WILLOW
SALEX	Salix exigua	SANDBAR WILLOW
SCHSCO	Schizachyrium scoparium	LITTLE BLUESTEM
SMISTE	Maianthemum stellata	STARRY FALSE SOLOMON SEAL
SOLHIS	Solidago hispida	HAIRY GOLDENROD
SORBU	Sorbus sp.	MOUNTAIN ASH
SPILA	Spiranthes lacera	SLENDER LADIES'-TRESSES
STELO	Stellaria longipes	STARWORT
TANHU	Tanacetum huronense	LAKE HURON TANSY
TARAX	Taraxacum officinae	DANDELION
TOXRA	Toxicodendron radicans	POISON IVY
TRIPR	Trifolium pratense	RED CLOVER
TSUCA	Tsuga canadensis	HEMLOCK
VACAN	Vaccinium angustifolium	BLUEBERRY
VACME	Vaccinium membranaceum	TALL BILBERRY

VEROF	Veronica officinalis	COMMON SPEEDWELL
VIORE	Viola renifolia	KIDNEY-LEAVED VIOLET
VIOSP	Viola sp.	VIOLET

APPENDIX B

Moss species codes used in NMS ordination diagrams.

species code	scientific name	common name
CERPUR	Ceratodon purpureus	purple horn tooth moss
CLAMIT	Cladina mitis	reindeer lichen
CLARAN	Cladina rangiferina	reindeer lichen
CLACHL	Cladonia chlorophaea	pixie goblet lichen
CLASPP	Cladonia sp.	goblet lichen
DICPOL	Dicranum polysetum	wavy broom moss
DICSCO	Dicranum scoparium	broom moss
HEDCIL	hedwigia ciliata	white-tipped moss
HYLSPL	Hylocomium splendens	stair step moss
PELCAN	Peltigera canina	dog tooth lichen
PELSP	Peltigera sp,	lichen
PELREF	Peltigera rufescens	felt lichen
PLACIL	Plagiomnium ciliare	many-fruited thread moss
PLACUS	Plagiomnium cuspidatum	toothed plagiomnium moss
PLESCH	Pleurozium schreberi	red-stemmed moss
POLJUN	Polytrichum juniperinum	hairy cap moss
POLPOL	Polytrichum piliferum	awned hairy cap moss
PTICRI	Ptilium crista-castrensis	ostrich plume moss
PTISP	Ptilium sp.	plume moss
RHYTRI	Rhytidiadephus triquetris	shaggy moss
SANSP	Sanionia sp.	sickle moss
THUREC	Thuidium recognitum	fern moss
UNKSP	unknown sp.	unknown sp.

GRAND CONCLUSIONS

Faced with an incident of orchid poaching and concerns of other human impacts on the habitat of rare plants in Pictured Rocks National Lakeshore, this study was undertaken to assess the current status, demography, and habitat preferences of *Calypso bulbosa* and *Cypripedium arietinum* at the Grand Sable Dunes.

Based on extensive ground surveys in 2007-2008, the minimum population size of *Calypso bulbosa* in the Grand Sable Dunes (GSD) was 321 plants. These orchids were found in three different populations covering a total area of <0.4 ha in jack pine-spruce-fir islands in the GSD. Over 100 *Calypso* were monitored in eight, 4 x 4-m permanent plots for six years. The population declined 40.5% from 2008-2014, giving an intrinsic rate of change (r) of -0.150. If the decline continues at this rate, the GSD population will be extirpated in roughly 25 years. Several sources of orchid mortality were identified in this study, notably herbivory (mainly slugs and snowshoe hare).

The habitat models of *Calypso* show that it is strongly associated with several other orchids (e.g. *Goodyera oblongifolia, Listera convallariodes, Cypripedium arietinum, and Plantanthera* spp.), *Abies balsamea*, *Viola blanda*, *Vaccinium membranaceum*, *Linnaea borealis*, and *Mitella nuda*. Very strong associations were also noted for several mosses and lichens, such as *Ptilium crista-castrensis, Plagiomnium ciliare,* and *Pleurozium schreberi. Calypso* favored moist swales and valley bottoms of older jack pine (>60 years-old), with a significant component of spruce (*Picea abies*) and fir (*Abies balsamea*). A discriminant analysis found that coarse woody debris (logs >10 cm diameter) was the single best variable separating *Calypso* from non-*Calypso* habitat. The depressions favored by *Calypso* are subject to cold air drainage at night and had nighttime lows 2.3 C cooler (June 2008) than non-*Calypso* sites; heavy shading keeps the daytime highs 1.8 C cooler than non-*Calypso* habitat.

Cypripedium arietinum is extremely rare throughout its range, but my estimates for the GSD population exceed 3.5 million plants, and they appear to be expanding their distribution westward. The habitat models showed the *C. arietinum* was most closely associated with *Pinus banksiana* in the overstory; and many ericaceous shrubs (especially *Chimaphila umbellata*), other orchids (e.g. *Spiranthes lacera*), and mosses (especially *Pleurozium schreberi*) in the understory. *C. arietinum* abundance declined in areas of heavy woody undergrowth of *Abies balsamea.*

The Grand Sable Dunes is an important stronghold for *Cypripedium arietinum*: its population may rival the combined total for all other sites in its range. This provides an opportunity to better understand the demographic forces that lead to population changes, and a benchmark against which to compare the genetic issues associated with smaller, isolated populations that are so pervasive across its range. Smaller populations are too easily affected by stochastic events, such as local disturbances, that can radically affect their status. Effects of climate change, for example, may be easier to assess in a large population where long-term trends can be monitored in a series of permanent plots. Other opportunities exist to study poorly understood aspects of basic biology, such as mycorrhizal associations, seed dispersal, and vegetative spread. Finally, this population may be suitable as a source of propagules for reintroduction of the orchid in former or potential new habitats.

Among the highest management priorities for both orchids should be an increase in monitoring for human disturbance, especially at high risk populations near trails. Education efforts and an enforcement presence should be considered in some areas. Baiting and trapping invasive slugs around *Calypso* populations may be necessary to prevent herbivory and subsequent stress or mortality. Climate change will likely have a tremendous impact on the Grand Sable Dunes and its plant communities, mainly through its effects on Superior's lake level. Current and projected lowering of lake levels favor dune quiescence and forest expansion. In the short-term this may favor both *Calypso bulbosa* and *Cypripedium arietinum* at the expense of many rare open dune species (e.g. *Cirsium pitcheri*). The long-term effects of climate change on these orchids and their associated mycorrhizae are unknown.