RESEARCH ARTICLE

Monitoring *Lesquerella filiformis* Rollins (Missouri bladderpod): Application and Evaluation of a Grid-based Survey Approach

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ABSTRACT: Wilson's Creek National Battlefield in Republic, Missouri, protects a large concentration of the rare, glade-endemic, winter annual *Lesquerella filiformis* Rollins (Missouri bladderpod; scientific nomenclature follows USDA PLANTS Database). A sustained monitoring effort provided the opportunity to assess a grid-based survey approach featuring the use of visual density class estimates. Population size intervals, which include the uncertainty associated with the use of density classes, were compared to sample-based population size estimates from a computer simulation of the *L. filiformis* population. A 100% population size interval was similar to a sample-based 95% confidence interval and much narrower than a 99.9% confidence interval. If the distributions of observations within density classes are not symmetrical, however, a sample-based approach will yield more accurate point estimates. Observers frequently underestimated plant densities when using density classes, although counts of plants provided correction factors. Following recalibration of the density class scale, the grid-based survey approach supported the calculation of population metrics based on historical occupancy and abundance in individual grid cells. For some rare plant populations, conservation practitioners may determine that the advantages of such cell-based metrics outweigh the disadvantages of uncertainty and observer error stemming from the grid-based survey approach.

Index terms: grid, *Lesquerella filiformis*, Missouri bladderpod, monitoring, rare plants

INTRODUCTION

A comparison of the advantages and disadvantages of sampling and census (i.e., complete count) approaches to variable measurement is a standard of the biological monitoring literature (Elzinga et al. 2001; Sutherland 2006). Sampling provides parameter estimation with a measure of variance and permits extrapolation to the study population. A census may be appropriate for species that occur at relatively low density, are easily detected, and occur in sites where the entire area of interest can be covered (Elzinga et al. 2001; Sutherland 2006). Rare plant populations with very specific habitat requirements may be the best candidates for meeting these conditions. Such populations are monitored in disproportionately high numbers because of conservation concerns.

Certain design features can make plant censuses more practicable. A grid may increase plant detection and facilitate repeatable, systematic searches of the population (Greig-Smith 1983; Menges and Gordon 1996; Kettle et al. 2000; Sutherland 2006). For a census, logistical considerations that balance costs of permanent plot establishment with search efficiency often determine grid cell size. Fewer, larger plots require less time and infrastructure to set up initially and to locate during monitoring, but may produce less accurate counts. In order to cover exhaustively the area of interest as required for a census, visual abundance classes typically reduce the effort required to completely enumerate plants (Menges and Gordon 1996; Elzinga et al. 2001). Visual abundance classes are widely used in vegetation studies to estimate plant cover (Stohlgren 2007) and have been used to estimate density (McAuliffe 1990; Ward et al. 1993). The uncertainty associated with this trade-off of precision for speed decreases the resolution available to detect demographic changes.

Along with increased uncertainty, visual abundance estimates may suffer from substantial observer bias (Greig-Smith 1983; Elzinga et al. 2001). For some plant species, visual cover estimates from one sampling event by a single observer at a 1-m scale exceeded 20% of the mean estimated during nine sampling periods over a 10-day period (Kennedy and Addison 1987). Cover estimates to the nearest 5% in three plot sizes (4 m^2, 50 m^2, 200 m^2) varied from approximately 5% to 15% for individual observers, while differences among observers ranged from 10% to 20% (Sykes et al. 1983). Differences tended to be systematic, allowing correction factors to improve estimates. In one study, the coefficient of variation of species cover estimates among five observers in plots from 0.001 m^2 to 4 m^2 ranged from 0 to 225% (Klimeš 2003). Given the prevalence of observer error in using visual cover classes, error rates and magnitudes should be determined and reported (Kercher et al. 2003).

A monitoring protocol ideally provides an unbiased, statistically powerful, and

cost effective approach to detect change in biological populations (Gibbs et al. 1998). At Wilson's Creek National Battlefield, monitoring of Missouri bladderpod (*Lesquerella filiformis* Rollins; scientific nomenclature follows USDA PLANTS Database; USDA, NRCS 2008), a federally threatened species, is intended to provide robust indicators of population condition that inform management decisions. In this study, we compared the *L. filiformis* population size estimates from comprehensive surveys based on density class estimates to sample-based estimates from computer simulations of these same populations. We also evaluated observer error associated with the use of density class estimates and suggested methods for increasing their accuracy. Finally, we described *L. filiformis* distribution and abundance using grid cell-based metrics that may supplement population size as indicators of population condition.

METHODS

Lesquerella filiformis life-history

Lesquerella filiformis is a small (10 to 20 cm tall) winter annual in the mustard family (Brassicaceae) (Rollins and Shaw 1973). The species is federally listed as threatened and known only from a few counties in Missouri and Arkansas (U.S. Fish and Wildlife Service 2003; NatureServe 2007). Individual plants often have multiple stems arising from a small (1 to 4 cm) basal rosette. *Lesquerella filiformis* habitat includes limestone substrates of open cedar glades, barrens, and limestone outcrops in open woods (Rollins and Shaw 1973). Plants produce yellow flowers and globose siliques in mid-April to May. The plants die by late June and seeds lie dormant through the summer. Seeds that break dormancy germinate in the late summer or fall. Rosettes remain green throughout the winter and produce flowering stems in the spring. Rosette survivorship depends on microhabitat and year (Thomas 1996). Indeterminate flowering allows *L. filiformis* to reproduce prolifically under favorable conditions.

Field surveys

Bloody Hill Glade (BHG), a large limestone glade at Wilson's Creek National Battlefield in Republic, Missouri, supports a relatively large *L. filiformis* population (U.S. Fish and Wildlife Service 2003). Using permanently installed markers at the vertices, BHG was divided into an orthogonal grid of 963 5-m x 5-m cells. In 1997, 1998, 2003, and 2005, observers estimated *L. filiformis* density in all cells using a density class scale: 0 = no plants; 1 = 1-9 plants; 2 = 10-49 plants; 3 = 50-99 plants; 4 = 100-499 plants; 5 = 500-999 plants; and 6 = 1000-4999 plants (M. Kelrick, unpubl. data). The gaps in sampling years resulted from tests of alternative methods during these years (1999-2002 and 2004). In 2006, the sample area was reduced to assess the accuracy of density class assignments in areas of high plant abundance (see *Accuracy of density class estimates*). Surveys were conducted in mid-April to early May when most plants were in flower.

Population size intervals

Lesquerella filiformis population size was estimated using the density class endpoints. Low and high ends of the estimated density class assigned to each occupied cell were summed, respectively, to calculate the endpoints of this population size interval, referred to as "PSI_{100}". Assuming correct density class assignment, this interval contains the actual population size (i.e., is similar to a 100% confidence interval). Non-overlapping $PSIs_{100}$ were considered significantly different.

Computer simulated estimates of population size

We used the software program SAMPLE to simulate taking a simple random sample from this population. SAMPLE (available at http://www.lsc.usgs.gov/AEB/davids/acs/) is an application that was developed as a tool to simulate conventional and adaptive sampling designs. SAMPLE inputs sample or population data, depending on whether an analysis or simulation is desired. In this application, density classes were converted to counts by generating a Poisson random deviate with expected value equal to the category midpoint. A separate model population was created for each sampling year (1997, 1998, 2003, and 2005). Details of this procedure and full results of the simulations for additional sampling designs are in Morrison et al. (in press).

For comparison with $PSIs_{100}$, we calculated 95% confidence intervals (CI_{95}) using the population mean and variance of those means estimated from 1000 replications with a ~10% sample size (n = 96). A simple random sample of this size was found to yield relatively precise density estimates (Morrison et al., in press). Simulations showed that sampling efficiencies for all designs evaluated (including adaptive ones) were similar (Morrison et al., in press), so that comparison with a different sampling design would yield conclusions similar to those we report here. Based on the knowledge of field effort required, observers could expect to complete a 10% sample of BHG in 72 person hours, approximately the same amount of time required to visually estimate density in a comprehensive survey of all 963 cells.

Accuracy of density class estimates

We assessed the accuracy of density class estimates in 1998 and 2006; after cells were assigned to density classes by visual observation, they were revisited and censused. Forty randomly chosen 5-m x 5-m cells and fifteen 5-m x 5-m cells with known high plant densities were selected in 1998. In each cell, observers counted plants in defined 1-m^2 sections. In 2006, the 477 cells containing the great majority of *L. filiformis* plants on BHG were subdivided into fifty-three 15-m x 15-m strata. A single 5-m x 5-m cell was randomly selected from each stratum. An additional nine cells were purposively selected to bring the total number of cells assessed for each density class to at least 10. Observers divided cells into five 1-m-wide lanes and counted plants at ground-level in these defined sections. We used a ~10% error factor to eliminate misclassifications resulting from densities occurring near density class end points. For example, an estimate between

50 and 99 plants (density class 3) was considered correct for counts between 45 and 109 plants.

Cell-based measurement of occupancy and abundance patterns

To allow reliable year-to-year comparisons of occupancy and abundance in individual cells, the density class scale was recalibrated. Density classes 1-6 were combined and relabeled: classes 1 and 2 (1 to 49 plants) became class 1; classes 3 and 4 (50 to 499 plants) became class 2; and classes 5 and 6 (500 to 4999 plants) became class 3. We then calculated the following metrics: (1) the percentage of cells occupied at least once during the four sampling periods, (2) the percentage of those cells occupied during each sampling period, (3) the percentage overlap in cell occupancy for all pair-wise year combinations, (4) the percentage of cells in each density class attaining historic maximum and minimum density class for each sampling period, and (5) the distribution of persistence by maximum density classes over all sampling periods. Overlap in cell occupancy was calculated as the number of cells occupied in common during two years, relative to the number of cells occupied during the year with the lesser number of occupied cells expressed as a percentage. Persistence for a cell was defined as the number of sampling periods out of four in which a cell was occupied. A cross-tabulation between maximum and minimum density class for each cell was calculated. The relationship between maximum abundance and persistence in grid cells was evaluated with a chi-square test for independence (SPSS 14.0 for Windows; SPSS 2005). Because every cell was observed in every year, differences detected in analyses of these five metrics were actual differences.

RESULTS

Density class distribution

The *L. filiformis* density class distribution varied among the years of this study (Figure 1). The PSI_{100} low and high endpoints increased from 1997 to 1998 and from 2003 to 2005 (Figure 2). In general, the number of unoccupied cells decreased with increasing PSI endpoints, and the number of cells in density classes 2, 3, and 4 increased (Figure 1). The year of highest plant abundance, 2005, included the greatest number of cells characterized as density class 5 and was the only

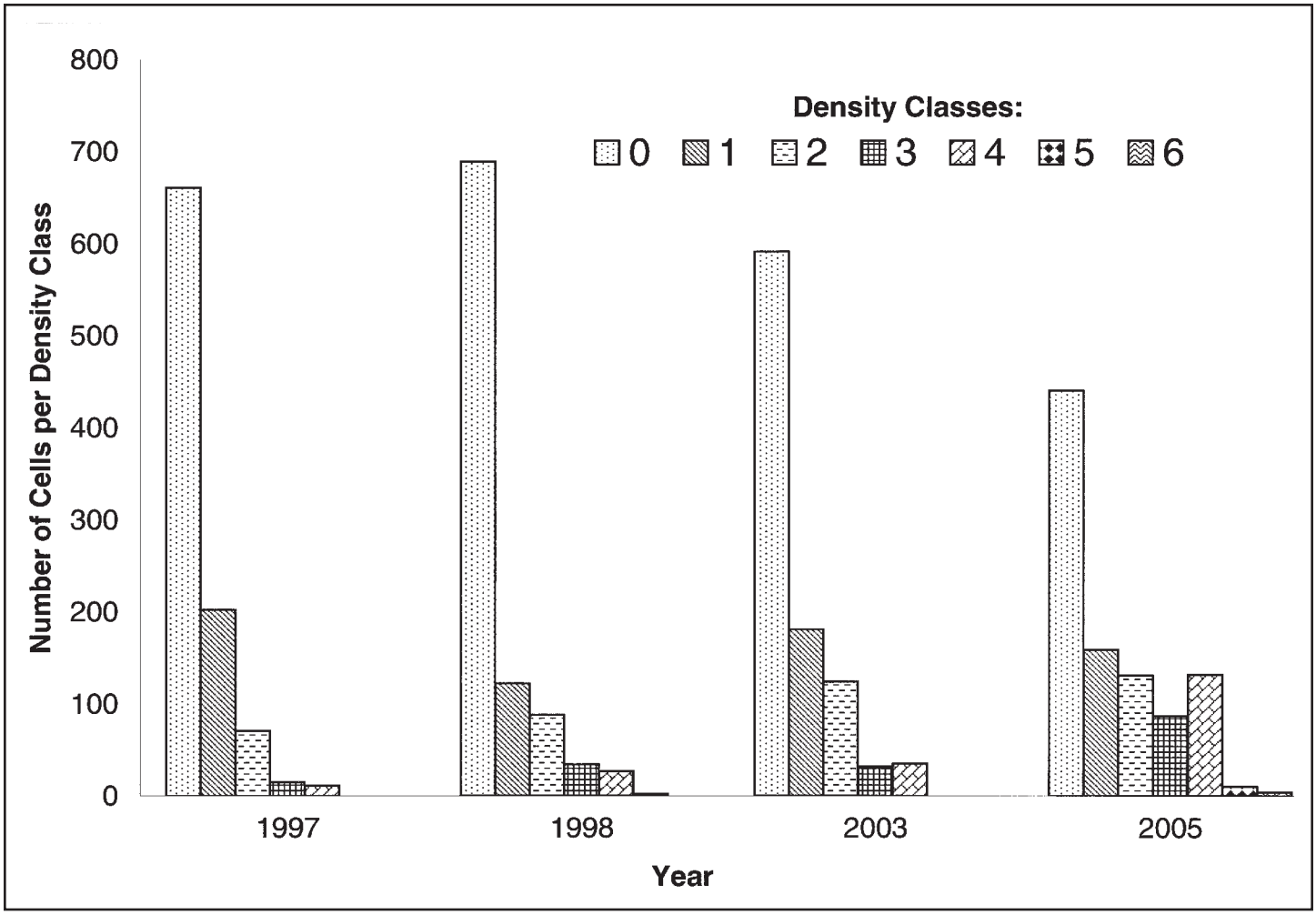

Figure 1. Distribution of *Lesquerella filiformis* density classes at Bloody Hill Glade, Wilson's Creek National Battlefield: 1997, 1998, 2003, and 2005. Density class scale: 0 = no plants; 1 = 1-9 plants; 2 = 10-49 plants; 3 = 50-99 plants; 4 = 100-499 plants; 5 = 500-999 plants; and 6 = 1,000-4,999 plants.

period with class 6 densities. Although 1997 included more occupied cells than 1998, the greater number of cells in density classes 3, 4, and 5 in 1998 resulted in its broader PSI_{100}.

Comparison of population size intervals

PSI_{100} and CI_{95} intervals differed little with regard to detection of change in *L. filiformis* population size among sampling periods (Figure 2). Ranges of the $PSIs_{100}$ were slightly narrower than those of CI_{95} calculated from computer simulations for 1997 and 1998, but were slightly broader for 2003 and 2005. Based on PSI_{100}, *L. filiformis* population size at BHG was smaller in 1997 and 1998 than in 2005. The 2003 PSI_{100} overlapped extensively with the PSI_{100} for 1997 and 1998, but only very slightly with the 2005 PSI_{100}. CI_{95} indicated that *L. filiformis* population size was smaller in 1997, 1998, and 2003 than in 2005; the CI in 2003 overlapped with 2005 to a very slight degree. If a 99.9% CI was used, which may be a more accurate comparison since PSI_{100} essentially functions as a 100% CI, the $CI_{99.9}$ would be ~71.0% wider than the CI_{95}. The PSI_{100} midpoints were 7,516.5; 15,324.5; 17,429.5; and 70,168.0, while CI_{95} midpoints were 7,704.0; 15,648.8; 17,815.5; and 70,058.2 for 1997, 1998, 2003, and 2005, respectively.

Accuracy of density class estimates

The accuracy of visual estimates varied greatly among density classes, ranging from 20% to 100% in 1998 and from 40% to 91.7% in 2006 (Table 1). All misclassifications underestimated plant density. In 1998 and 2006, actual density classes were usually within one density class and always within two density classes of the estimated density class.

Cell-based measurement of occupancy and abundance patterns

The calculation of PSI_{100} requires exhaustive sampling, but does not require the use of permanent cells. The permanently marked cells in a grid, however, allowed occupancy changes in individual cells to be recorded. On BHG, *L. filiformis* occupied 575 of 963 (59.7%) cells for at least one of four sampling periods (Figure 3). The number of cells occupied during each

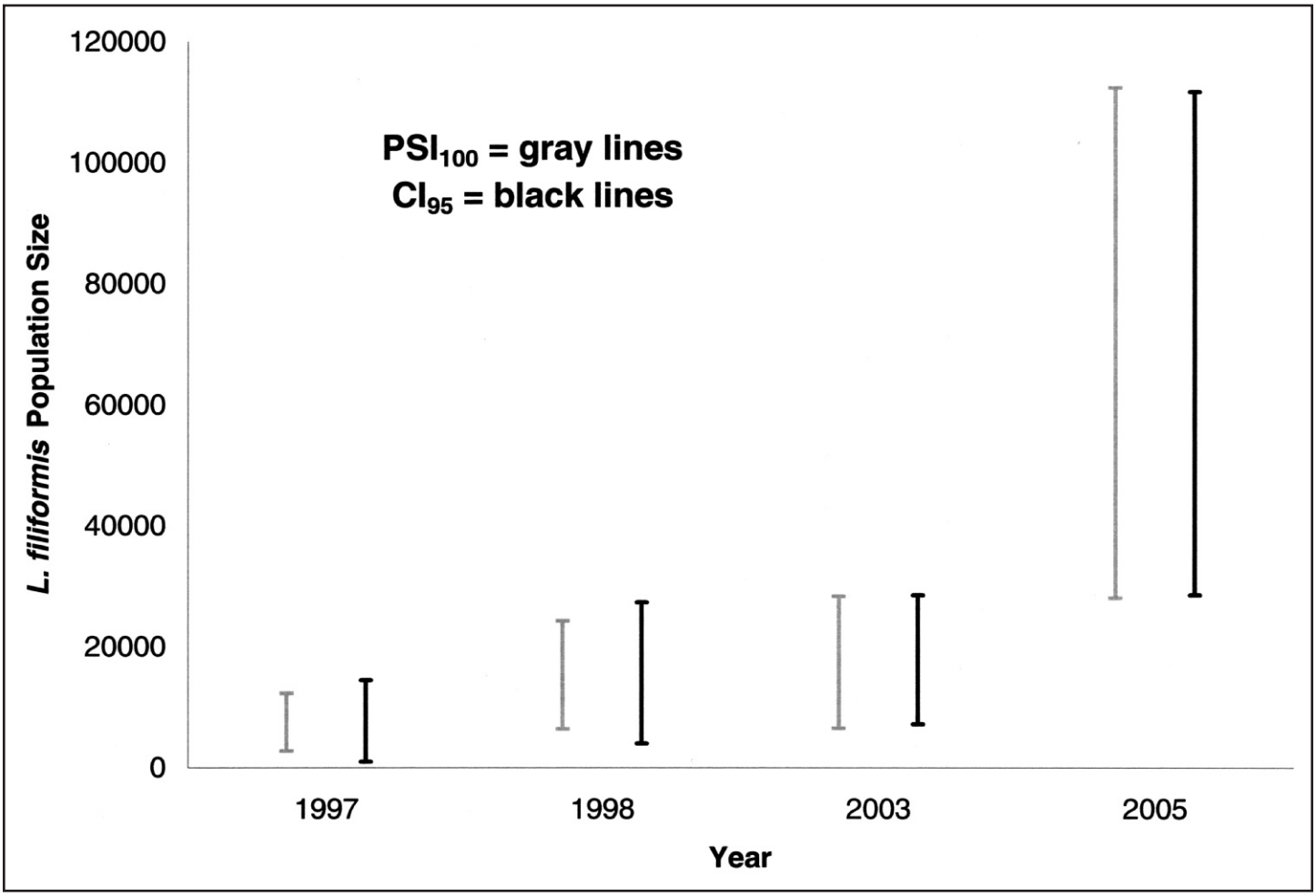

Figure 2. A comparison of population size intervals (PSI_{100}) from the comprehensive grid-based survey using visual density class estimates vs. from 95% confidence intervals (CI_{95}) based on a ~10% sample of a computer-simulated *Lesquerella filiformis* population at Bloody Hill Glade, Wilson's Creek National Battlefield.

Table 1. Accuracy assessment of density class estimates for *Lesquerella filiformis* at Bloody Hill Glade, Wilson's Creek National Battlefield in 1998 and 2006. Density class scale: 0 = no plants; 1 = 1-9 plants; 2 = 10-49 plants; 3 = 50-99 plants; and 4 = 100-499 plants.

Estimated density class	N	% correct classification	% of errors within 1 density class	% of errors within 2 density classes
		1998		
0	28	92.90%	50.00%	50.00%
1	8	85.70%	100.00%	0.00%
2	12	41.70%	57.10%	42.90%
3	5	20.00%	100.00%	0.00%
4	3	100.00%	0.00%	0.00%
		2006		
0	10	80.00%	50.00%	50.00%
1	15	60.00%	100.00%	0.00%
2	10	70.00%	100.00%	0.00%
3	15	40.00%	100.00%	0.00%
4	12	91.70%	100.00%	0.00%

sampling period as a percentage of cells occupied cumulatively during all four sampling periods was 52.0%, 47.5%, 64.7%, and 91.0% in 1997, 1998, 2003, and 2005, respectively. In an evaluation of overlap in cell occupancy, 72.5% of the cells occupied in 1997 were also occupied in 1998, the year of lower total occupancy. Overlap in cell occupancy for additional years were as follows: 1997-2003 = 77.9%, 1997-2005 = 94.7%, 1998-2003 = 79.1%, 1998-2005 = 94.5%, 2003-2005 = 92.2%. Persistence in grid cells is illustrated in Figure 3.

Changes in maximum and minimum density classes in individual cells provided another perspective on *L. filiformis* population dynamics. Density in occupied cells was generally not stable over time (Table 2). In cells with a maximum density class estimate of 1, 11.2% of cells maintained that class during all sampling periods. Density class 1 cells also showed a greater tendency (88.8%) to remain unoccupied during some years compared to cells with maximum density classes 2 (49.8%) and 3 (6.7%). The percentage of cells attaining historic maximum density class varied among years and did not strictly track PSI_{100} endpoints (Table 3 and Figure 2). However, the percentage of cells attaining the historical maximum was highest for all density classes during 2005, the sampling period with the largest population size. Finally, increased persistence was associated with higher maximum density in grid cells (Table 4, χ^2 test of independence, $\chi^2 = 201$, $p < 0.0001$, d.f. = 6).

DISCUSSION

Comparison of population size intervals and sampling estimates based on computer simulation

PSI_{100} offered a method to compare differences in *L. filiformis* population size that accounted for the uncertainty that visual estimates introduce. The uncertainty associated with comprehensive surveys of *L. filiformis* based on density class estimates (i.e., width of the PSI_{100}) only slightly exceeded error due to the expected variance determined from a simulated simple random sample of the population (i.e., width of CI_{95}) during two of four years (Figure 2). As population size increased, PSI_{100} was slightly less apt to detect changes in *L. filiformis* abundance than CI_{95}. (PSI_{100} necessarily increases in relation to population size.) During 2005, the year of highest *L. filiformis* abundance, PSI_{100} was only 8% wider than the corresponding CI_{95}. Compared to a simulated $CI_{99.5,}$ however, *L. filiformis* PSI_{100} ranges were much narrower. While PSI_{100} effectively renders an absolute population size range, asymmetrical distributions of actual values within density classes may prevent the midpoints of population size intervals from serving as good approximations of sample means. In such cases, a sample-based design may provide more accurate point estimates. The choice of which method to use may depend upon whether an accurate point estimate or the degree of uncertainty associated with

Table 2. Percentages of grid cells in minimum *Lesquerella filiformis* density classes per maximum density classes during four sampling periods (1997, 1998, 2003, 2005) at Bloody Hill Glade, Wilson's Creek National Battlefield (n = 963). Density class scale (combined): 0 = no plants; 1 = 1-49 plants; 2 = 50-499 plants; and 3 = 500-4,999 plants. Dashes indicate false combinations (i.e., minimum density class cannot exceed maximum density class).

	Minimum density class			
Maximum density class	0	1	2	3
0 (n=388)	100.00%	--	--	--
1 (n=321)	88.80%	11.20%	--	--
2 (n=239)	49.80%	49.80%	0.40%	--
3 (n=15)	6.70%	86.70%	6.70%	0.00%

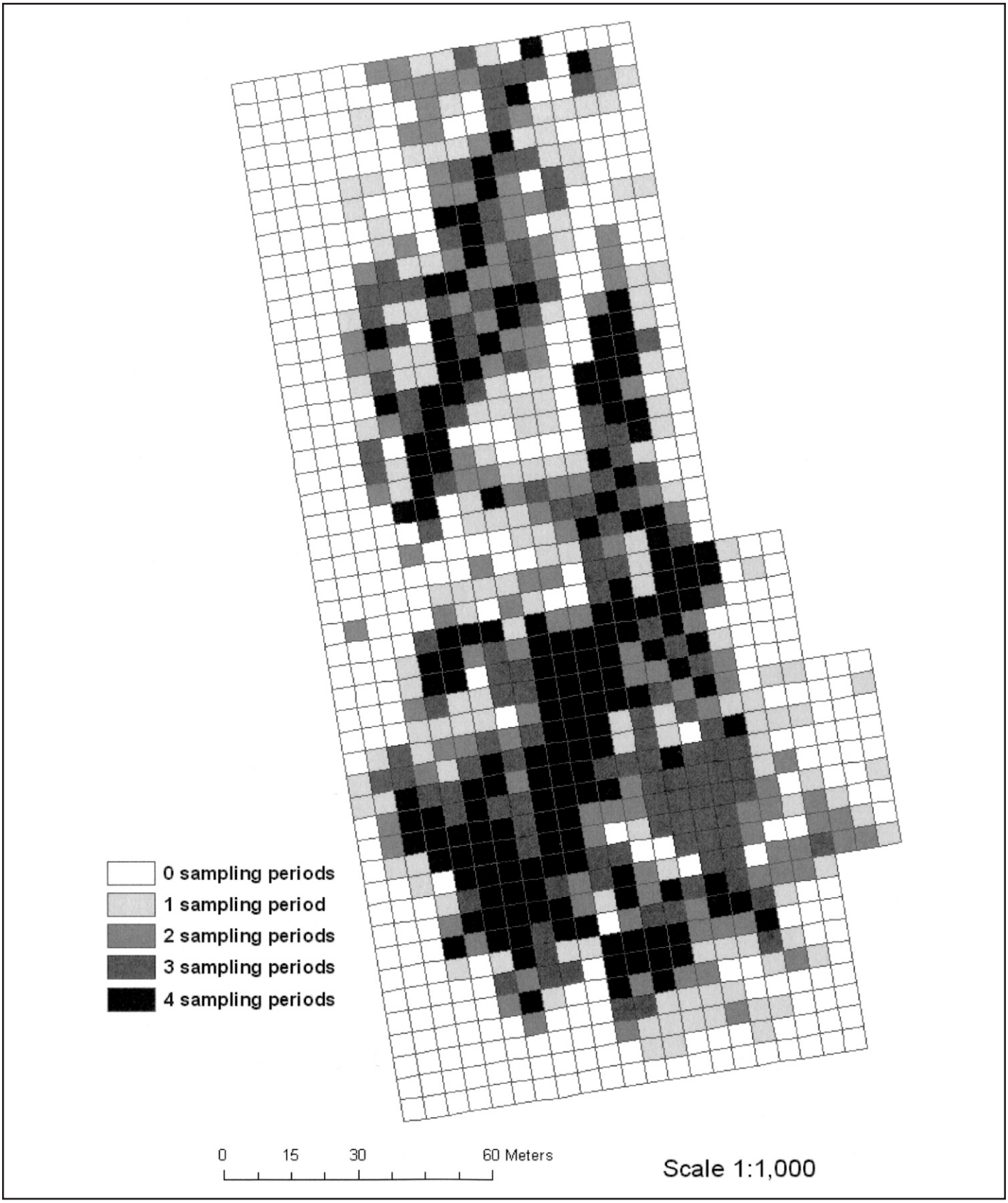

Figure 3. Map of *Lesquerella filiformis* persistence during four sampling periods (1997, 1998, 2003, and 2005) at Bloody Hill Glade, Wilson's Creek National Battlefield. A total of 388, 152, 124, 129, and 170 cells were occupied during no, one, two, three, and four sampling periods, respectively.

Table 3. Percentage of cells attaining historic maximum *Lesquerella filiformis* density classes for cells with at least one occurrence during four sampling periods (1997, 1998, 2003, and 2005) at Bloody Hill Glade, Wilson's Creek National Battlefield. Percentages are based on the number of cells in each maximum density class. Density class scale (combined): 1 = 1-49 plants, 2 = 50-499 plants, 3 = 500-4,999 plants.

Maximum density class	1997	1998	2003	2005
1 (n=321)	34.30%	28.70%	66.70%	85.40%
2 (n=239)	10.50%	21.30%	23.00%	91.20%
3 (n=15)	6.70%	13.30%	0.00%	93.30%

the estimate is deemed more important.

Accuracy of density class estimates

In addition to uncertainty, we found significant observer error connected with density class estimates. Observers systematically underestimated *L. filiformis* densities at relatively high rates for the majority of density classes. Field observations suggested that diminutive plants growing in marginal habitats were overlooked most often. The consistent directional error is advantageous in that estimates of population size are inherently conservative relative to management thresholds. Error rates from accuracy assessments can also be used as correction factors to reassign cells' density classes. A PSI_{100} can then be recalculated from the corrected density class distribution. In this study, PSI_{100} widths would increase following such correction.

In order to improve comparisons within individual cells across years, collapsing density classes increased the accuracy of density class assignment. Based on the 2006 accuracy data (Table 1), combining density classes 1 and 2, as well as 3 and 4, increased the correct density class assignment to 88.0% and 96.3%, respectively. Taken with correct assignment at 80.0% for density class 0, these percentages appear to be reasonable for ecological data. In this case, improved accuracy comes at the cost of increased uncertainty and reduced resolution to detect change in *L. filiformis* densities.

Cell-based measurement of occupancy and abundance patterns

With only ~60% of grid cells at BHG occupied during the four sampling periods, *L. filiformis,* like many plant species of Ozarks glades (Baskin and Baskin 2000a; Ware 2002; Nelson 2005), demonstrated clear preferences for certain areas of the glade. Although the percentage of occupied cells may increase in future years, all habitat on BHG is clearly not of equal suitability to *L. filiformis*. Furthermore, overlap in cell occupancy showed that cells occupied in a high abundance year, such as 2005, included the majority of cells occupied during lower abundance years. Even years of lower abundance (e.g., 1997-1998) exhibited only modest differentiation in cell occupancy. Occupied patches in the glade mosaic did not simply shift independently across years. Rather, habitat use shifted annually due largely to occupancy increases or decreases in a set of inhabitable patches.

Several patterns in historic *L. filiformis* abundance data were also noted. Despite the relatively wide density classes, fluctuation in plant densities was detected (Table 2). Such patterns are typical of annual plants (Bush and Lancaster 2005), including the congener *Lesquerella lyrata* Rollins (Baskin and Baskin 2000b). In addition, cells with higher maximum density supported plants more consistently through time (Table 4). Conditions promoting high maximum abundance apparently also support more reliable establishment. Increased establishment might result from increased rates of reproduction or germination. In the desert annual *Lesquerella fendleri*, seed set increased with increasing plant density (Roll et al. 1997). This relationship also suggested that tracking persistence alone may provide a robust indicator of habitat supporting high *L. filiformis* density. Finally, the ranges of the percentage of cells attaining maximum historical abundance was wide enough to serve potentially as an additional indicator of population condition (Table 3). It should be noted that while the error rates for cell-based changes in density class can be calculated, such errors will usually be disregarded for the purposes of long-term monitoring. If such errors are unacceptable, alternative monitoring approaches should be considered.

Table 4. Relationship between *Lesquerella filiformis* maximum density class and persistence for cells with at least one occurrence during four sampling periods (1997, 1998, 2003, and 2005) at Bloody Hill Glade, Wilson's Creek National Battlefield. Percentages are based on the number of cells in each maximum density class. Density class scale (combined): 0 = no plants; 1 = 1-49 plants; 2 = 50-499 plants; and 3 = 500-4,999 plants.

	Persistence (# years occupied)			
Maximum density class	1	2	3	4
1 (n=321)	43.30%	28.30%	17.10%	11.20%
2 (n=239)	5.40%	13.40%	31.00%	50.20%
3 (n=15)	0.00%	6.70%	0.00%	93.30%

Overall assessment of the grid-based *Lesquerella filiformis* monitoring survey

The grid-based survey method provided an estimate of *L. filiformis* population size, as well as cell-based occupancy and abundance metrics. PSI_{100} provided reasonably precise population size estimates without requiring complete counts. The data also allowed population measurements to be explained in the context of historic occupancy

(total number of cells occupied during all sampling periods, overlap in cell occupancy between years, persistence) and abundance (maximum and minimum abundance for all sampling periods). The resolution of the recalibrated density class scale was still sufficient to detect differences in plant density. Finally, exhaustively sampling all grid cells in the survey produced a map of the population with the minimum mapping unit equal to the grid cell size (Figure 3). Aside from the amenability of these data to spatial analysis, the simple visual display of *L. filiormis* density may identify important plant clusters or problems at specific locations. These maps may be especially valuable to natural area managers charged with protection of rare plant populations. In these cases, conservation practitioners may decide that the benefits of the comprehensive grid-based survey approach compensate for the attending uncertainty and observer error associated with visual density classes.

While monitoring demands a connection with management actions, setting relevant thresholds for initiating actions is often difficult (Elzinga et al. 2001). Fluctuating population sizes in annual plants such as *L. filiformis* further complicate such decisions. Because *L. filiformis* population size at Bloody Hill Glade is highly dynamic, available data provided the most obvious basis for establishing management thresholds. The large population in 2005 suggests that the lowest population metrics from previous years may provide safe thresholds. The grid-based survey also allows natural areas managers to establish thresholds for individual cells. For example, management may focus on cells attaining a new minimum density or on cells unoccupied for a sequence of years after being previously occupied. In each cell, the availability of suitable habitat, prevalence of human disturbance, and encroachment of invasive plants should be evaluated (Thomas and Willson 1992; Thomas 1996; U.S. Fish and Wildlife Service 2003). Choices of population metrics and selection of management thresholds, especially for annual plants, must be considered in relation to seed viability and seed bank dynamics. Dormant *L. filiformis* seeds are expected to remain viable for four calendar years (M. Kelrick, pers. obs.).

The monitoring approach presented in this study is most applicable for rare, patchily-dispersed plants occurring on small, restricted habitats, such as glades, serpentine barrens, and small isolated wetlands. While this approach may be less applicable at larger spatial scales, the density class scale can be adjusted and evaluated using the approach presented. GPS receivers with sub-meter accuracy may allow grids to be searched without the intensive investment in permanent cell establishment.

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