Establishing New Patches of Mimulus gemmiparus in Staunton State Park

Assisted establishment as a tool for reducing the risk of extinction

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Abstract: This study evaluates the feasibility of establishing new patches of *Mimulus gemmiparus* as an active approach to reducing the threat of extinction to this rare species. Bulbils were collected from extant populations at Staunton State Park in 2011, and propagated over the winter. About 9,000 propagated bulbils were used to establish 16 new patches of plants in 2012. These were monitored for two years, tracking plant numbers and 14 specific habitat parameters to determine success. Establishment of 6 out of 16 patches was deemed successful based on rapidly increasing plant numbers. An additional four patches were considered marginal with plant numbers maintaining but not rapidly expanding. The species is very amenable to translocation, and establishing new patches of the plant is feasible. We conclude that assisted establishment is a very practical means for reducing the risk of population extirpations and a viable tool for managing the threat of extinction.

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Introduction

The budding monkeyflower, *Mimulus gemmiparus,* is one of the rarest plants in the state of Colorado. There are presently only nine known populations worldwide, all of which are in Central Colorado mountain areas. The spatial extent of these populations is very small. The entire land area known to be occupied by the species is less than 100 m^2. Each population consists of one or more spatially distinct patches of plants which are typically very small in size, rarely larger than one square meter. Demographically, patches of *M. gemmiparus* are the relevant unit for studying population dynamics of this species and for evaluating the persistence of populations (Beardsley and Steingraeber 2013). The patch, rather than a single plant, represents the demographic "individual" and since reproduction is completely vegetative (or nearly so), the patch also represents an appropriate genetic individual as well.

The greatest threats to *M. gemmiparus* populations, and therefore to the species at large, are stochastic and generally unpredictable events that could rapidly wipe out individual patches, including both natural and human-caused disturbance such as fire, flood, drought, trampling, development, *etc.* (Steingraeber and Beardsley 2005). Each patch has a significant probability of being extirpated by one of by one of these disturbance factors each year. The risk of extinction of an entire population is therefore, in the simplest terms, a function of this probability of individual patch extinction divided by the number of individual patches (Beardsley and Steingraeber 2013).

Efforts at protecting the species from extinction may address either the number of patches or threats to those patches. It is easier to manage the number and distribution of patches than it is to manage the threats facing individual patches since these threats are related to stochastic and unpredictable disturbance events. In our 2013 report, we concluded that the threats related to natural and artificial disturbance can be managed to some degree with land protection and good stewardship, but a more effective approach to conservation of the species would be to actively increase the number and distribution of patches, especially in populations that presently contain few patches. The recommended approach, which involves establishing new patches by planting *M. gemmiparus* at likely locations in the wild, can be described as "assisted establishment."

The aim of this study is to assess the feasibility of this approach by attempting to increase the number and distribution of patches within the two populations at Staunton State Park. In 2011, the Colorado Natural Areas Program (CNAP) , within Colorado Parks and Wildlife, partnered with the Colorado State University (CSU) department of biology and EcoMetrics to implement a *M. gemmiparus* assisted establishment project at Staunton State Park. The project was treated as a two-year field experiment, summarized by this report, in which we evaluate the success of establishment efforts as well as defining optimal habitat conditions for establishing the species.

Methods

Patch Establishment Methods

Details of the assisted establishment methods and monitoring protocol are described in a separate report (Beardsley 2012) and summarized here. We collected about 420 bulbils[1] from the native patches of plants in both the Black Mountain Creek (BM) and North Elk Creek (EC) populations in 2011 and propagated these separately in a greenhouse at CSU. Propagation efforts resulted in about 10,000 bulbils, 9,000 of which were used to plant 16 new patches, seven in the BM population and nine in the EC population, on June 27 and 28, 2012. To maintain genetic integrity of the separate populations, new BM patches were planted with bulbils propagated from BM parent material, and EC patches with bulbils from EC parent material. When planting bulbils, we simply scattered them on the ground at the site allowing them to settle naturally onto the substrate. On sites where moss or litter was present, we gently pressed the bulbils into the medium to help insure that they would have soil contact.

Planting efforts resulted in two new potential "subpopulations" in the Black Mountain Creek drainage and two in the North Elk Creek drainage. Subpopulations are groups of patches in close proximity. Each patch has one or more associated sample sites. The 39 sample sites in this study are the specific areas within which bulbils were planted, and each represents a very specific set of habitat conditions. Four of the 39 sample sites are part of native *M. gemmiparus* patches where no planting took place, and in these cases the site is the area containing existing plants. The organization of patches and sample sites is shown in Table 1. Figure 1 shows the location of populations and subpopulations within the Park.

The number and density of planted bulbils varied by sample point as shown in Table 2. Note that all of the planting densities in this study are much less than the density of bulbils typically observed in native populations which tends to be greater than 100 bulbils/dm^2.

[1] A bulbil is the vegetative propagule by which this annual plant reproduces and survives through the non-growing season. Bulbils are produced by adult plants during the growing season, and when the plant dies, the bulbils are dispersed much the same way seeds would be. *M. gemmiparus* survives winter in the bulbil stage which then can germinate in spring to grow into a new adult. Though its anatomical and genetic origin is quite different, a bulbil is the functional equivalent of a seed.

Table 1: Patches are grouped into "subpopulations" within the two populations. Each patch has one or more associated study points. The native patches are shown in purple.

Popl.	Group (sub-popl.)	Patches		Sample sites
BM	Z	*Native*	*Z-0101*	*Z-01*
			Z-0202	*Z-02*
			Z-0303	*Z-03*
	A	New	A-0103	A-01, A-02, A-03
			A-0407	A-04, A-05, A-06, A-07
			A-0809	A-08, A-09
			A-1012	A-10, A-11, A-12
			A-1313	A-13
	B		B-0101	B-01
			B-0203	B-02, B-03
EC	Y	*Native*	*Y-0101*	*Y-01*
		New	Y-0206	Y-02, Y-03, Y-04, Y-05, Y-06
	E		E-0102	E-01, E-02
			E-0307	E-03, E-04, E-05, E-06, E-07
			E-0809	E-08, E-09
			E-1010	E-10
	F		F-0101	F-01
			F-0202	F-02
			F-0303	F-03
			F-0404	F-04

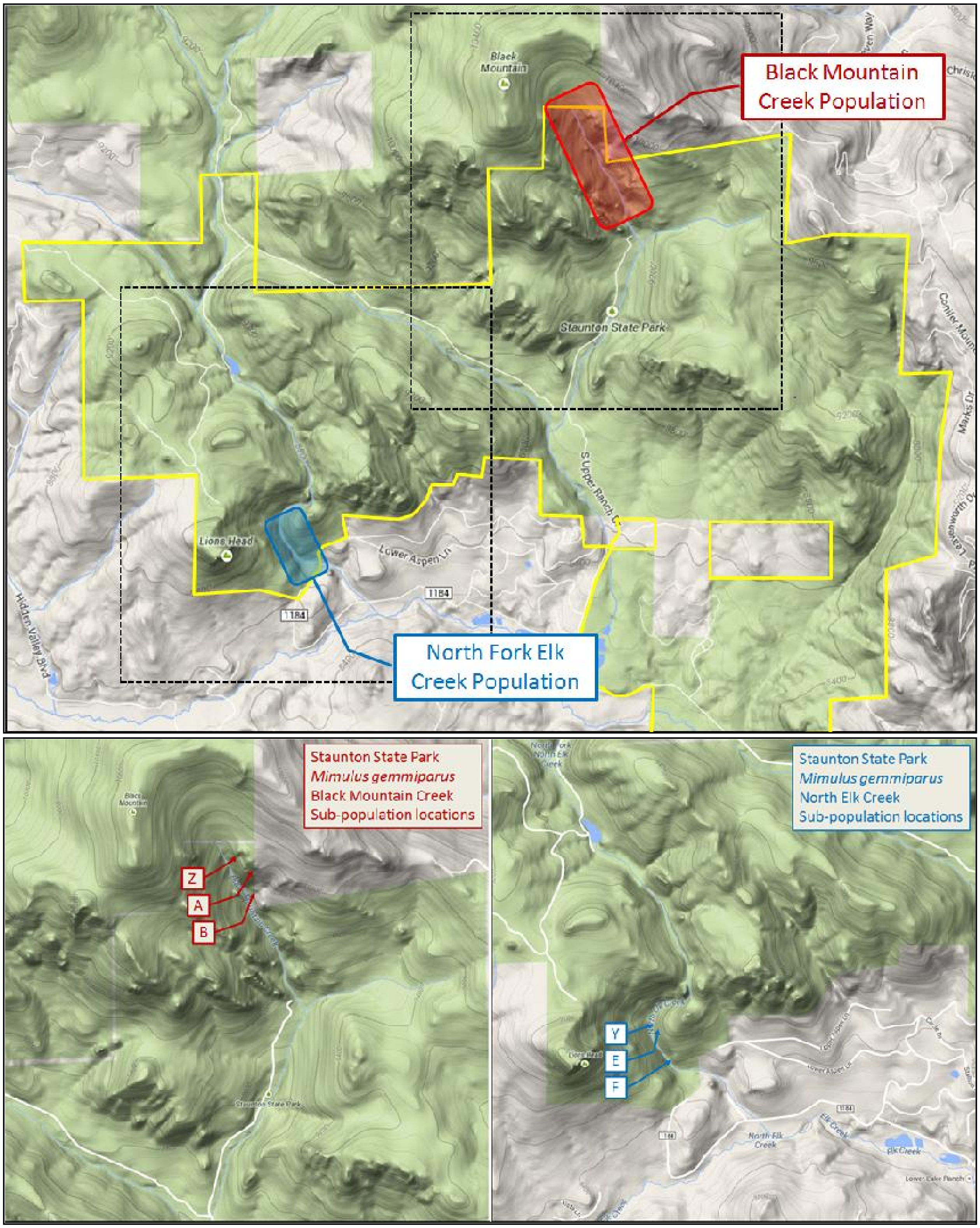

Figure 1: The upper map (Google Maps) shows a rough outline of Staunton State Park (yellow) and the locations of the BM and EC populations. The lower maps highlight areas within the dashed lines to show the rough location of groups of patches (subpopulations).

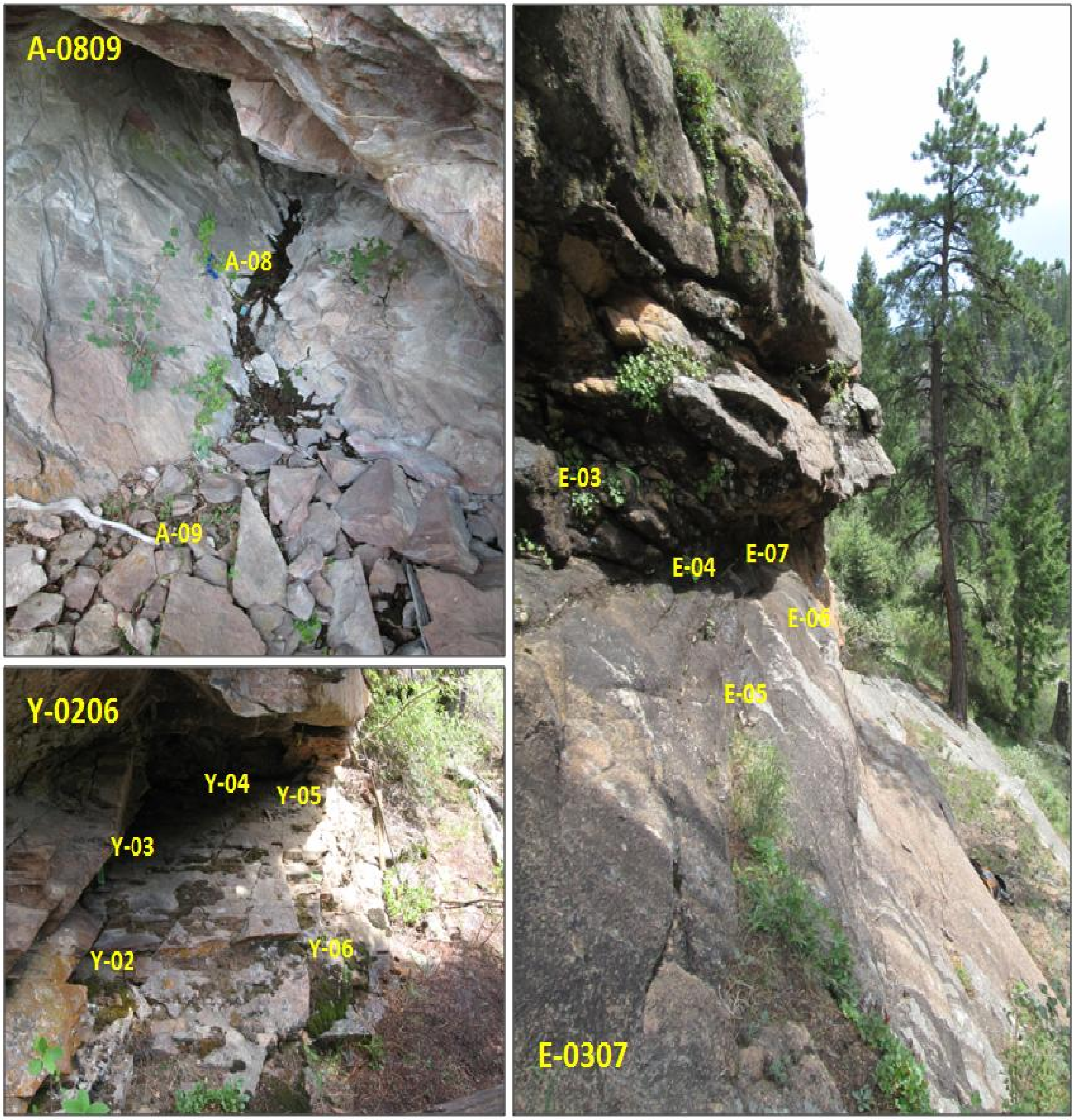

Figure 2: These photos show some of the patches that have multiple sample sites. We established multiple sample sites within a patch when there was a significant habitat variation within it. In these examples, key habitat parameters vary from one site to another within the relatively small patch area.

Table 2: The number and density of planting is shown for each sample point in a new patch.

BM Population			
Site ID	# bulbils planted	area (dm^2)	density ($\#/dm^2$)
A-01	100	3	33
A-02	200	8	25
A-03	200	4	50
A-04	400	20	20
A-05	200	8	25
A-06	200	30	7
A-07	100	30	3
A-08	500	36	14
A-09	1500	168	9
A-10	150	3	50
A-11	250	40	6
A-12	50	1	50
A-13	200	63	3
B-01	300	18	17
B-02	200	9	22
B-03	100	48	2

EC Population			
Site ID	# bulbils planted	area (dm^2)	density ($\#/dm^2$)
Y-02	60	2	30
Y-03	180	8	23
Y-04	60	2	30
Y-05	60	8	8
Y-06	180	32	6
E-01	300	40	8
E-02	60	3	20
E-03	60	2	30
E-04	60	8	8
E-05	60	1	60
E-06	60	3	20
E-07	240	24	10
E-08	120	3	40
E-09	150	4	38
E-10	30	3	10
F-01	300	5	60
F-02	720	100	7
F-03	800	45	18
F-04	800	48	17

Demography Methods

The growth form and reproductive strategy of *M. gemmiparus* make it particularly amenable for monitoring demographics (Figure 2). This is because it is iteroparous, and reproduction is a direct function of growth (Beardsley 1997). These plants grow by producing nodes, and the number of nodes is a direct measure of the demographic stage of an individual plant. More importantly, two bulbils form on each node (one bulbil forms in each of the two opposite leaves) except for the first (Weber 1972), so the potential reproductive output of any individual can be calculated at any time by the formula: $b = 2(n - 1)$, where *b* is the number of bulbils (reproduction) and *n* is the number of nodes. The number of nodes is therefore a direct measure of vegetative reproduction as well as a good representation of demographic stage.

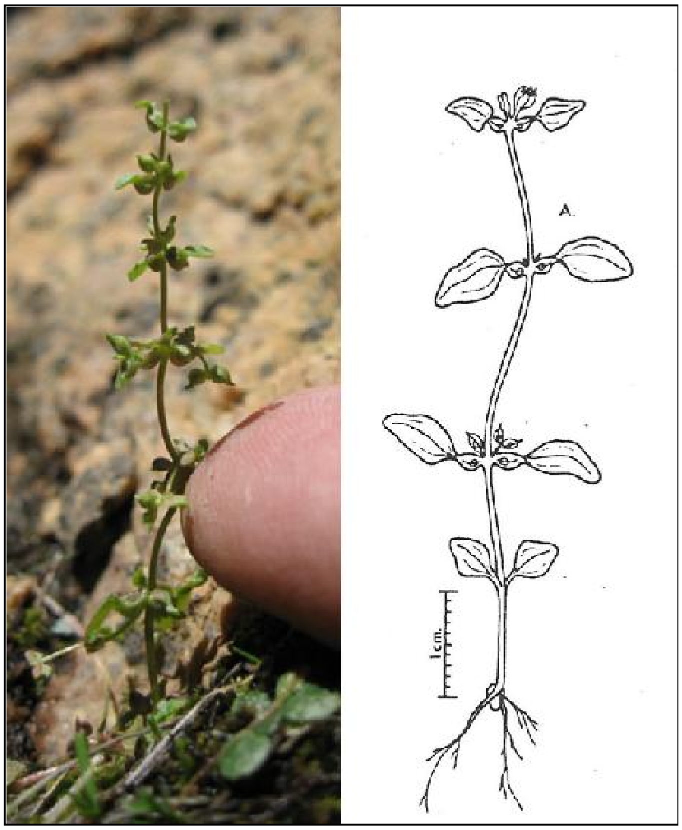

Figure 3: ***M. gemmiparus*** **reproduces by producing two vegetative propagules (bulbils) per node as it grows. Each pair of opposite leaves represents one node, and the leaves of each node except the first contain a bulbil, which are seen as the swollen petioles. Thus, the number of nodes is a measure of both the demographic stage and degree of reproduction. The diagram is from Weber (1972).**

Our strategy for tracking population numbers for this species is to estimate the number of bulbils present through time, and as described above, this can be calculated directly from demographic stage. For this study, we visited all of the sites to complete demographic profiles for each of the 39 sites on the following dates: 6/27/12, 7/14/12, 8/10/12, 9/14/12, 6/21/13, 8/5/13, and 9/8/13. For each site, the profile was made by counting or estimating the number of individuals in various stage classes, defined by the number of nodes present. We also estimated the number of additional bulbils that were produced during the season that had already dehisced from their parent plants based on clues such as the number and density of dried stems and demographic profiles from earlier in the season. The Estimated number of dehisced bulbils was added to the total number of bulbils calculated from the demographic profile to give a total estimate of the number of bulbils produced for the season at each site. The result is a tally of the number of individuals (bulbils) for each site and sample date.

Habitat Methods

On each of the sampling dates, we collected data to characterize 14 different physical habitat parameters at each of the 39 sample sites. Each parameter was scored on a relative scale using a numerical rating system of 1 (low) to 5 (high). A brief description of the 14 parameters follows, and guidelines for assigning relative ratings are provided in Table 3.

1. **Site size** - This is the physical area of the site, approximated by estimating average length and width, subjectively accounting for irregularities in shape.
2. **Overhang protection** - This parameter provides a measure of the degree to which the site is situated under an overhang, or ceiling of rock. It takes into account both the depth of the overhang (position relative to the front edge) and height of the ceiling above ground.
3. **Cliff association** - This parameter is a measure for the degree to which the patch is associated with a rock cliff. It is a categorical descriptor that describes the angle of the associated cliff.
4. **Aspect -** All of the patch sites in this study are on hillsides or cliffs. Aspect describes the direction of exposure of the slope, relative to due north.
5. **Hydrology** - This parameter is a measure of the typical degree of moisture
6. **Microtopography** - Microtopography is a measure of the roughness of the site, at the scale of an average adult plant (~10 cm).
7. **Substrate type** - This parameter is essentially a measure of the texture of the substrate or soil upon which the plants could establish.
8. **Soil depth -** The mean depth of soil at a patch can be directly measured or estimated.
9. **Moss presence** - The percent cover of moss within the perimeter of the site is visually estimated.
10. **Other herb presence** - Proportion of the patch site covered by herbaceous plant species other than *M. gemmiparus*. This may be considered a coarse measure of the amount of direct interspecific competition.
11. **Mean light intensity** - This is a measure of the net amount of light that the site receives during the growing season expressed as mean light intensity in lumens/ft^2. We directly monitored the intensity of light at 13 of the study sites using data logging sensors , and could calculate values for this parameter directly. For sites without sensors, we estimated relative mean light intensity at the site by comparing the aspect and degree of shading to nearby sites with sensors.
12. **Direct sun frequency** - This parameter is a measure of the average amount of time that the site receives direct sunlight during the growing season expressed as hours/day. This value is calculated directly on the 13 sites with sensors and estimated at sites without sensors.
13. **Mean temperature** - This parameter is a measure of mean temperature through the growing season. Direct measurements were made on the 13 sites with sensors, and relative ratings were estimated at sites without sensors.
14. **High temperature frequency** - This parameter rates the amount of time that the site experiences especially high temperature, expressed as h/day when temperature exceeds 90°F. Direct measurements were made on the 13 sites with sensors, and relative ratings were estimated for sites without sensors.

A report sheet was prepared for each sample point to record all demographic and habitat data, including narrative summary descriptions of each site.

Table 3: Guidelines for assigning rating values for the 14 physical habitat parameters.

Rating	1	2	3	4	5
Patch size	< 100 cm^2	100 - 300 cm^2	300 - 1000 cm^2	0.1 - 1.0 m^2	> 1.0 m^2
Overhang protection	none	low (edge, high ceiling)	mod (edge, low ceiling or deep, high ceiling)	high (low ceiling or very deep, high ceiling)	> 30 cm deep, low ceiling
Cliff association	none (open area)	boulder association	Slab (less than vertical cliff)	near-vertical cliff	overhanging large cliff
Aspect	N	NE, NW	E, W	SE, SW	S
Hydrology	consistently dry	intermittently moist	frequently moist	consistently moist	consistently saturated
Microtopography	flat	coarse sand	litter, small gravel	gravel interstices	cobble interstices, crevices
Soil type	none	gravel	sand	sand/fine mix	fine mineral
Soil depth	none	< 1 cm	1-2 cm	2-5 cm	> 5 cm
Moss presence	none	10% of patch	30% of patch	60% of patch	90% of patch
Other herbs	none	10% of patch	30% of patch	60% of patch	90% of patch
Mean light intensity	< 100 lumen/ft^2	100 - 249 lumen/ft2	250 - 449 lumen/ft2	450 - 699 lumen/ft2	> 700 lumen/ft2
Direct sun frequency	avg < 0.1 h per day > 1000 lumen/ft^2	avg 0.1-0.5 h per day > 1000 lumen/ft^2	avg 0.5-1 h per day > 1000 lumen/ft^2	avg 1-2 h per day > 1000 lumen/ft^2	avg > 2 h per day > 1000 lumen/ft^2
Mean temp	50.0 - 51.9 °F	52.0 - 53.9 °F	54.0 - 55.9 °F	56.0 - 57.9 °F	58.0 - 59.9 °F
High temp frequency	no temps > 90 °F	avg < 0.5 h per day temps > 90 °F	avg 0.5-1 h per day temps > 90 °F	avg 1-2 h per day temps > 90 °F	avg > 2 h per day temps > 90 °F

Results

Demography and establishment

The estimated number of *M. gemmiparus* bulbils produced for the season up to each sample date is provided in Tables 5 and 6 for the sites in the Black Mountain Creek and North Elk Creek populations, respectively. The number of bulbils calculated for the 9/8/2013 sampling date was taken as the total number produced in the second season. For each site then, we compared the total number of second-season bulbils to the number that were originally planted to calculate the **percent replacement** as a metric to represent the degree to which population numbers are expanding (or contracting) at each sample site relative to the number planted.

$$Percent\ replacement = \frac{number\ of\ bulbils\ after\ two\ seasons}{number\ originally\ planted}$$

In general, less than 100% replacement indicates a shrinking population and greater than 100% a population that is growing in number, though a direct interpretation is complicated due to natural variability and fluctuation in population numbers. Acknowledging this uncertainty, percent replacement is used as a basic indicator of the potential for successful establishment. Sites that are actively expanding in number are generally more likely to become established compared to those that are not. We used percent replacement to rate the potential for successful establishment according to the guidelines in Table 4. Calculated replacement values and the corresponding success ratings are given in Table 7.

Table 4: Guidelines used for rating the potential for successful establishment based on observed demographic expansion as measured by percent replacement.

Rating potential for successful establishment based on demographic expansion		
% replacement (number of bulbils)	rating	descriptor
No germination	0	none
< 20%	1	very poor
20% - 60%	2	poor
60% - 120%	3	marginal
120% - 200%	4	good
> 200%	5	very good

Table 5: Results of demographic analysis for *M. gemmiparus* sample sites in the Black Mountain Creek population. All data are estimated number of bulbils. "# planted" is the number of bulbils we planted at the site in 2012. "Est. # produced" is the number of bulbils present on living plants at the time of the survey or presumed to have already dehisced from plants that grew that season. Similarly shaded sites are within the same patch. Native patches are shown with purple text.

Popl.	Group (sub-popl.)	Patch		Point	# Planted	Est. # bulbils produced for the season					
					06/28/12	07/14/12	08/10/12	09/14/12	06/21/13	08/05/13	09/08/13
BM	Z	Native	Z-0101	Z-01	0	200	240	1500	0	940	1500
			Z-0202	Z-02	0	0	0	0	0	0	0
			Z-0303	Z-03	0	0	540	2500	5800	3000	5000
	A	New	A-0103	A-01	100	0	20	100	20	60	120
				A-02	200	0	0	12	60	150	450
				A-03	200	0	30	40	0	0	0
			A-0407	A-04	400	0	0	0	0	0	0
				A-05	200	0	0	0	0	0	0
				A-06	200	0	0	0	0	0	0
				A-07	100	0	0	0	0	0	0
			A-0809	A-08	500	0	0	120	0	280	400
				A-09	1500	0	90	200	0	1200	2800
			A-1012	A-10	150	0	80	120	0	0	0
				A-11	250	0	80	200	0	2300	4600
				A-12	50	0	16	30	0	60	120
			A-1313	A-13	200	0	40	50	0	0	0
	B		B-0101	B-01	300	0	230	500	70	840	1500
			B-0203	B-02	200	0	0	50	0	0	0
				B-03	100	0	0	30	0	0	0

Table 6: Results of demographic analysis for *M. gemmiparus* sample sites in the North Elk Creek population. The data and format are similar to Table 5.

Popl.	Group (sub-popl.)	Patch		Point	# Planted	Est. # bulbils produced for the season					
					06/28/12	07/14/12	08/10/12	09/14/12	06/21/13	08/05/13	09/08/13
EC	Y	Native	Y-0101	Y-01	0	400	9000	10000	4200	8000	12000
		New	Y-0206	Y-02	60	0	0	0	0	0	0
				Y-03	180	0	0	0	0	0	0
				Y-04	60	0	0	0	0	0	0
				Y-05	60	0	0	0	0	0	0
				Y-06	180	0	0	0	0	0	0
	E		E-0102	E-01	300	0	0	0	0	0	0
				E-02	60	0	0	0	0	0	0
			E-0307	E-03	60	0	0	0	0	0	0
				E-04	60	0	0	0	0	0	0
				E-05	60	0	0	0	0	0	0
				E-06	60	0	16	60	0	0	0
				E-07	240	0	0	0	0	210	300
			E-0809	E-08	120	0	0	0	0	0	0
				E-09	150	0	0	0	0	0	0
			E-1010	E-10	30	0	66	252	16	30	30
	F		F-01	F-01	300	0	60	380	310	2100	3600
			F-02	F-02	720	0	340	1050	20	620	1200
			F-03	F-03	800	0	0	100	0	160	1200
			F-04	F-04	800	0	0	100	0	400	900

Table 7: Calculated percent replacement values and corresponding establishment success rating scores for sites and patches in both populations.

BM Population					
Results by site			Results by patch		
Site ID	% repl.	success rating	Patch ID	% repl.	success rating
A-01	120%	3	A-0103	114%	3
A-02	225%	5			
A-03	0%	1			
A-04	0%	0	A-0407	0%	0
A-05	0%	0			
A-06	0%	0			
A-07	0%	0			
A-08	80%	3	A-0809	160%	4
A-09	187%	4			
A-10	0%	1	A-1012	1049%	5
A-11	1840%	5			
A-12	240%	5			
A-13	0%	1	A-1313	0%	1
B-01	500%	5	B-0101	500%	5
B-02	0%	1	B-0203	0%	1
B-03	0%	1			

EC Population					
Results by site			Results by patch		
Site ID	% repl.	success rating	Patch ID	% repl.	success rating
Y-02	0%	0	Y-0206	0%	0
Y-03	0%	0			
Y-04	0%	0			
Y-05	0%	0			
Y-06	0%	0			
E-01	0%	0	E-0102	0%	0
E-02	0%	0			
E-03	0%	0	E-0307	63%	3
E-04	0%	0			
E-05	0%	0			
E-06	0%	1			
E-07	125%	4			
E-08	0%	0	E-0809	0%	0
E-09	0%	0			
E-10	100%	3	E-1010	100%	3
F-01	1200%	5	F-0101	1200%	5
F-02	167%	4	F-0202	167%	4
F-03	150%	4	F-0303	150%	4
F-04	113%	3	F-0404	113%	3

The distribution of patches by establishment potential rating is shown in Table 8.

Table 8: Distribution of the 16 planted patches among the five categories of establishment success rating

establishment potential rating		# of patches	% of patches	Cumulative %
5	**Very good**	3	19%	19%
4	**Good**	3	19%	38%
3	**Marginal**	4	25%	63%
2	**Poor**	0	0%	63%
1	**Very poor**	2	13%	75%
0	**None**	4	25%	100%
Total		16	100%	

Six of the 16 planted patches (38%) show good or very good potential for successful establishment based on the percent replacement indicator. Three (19%) are marginal. The potential of successful establishment is rated very poor or worse for the remaining six patches (378%). In two of those patches, a few plants germinated and grew in 2012, but no plants were observed in 2013. In the other four patches, no plants were observed in either 2012 or 2013.

Habitat Parameters

Habitat parameter results are presented as a matrix alongside establishment success data in Table 9. A detailed statistical analysis of habitat data is beyond the scope of this study, but the matrix provides a visual way to detect relevant patterns and correlation between habitat parameters and establishment success across the sites. Apparent patterns of correlation for each habitat parameter are described briefly below.

Patch size

There is no discernible pattern of correlation between site size and establishment success. Patch size is evenly distributed about the successful and unsuccessful sites.

Overhang protection

Overhangs are usually described as a key site characteristic for *M. gemmiparus*, but these results suggest that populations do not necessarily have to be intimately associated with them to be successful. While two of the three native sites do have high ratings (5) for overhang protection, the native EC site is not under a protective overhang and only rated 3. Successful experimental sites have ratings that range from 2 to 5 for this parameter, which means that there is some sort of overhang present at these sites, but the degree of protection may be minimal. Plants may be near the edge of the overhang, or it may be tall and shallow. None of the sites planted in areas with no overhang at all had any bulbils germinate.

Table 9: A matrix for comparing habitat parameter scores and success ratings. The degree of cell shading indicates the magnitude of the parameter value. Sites (rows) are ordered by the degree of establishment success. Arranged this way, parameters that are correlated with establishment success should show a vertical pattern of score distribution.

Site ID	Site size	Overhang	Cliff assoc.	Aspect	Hydrology	Microtopo.	Substrate	Soil depth	Moss	Other herb	Mean light	Direct sun	Mean temp.	High temp	Success	% Repl.
Y-01	4	3	3	2	2	1	2	3	4	1	4	2	5	5	Native	
Z-01	3	5	5	4	3	5	2	3	1	3	2	2	4	2	Native	
Z-03	3	5	4	2	2	1	5	3	4	1	2	2	4	2	Native	
A-11	4	3	5	4	4	5	5	4	3	2	4	5	3	3	5	1840%
F-01	2	3	5	3	3	4	5	3	2	3	4	2	5	2	5	1200%
B-01	4	4	4	3	4	2	5	3	1	2	5	5	4	3	5	500%
A-12	1	5	5	3	4	1	3	3	1	3	3	2	3	2	5	240%
A-02	3	2	4	3	3	5	5	2	4	4	2	1	3	2	5	225%
A-09	5	2	4	4	4	3	4	3	3	2	3	3	3	2	4	187%
F-02	4	3	5	2	4	2	5	3	3	3	3	3	3	2	4	167%
F-03	4	3	5	2	4	3	4	4	4	3	2	2	2	1	4	150%
E-07	4	5	4	3	2	2	2	3	3	1	1	1	5	1	4	125%
A-01	2	2	4	3	3	4	5	4	4	2	2	1	2	2	3	120%
F-04	4	3	5	2	4	3	4	4	4	3	2	2	4	2	3	113%
E-10	2	3	3	3	4	2	3	2	4	3	3	3	5	3	3	100%
A-08	4	5	5	4	4	3	4	2	4	2	1	1	1	1	3	80%
A-10	2	3	5	4	5	1	4	1	4	2	4	5	3	3	1	0%
A-13	4	5	3	2	2	2	2	5	4	1	2	2	3	1	1	0%
B-02	4	3	5	3	5	3	3	4	3	1	1	1	2	1	1	0%
B-03	4	3	5	2	5	1	5	4	5	2	1	1	2	1	1	0%
E-08	2	4	3	3	1	2	2	2	1	2	1	1	5	1	0	0%
E-09	3	3	3	3	2	2	3	2	5	3	3	3	5	3	0	0%
E-01	4	5	3	3	1	1	2	5	1	3	4	2	4	3	0	0%
E-02	2	1	3	3	1	2	2	3	3	1	5	5	5	5	0	0%
E-03	1	1	3	3	2	3	4	3	3	1	5	5	5	5	0	0%
E-04	3	5	3	3	4	1	4	3	3	1	2	1	5	1	0	0%
E-05	1	5	3	3	3	2	3	2	5	1	1	1	5	1	0	0%
E-06	2	4	3	3	3	2	2	2	2	1	3	3	5	3	0	0%
A-03	2	3	4	3	3	3	2	4	2	3	2	1	3	2	0	0%
A-04	4	4	2	5	5	4	2	5	1	2	2	2	2	1	0	0%
A-05	3	4	2	5	5	4	2	5	1	2	2	2	2	1	0	0%
A-06	4	1	1	4	5	4	2	5	1	2	5	5	4	5	0	0%
A-07	4	1	3	4	5	4	2	5	1	2	5	5	4	5	0	0%
Y-02	2	4	3	2	2	1	3	2	4	1	3	1	3	1	0	0%
Y-03	3	5	3	2	3	1	3	2	5	1	2	1	2	1	0	0%
Y-04	2	5	3	2	2	1	3	2	2	1	2	1	2	1	0	0%
Y-05	3	3	3	2	2	1	3	2	4	1	3	1	2	1	0	0%
Y-06	4	2	3	2	1	1	3	2	3	1	4	3	4	5	0	0%
Z-02	5	4	5	4	5	1	2	3	2	3	2	4	4	3	Native?	

Cliff association

Cliff association appears to be an important habitat factor, though the relative steepness of the cliff may not be critical. All of the native and successfully established sites in this study are closely associated with cliff faces. Cliffs associated with successful sites tend to be nearly vertical or overhanging, but two successful sites, including the native EC site, are associated with lower-angle rock faces. Our study included only three sites that had no cliff association, and none of these sites had any plant growth. Most of the unsuccessful sites are associated with lower-angle cliffs.

Aspect

There is no particular pattern relating aspect to establishment success. The successful sites are more or less evenly distributed among the various compass directions, except for due north and south. That is, all of the successful sites have aspects from NE to SE or SX to SW, including due east and west. However, this study does not include any sites with due north exposure and only two sites with south exposure, so these aspects were not effectively tested in the study.

Hydrology

The presence of a constant water supply or seep is considered a primary habitat requirement for *M. gemmiparus*. In this study, we found that consistency of water through the season is not a necessary prerequisite for success, however. The observed pattern suggests that sites with mid-range moisture values are most successful. While the most successful experimental patches did tend to be those with either frequent or consistent moisture, two of the three native patches were only intermittently moist. None of the extremely dry or wet sites had successful establishment. While having some periods when the soil is moist must be important for establishment, too much water appears to be detrimental. Consistently saturated sites often had standing water for much of the growing season, and none of these had any germination or plant growth.

Microtopography

There appears to be some pattern relating success to microtopography, but relationship is complicated. The successful sites represent both extremes (flat and smooth to rough cobble interstices and crevices). Because all three of the roughest sites were successful, it would seem that cobbles and crevices are beneficial, but at the same time two of the three native sites have the smoothest microtopography. The relationship is unclear.

Substrate

There is no discernible pattern of correlation between substrate and success. Plants successfully established in sites with fine mineral soil, sand, and gravel substrate.

Soil depth
All of the successful sites have thin soil. 11 of the 12 sites with "good" success ratings (≥ 4) have soil shallower than 2 cm. The odd site in that group has slightly deeper soil that is still less than 5 cm. None of the sites with soil deeper than 5 cm were successful.

Moss presence
There is no discernible pattern of correlation between moss presence and establishment success.

Other herb presence
There is no discernible pattern of correlation between the presence of other herbaceous plants and establishment success. This pattern was not tested well in this study because only one site had more than 30% herbaceous cover. It so happens that this site, which has about 50% cover, is among the top eight sites in terms of establishment success.

Mean light intensity
Mean light intensity does not appear to be highly correlated with establishment success, but the results do suggest a possible minimum threshold. None of the sites with very low light (mean < 100 lumen/ft^2) were successful. But above that level, a wide range of mean light intensity values is represented in the successful sites, from about 200 to more than 1000 lumen/ft^2. The full range is also expressed among the unsuccessful sites.

Direct sun frequency
The pattern for amount of direct sunlight is especially unclear. All of the native sites and most of the successful establishment sites see very little direct sun (about 0.3 hours per day on average), suggesting a possible threshold above which establishment is hindered. In spite of this, two of the most successful establishment sites have a high frequency of direct sun exceeding 2 hours per day on average. There is really no discernible pattern of correlation between direct sun frequency and establishment success.

Mean temperature
In general, the more successful experimental sites tended to be those with relatively warmer temperature regimes (mean temperature 54°F - 60°F). The correlation is weak, though, as two of the sites with moderate to good establishment had mean temperature values less than 54°F. The entire range of mean temperature values is represented among the unsuccessful sites, so mean temperature does not appear to be a good determining factor for establishment.

High temperature frequency
There is no discernible pattern of correlation between the frequency of high temperatures and establishment success. Most of the successful sites have, on average, approximately 0.5 hours per day when the temperature exceeds 90°F in the growing season, but the native EC site

averages more than 2.0 hours per day. This is strong evidence against what otherwise looks like a potential threshold against long periods of high temperature. The entire range of high temperature frequencies is represented among both the successful and unsuccessful sites. There is no definitive pattern of correlation.

Conclusions

Establishing new patches

Assisted establishment is a practicable method to minimize the risk of *M. gemmiparus* population extinctions by increasing the number of viable patches. We already determined that propagating plants is easy, and this study demonstrates the feasibility of establishing them in field.

Viable plant material is easy to collect. For this project we obtained 420 bulbils from the field in a short visit without any significant disturbance to the native populations. The bulbils are easy to germinate and grow in a an *ex situ* setting. The plants grow and produce propagules quickly, and they require little to no specialized attention. Without any specialized methods or equipment, Dave Steingraeber was able to produce more than 10,000 viable bulbils by growing plants for about 12 weeks in an office at CSU during the winter of 2011/12. Similarly, the propagules require no specialized methods for storage or transport. We have stored bulbils as long as a year in a common refrigerator and found them to be fully viable as long as the container is not airtight. Transport simply requires basic precautions to avoid moisture or extreme temperature. The amenability of the species to collection, propagation, storage, and transport was already known, but this study provides a demonstration of it in practice.

The more important result of this study is a demonstration of the feasibility of establishing new patches of the plant in nature. With little effort and minimal expense, we established seven new patches of *M. gemmiparus* in two different populations, and three additional patches have some potential for becoming established as well. Out of 16 attempts, only 6 (37%) were deemed unsuccessful by the standards set in this study.

These establishment rates are much greater than the success rates typically reported for rare plant reintroduction and translocation experiments. In a recent survey, Godefroid, *et. al.* (2011) report establishment rates of 33% for these types of experiments when simple survival of transplants is considered, and success rates are much lower when reproduction and survival through multiple generations are considered. The greater success rate observed in this study for *M. gemmiparus* is more a factor of the species itself rather than any special translocation methods or techniques. We did not employ any special treatments such as site preparation, supplements, or extra protection in these translocations. The species is biologically amenable to translocation as long as habitat of the receiving sites is appropriate.

Godefroid, *et. al.* (2011) also warn about "*overly optimistic evaluation of success based on short-term results.*" We acknowledge the fact that two years is a short time frame to verify successful establishment of plants in the field. For most plant reintroduction and translocation experiments, the researchers recommend decades-long monitoring to verify success. Success standards are based on self-sustainability of established plant populations, and for most types of plants longer term studies are necessary to determine reproductive success and the ability of populations to sustain numbers through multiple generations. Though it only spanned 2 years, the period of this study did cover two full generations, and it focused on reproductive success. Even by these stricter standards, it is clear that six of the 16 experimental patches successfully established, and given the rates of increase in plant numbers at these sites, they are more than likely sustainable. To definitively answer the question of long term sustainability we highly recommend continued plant demography monitoring. Long-term monitoring for this purpose can be simple and inexpensive. Two field trips to the sites per year between late July and mid-September would suffice to obtain population number estimates that are adequate for tracking sustainability.

Habitat considerations

A secondary goal of this study was to better quantify the optimal habitat conditions for establishing new patches of *M. gemmiparus*. Results for this portion of the study are largely inconclusive. We identified 14 habitat parameters based on what we thought to be some of the most important factors, and selected sites to reflect a range of conditions for each of them. The study was not intended to be statistically robust, but even at the cursory level, correlations between habitat parameters and establishment success were difficult to make. For most of the parameters, successful patches exhibited a wide range of conditions, which indicates that the species is "not very particular" when it comes to these specific parameters. It was able to establish in a wide range of conditions. Pinpointing optimal ranges for specific habitat parameters has been difficult.

All past studies and species descriptions suggest that periods of very moist or saturated soil are important, but it appears that too much water is a problem. None of the sites that were perennially saturated or had standing water were successfully established. Optimal hydrological conditions appear to be sites that are only periodically saturated or, at most, consistently moist with no long periods of standing water.

Similarly, we suspected that some soil is necessary for establishment, but found that too much soil may be detrimental. Successful sites tended to have very shallow soil, typically less than two cm. The importance of shallow soil may be that it limits other species and therefore reduces interspecific competition, as we suspect *M. gemmiparus* is generally a poor competitor. Correspondingly, none of the successful sites had cover values for other herbs greater than 30%.

We are also fairly confident that cliff association is important, but have little data to support this supposition except for the fact that nearly all of the native patches of the species are on or adjacent to cliff faces[2]. We did not adequately test the importance of cliff association in this study since the study included only one patch that was not on or adjacent to a cliff. We also have no good explanation at this time for why cliff association is important, especially since there seems to be little preference of plants for specific aspect or angle of the associated rock face. It may be that a cliff is important for providing overhang protection, and in this study all of the successful patches were associated with some sort of overhang. The degree of protection provided by the overhang seemed to vary, however, with some patches deep within them and others existing near the edge or simply near steep walls that provided no real ceiling.

We were unable to determine any definitive patterns for other seemingly key variables such as microtopography, substrate, light regime, or temperature. This was a bit of a surprise. The extremely limited range of *M. gemmiparus* in nature suggests specific habitat tolerances, but at least in terms of these key variables the species responds more like a generalist. It is not limited by strict tolerances. On the other hand, tolerance to a fairly wide range of abiotic conditions in the field is corroborated by our earlier work on the species in the laboratory (Beardsley 1997).

At this point, our best recommendation for selecting sites for potential establishment or translocation is to focus on those that are adjacent to cliff faces with some degree of overhang, shallow soil that is moist or occasionally saturated but not perennially wet, and low herbaceous cover. If these conditions are met, and the site is within the proper geographic and elevation range, then establishment will probably not be limited by microtopography, substrate, light, or temperature.

Assisted establishment as a conservation tool for *M. gemmiparus*

The primary purpose of this study is to determine whether assisted establishment is a feasible conservation tool for protecting *M. gemmiparus* against the threat of extinction. The results are optimistic with respect to practical considerations (it is simple and inexpensive to propagate, disperse, and monitor *M. gemmiparus*) and also with respect to plant response

[2] One major exception had been the patches of plants in the Horseshoe Park population in Rocky Mountain National Park. The patches making up this population existed among boulders and cobble on recently-formed alluvial fan and on point bars along Fall River. These patches have apparently gone extinct, as no plants have reported there for several years.

(establishment success rates were high). We conclude that the approach is feasible and that it could be a valuable tool for protecting the species.

Assisted establishment is a particularly effective tool for *M. gemmiparus* due to its unique biology. The species will benefit from the establishment of new patches by directly reducing risk of extinction at the population and species level. In our surveys of *M. gemmiparus* over the past 25 years (Beardsley and Steingraeber 2013, Steingraeber and Beardsley 2005), we discovered that surrounding any of the extant populations there are many areas with suitable habitat that could potentially be occupied by the species. For instance, in our survey of Staunton State park (Beardsley and Beardsley 2007) we identified hundreds of locations where the plant could potentially thrive. Yet in that survey we found just one site that was actually occupied by the species. It appears that *M. gemmiparus* can readily become established in suitable habitat, and this is corroborated by the results of this study. Expansion of the number of patches, therefore, is likely limited by the dispersal ability of the species rather than a lack of habitat or difficulty in establishment. In short, there are many "empty patches" (suitable habitat with no plants) in each population, and the species would likely establish in these patches "*if only it could get there."* Translocating plants to viable habitat is really not much more than assisting dispersal. It provides an artificial means by which the plants can expand into neighboring habitat patches.

How does this reduce the threat of extinction? M. gemmiparus is very rare; there are only nine small populations at this time. Each population is therefore a very significant portion of the entire range of the species. In the introduction to this report, we described that the risk of extinction of any *M. gemmiparus* population is a function of the probability that any individual patch is extirpated divided by the number of patches in the population. It is difficult to reduce the risk of extirpation for individual patches since the threats are related to stochastic and unpredictable disturbance events. But increasing the number of patches in a population is not very difficult. Most populations of *M. gemmiparus* contain only one or very few patches, so increasing the number of patches would have disproportionately large reductions of risk at the population level. Translocating plants to some of the many "empty patches" within these populations in an assisted establishment program is a simple and easy way to actively reduce the risk that the population will be extirpated.

One can think of the problem by analogy to a common idiom if you can imagine individual plants as eggs and patches as baskets. No matter how careful one is, there is always a risk of dropping a basket if one has to carry it somewhere, and like the risk of losing patches to stochastic disturbance events, there is only so much one can do to minimize that risk. Most populations of *M. gemmiparus* have few patches. That is, they are storing all their eggs in one or a few baskets. Assisted establishment is essentially a way to help each population spread more eggs among more baskets.

Moving forward

Given the benefits in reducing the risk of extinction, the relative simplicity, and low cost of the approach, we recommend expanding the assisted establishment approach for *M. gemmiparus* both within Staunton State Park and at other high risk populations. With the exception of one population (the Saint Vrain population in Roosevelt National Forest) all of the populations have very few patches, are at high risk, and could therefore benefit from this method.

Godefroid, *et. al.* (2011) concluded their review of plant reintroduction studies as follows: "*We therefore conclude that the value of plant reintroductions as a conservation tool could be improved by: (1) an increased focus on species biology; (2) using a higher number of transplants (preferring seedlings rather than seeds); (3) taking better account of seed production and recruitment when assessing the success of reintroductions; (4) a consistent long-term monitoring after reintroduction.*" In moving forward, we recommend that future efforts heed the advice in these suggestions.

Future efforts in *M. gemmiparus* assisted establishment should include longer-term monitoring extending up to ten years or more (recommendation 4). If the monitoring programs use demographic methods like those we used in this study, then bulbil recruitment will be adequately accounted for (recommendation 3). Monitoring does not need to be expensive, one can easily prepare adequate demographic profiles for dozens of patches in one or two field days each season. When establishing new patches, we recommend planting much higher densities of bulbils than we used in this study (recommendation 2). Bulbils can be propagated very easily and inexpensively in unspecialized facilities, and mass-producing bulbils would not be cost prohibitive. Finally, assisted establishment would benefit from a focus on species biology (recommendation 1).

In this report, we describe the basic habitat requirements of *M. gemmiparus* and what types of sites would be most suitable for establishment. Further analysis of specific habitat requirements may help us optimize site selection in the future, but for now we are confident that if the few key habitat components we described are present, then there is a good chance plants will establish. Because plant material is easy to come by and planting methods are so undemanding, the most cost-effective approach is probably to continue in the vein of this study by targeting as many sites for establishment as possible. Obviously, it is good to be as efficient as possible, but there is little at stake if some sites fail to establish. We therefore recommend establishing *M. gemmiparus* to as many sites as possible in carefully monitored studies.

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