

Summary Report on the 2007 Pilot Study Examining
Possible Impacts of Cattle on Pumice Moonwort (*Botrychium pumicola*)
at Sand Springs Pasture, Deschutes National Forest, Oregon



A report prepared for the U.S. Forest Service, Bend/Ft. Rock Ranger District

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Introduction

Botrychium pumicola, pumice moonwort, is an Oregon state Listed Threatened plant (ORNHIC 2004) and a Forest Service Region 6 Sensitive Plant Species that occurs on Deschutes National Forest, as well as Fremont/Winema National Forests, Prineville District (Bureau of Land Management) and Crater Lake National Park. There are 27,560 pumice moonwort individuals estimated to exist currently.

A total of 4,815 pumice moonwort plants, constituting approximately 17% of the global population, are known to occur in the Sand Springs Pasture on Deschutes National Forest. To protect the pumice moonwort, the Sand Springs Pasture is being grazed using a deferred rest rotation system with three years of grazing and one year of rest specified (Sand Springs Allotment Annual Operating Instructions 2006). During two of the three grazing years, cattle are to be released after August 1 (after moonwort plants produce and release their spores). During the other grazing year, cattle may be present at Sand Springs Pasture during the main emergence, growth and reproduction period for pumice moonwort (June and July). Actual (implemented) rotations may vary, however. In recent years, rest periods have been more frequent for this pasture. In 2006, there were no cows released at the Sand Springs Pasture (it was a rest year). In 2007, cattle release was scheduled to occur later in the season. Cows were present for a short period in June at both Mahogany Butte and Water Trough 22, however, while cattle were being moved through the area.

In this context, the Forest Service is monitoring pumice moonwort plant numbers and vigor at the Sand Springs Pasture to investigate possible impacts of cattle grazing. Our objectives for this study are the following:

Management objective: We want to maintain a stable population of pumice moonwort, *Botrychium pumicola*, at the Sand Springs Pasture with at least 4,815 individuals over the next ten years.

Monitoring objectives:

1. We want to be able to detect a 50% decline in the numbers of pumice moonwort plants within two focal areas (subpopulations) in the Sand Springs Pasture and inside the fenced Bureau of Land Management plot between any two years during a 10-year study period (2008-2017) with 90% confidence at a significance level of 0.10.
2. We want to be able to detect a 50% difference in the number of pumice moonwort plants in the Sand Springs Pasture that are grazed (unfenced areas), plants that are released from grazing (fenced areas), and plants that have not recently been grazed by cattle (BLM plot). We want to be 90% confident of detecting a difference in plant numbers among treatments at a significance level of 0.10 during each year of the 10-year study period (2008-2017).
3. We want to be able to estimate the proportion of each subpopulation that is reproductive (producing spores) during each year of the study.
4. We want to determine if there are differences in the proportions of reproductive individuals through time in the fenced and unfenced areas.

If sufficient time is available next year, data will be collected on plant sizes and we will include the following additional objective:

5. We want to determine if there are differences in plant sizes through time in fenced and unfenced areas. The length of each moonwort's sterile blade (trophophore) will serve as a measure of plant size. We want to be 95% confident of detecting size differences at a significance level of 0.05.

This report describes the pilot study performed in 2007 and the results of the first year's data collection. This report describes the long-term monitoring design chosen to investigate the possible impacts of cattle presence on pumice moonwort plants. Note that the monitoring objectives were changed in 2007 to balance the need for scientifically rigorous research with the need for efficiency. Pumice moonwort emergence can vary greatly from year to year. We increased the minimum detectable change in moonwort plant numbers from 30% to 50% in order to keep the study practical (limit the number of quadrats sampled) while examining this dynamic plant.

Methods

Researchers conducted field work in May, June and July of 2007. Charmane Powers, Jeanne Sargent and I first visited the three potential Sand Springs Pasture moonwort subpopulations: one located south and southwest of Mahogany Butte (referred to as "Mahogany Butte"), one located near a water trough set out for cattle (referred to as "Water Trough 22") and one located near a site formerly used for silviculture research (referred to as the "Silviculture Site"). We discovered that moonwort densities were lower in 2007 than those estimated in 2006 at all three sites. We selected Mahogany Butte and Water Trough 22 subpopulations for this study based on plant densities and distributions.

We selected the sampling universe at Mahogany Butte and Water Trough 22 to encompass the greatest total number of plants while still being practical for monitoring. We selected two sampling areas within each site: one to be fenced and one left unfenced. We subjectively chose sampling locations using the following criteria:

- the areas contained typical moonwort habitat,
- moderate to high densities of moonworts were present,
- the areas are regularly used by cattle during the grazing season, and
- within each site, the two sampling areas were similar to each other in geographic and biological features (such as topography, substrate, number and size of woody plants present, and associated plants).

We did not select areas that were subject to the heaviest cattle use (such as the area closest to the water trough), since there were no moonworts present there. We placed wooden stakes in the corners of sampling areas and placed Forest Service rare plant monitoring stakes (metal) at each end of the midlines of the two unfenced sampling areas, taking care so that stakes were placed flush to the ground (in a cattle-friendly manner). We also flagged the approximate location of the

two fences in May and June. Fences were constructed at Mahogany Butte and Water Trough 22 in July of 2007.

Charmane and I selected quadrat sizes and shapes based on plant densities at each of the subpopulations and based on information gained through computer modeling performed in 2006 (Raven 2006). To determine plant densities, we flagged plants and made a detailed map of plants found in focal areas (10 m. x 10 m. or 20 m. x 20 m. in size) at each of the two sites. I calculated the mean distance between clumps and then selected the minimum length of the long edge of study quadrats. We then counted plants in nested pilot quadrats, recording specific plant locations so that we could determine plant densities in quadrats with different sizes. The largest pilot quadrat sizes were 2 m. x 40 m. at Mahogany Butte and 2 m. x 100 m. at Water Trough 22. The distribution of plants at Water Trough 22 was particularly patchy and moonwort density was lower in some portions of the site than densities observed at Mahogany Butte. Given those two population features, we also examined the impacts of placing permanent quadrats in two different orientations (roughly north-south vs. roughly east-west) to ensure that we can meet the desired sampling requirements (ability to detect a 50% change in the numbers of plants present in different years and ability to distinguish a 50% difference in plant numbers among treatments). I used the software STPLAN to determine the necessary sample size (number of plots) for each subpopulation.

Once we established quadrat sizes and shapes, we selected permanent quadrat locations within the sampling areas using a random numbers table. We placed large nails with quadrat locations marked on them at the corners of selected permanent quadrats. We established 20 permanent quadrats at Mahogany Butte (10 each in the fenced and unfenced areas). Permanent quadrats at Water Trough 22 will be established in 2008.

At Mahogany Butte, we searched for moonwort plants in selected quadrats and placed a round metal tag with a unique number approximately 3" to the north of each plant. When several plants formed a tight clump, we placed tags near each individual plant (but not necessarily to the north). We mapped plant locations for each quadrat. We also recorded plant locations within the quadrats and plant reproductive status. We defined an individual plant as a plant apparently emerging from a single base (the common stalk was sometimes difficult to see when it occurred under the pumice substrate). Plants were recorded as being fertile if a sporophore was present or non-fertile if no sporangia were present. We determined that a plant was inside the quadrat if the base (common stalk) of the plant was in. Charmane and I made judgment calls about plants emerging right on quadrat borders. Two fenced quadrats (30-32 and 38-40) were only searched once for plants. All other quadrats were searched on several dates in June and July. Data on reproductive status were not collected in two fenced quadrats (30-32 and 38-40).

I used STPLAN software to determine appropriate quadrat shapes and sample sizes. I used SYSTAT software to analyze plant numbers and reproductive status data. I performed t-tests to compare plant numbers in the fenced and unfenced areas at the two sites. I performed a Pearson chi-square test to compare proportions of fertile plants in the fenced and unfenced areas at Mahogany Butte. For 2007 t-tests and the chi-square test, I considered p-values equal to or lower than 0.05 to indicate significant differences.

Results

We estimated that pumice moonwort density at the Silviculture Site was about 0.05 plants/m² (based on the total estimated number of plants inside the silviculture fence and plant counts in a focal area inside the fence that contained the highest density of moonwort plants). We mapped and counted plants in focal areas at Mahogany Butte and Water Trough 22. We initially estimated that moonwort density was 0.4 plants/m² at Mahogany Butte and ranged from 0.3 to 0.9 plants/m² at the Water Trough 22 site. Plant distribution at Water Trough 22 appeared to be patchier than at Mahogany Butte.

The quadrat sizes at Mahogany Butte that appeared to best meet project objectives were 1 m. x 40 m. and 2 m. x 40 m. quadrats, based on pilot data collected this year. Charmane chose the 2 m. x 40 m. quadrats since Use of quadrats that size would require the least additional data collection this year. Pilot data in quadrats that were 1 m. x 100 m. in size best met project objectives for the Water Trough 22 site. We selected two study areas (sampling universes) for each site: one fenced and one unfenced. Fences were constructed in July of 2007. Study areas were 40 m. x 70 m. at Mahogany Butte and 70 m. x 100 m. at Water Trough 22. We established 20 permanent quadrats (10 fenced, 10 unfenced) at Mahogany Butte. A minimum of twenty permanent quadrat locations for the Water Trough 22 site have been selected on paper and will be established on the ground in 2008.

Baseline data collected from permanent quadrats at Mahogany Butte indicated that initial plant numbers did not differ significantly between fenced and unfenced quadrats (Table 1; pooled variances t-test, $p = 0.163$). We found a total of 304 plants in the 10 unfenced quadrats (mean of 30.4 plants per quadrat ± 5.1 plants standard error) with a corresponding estimated density of 0.4 plants/m² for the unfenced area. We found a total of 413 plants in the 10 fenced quadrats (mean of 41.3 plants per quadrat ± 5.5 plants standard error) with a corresponding estimated density of

Table 1. Baseline data on plant numbers collected from 2 m. x 40 m. quadrats at the Mahogany Butte site in 2007. Quadrat numbers refer to distance in meters along the 70 m. side.

Unfenced Quadrat	# of Plants		Fenced Quadrat	# of Plants
0-2	45		0-2	27
6-8	28		2-4	45
8-10	8		12-14	23
16-18	45		14-16	14
38-40	43		20-22	57
42-44	15		26-28	46
44-46	55		30-32	66
46-48	29		38-40	27
62-64	24		46-48	50
64-66	12		64-66	58
Total #:	304		Total #:	413

0.5 plants/m² in the fenced area. Two fenced quadrats (30-32 and 38-40) were only searched once for plants. All other quadrats were searched on several dates in June and July.

Baseline data indicated that the proportions of fertile moonwort plants did not differ among fenced and unfenced quadrats at Mahogany Butte (Pearson chi-square test, $p = 0.86$). We observed that 88% of fenced plants were fertile and 88.5% of unfenced plants were fertile. We did not have data on the proportions of fertile plants for two fenced quadrats (30-32 and 38-40).

Post-hoc evaluation of study design:

Analyses using the software STPLAN indicate that the minimum detectable declines we can statistically distinguish with the current study design at Mahogany Butte are a 46% decline in plant numbers through time for unfenced moonwort plants and a 39% decline for fenced moonwort plants. Those minimum detectable declines meet the project objectives. Since those are minimum detectable declines, we can detect a 46% or greater decline in unfenced plant numbers and a 39% or greater decline in fenced plant numbers.

If we wish to detect either an increase or a decrease in plant numbers (instead of changes only in one direction), this study can detect a 54% or greater change for unfenced plants and a 43% or greater change for fenced plants. The addition of four additional quadrats at Mahogany Butte (two quadrats each at the fenced and unfenced areas) would allow us to detect a 50% or greater change (increase or decrease) in plant numbers.

Preliminary summary of Water Trough 22 data:

Pilot data collected at Water Trough 22 indicated that initial plant numbers did appear to differ among fenced and unfenced quadrats (Table 2; separate variance t-test, $p < 0.001$). We estimated moonwort density in the fenced area to be approximately 0.2 plants/m², while the density in the unfenced area was estimated to be approximately 0.5 plants/m². Note that the data we collected in 2007 are only preliminary and do not constitute a full set of baseline data, as were collected at Mahogany Butte. Note also that pilot data collected from Water Trough 22 in 2007 do not represent samples from fully random locations since I included data from both 1 m. x 100 m. quadrats nested within the 2 m. x 100 m. quadrats (see pairs of quadrats listed in Table 2).

Post-hoc analyses of pilot data indicate that data collection from a total of 20 permanent quadrats at Water Trough 22 (ten fenced quadrats and ten unfenced quadrats) will allow us to meet project objectives, provided that we find as many plants next year (in other words, plant means do not decrease significantly) and plant patchiness is the same or better next year (in other words, standard deviations do not increase significantly). Data collection from 20 permanent quadrats is predicted to allow us to detect a 50% or greater decline in plant numbers. If we want to detect either an increase or decrease in plant numbers, we would need to establish 26 permanent quadrats (13 at fenced and unfenced areas).

Table 2. Preliminary data (not baseline data) on plant numbers collected in 2007 from the Water Trough 22 site. Quadrat numbers refer to distance in meters along the 70 m. side.

Unfenced Quadrat	# of Plants		Fenced Quadrat	# of Plants
38-39	42		6-7	8
39-40	66		7-8	11
60-61	68		8-9	12
61-62	64		9-10	7
62-63	65		32-33	28
63-64	45		33-34	20
66-67	39		42-43	42
67-68	30		43-44	29
			68-69	17
			69-70	19
Total #:	419		Total #:	193

Discussion

Botrychium pumicola is a challenge to sample for several reasons. Plants are small and cryptic and can occur under the foliage of other plants (such as sagebrush). This characteristic is relatively easy to address through repeated searches (searches on different days) by several people. Pumice moonwort is also challenging because it can be patchily distributed within a site and plant numbers can fluctuate greatly from year to year. High spatial and temporal variation can result in large standard deviations, requiring a land manager to examine more plants over a larger geographic area in order to make strong conclusions about what is happening biologically and to make the best management recommendations.

Our greatest challenge this year was in selecting areas with comparable initial numbers of pumice moonwort plants. Pumice moonwort distribution within the Water Trough 22 site was quite patchy in 2007. Previous mapping was useful but not detailed enough to use for selection of the study areas, so we spent time searching for large areas with similar plant densities. It is possible that when the full study is set up in 2008, there will be similar numbers of plants present at the fenced and unfenced areas at Water Trough 22.

For this study, it would be ideal to have similar plant numbers in fenced and unfenced areas initially, as this would simplify both the study design as well as future interpretation of any changes observed through time. Having similar numbers initially is not absolutely necessary, however, since we are most interested in detecting changes in plant numbers. We are more interested in the magnitude and direction of changes in plant numbers through time than we are in the absolute numbers of plants at the site.

Plant numbers at Mahogany Butte were statistically indistinguishable, however note that the statistical test's p-value was low ($p = 0.16$). In other words, initial plant numbers were closer to being statistically different than if the p-value had been 0.8 or 0.9. (P-values closer to 1.0 indicate more similar data sets.) We did not search two fenced quadrats at Mahogany Butte a second time. A second search could have resulted in the discovery of additional plants, driving the p-value lower and leading to the conclusion that initial plant numbers do differ.

Initial plant numbers were statistically higher in the unfenced Water Trough 22 study area and numbers were close to being statistically higher in the fenced Mahogany Butte study area. Since higher initial numbers were observed in the unfenced area at one site and the fenced area at the other, trends or differences at the two sites might balance each other out. In other words, when we combine the data from the two sites in the full study, it is possible that initial numbers in the two treatment types (release from cattle grazing, continued cattle grazing) will be similar. At this time, this is only speculation, since we have not collected the full data set yet. Additionally, plant numbers in any given area might be quite different in future years.

A few comments on plant size data collection:

It is very likely that the number of plants included in this study will be over 1,000, posing a possible challenge for data collection on plant sizes. Measuring all study plants could require a significant resource commitment. There are options for sampling fewer plants. One of the best options would be to measure a portion of randomly selected plants (specific percentage to be determined by Charmane). This is relatively easy to do in a study with tagged plants. A second option would be to systematically select plants along a quadrat. In either situation, it will be important to sample from the same plants every year of the study, provided they are present.

A few comments on future statistical analyses:

Since we selected quadrats with different sizes, corrections will be needed when performing statistical tests comparing plant numbers from the two sites. Quadrats at Mahogany Butte have a total area of 80 m^2 while quadrats at Water Trough 22 will be 100 m^2 in size. Before performing statistical tests, each individual plant number from a Water Trough 22 quadrat must be multiplied by 0.8 to correct for the different areas (convert to the number of plants observed to the equivalent 80 m^2 area). Here is an example:

Quadrat 1 at WT22 has 30 plants. We convert that to 24 plants (30×0.8).
Quadrat 2 at WT22 has 56 plants. We convert that to 44.8 (56×0.8).

We still present the summary data using actual numbers (mean in this example would be 43 plants, not 34.4 plants), but we use the converted numbers in statistical tests to get the correct p-values and to make conclusions about any differences observed. Converted numbers should not be rounded to the nearest whole number.

Acknowledgments

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Literature Cited

2006 Sand Springs Allotment Annual Operating Instructions. US Forest Service report, Deschutes National Forest (in-house). 12 pp.

Raven, A. 2006 Experimental Design to Examine Possible Cattle Impacts on Pumice Grapefern, *Botrychium pumicola*, at Sand Springs Pasture, Deschutes National Forest, Oregon. An unpublished report submitted to the USFS. 10 pp.

Cover Images:

Upper left, *Botrychium pumicola*, pumice moonwort, in its native habitat

Upper right, Charmane Powers (left) and Jeanne Sargent (right) searching for moonworts

Lower left, Christa von Behren recording data

Summary of work completed in 2007:

- 1) We selected two Sand Springs Pasture pumice moonwort subpopulations to examine (Mahogany Butte and Water Trough 22) and determined the sampling universe for each.
- 2) We selected two sites within each subpopulation, one to be fenced and one left unfenced. Fences were constructed in July of 2007.
- 3) We collected data on plant numbers and distributions (patchiness) at Mahogany Butte and Water Trough 22 and selected quadrat sizes and numbers that will satisfy the project objectives.
- 4) We established 20 permanent quadrats (10 fenced, 10 unfenced) at Mahogany Butte, marked individual plants, and collected baseline data on plant locations and reproductive status.

Summary of work to be completed in 2008:

- 1) Establish permanent quadrats at Water Trough 22. Quadrats can be established prior to moonwort emergence in 2008. Search for moonworts, tag plants, map plant locations and record data on plant locations, reproductive status and plant sizes. Estimated time needed: 30 hours (3 field days).
- 2) Search for moonworts at Mahogany Butte (find previously located plants and search for new plants). Map any new plants found and record data on their locations. Record data on reproductive status and plant sizes of all moonworts in quadrats. Estimated time needed: 15 hours (1.5 field days).
- 3) Establish the study area at the BLM site. Search for moonworts and place identification tags near them. Record data on plant locations, reproductive status and plant sizes, and map plant locations with the study area. This will be a census, so there will not be quadrats. Estimated time needed: 10 hours (1 field day).
- 4) Search for late-emerging plants in all permanent quadrats at the three sites. Estimated time needed: 15 hours (1.5 field days).
- 5) Summarize and analyze the data, including a comparison of plant numbers and reproductive status for the two years of data collected at Mahogany Butte and comparisons of data on plants not recently grazed by cattle (BLM site), plants released from cattle grazing (fenced quadrats combined) and plants currently subject to cattle grazing (unfenced quadrats combined).

Estimated times are based on 10-hour field days (time actually spent in the field, not including traveling).