Optimal Monitoring of Rare Plant Populations
II: Data Collection and Analysis

Report for the USDA Forest Service

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Preface

The Chicago Botanic Garden was contracted by the USDA Forest Service to develop a technical manual for rare plant monitoring methods to complement our previous work, Optimal Monitoring of Rare Plant Populations: Report for the USDA Forest Service (Tienes et al. 2010). The Forest Service currently lacks consistent guidelines for monitoring rare plant populations, and monitoring methods often vary across Forest Service units. This lack of consistency makes comparisons across years and across regions very difficult. The resulting manual highlights consistency and efficiency while providing guidelines for data collection and analysis when using each intensity level of monitoring as described in Tienes et al. (2010).

Our first step in this project was to review the literature on current data collection and analysis techniques for each of the monitoring methods presented. The BLM publication, *Measuring and Monitoring Plant Populations* (Elzinga et al. 1998), was used as the base reference for many of the data collection methods. Rather than describing each of the many techniques from this reference, a few of the most user-friendly and efficient techniques were chosen to highlight in detail. The data analysis references *Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis* (Morris and Doak 2002) and *Matrix Population Models: Construction, Analysis, and Interpretation* (Caswell 2001) formed the basis of our discussions on count-based population viability analysis (PVA) and matrix population modeling, respectively. These resources were supplemented with additional literature and personal experience in the field to create a list of commonly and effectively used techniques of data collection and analysis for each monitoring intensity level.

The data collection methods chosen were then organized based on the monitoring intensity level for which they were most applicable.

- Level 1, or Inventory Monitoring, is the least time and resource intensive level of monitoring. For this level we focused on two different techniques; presence-absence monitoring and conducting a complete population census.
    - Presence-absence monitoring is a quick and straightforward method that provides very basic data
    - A complete population census, when paired with a count-based population viability analysis (PVA), is more informative and provides an estimate of the extinction risk, and is therefore somewhat predictive of population trends. We discuss how to complete an informative count-based PVA with 6-10 years of population census data.
    - For situations where more accurate predictions are needed, we describe how to complete a census with enhanced count data (Level 1.5) using little more time or resources. We have also included three case studies involving different species as examples of well-structured Level 1 and 1.5 monitoring programs.

- Level 2, or Survey Monitoring, is slightly more time and resource intensive than Level 1 and is best to use when tracking population trends or investigating the effects of management actions. In Level 2 Monitoring, one of three different population attributes is measured and monitored, based on the species of interest and monitoring objectives. We included information on collecting density, frequency, and cover data, along with information on when each attribute is most appropriate. There are a number of data analysis techniques that can be used with Level 2 monitoring data, and the one chosen will depend on the monitoring objective, either to track trends in a population or to investigate the effects of management actions.

- Finally, Level 3, or demographic monitoring, is the most time and resource intensive level of plant population monitoring. In this level we have included instructions on how to collect demographic data and when it is most appropriate for a monitoring program. Demographic data are best analyzed using matrix models. If you lack matrix-modeling software, a version of this type of analysis can be done in Microsoft Excel, using a free add-in called PopTools (Hood 2010, URL http://www.poptools.org). We have included instructions for running this analysis along with screen shots and an Excel template to make the analysis more user-friendly. A case study has also been included to illustrate an example of how this type of monitoring program has been used for the rare plant, *Lespedeza leptostachya*.

The intensity of the data collection effort increases as you move from Level 1 to Level 3 monitoring, however, the levels are not necessarily exclusive. The levels tend to build upon each other; however each level of monitoring provides you with different types of data that can answer different questions. For example, Level 2 and Level 3 monitoring provide additional data, such as plant density, changes in spatial cover, or transitional probabilities in a demographic context, for a population rather than the simple count provided in Level 1 data schemes. In addition, Level 2 and Level 3 data are often collected only on a subsample of the population, rather than on every individual, except in the cases of very small populations. Overall, Level 1 monitoring establishes a baseline of the number of plants in a population as a whole, Level 2 monitoring investigates trends in populations, often using subsampling methods, and level 3 monitoring follows selected individuals over time in order to calculate population metrics like survival, reproductive output, and extinction risk.

When choosing which monitoring level and technique to use, it is important to consider not only the questions you hope to answer, but also the details of the species of concern itself. A well-defined monitoring or management question is critical in determining which monitoring techniques will be the most helpful, however, not all techniques work equally well for all species and in all habitats. Knowing the plant life history, morphology, phenology, etc., as well as information on its habitat and associated species can help top determine the feasibility and costs of the different levels of monitoring. This will help to ensure that your monitoring program will be as efficient and informative as possible.

Introduction

The Chicago Botanic Garden created this technical manual with input from the USDA Forest Service and a technical advisory group to increase consistency and efficiency across Forest Service rare plant monitoring programs. It was designed to work in conjunction with Optimal Monitoring of Rare Plant Populations: Report for the USDA Forest Service (Tienes et al. 2010) to design and carry out a rare plant monitoring program. The 2010 report focused on how to choose a monitoring technique based on monitoring objectives, species, and habitat. This manual takes the next step, guiding monitors through the technical aspects of data collection and analysis. This manual is helpful for both planning and carrying out a new monitoring project as well as streamlining an existing project and/or analyzing already established long-term data sets.

Multiple data analysis strategies are included in the manual, based on the amount and type of data collected. The suggestions provided emphasize maximizing consistency and efficiency in the face of limited budgets. In addition to providing advice for developing an efficient monitoring study, this guide promotes consistent, well-documented methods across the Forest Service and aids in comparisons across regions. This will become increasingly important as species face an uncertain future amid numerous anthropogenic modifications; for example, climate change altering the distribution and abundance of habitat required of many rare species. Making the results of monitoring programs available in reports, will allow other botanists to learn from and collaborate with the Forest Service to better understand the status of rare species.

The general outline described for designing and implementing a monitoring program is as follows:

1. **Determine monitoring technique** using Optimal Monitoring of Rare Plant Populations: Report for the USDA Forest Service (Tienes et. al 2010)
2. **Assess data collection tool options** including plot and transect design and sampling strategies
3. **Design data sheet** using the recommendations discussed on page 6.
4. **Collect data**
5. **Analyze/Report data**

The methods detailed in this report increase in intensity as the manual progresses. The data collection topics presented include: presence/absence monitoring, population census (Level 1), enhanced population counts, monitoring frequency, density, and cover with quadrats or transects (Level 2), and following individual plants in a demographic study (Level 3). This manual also walks through two analytical techniques, a count-based PVA, for use with population census data (Level 1), and matrix modeling, for use with demographic monitoring data (Level 3). Step-by-step instructions on how to complete these analyses in Microsoft Excel (with the free add-in PopTools for demographic analysis) are included. Sample data sheets and templates, as well as case studies with fully worked examples are included to aid in understanding each component of a full monitoring program.

SECTION I: Data Collection and Sampling Design

Data Collection Methods

When collecting data, one of the most important factors to consider is controlling for observer bias, especially in situations with frequent workforce turnover. This concept, and techniques for correcting for this type of bias in data collection are discussed in more detail when discussing sampling units (plots, quadrats and transects). In this section, we summarize the three most common methods for recording data in the field: using an electronic device (laptop, GPS unit, etc.), voice recorder, or paper data sheets. Regardless of the method used, care should be taken at all stages of data entry to ensure accuracy. Data transcription into formats needed for statistical analyses must be verified at each step to catch errors in data entry.

Electronic Data Collection

Collecting data on a laptop, GPS unit, or other electronic device is efficient and eliminates the additional step of data entry. This type of combined data collection and entry reduces the possibility for human errors caused during data transfer and can keep data more organized. Many of the electronic options available are extremely durable for work in the field (though care must be taken if collecting data in inclement weather). In addition, data dictionaries on electronic devices can be designed in a way that fields must be populated before moving on, helping to ensure that no data are missed. Disadvantages to electronic devices include cost, set up, training, battery power limitations, difficulty reading displays in very high or low light, required periodic backing up of data and updating of software, and the fact that some devices can be heavy or awkward to take into the field. It is recommended that paper printout or PDF backups are included in any project that relies on electronic devices, which may suffer from technical difficulties.

Voice Recorders

Recording data using a voice recorder can be very helpful when conducting monitoring alone, allowing for the efficient collection of multiple data points and long descriptions of populations or habitats. However, transcribing data from such recordings can be time consuming. Additionally, technical problems in the field can result in the loss of data. Voice recorders also lack real time visual data, such as maps, data already collected, etc., to review while in the field.

Paper Data Sheets

Paper data sheets are the most inexpensive and lightweight option for data collection; therefore they work well for programs involving rough terrain, large groups of monitors, and/or tight budgets. Data sheets can be printed on Rite-in-the-Rain™ paper (J.L. Darlington, Co, Tacoma, WA), www.riteinrain.com and available from field supply companies. Data sheets are easy to customize, and when well designed, they can

contribute to consistency of data collection. Designed data sheets can be photocopied onto Rite-in-the-Rain paper. Entering data on paper sheets requires little training, but care must be taken to avoid loss of data through sloppy handwriting. Pencil is recommended for recording on paper data sheets, as many ink options can smear or run if pages get wet and some inks fade with time and exposure.

Designing a Data Sheet

A well-designed data sheet can save time and improve the quality and consistency of data collected among observers and across years. An easy, yet often overlooked step in data collection is to make sure that all data needed has been collected and that all data cells have been populated prior to leaving a site. Elzinga et al. (1998) offer the following suggestions when designing a data sheet for a monitoring project:

- Include all relevant details and only relevant information (minimize unnecessary information that can clutter data sheets, but do leave room for notes)
- Instruct observers to write legibly, as another person may enter the data for analysis
- Sheets should be organized so that as much data as possible are numeric
- The use of species codes (first three letters of genus name + first three letters of species name) or USDA PLANTS codes can be efficient when monitoring multiple species, remembering to remain consistent throughout a project
- Provide guidance for the types of additional information that could or should be collected from observations and designate where to record these details, usually in a designated 'Notes' section

What to include in an effective data sheet:

- Study location
- First and last names of all persons in the team collecting data that day
- Full name of data recorder
- Date (including year) and time of data collection
- Sampling method, including transect or plot # and location
- GPS coordinates (if applicable) including datum, projection and accuracy
- List of plant names (cite authority used to ID plant if needed)
- EO #s where appropriate
- All data that are required to answer your monitoring questions(s) or evaluate your monitoring goal(s) including appropriate summary details for easy coding of variables, e.g., stages, height classes, cover classes, etc. (1=seedling, 2=juvenile, 3=vegetative, 4=flowering, 5=fruiting, etc.)
- Be sure to populate all data cell fields, even if zero, to confirm the data for that cell was collected, and if data are not collected indicate why in 'Comments' or 'Notes' section

Spreadsheets and Databases

The examples provided in this manual use Microsoft Excel (2010) for data organization and analysis, as it is widely available, user-friendly, and has statistical analysis capabilities. Please note that the menus, tools and formatting used in our examples may vary slightly in newer versions of Excel. The addition of the free add-in, PopTools (available for download at www.poptools.org), allows for basic analysis for all types of monitoring in one program. After becoming familiar with Excel commands and formulas, data entry and analysis can be very time-efficient.

An additional benefit to using Excel spreadsheets is that they are easily transferred into many different relational database programs. A relational database uses multiple tables to store information from large and complex data sets. The tables are all related so that data can be accessed or reassembled in multiple ways without having to reorganize the tables themselves. Relational databases provide an easy way to keep large, complicated data sets organized and facilitate the access of the portions of the data set that are needed for any specific analysis or question.

Examples of relational database software include Microsoft Access, Dbase, Paradox, and Oracle. While Microsoft Access is often readily available for most PC users (it comes as part of the standard Microsoft Office Suite), it is not particularly user-friendly and takes some training to be used efficiently.

When dealing with electronic data, it is important to keep files organized and easy to find. It is best to use a consistent method for naming files and folders that includes important information such as species name, site name and/or date. This allows the files to be easily found by anyone involved with the project. In addition, a "Read me First" file outlining the names and locations of all pertinent information and files relating to each project can be extremely valuable. Files should also be backed up to prevent data loss.

Determining Layout of Sampling Units

Deciding how and where to set up sampling locations is one of the most critical steps in conducting a monitoring program. Sampling locations may be based upon plots, quadrats and/or transects. A plot, referred to as "macroplot" in Elzinga et al (1998), is a polygon, usually rectangular or square, bounding the target population to be monitored. Used when a population is small (both in terms of census size and/or spatial extent), a plot can encompass either a sample of a population, or the vast majority of individuals in the densest part of the population. It is important to distinguish between the two, because the setup will determine the type of inference your study may allow. A randomly placed plot contains a random sample of the population will allow you to infer the results of your study to the whole population. A plot that is placed non-randomly to encompass a portion of the population will only allow you to infer your results to the portion of the population that you included in the plot.

It may be difficult, to virtually impossible; to place plots randomly in a population of rare plants and have an adequate sample size. Placing a plot, or a series of plots, in the densest portion of the population that encompasses the vast majority of the population may be the most efficient means of demarcating your target individuals. It is important to select the placement of transects and quadrats in way that avoids bias and ensures that measurements are accurate and repeatable. In small populations where you are monitoring the entire population, this step is unnecessary.

Sampling Individuals

Many populations are too large to measure all or most of the individuals, therefore it is necessary to find a way to sample the population. It can be very difficult to obtain a sample of rare plants, simply because they are rare and are often scattered across the habitat non-uniformly. Therefore, it is necessary to find a sampling method that is statistically rigorous, depending upon the actual application. One thing that you will want to determine early in the design process is whether or not you will need to extrapolate from your results to other non-targeted plants or populations. For example, if you are monitoring a group of plants next to a new trail to determine their response to the trail, you may not have to infer this response to plants that do not grow next to a trail. If, however, you wish to measure the response of a population to a management activity, and then apply what you have learned to other populations of the same species, then you must use an appropriate sampling scheme which contains a random element.

The best sampling design provides a sample that is unbiased and representative of the population as a whole and results in a data set for which analysis is straightforward. A **Simple Random Sample** (SRS) is considered the most effective and efficient way to sample a population, particularly one that is uniformly distributed across a geographic area of interest. An SRS results in a sample that is unbiased, and is highly likely to be representative of the entire population. However, individuals with rare traits may not be included in these samples, leading to sampling error, which can never be eliminated, only reduced. Additionally, an SRS results in a dataset that is amenable to parametric statistical analysis such as t-tests, One-Way ANOVA and Regression analysis.

An SRS requires a known data frame, or group of known or identified individuals. A straightforward way of obtaining a data frame of known individuals is to simply conduct a count of the population by dropping pin flags next to every plant observed. A random number generator is then used to obtain a list of random numbers to "walk off" your sample. For example, if you want to include half of the censused plants, you could use the random number generator under the Data Analysis menu in Excel (found in Tools) to generate a list of randomly generated 1's and 2's with a uniform distribution, resulting in an equal, or nearly equal, proportion of each. Walking through the population and picking up flags corresponding to the 1's would randomly leave flags next to approximately half the plants. Pin flags are then replaced by numbered tags to complete the sample design.

A simple random sample may be particularly efficacious where individuals are uniformly distributed across a habitat, however, it can lead to over/under sampling individuals in a population that is clumped or that is distributed in response to an underlying environmental gradient. In contrast, a **Systematic Sample** provides each individual in the population a known and equal probability of selection. Beginning with a data frame of identified individuals, the first individual is chosen at random, this is known as a "random start." All subsequent individuals are chosen systematically, over a regular interval, such that all remaining individuals have an equal chance of being selected. For example, if you wanted a sample of 10 individuals out of a population of 100, $1/10^{th}$ of the known data frame, the first individual would be chosen at random out of individuals 1-10. For example, if the random start is individual 6, and you want $1/10^{th}$ of the population, the interval would be 10, and the sample would then be comprised of individuals 6, 16, 26, etc.

Systematic sampling is particularly effective along a transect, where the random start could either be the length of measure, say 6 meters, or plant number along the length of the entire transect as outlined above (also see Figure 3.1). If plants are scattered such that they do not intersect the transect line, you can use the line as a center to establish a transect of an appropriate width; these are often referred to as belt transects. Systematic sampling is particularly useful when the characteristic of interest is exhibited by individuals; e.g the association of floral production relative to plants size, or survivorship. These individual characteristics, or demographics, are the very responses that we are measuring when building demographic models (Discussed in Section IV).

In some cases, systematic sampling does not have to occur with a random start. When monitoring a population of thistles for seed predating weevils, for example, the plant is used as the sampling unit, and it is systematically determined which plants will be included in the observations. Every third plant, or every i^{th} plant, would be systematically sampled and observed for the presence of weevils. Then the number of weevils per plant would be counted, and an average for your observations obtained. However, it cannot be inferred from this observation that plants other than those sampled have a similar level of seed predation. If you obtained multiple samples across several populations, you might, however, detect a trend in the level of infestation across the landscape where the observations were made.

Another means of achieving a systematic sample would be to implement a grid-based design. This may be implemented on any scale, from centimeters to kilometers, depending upon the monitoring objective. While it is possible to implement a small-scale grid design in the field using meter tapes and pin flags to mark the grid corners and then determine the sampling location, it is better to design the grid and sampling scheme on a map, and then implement that design in the field. In the grid in Figure 1.1, for example, 35 grid locations that fall within the boundary were used, with a random start of 6. To sample 1/3 of the grid, we would systematically sample every third grid corner, resulting in a sample size of 11 points. A grid of this nature could be a useful way of evenly distributing plots for a vegetation survey, and can be very useful for creating a unified sampling design to monitor multiple responses to management. For example, the grid could be used simultaneously for bird counting stations, locations for canopy photos or soil cores. Another way of utilizing the grid would be to sample at the centroid (star) of a square (blue) where two grid corners are selected systematically. Designing a grid system in a GIS and using hand-held GPS devices to locate the grid corners to be sampled would increase the precision during implementation.

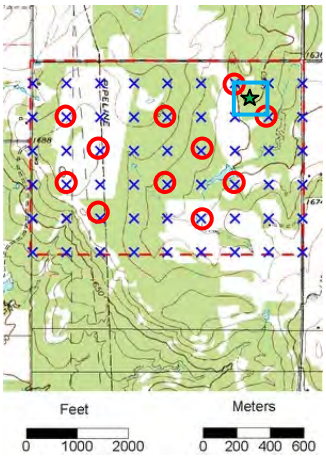


Figure 1.1. A set-up of a grid system used to systematically create plots in an area, where the grid points are used as corners to create a plot.

A **Cluster Sample** is a useful way to obtain a sample when it is difficult or impossible to obtain a random sample of a large population that might be clumped or otherwise scattered across the landscape in a non-uniform pattern. Cluster sampling is particularly useful when the characteristic of interest is exhibited by individuals, say the association of floral production relative to plants size, or survivorship. Again, these individual characteristics, or demographics, are the responses that we are measuring when building demographic models (Discussed in Section IV). Cluster sampling is most effective when the clusters are very similar to each other, while the units within each cluster are highly variable. One means of obtaining a cluster sample is to sample the population via quadrats or transects (Discussed in Section III)

If the population or study site is situated in such a way that there are obvious groupings that have slightly different environmental characteristics, a **Stratified Sample**, sometimes called a **Two-Stage Sample** may be the most appropriate. Given a population of rare plants that are scattered on two different hillsides, for example, the sample would be comprised of different locations, or "strata." To have the statistical power to fully infer your results from one hillside to another, you would need to take a random sample from each location – the first stage of the sampling is identifying the strata, and the second stage would be taking a random sample within each strata. Stratified sampling requires the application of a different calculation to obtain the total sample mean and variance, however. Essentially, the mean of each strata would be

obtained independently, and then the total mean would be calculated based upon the proportion of plants found in each population.

There are many excellent resources available on the web and in textbooks to help design and implement an appropriate sampling scheme, and a more comprehensive coverage of sampling design and data analysis is beyond the scope of this manual. A list of potential sources is available in Appendix A. Also, it may be useful to consult with a biostatistician early in your investigation to ensure that your design has the statistical power necessary and to ensure that you will be able to answer your monitoring questions with the data you are collecting. As we have stated elsewhere, a pilot study is highly beneficial to ensure the quality and power of your data. One of the best investments you can make to assure success is a consultation with a biostatistician at the end of the pilot study. Having data in hand from a pilot study is advantageous because any problems with sample size or parameters measured will be apparent, but you will not have invested an inordinate amount of time in a faulty design.

Sampling Quadrats

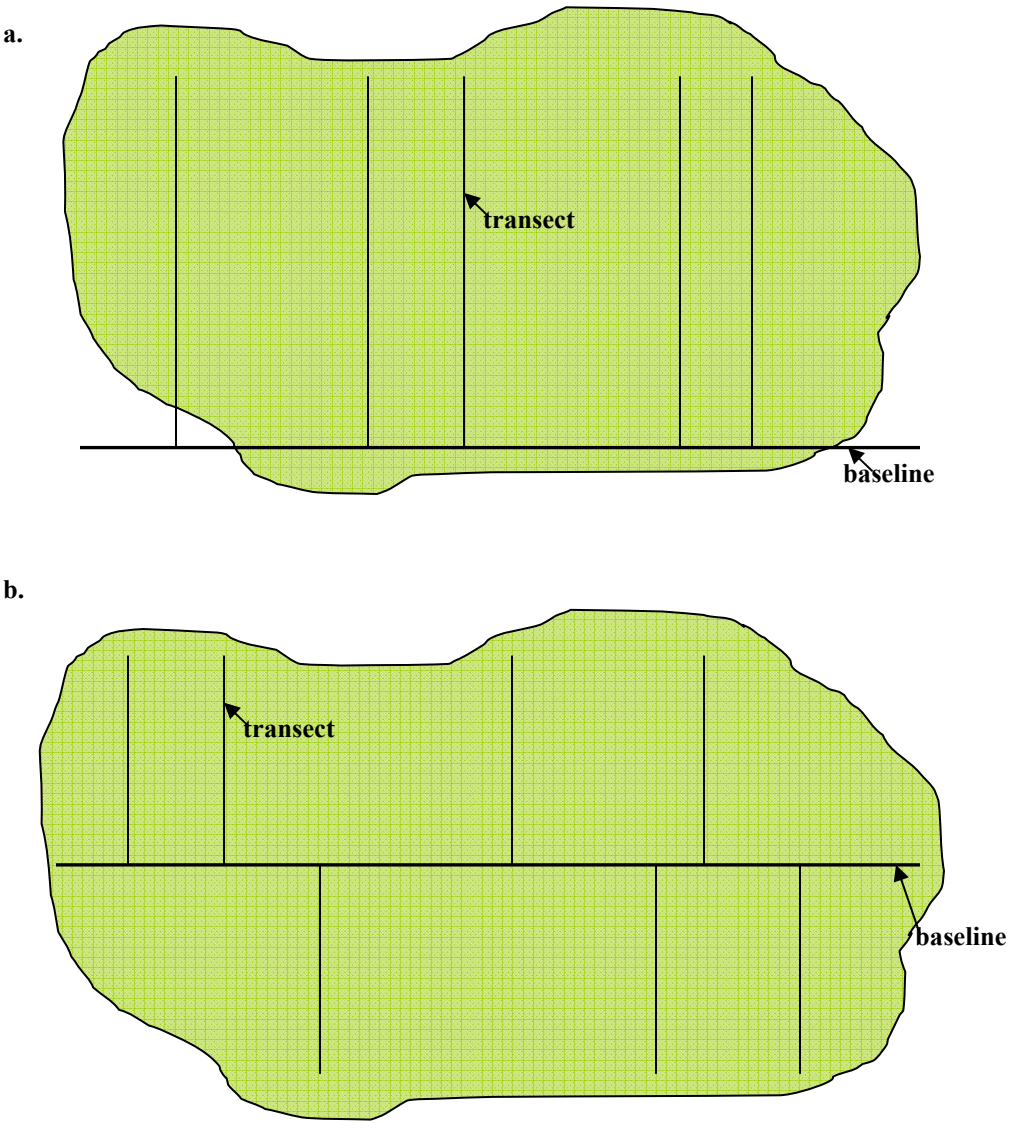
The location of quadrats in a population will greatly impact the outcome of a monitoring project. Quadrats should be positioned randomly with proper dispersal of the quadrats throughout the population to obtain the most powerful and broadly useful sample (Elzinga et al. 1998). Random sampling ensures that calculated probabilities and statistical inferences about the population are likely to represent the population truly as a whole, which in turn will impact the ability to make conservation decisions that are likely to achieve desired goals. Once the location of quadrats has been determined, a permanent marker should be placed somewhere in the plot (usually on a corner or in the center), and the location of the marker should be recorded so that data can be collected in the same locations over time. Such markers should be visible so they can be readily found, but in many cases, cannot be conspicuous. Use of GPS units or points that can be found using triangulation can be for relocating permanent markers. When collecting data within a quadrat it is helpful to use pre-made frames, which can be easily constructed from PVC pipe. These frames are inexpensive, lightweight, can be broken down and are easy to transport from site to site (determining size and shape of quadrats is discussed further in Section III).

Transects

When using transects as the sampling unit in a monitoring project, the length should accommodate most of the variability within the population of interest. One common method used to determine where to place transects is the baseline method (Coulloudon et al. 1999), where a baseline is established across the long edge of the area or population by stretching a tape between two stakes. Individual transects are then run perpendicular to the baseline at randomly- (using a dice roll, number table, or other similar method) or strategically-chosen points along that baseline. Transects can be run all in the same direction from the baseline, if the baseline is run along an edge of the population, or they can be run in two opposite directions from the baseline, if the

baseline is run through the middle of the population (see Figure 1.2). Once transects have been placed, quadrats or points can then be located along those transects using a similar procedure to the one used when locating the transects along the baseline.

Figure 1.2. Diagram of the baseline method of placing monitoring transects across a population, placing transects randomly along the baseline. Based on the site the transects can be run in one direction (a) or two opposite directions (b) from the baseline.



Marking and Relocating Plants, Plots and Transects

When the objective of your monitoring design requires you to revisit a site year after year, or even within the same year, it is important to implement a method of marking and mapping the site. The method employed will depend upon the longevity of the monitoring effort: the longer the monitoring plan, the more permanent the marking and mapping needed. Background vegetation and overall site conditions also play a role, and good record keeping is vitally important.

When a monitoring plan calls for following individual plants, you will need to mark each individual uniquely, and also have a plan for relocating either the plants or the material marking their location. In some applications, using plastic pot tags used in the greenhouse industry may be adequate or even desirable. If this is the case, a code or number may be hand-written on the tag to identify the plant and any characteristics desired. Even under full canopy during the field season, however, hand-written tags are subject to fading, especially from UV light. In our experience, it is best to either use pencil or a thick-pointed pen with permanent ink. Fine Point Black Sharpie pens by Sanford are ideal for this application. You may want to avoid the Ultra Fine Point pens by the same maker if your tags will be in full sun, and colored Sharpies should be banned from your field kit, however attractive the idea of color coding your tags might be. Even under fully closed canopy, colored Sharpies are highly likely to fade into oblivion causing you to lose data continuity between site visits.

If your budget allows, pre-numbered metal tags are available through suppliers to the forestry industry such as Forestry Suppliers or Ben Meadows. They also have small aluminum tags that you can write on, and Paw Paw Everlast has hang tags which may be written upon with a wax pencil. In practice, however, we have found the pre-numbered aluminum tags to be ideal for marking plants, plots and transects. They are available both in silver or blue, with the blue tags generally easier to find visually, especially when the study site is in full sun. You can order the tags in sets of numbers from 1-1000, 5000-6000 etc. If you have more than one site, or more than one project, designating a range or sequence of numbers to a site or project is a good practice.

Figure 1.3. A photo showing a plant marked with a flag and pre-numbered aluminum tag.

Depending upon the life form and habitat of the target species, tags may be deployed in a number of ways. Aluminum tags are often simply nailed into the trunk of tree, for example, or wired onto a branch of a woody or shrubby species. If your population is situated in a place where pin flags may be left in place, they may be used as a tag holder by simply creating a loop to hold a numbered metal tag (Figure 1.3). This can be done where the flags will not draw attention to a population of a sensitive species or area, where there is no expectation of proscribed fire, where the plant will only be

marked for a short duration, and/or where the plants would otherwise be difficult to locate in the surrounding vegetation. Marking seedlings may require less permanent methods as mortality can be quite high. Using toothpicks or other small markers to designate where seedlings emerged may be enough to census seedling number, and indicate survivorship over the time frame of the next site visit, whether that is between years, or across a single field season.

Figure 1.4. A photo showing a plant marked with a pre-numbered aluminum tag "nailed into the ground".

For more permanent placement, the tags may be "nailed to the ground" next to the plant using stainless steel nails (Figure 1.4), or using a wire loop around the base of a woody stem or on a substantial branch. Using a stainless steel nail is best practice, as they are inert, and placement of the nail is important to decrease the likelihood of damaging the root system or other underground parts such as rhizomes or tubers. Depending upon the belowground morphology of your species, placing the tag a few centimeters away from the plant may be prudent. In one study site, we place the tag 2 cm due north of each study plant. In practice, however, it may be difficult or time consuming to determine due north, so placement as close as practicable to the plant may be preferable. In one application, we have used plastic cable ties to attach tags to the taproot of a plant in a dune system. We were then able to bury the tag under the sand so as not to attract people and to disguise the study site.

Metal tags have an additional advantage over plastic or other material: they can be relocated using a metal detector, which significantly increases the recapture rate. This is particularly true where litter builds up and buries the tag, such as in habitats or sites where proscribed fire intervals are infrequent, or where the tag is purposely buried to disguise the study site or decrease the likelihood of tag movement or inadvertent removal.

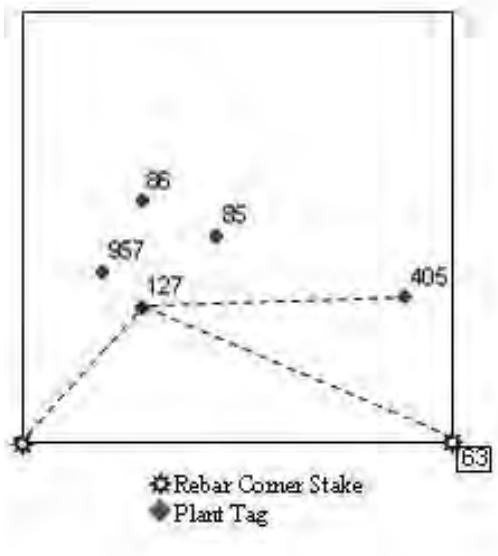
Be aware that tags may have to be replaced or repositioned periodically. Repositioning is necessary when plants have creeping rhizomes that lead to "foraging" across the habitat. Some plants may move a few centimeters a year, so careful annual repositioning may be necessary to keep up with the creeping nature of plant movement. Despite best efforts to the contrary, tags often go missing, usually in an unexplained manner. Therefore, having an additional method to relocate the plants that does not depend upon visual recapture of the tag is necessary. Regardless of the method of tagging a plant, the best way to facilitate relocation is to map all study plants, and determine a method to use the map to relocate plants and/or tags in the field. Depending upon the technology you have available, high-accuracy GPS is perhaps the most efficient way to map tagged individuals, particularly when plants are widely scattered across the site or where plot size is very large (>5m^2), although having very high accuracy GPS coordinates to map individuals in small plots (1m^2) is also very useful.

The accuracy of using GPS coordinates will vary depending upon the plot size and the method used to obtain the coordinates.

Trimble makes differential GPS units that vary between low accuracy to very high accuracy. The expense increases with increasing accuracy, of course, and the technology changes rapidly. TopCon also makes a very high accuracy GPS, or survey grade, unit. Both Trimble and TopCon have units that provide "real-time" very high accuracy (to the decimeter), when you have a cellular phone connection. You also have the option of "post-processing" your GPS data, to correct for measurement inaccuracies that occur in the field when there is no cellular phone connection. We have found that mapping with high accuracy, post-processing the data, and using maps generated from these data to relocate plants in the field is very successful. Using a high accuracy GPS unit in the field is also very useful when relocating plots.

In Figure 1.5, a simple plot map was generated using high accuracy GPS coordinates for the plant tags and the corner stakes. Plots are always oriented north-south and the plot is always identified with a tag in lower right corner. Two pieces of rebar are pounded into the ground to permanently mark the plot corners. The square represents a meter-square plot that is identified in the field by laying a meter-square plot frame, built from ½ inch PVC pipe, flush with the plot corners. Tags are either visually relocated by a thorough search of the plot, and/or using the plot map relocated by searching the area indicated by the dashed lines. The dashed lines represent a meter-stick or measuring tape used to triangulate the location of a tag from known permanently marked locations, such as the plot corners or another plant. By using two measurements from the corners simultaneously, it is possible to quickly locate hidden or obscured tags. If necessary, the distance to a third known location may be used. The area indicated may then be searched with a metal detector if the tag cannot be visually located. Using GPS coordinates to generate a plot map such as this has the added benefit of accurate distance measures between the objects. This data can also be generated on the fly using the handheld units.

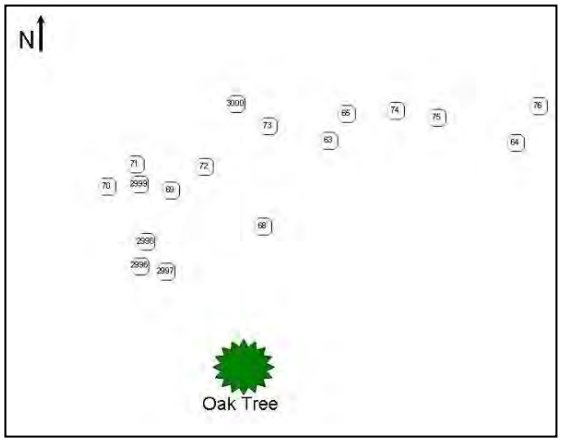
Figure 1.5. Plot map using high accuracy GPS coordinates for plant tags and corner stakes.



In the past, we have utilized a plotless technique where a permanent stake was placed in the center of a population or group of plants, and the distance as well as angle from the stake was measured for each plant. This technique is particularly useful in a dynamic environment such as dune or beach, where shifting sands may render individual tags unfeasible. It is also useful in situations where a population of a sensitive species is highly visible, and placing permanent tags would draw too much attention to the study

site. If such a plotless technique is employed, however, using two or three permanent stakes and triangulating among them is far more effective in relocating individuals than using a single center stake distance and angle.

Figure 1.6. A map of several plots in relation to an oak tree being used as a monument.



All of the techniques described here to mark and map individual plants can also be employed to mark and map both plots and transects. However, there is an increased need to ensure that plot and transect markers don't move, even in dynamic environments such as a dune. Plot corners and transect monuments need to be as permanent as possible, similar to a survey marker or monument. The easiest and least expensive way to monument a plot or transect is to simply use an object that is already in place, such as a tree or sign post (Figure 1.6). Generally, this will only monument one point of a plot or transect, so another method is to use a piece of rebar pounded into the ground to mark corners or endpoints of plots and transects, respectively.

The length of rebar used should reflect the conditions at the site, in particular the soil texture and depth, as well as the desired visibility of the marker. For example, using a 1.5-meter stake will be fairly visible, even if 10cm are below ground. This can be very useful, even desirable, depending upon the situation. Some investigators paint the top of the stakes to make them more visible, or tie flagging tape to them for ease in relocation. In a highly visible location, however, this may be considered an eyesore, or put the investigation or population in danger of trampling or vandalism. In this case, rebar may be placed such that it is just a few centimeters above the soil surface, which can aid in relocating by sweeping a metal detector or a foot over the area.

In some applications, using magnetized survey markers or nails may be useful. In particular, where visual relocation may be problematic, or in dynamic habitats such as stream sides or dunes, specialized magnetic markers such as Deep-1 (www.berntsen.com/Go-Shopping/Utilities/DEEP1-Magnets-for-Utilities) can be relocated with a magnetic locator even when buried a meter under debris or shifted soil.

Statistics

We have written this manual making the assumption that users have a least a basic understanding of statistics and data analysis. Further, we have made the assumption that the user is interested in monitoring the responses of a rare plant population to management or some environmental effect that is similar to some form of treatment in an experimental setting, although we do not expect users to implement an experimental paradigm. In other words, we expect that users are interested in monitoring outside of

an experimental setting, or that users might be observing what might be considered to be a "natural" experiment, without any form of manipulation. What we hope to provide is the statistical framework necessary to look at trends and responses of populations, rather than a comprehensive discussion on statistics, which is beyond the scope of this manual. Additionally, any data collection scheme and analytical design can be improved. It is always a good idea to consult with a statistician before beginning a project. It is easier to change the data collection protocols before too much data has been collected, than it is to change them in mid-study. It is also easier to change collection protocols than attempt to analyze data that is inappropriate to answer the question you would like to address.

In this context, we expect that users will implement a monitoring design that will be analyzed using a parametric framework, as well as some non-parametric approaches that might prove useful in understanding trend data. Parametric analysis assumes that data were sampled from a population that has an inherent and known distribution, from which the parameters of the distribution are subsequently estimated from the sampled data. Parametric analysis refers to the statistical tests and theories that are commonly used in basic statistics courses and applied in many ecology courses, such as analysis of variance or regression analysis. For example, the most common parameter used to describe a characteristic or feature of a population is a mean and variance, where the variance is calculated from a sample of continuous data that has been drawn from a population that is normally distributed.

Monitoring Population Changes in Response to Management

Monitoring a population might entail trying to determine the response to a management treatment where the aim is to increase reproduction. The monitoring design might call for a count of flowers, fruits and/or seeds in managed versus unmanaged plots or areas. Appropriate parametric statistical tests might include analysis of variance (ANOVA) or t-tests if the sample size is small, while an appropriate non-parametric test might be a chi-square goodness of fit test on the count data. In Figure 1.7, the total number of plants in grazed versus ungrazed plots were counted to determine if grazing increased total plant number. As these were paired samples, and the number of plots (our sample unit) in each pair is less than 30, a t-test for paired samples is an appropriate test and can be done under the data analysis tool add-in in Excel. In this instance, we are interested in determining if grazing either increases or decreases total plant number, so a two-tailed test is the appropriate choice.

Table 1.1. The total numbers of plants in grazed versus ungrazed plots. The results of the t-test for paired samples show that the means in the grazed versus the ungrazed are significantly different

Plot_ID	GrazingRX	Tot_Plants_Plot	Plot_ID	GrazingRX	Tot_Plants_Plot
73	Grzd	2	68	UnGrzd	0
74	Grzd	0	69	UnGrzd	1
75	Grzd	5	70	UnGrzd	3
76	Grzd	0	71	UnGrzd	0
77	Grzd	1	72	UnGrzd	1
80	Grzd	1	78	UnGrzd	0
84	Grzd	3	79	UnGrzd	1
85	Grzd	2	81	UnGrzd	2
86	Grzd	4	82	UnGrzd	0
87	Grzd	7	83	UnGrzd	2
88	Grzd	5	89	UnGrzd	2
91	Grzd	2	90	UnGrzd	0
95	Grzd	2	92	UnGrzd	1
96	Grzd	4	93	UnGrzd	5
97	Grzd	3	94	UnGrzd	0

t-Test: Paired Two Sample for Means

	Grazed Plots	*Ungrazed Plots*
Mean	2.733333333	1.2
Variance	3.923809524	2.028571429
Observations	15	15
Pearson Correlation	0.501288849	
Hypothesized Mean Difference	0	
df	14	
t Stat	3.360005081	
P(T<=t) one-tail	0.002335036	
t Critical one-tail	1.761310136	
P(T<=t) two-tail	**0.004670071**	
t Critical two-tail	2.144786688	

You might decide to ask a different question at the beginning of your monitoring design, or you might not have the resources to count every plant in the monitoring plots because of the field time involved, or because the species is cryptic or difficult to identify to species at every life history stage. In this case, you might instead record only

the presence/absence of the plant in each plot. Using the same data in the table in Figure 1.7, the ratio of presence/ absence in grazed versus ungrazed plots would be 13/9. At the beginning of the investigation, each of the 15 grazed and ungrazed plots contained at least one plant. A contingency table analysis undertaken with a Chi-square test or Fisher's Exact Test would be useful here. This results in a p-value = 0.215, meaning that there is no detectable difference in Presence/Absence of plants in grazed versus ungrazed plots. We could increase our ability to detect a difference by increasing the sample size. If we had measured another variable, such as grass height resulting from the grazing or an estimate of the percent cover removed by grazing, we might instead opt for a logistic regression on Presence/Absence.

Table 1.2. A Contingency table analysis using the grazed versus ungrazed data from Figure 1.7. The results show no detectable difference in the Presence/Absence of plants in each kind of plot.

	Absent	Present	Total
Grzd	2	13	15
UnGrzd	6	9	15
Total	8	22	30

Test statistic	Value	Df	Prob
Fisher exact test (two-tail)			0.215

Monitoring Population Changes Over Time

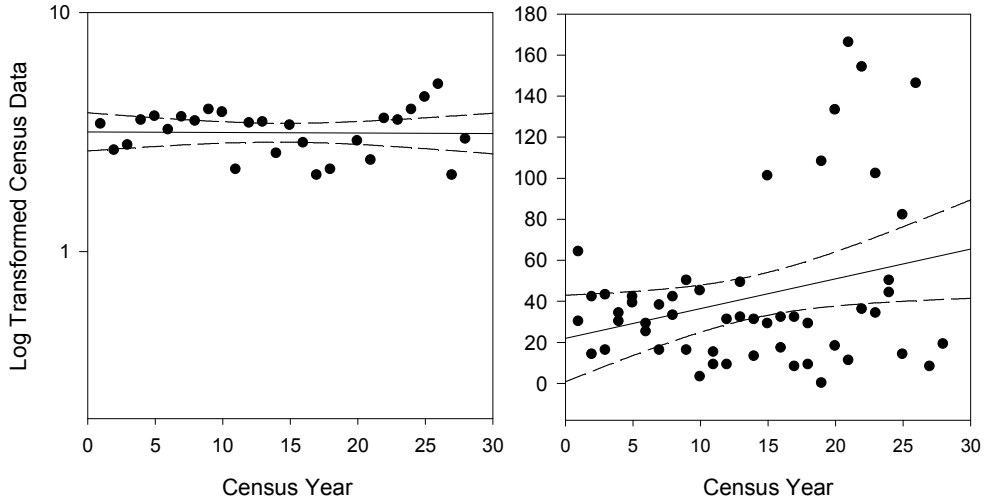
Detecting population changes over time is quite problematic for many monitoring programs. Several things account for the difficulty, including misidentification of the target species, difficulty in distinguishing cryptic species or life-history stages, inaccurate and/or imprecise count data, high variability in count versus estimated counts among different investigators or monitors, and data collected across unequal intervals. Seedlings or juveniles of many, if not most, plant species are difficult to detect and to identify, leading to underestimated counts of over population size. In theory, if surveys are done at the same time for each data collection interval, and the life history stage is highly detectable, for example, an endangered orchid monitored during the period of highest flowering, estimates of such highly visible stages may be reliable. In practice, however, it may be logistically difficult to implement a monitoring design that is perfectly repeatable across sampling intervals or monitoring teams, especially when multiple sites are being monitored, because of errors of variation in detection.

Ensuring that a monitoring program will have the capacity to detect a change in population status requires a commitment to good data collection strategies. Increasing the duration of the monitoring program from a few years to a decade or more will vastly improve the power to detect trends, and 15 years seems to be "the sweet spot" allowing several approaches to be fruitful. Humbert et al (2009) recommend a minimum of a 10-year time frame, with a minimum of 5 collection intervals. In addition, if a monitoring program cannot be conducted thoroughly every year, it is better to conduct surveys in alternate years or every three years, and devote the effort to precise counts in those years, as opposed to diluted effort annually. Having equal intervals may also help in this regard. Therefore annual, biennial or triennial counts should be maintained to increase the power to detect changes.

Analyses of trends in these data are predominately accomplished via loglinear regression of the count data over time. Loglinear regression is the preferred method of analysis because the relationship between the dependent and the independent variable is converted from a multiplicative relationship into an additive relationship during the log transformation (Humbert et al, 2009; Nur, et al 1999). In addition, log transforming the variables will often normalize the distribution, and therefore avoid violating the assumption of normally distributed data when using parametric data analysis.

In Figure 1.9 below, a population of *Lespedeza leptostachya* that has been censused over many years shows significant variation in counts over time, ranging from 3 to greater than 166. Loglinear regression, shown in the left panel, overcomes the tendency of the regression line to be overly influenced by growth in the latter years of monitoring, and reveals that the population has a tendency to be more or less stable over time. In Appendix B, we use the same data and show that the population only has a 30% chance of going extinct over the next 100 years, providing another means of assessing the stability of this population.

Figure 1.7. A loglinear regression of a population of Lespedeza leptostachya that has been censused over many years, showing that the population has a tendency to be fairly stable over time.



Population Trajectories and Projection Models

Population Viability Analysis (PVA) provides a method to examine the general trajectory of a population. In particular, PVA seeks to address one of two questions: 1. Is the population likely to be there in the future; and 2. Is the population size stable? The first question seeks to quantify the probability that a population will be in the same location at some point in the future by determining the likelihood of extinction. The second question seeks to understand something more about the populations by quantifying the number of juveniles, reproductive and senescent individuals and their contribution to the population growth rate over time. Both approaches are trying to determine if the population is stable over time, or if it is growing or shrinking. A shrinking population has a lower growth rate and a higher probability of extinction than a population that is growing or stable.

There are several ways to undertake a PVA, depending upon the type of data collected, the population size and the ability of an investigator or monitor to access software. In this guide, we present two ways to undertake a PVA using Excel spreadsheets and Excel add-ins that are freely available to download. We show you how to obtain and use count data to undertake a count-based PVA using the equation functions in Excel to explore the probability of extinction. We also show you how to obtain more detailed demographic data on a sample of individuals in a population to build a population trajectory model using PopTools, a freely available add-in for Excel. Population trajectory models allow you to explore both the growth rate of a population, and the contribution to growth provided by juveniles and currently reproductive individuals by exploring the probability of survival and reproduction of individuals based on either their size or their life stage.

A PVA is only as good as the data. Even a biologically sound model cannot make up for a too-small data set. Sparse data will decrease precision in estimates of both population growth rates and in extinction probabilities (Morris and Doak, 2002). Pooling data collected across years can be used to overcome data sets that result in too few individuals in each size or stage class (Morris and Doak, 2002). In addition, it is important to bear in mind that the simple matrix models presented here do not take density-dependence into account, and vital rates for many species will certainly be affected by density. However, meta-analysis of matrix projection studies of 20 plant species revealed that there is little evidence that density-dependent models substantially improve the ability of matrix models to forecast future population growth (Crone et al, 2012).

Population Viability Analysis can be used to guide management decisions such as proscribed burning regimes (Evans, Menges, Holsinger, 2008; Kaye, et al, 2001; Caswell, and Kaye, 2001); deer control (Knight; 2004; Vitt et al, 2010); brush removal (Vitt et al, 2010), how many individuals to use to augment an existing population (South et al, 2000) and setting limits on the harvest from a population (Endress et al, 2006;Ticktin, 2004). In addition, the Recovery Plans of many Federally listed species specify a minimum number of viable populations before a species can be considered

"recovered," and Habitat Conservation plans often contain a monitoring requirement. PVAs are often the simplest way to summarize monitoring data in a manner that can "use the past to estimate the future." Most plant ecologists tend to use population growth rates, rather than extinction rates, as their metric of population viability (Crone et 20111). Indeed, it seems self-evident that populations with a rapidly declining growth rate are likely to go extinct, while one with a rapidly increasing growth rate are likely to be viable (Crone et al, 2011).

Most monitoring programs are generally too short to capture all of the variability experienced by a population, and increasing data collection across addition years increases the ability of matrix analysis to forecast outcomes of the populations under study (Crone et al, 2012). For example, an extreme drought that causes high mortality in the usually long-lived adults, or early spring rains coupled with mild temperatures that increases the germination rates of seeds in a long-lived seed bank may only rarely be captured during a sequence of data collection. Either of these examples of environmental stochasticity will change the dynamics of a population in ways that a short-term monitoring program might miss. In addition, we only present examples of deterministic matrix models in this manual, while models that explicitly attempt to include stochasticity are more realistic (Crone et al, 2012).

A meta-analysis of matric models on 20 plant species found that growth grates tended to be over estimates (Crone et al 2012), and did not successfully forecast the futures of the populations under study. Therefore, it is best to interpret the results of a PVA as a general tendency, either for a long-term decline, or a short-term spike in the growth rate. Populations, by their very nature, are quite dynamic and the environment is experiencing rapid changes due to drivers beyond our immediate control such as climate change. Indeed, the meta-analysis undertaken by Crone et al (2012) revealed that future-casting of population fates generally failed because the environment of the study period was different from that of the model outcome evaluation period. This does not negate the value of matrix models, however (Crone et al 2011; Crone eta al 2012).

It is important to appreciate that the results of a PVA are best interpreted as a relative population growth rate or risk of extinction, rather than providing an absolute "answer" about what to expect in future (Morris and Doak, 2002; Crone et 2011; Crone et 2012). Indeed, obtaining an estimate of extinction risk or population growth rate can be an useful means of assessing the trajectory of a population with or without management inputs. The trick is to monitor a population long enough and well enough to incorporate some of this dynamism into your PVA and then interpret the results "as if" the environment is stable, while understanding that, in reality, things are going to change.

Type I and Type II Errors

The aim of any statistical analysis is to test if two or more samples are truly different from each other, and there are two ways that such a test can fail. The first type of error, Type I, is essentially a false positive, where the test results lead to the conclusion that the two samples are different, when in fact they are not. The second type of error, Type II, is a false negative, where the tests results lead to a conclusion that the two samples are not different, when in fact they are. All statistical tests have some chance of resulting in either a Type I or a Type II error. Our job in creating a monitoring program, complete with an appropriate sampling scheme leading to a statistically powerful sample size and the correct test statistic, is to reduce the probability of generating either type of error.

Sampling design will greatly affect your ability to avoid both types of error, and you should consider consulting a statistician before you implement a monitoring program, or soon after you have a year's pilot data, to ensure that your design will enable you to detect changes in your population(s) and/or answer your management efficacy questions. In the above grazing sample, the number of plots was limited to 30, 15 grazed and 15 ungrazed to limit field time. If the design had called for observing presence/absence only, we would not have had the statistical power to detect a difference in management (a Type II error). Increasing the number of plots may have allowed us to detect a difference while recording only presence/absence data, but only taking full counts of all plants in the plots would allow us to detect a difference given our small sample size.

In the same study, however, we did not have enough statistical power to detect if the number of juvenile (non-reproductive) plants was higher in the grazed plots, which was a response of considerable interest because it would have indicated successful seedling establishment under the management treatment. The trend towards increasing the number of juveniles was there, and a t-test on these data resulted in a p-value of 0.06. This was very likely a Type I error, resulting from high variance around the mean because of a small sample size. In this particular case, because of the small sample size and the p-value close to 0.05, it is likely there was a difference that we were unable to detect statistically. Indeed, subsequently increasing the number of plots did result in a statistically significant difference in the number of juveniles in treated versus untreated plots.

To ensure that you have the power to detect the responses of interest, it is important to consider a power analysis before you fully implement your design (e.g. Lehtilä et al, 2006; Orrock et al, 2006; Thomas, 1997), Seed predation, not seed dispersal, explains the landscape-level abundance of an early-successional plant. Journal of Ecology, 94: 838–845). Analyzing the first year's data as a pilot study will also provide insight into your ability to avoid both Type I and Type II errors.

Detectability

The ultimate reason to initiate a rare-plant monitoring program is to detect changes or responses in a population over time. All methods of detecting such a change rely on the ability of an observer to see what they are monitoring. Many plant species are difficult to distinguish from the background vegetation unless they are in flower, and most species have one or more life history stages that are very difficult to distinguish from the background vegetation. Some species have stages in their lifecycle that are entirely below-ground, and some will even experience a dormant year without an above-ground appearance only to reemerge in a subsequent years.

Given that our ability to detect changes in populations is often limited when their populations, and thus sample sizes, are small, detectability of individuals in the population will affect our results and conclusions. The impact of detectability on the accuracy of a count can be presented as follows:

$$E(C_i) = N_i \, p_i,$$

where C_i denotes the count, N, the true abundance, and p_i the detection probability, all associated with time and location *i* (Nichols et al, 2000). When an estimate of the probability of detection is included as part of the monitoring design, a more realistic estimate of abundance or population size is possible.

Alternatively, a monitoring program can be designed with the assumption that the detection probability will be the same at different times, locations and by different observers. This is generally the default assumption made by most plant monitoring programs, as it is quite unusual for a rare plant monitoring program to explicitly include detectability in the sampling scheme.

SECTION II: Level 1 Monitoring

Level 1 Monitoring

Level 1 monitoring is most appropriate when collecting baseline data for a population or species and encompasses both presence/absence approaches

- Level 1A; monitoring to see *if* a species/population or species is present) and complete or estimated population censuses
- Level 1B; monitoring to see *how much* of a species/population is present).

Level 1A Monitoring – Presence/Absence Monitoring

Number of People Required: 1-2
Estimated Field Days/Year Required: 1-2 per site (this will vary depending on species monitored)

Level 1A monitoring is the least time- and resource-intensive level of monitoring and is most appropriate when the objective is to acquire *baseline data* on populations. A simple presence/absence monitoring scheme is appropriate for assessing the basic status of populations or species, and is often accomplished using a meander search method. A timed meander search involves walking 'randomly' through a site or plant association and noting each new species or new occurrence of the monitored species for a set period of time (Lancaster 2000). Setting a time limit for the meander search assures increased consistency in sampling intensity among sites. It has been shown that when budgets are small, collecting presence/absence data can more reliably capture population decline than collecting abundance data due to the decrease in variability among estimates in decline rates (Joseph et al. 2006). It should be cautioned that this method may not be sensitive enough to detect small amounts of decline in the time desired to trigger a change in management.

Data Collection

Using a presence/absence technique requires very little time or resources, as the only required skill is the ability to identify the focal species correctly. Presence/absence monitoring is most efficient to use with easy-to-detect species due to the rapid nature of this technique. It will be less efficient and therefore less effective for species with low detectability, *ie*: those that are cryptic, difficult to identify, or difficult to distinguish from closely related congeners. This type of monitoring is helpful in cases where many small populations need to be monitored. It takes less time to assess the presence of a species than to estimate its abundance, which allows for assessments at numerous sites in a short period of time. It is important to keep sampling intensity and scale constant through repeated measures of presence/absence if you hope to compare measures from different years or different locations. These data can be collected while other monitoring activities are being carried out, with little extra time or effort exerted. Some key points about Level 1A monitoring are listed below:

Areas to search for species

- Previously known locations
- Historical locations (determined from herbarium or other records)
- Areas with high-quality, appropriate habitat
- Areas where you may already be monitoring another similar or commonly-associated species

Data to collect

- Presence or absence of species or individuals
- Additional information can include:
    - date of monitoring
    - observer first and last name(s)
    - time spent at site
    - general notes on habitat conditions
    - presence of exotic species (invasive plant species, earthworms, etc.)
    - immediate threats
    - a survey map showing the general survey track
    - a general estimate of population size

Additional Recommendations

- Search for plants during the time of year when they are most visible (usually when flowering/fruiting) making sure the timing is consistent from survey to survey
- Record GPS coordinates, this will aid future searches, and be used to monitor spatial and temporal changes in the species/population
- Take photographs from a designated photo point to help future searches and help to qualitatively compare habitats/sites over time
- Use witness trees or other landscape features to adequately describe the location and cut down on time lost trying to relocate poorly marked populations

<u>Data Analysis</u>

Recording simple presence/absence data for a single population provides no means for detecting trends, unless the site is routinely inventoried, multiple sites or sub-populations are inventoried, or other habitat data are collected in tandem. It is also possible to use these data to understand local spatial population dynamics over time if multiple locations or sub-populations are included in routine monitoring. If the number of populations decreases, this can be evidence that the species may be in decline, and it should be a candidate for a more intensive monitoring scheme. As a presence/absence monitoring scheme is implemented, it should be noted that the number of recorded populations would be expected to increase initially due to new populations being found from expanded searches (Menges and Gordon 1996). In addition, to maintain precision in the estimated number of populations present, suitable habitat with recorded absences

should continue to be monitored for presence absence to obtain information about the spatial dynamics of the species, as well as to accurately observe the number of populations.

Taking GPS coordinates will help track changes in the number of populations and their locations, and if time and resources allow, each population should be mapped as a GPS polygon. If time, or the terrain, does not allow, a gps point should be taken, and the extent of the population may be recorded on the data sheet, or hand-drawn on a topo map. When hand-drawn on a topo, the polygon can be digitized into a GIS at some point in the future should this become desirable. Recording GPS points along the cardinal directions at the perimeter of the population can also be used to create a polygon such as an ellipse in a GIS. These spatial data help to detect changes in spatial distribution, or the spatial extent occupied by a population, sometimes called occupancy, which may assist managers further assess threats to population persistence. These data can also be mapped along with different environmental or topographical data, allowing for a greater picture of where the populations occur, where they are disappearing, and what variables (temperature, precipitation, elevation, etc.) correlate with those occurrences. Using widely available spatial datasets that cover hydrological features, topography, elevation and/or soil profile, spatial analysis in a GIS have the power to provide a more detailed picture of the habitat requirements for a given species, or correlated with a pattern of appearances/disappearances over time to better understand the species. For example, species that respond to seasonal flooding are likely to be found at slightly different elevation or slope in different years as they respond to fluctuations in inundation patterns.

These types of inventory data, especially when properly georeferenced with GPS coordinates, can also provide powerful insight into the distribution of the species as a whole, when aggregated with similar data collected across the species range. Data housed in state Natural Heritage inventories, especially when aggregated into conglomerated data sets such as those managed by NatureServe or GBIF have the potential to be used in GIS-based modeling projects that can determine the potential as well as realized distribution of a species. Such modeling efforts can also help establish the particular niche which they occupy, nearby habitat that may contain undiscovered populations, and their potential response to rapid environmental changes such as changes in climate. Such aggregated databases can also reveal species-wide declines in abundance across their range. Typically, absence data for most species are typically lacking, but can be very powerful when predicting distributions through modeling. This makes it important to collect and report absence data as well as presence data.

Level 1B Monitoring – Population Census Monitoring

Number of People Required: 2+
Estimated Field Days/Year Required: 1-2 per site (this will vary depending on species monitored)

Completing a population census requires more work than a presence/absence study, but provides greater detail on the status of a population or species. A major advantage to this approach is the ability to use data to perform a count-based Population Viability Analysis (PVA). PVA can be used to predict the future viability of a population or species. Collecting enhanced census data, where stage classes are recorded along with population census data (referred to as Level 1.5 monitoring in this manual; see page 35) requires little additional time or effort than a simple census, and can increase the precision and reliability of a PVA. Therefore, a strategic census provides strong foundational data that can easily be enhanced (to include a full demographic analysis, Level 3 Monitoring, if desired) to gain more predictive and explanatory power for your population or species.

Data Collection

When designing a population census, a number of parameters need to be considered, including when, where and how to collect the data. The monitoring objectives of each project will guide where and how a population census will be conducted. A practice census should be performed when possible, particularly when the group of monitors includes less experienced participants. Monitors should work in teams of at least two in order to decrease missed occurrences and generate consistent and reliable data (Henderson 2009). Photos representing each life history phase for which data are to be collected can help develop and hone search image skills for new investigators and serve as a reference in times of uncertainty.

Census locations

A census is most often effective when monitoring rare plants in known populations, rather than when searching for new or historical populations. The population census area should be clearly marked and mapped to enable relocation in following years (see discussion of Quadrats and Transects in Section I). It is also important to conduct censuses in a systematic manner that minimizes errors in data collection, including double counting. Attempting to census every individual in a population can be difficult when populations are large (>500) or when individuals are scattered over a large area. The difficulty in censusing a population dispersed across the landscape will be influenced by habitat type. In an open setting such as a grassland, meadow or dune with unrestricted sight lines, finding and counting plants across an area as large as several acre may be fairly straightforward, especially with the judicial use of highly colored pin flags. In other settings, dense woodland or shrubby thicket, with restricted sight lines, even an area 20 to 30 meters square will provide a challenge. In these situations, setting up plots with parallel linear transects (see Figures 2.1, 2.2, and 2.3) is often considered

the simplest and most effective method (Elzinga et al. 1998; Lancaster 2000; Henderson 2009). These plots should be established in a representative portion of the population in regard to general density and habitat conditions. These considerations help to avoid errors in collecting data, and provide a more robust picture of the status of the population or species.

Timing of Population Census

For population censuses to be the most time and resource efficient, they should be undertaken when the plants are the most visible, usually during flowering and/or fruiting. Because some species flower for only a short period, the timing of a census can be critical to recording accurate data. Conducting a census at the same time each year, or during the same phenological phase (peak flowering or fruiting), ensures that the censuses can be comparable across years. It should be noted that climatic differences among years (e.g. low snow melt, spring temperatures, summer droughts, etc.) can result in altered phenologies, making it important to note the dates and phonological phase during which monitoring is completed. Information including the emergence timeframe and average flowering and fruiting times should be described in the monitoring protocol, allowing easy notation of any departures from the normal time-frames. Not all plants flower each year, therefore, it is critical to be able to identify and count all stages of a species in order to achieve an accurate census number. Collecting data in this manner each year ensures that if any detection errors are made, they are consistent among years.

Life History and Census Unit

The life history details of the species being monitored must be incorporated into any monitoring program. Species with high dormancy rates and annual species with numbers that fluctuate greatly from year to year can be difficult to accurately track with a population census. Species with dormant or cryptic life stages can be underrepresented or underestimated with a population census due to the portions of the population not being counted each year.

Defining a clear census unit is one of the most important factors to consider when completing a census. Census units may include individual stems, ramets, genets, clumps, or some other unit depending on the life history of the species being monitored. The census unit must be well defined, easily identifiable, and consistent to ensure that the data are comparable across multiple years and observers. This can be complicated for certain species, especially those with clonal growth forms where individuals are difficult to define. The census unit, along with any age or size classes that will be omitted, should be well-documented to ensure that the data remain consistent over time. Any additional details of the life history of the focal species that may be pertinent (e.g. in some legumes, the first few true leaves are unifoliate rather than trifoliate) should be well known before beginning a population census to ensure data accuracy.

Duration and intensity of data collection

When the ultimate goal of a population census is to conduct a count-based PVA, a minimum of six consecutive years of data is recommended, but ten or more years is preferred (Morris et al. 1999), as shorter durations do not provide a reliable prediction of extinction probabilities. It is important to note that some genera may require a longer time period or less frequent monitoring (every 2 or 5 years) to adequately determine a trend. In order to determine the appropriate methods and time frames, the ecology and life/cycle of the target species should be well understood. While regular population censuses allow for calculating an extinction probability from a basic count-based PVA, enhanced census data allow for additional predictive ability when conducting a PVA. Enhanced census data include stage class or reproductive state (seedling, vegetative, reproductive, etc.) of each individual, data that can be collected with little additional time or effort (See Level 1.5 Monitoring).

The following is a general guide to performing a population census for a rare plant. These steps are a basic guideline and may need to be amended to accommodate the needs of the particular species or population being monitored.

Step 1: Familiarize yourself and your team with the focal plant and location.

- Identify where the monitoring should occur: known populations, areas and habitat where the species is likely to occur, etc.
- Identify the best time of year to monitor (plants are most visible, easily-identifiable)
- Identify the census unit to be used (individual stem, clump, etc.).
- Ensure every member of the team has a reliable search image of the plant (and/or provide photo reference cards)
- **NOTE Optional**: If recording stage class data, make sure all members of the team can recognize each of the life history stages of note (see Level 1.5 Monitoring)

Step 2: Identify and mark the area to be searched.

- Identify and flag population boundaries or individual census units
- Mark or map the area for successful relocation in subsequent years (permanent markers, GPS locations, etc.)

Step 3: Design a structured search of the area.

- When designing a complete census:
  - Parallel transects (~1m apart) are an easy and effective way to cover the area without overlap
  - A grid system or quadrats can also be used to break down the area (for ideas see Elzinga et al. 1998)
  - Flagging plants, first during a meander-search and then with another color flag once measured, is often helpful to avoid missing plants or counting them twice
- When using estimation methodologies:

- If population is very large or spread out, place several large plots, each small and close enough to be viewed from a single point
- Use classes of abundance rather than exact numbers to increase accuracy with classes smaller at the low end (e.g. 1-10, 11-50, 51-100, 101-200, 201-400, 401-800, etc.)
- Define a minimum number at which to begin estimation methodologies, preferably greater than 150-200.
- The goal should always be to have actual counts not estimations whenever possible because the increased precision increases your ability to detect changes in population size.

Step 4: Count and record all observed plants.

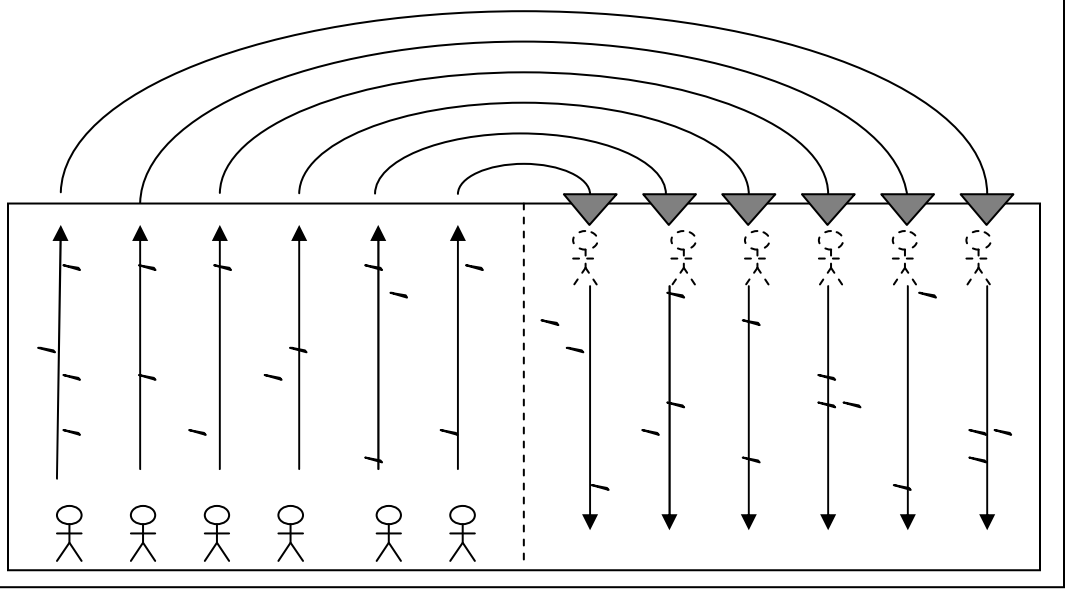
- Each member can record their own counts, or one official recorder can be designated, depending on the area being searched and the number of people involved

Examples of Field Protocols for Population Census

Lespedeza leptostachya
20+ years of data from Nachusa Grasslands, Franklin Grove, IL (Vitt)

- Censuses are completed in late August/early September after flowering and stage based demographic data are collected
- All observers are very familiar with the species and have a good search image
- Individual plants are not very dense and are located in well-known and well-defined areas.
- Participants form a line with ~1m between each person and walk parallel transects counting flowering and vegetative adults. Important: Due to dense surrounding vegetation, juveniles and seedlings are missed because they are not visible with this method (they are, however recorded in full demographic monitoring)
- At the end of each set of transects, the group flips and walks the opposite direction along parallel transects to cover the next section.
- One person has two handheld counters (one for vegetative and one for flowering plants, or one tally counter with multiple banks for entry) and the rest of the group calls out counts to him/her

Figure 2.1. Diagram of set-up for complete population census of *Lespedeza leptostachya* at Nachusa Grasslands



Oenothera haringtonii
4+ years of data, southeastern Colorado (Skogen)

- Censuses are completed at the end of each flowering season
- Because populations are small (<750) and surrounding vegetation is sparse, a complete census of all individuals is possible
- Parallel transects are marked off, and a team of monitors walk the transects and flag each plant they find
- Each person then turns around and walks back over the same transect, picking up the flags and counting the plants
- Separate counts are recorded for vegetative and reproductive plants

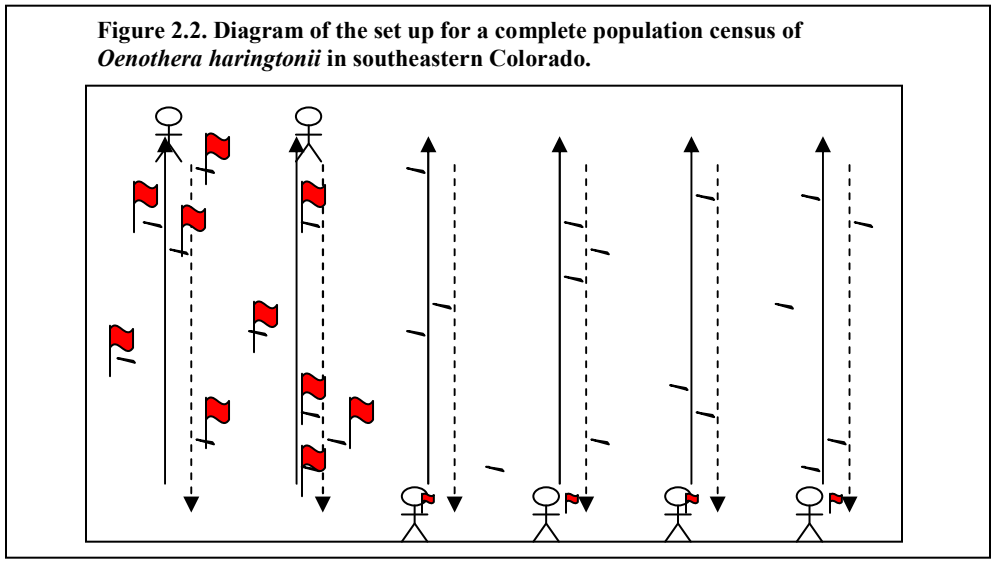
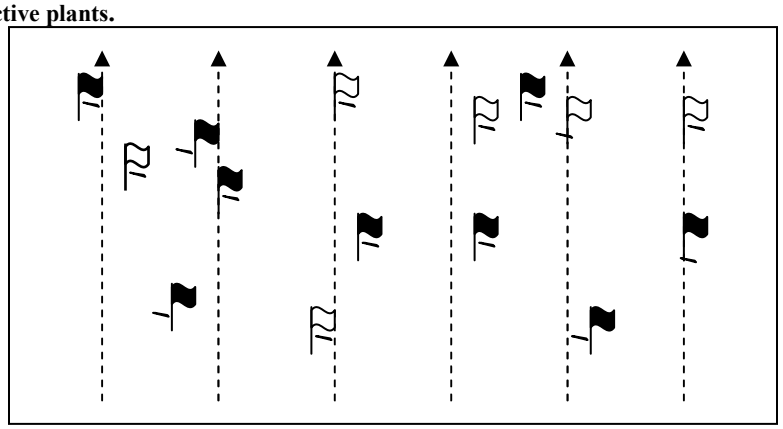


Figure 2.2. Diagram of the set up for a complete population census of *Oenothera haringtonii* in southeastern Colorado.

Cirsium pitcheri
2 years of data collection; Door Co., WI (Havens)

- Team members walk parallel transects and flag individuals with two different colors of flags (one for vegetative plants and one for reproductive plants)
- A picture is taken of each area while the flags are still in place to have a visual record of plant distribution for each year
- The flags of each color are counted and recorded
- This technique is good for areas of sparse vegetation and low population density

Figure 2.3. Diagram of a flagged population or *Cirsium pitcheri* for a complete census in Door Co., Wisconsin. White flags represent vegetative plants, while black flags represent reproductive plants.



Level 1.5 Monitoring (Enhanced Count Data)

Number of People Required: 2+
Estimated Field Days/Year Required: 1-2 per site (this will vary depending on species monitored)

In our previous report (Tienes et al. 2010), taking enhanced count data (recording the stage classes of plants during a population census) was highlighted as a monitoring technique that can provide some predictive power without the intensive time and resource investment required for a full demographic study. When choosing Level 1.5 monitoring, it is important to be mindful of the basic life history of your target species when viewing the distributions of stage classes. Long-lived perennials tend to have stable population sizes when there is a strong standing census of adults with good survivorship. In these cases it is expected to see an uneven distribution of large to small individuals in a population. Conversely, short-lived perennials would be expected to have a larger class of small and medium-sized individuals in a stable population.

Data Collection

For example, when conducting a census of a population of oak trees, recording a lack of seedlings from year to year might not be cause for great alarm. The overwhelming majority of seeds and seedlings die in long-lived species, with only the rare individual becoming a sapling. In addition, only a small portion of those saplings will grow into a large reproductive tree. Therefore, in the short term (5-10 years) trends in the adult population and survivorship are most important to provide insight into the viability of the population. However, in the long term (10-20+ years) a lack in seeding and sapling recruitment can be much more damaging to a population. For short-lived species, a lack in annual recruitment may be even more alarming for the future of the population. The standing stage distribution within a population can be informative for the viability of a population, regardless of whether there are management activities or not. As with most count-based data, however, consistency is a necessity.

There are two ways to record enhanced count data, either by simultaneously recording observations of stage classes during a walking census, or by taking random subsamples before the full census. Simultaneous recording of stage classes is most useful when individuals (including seedlings) are easily distinguished, for example, in sparse vegetation, or when the populations are very small. Taking random samples can be helpful when it is difficult to distinguish juveniles from the background vegetation. For example, many terrestrial orchids have strap-shaped juveniles that resemble grasses or other monocots, making them very difficult to see during a walking census. Smaller, vegetative plants of certain species can also be difficult to detect in areas with dense vegetation. Therefore, the smallest size classes or stages will likely be underestimated unless a formal sub-sampling scheme is utilized.

If it is important to capture stage data during a walking census, the use of permanent sub-sampling plots is advantageous. Randomly placing plots in populations of rare

plants generally does not capture enough plants to undertake a statistical analysis, but judicious use of random plots can reveal details about the stage distribution of a population that a census alone will not. For example, when trying to determine if fire is useful for increasing the seedling stage of a prairie species, 5-10 temporary plots could be placed in known high density areas that have been both burned and unburned. In these plots, a thorough search would be conducted for individuals in the smaller size classes and stages that would be missed during a walking census. While not a statistically rigorous sample, this technique will provide an idea about whether or not fire is having an effect on seedling production and recruitment. The results from this can then be used to instigate a more detailed study on this issue if warranted. These plots will be most useful when sampling long-lived species, as populations of short-lived species tend to be spatially dynamic, moving across the landscape in response to short-term environmental cues. In the case of short-lived species, a random subsample layout could include the use of a transect or random, temporary plots. We have used randomly placed transects to monitor seed predation in heads of *Cirsium pitcheri* on the dunes of Lake Michigan, for example. This allowed us to capture data on the flowers of randomly selected individuals in just a few hours.

Data Analysis (Levels 1B and 1.5)

Background

A count-based Population Viability Analysis (PVA) uses population census, or count data, to estimate the future extinction risk of a population. To undertake a robust data analysis, these censuses should be taken for a minimum of six years, but preferably more than ten years. In this analysis, simple census data are used to estimate population parameters, which are then used to calculate measures of population viability. The method of analysis described here was first proposed by Dennis et al. (1991) and is simple enough to be completed using basic statistical software or a spreadsheet program (Microsoft Excel is used in the examples presented here). Extinction risks are often strongly affected by environmental and stochastic factors not taken into account in a standard PVA, therefore, calculations of extinction risks should always be viewed cautiously (Crone et al. 2011). It should be noted that predicting extinction risks can be very problematic for plants specifically due to unobservable life stages and dormancy (Lesica and Steele 1994; Kendall and Nichols 2002).

A value that must be decided upon before a PVA can be completed is the quasi-extinction threshold (N_x) of the population. The quasi-extinction threshold is the estimated minimum number of individuals necessary to maintain a population below which it is likely to be considered critically imperiled (Ginzberg et al. 1982). This number is a characteristic of the particular species being monitored, as well as something managers can "set" in order to predict future viability. Problems like demographic stochasticity that affect extremely small populations, favor setting the quasi-extinction threshold somewhere between 20 and 50 individuals (Morris and Doak 2002). Additionally, because the effects of demographic stocahsticity differ depending on life-history strategies, the specified quasi-extinction threshold should also account for these differences (Fujiwara 2007). It has been shown that extinction risks caused by

demographic stochasticity increase with increased fecundity (Gilpin 1992; Kokko and Ebenhard 1996; Jeppsson and Forslund 2012) and decrease with delayed reproduction or an increased age of maturation (Jeppsson and Forslund 2012). Additional species characteristics, such as self incompatibility and Allee effects (Akcakaya 2000), may require that the quasi-extinction threshold is higher, up to 100 or more reproductive individuals. When dealing with species that are self-incompatible or short lived, a larger quasi-extinction threshold may be more appropriate, while a lower quasi-extinction threshold may be more appropriate for species that readily self and have a longer life span. However, for some species (long-lived perennials, shrubs, sub-shrubs, and trees), populations may persist at very low numbers (<50) for several years. In these cases, it may be appropriate to set the quasi-extinction threshold lower.

Box 1: Overview of count-based PVA calculations (modified from Morris and Doak 2002)

Definitions of variables can be found in Table 1, along with references to their locations in the Excel template for a sample count-based PVA.

Initial calculations

The first calculation to make when conducting a count-based PVA is the population growth rate ($\lambda_i = N_{i+1}/N_i$). Next, the continuous rate of increase for a population (r_i), can be easily calculated as the natural log of λ_i. These variables are calculated for each transition for which data have been collected. Two parameters that describe how the distribution of the population size will change over time 1) μ, the change in the mean of the normal distribution of the log of the population size, and 2) σ^2, the change in the variance of that same distribution, are then calculated from values of r_i and λ_i.

Calculating μ and σ^2

These parameters are calculated from r_i and λ_i, and the more years of data available, the more statistically robust these estimates will be. When a census has been completed on regular, yearly intervals, μ and σ^2 can be estimated directly as the mean and variance of r_i, respectively. If the census has been undertaken at irregular intervals, μ and σ^2 must be estimated using a linear regression. To do this, two new variables must be calculated, x_i and y_i, with $x_i = \sqrt{(t_{i+1} - t_i)}$, where t_i is the year in which the census was taken and $y_i = (r_i/x_i)$. After calculating these variables, a regression of y_i on x_i is performed, including forcing the intercept through zero. This can be done in Microsoft Excel by going to Tools>Data Analysis>Regression and filling in the appropriate **x** and **y** values, remembering to check the box "Constant is Zero" (Figure 2.4). The estimates of μ and σ^2 are then the values of the slope of the regression (A) and the residual mean squares (B), respectively (Figure 2.5).

Box 1 (Continued):

Figure 2.4: Screen shot from Microsoft Excel Regression tool.

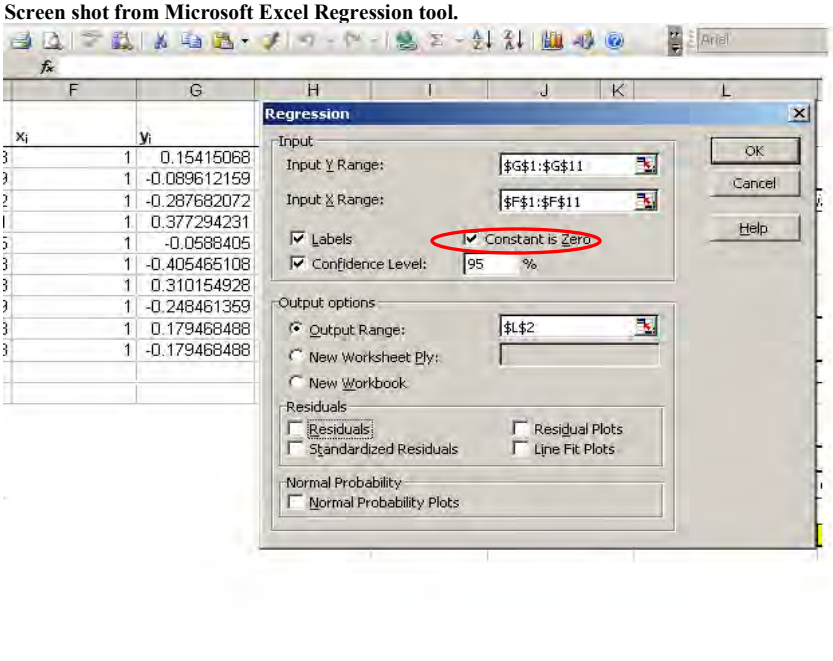


Figure 2.5: Regression output from Microsoft Excel showing the estimates of μ, the slope of the regression (A) and σ^2, the residual mean squares (B).

Regression Statistics	
Multiple R	0.1382003
R Square	0.0190993
Adjusted R Square	-0.0309007
Standard Error	0.5792855
Observations	21

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.130679659	0.1306797	0.389424	0.540023396
Residual	20	6.71143479	0.3355717		
Total	21	6.842114449			

B

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
1	0.078885	0.126410469	0.6240384	0.539656	-0.184802631	0.3425726	-0.1848026	0.3425726

A

Box 1 (Continued):

Because these measures of μ and σ^2 are only estimates, we want to calculate the upper and lower confidence intervals for each, to place the estimates in context. Confidence intervals are a measure of uncertainty and provide upper and lower values between which the true value of the variable is likely to lie. In order to calculate confidence intervals, two additional variables must be added to the analysis: ***α***, the confidence interval being solved for (usually 95% or α = 0.05), and ***q***, the number of data points included. Confidence intervals (CI) can then be calculated using the following equations in Microsoft Excel:

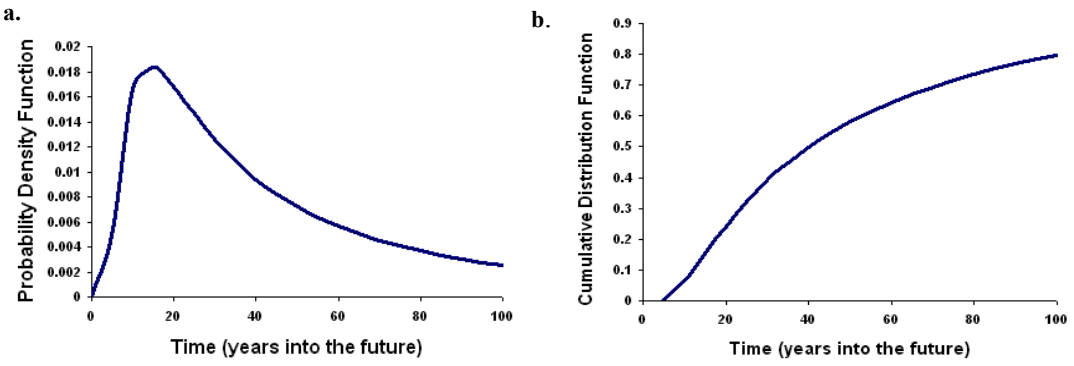
Upper CI for μ = (μ) + TINV(α,q-1)*SQRT(σ^2/q)
Lower CI for μ = (μ) - TINV(α,q-1)*SQRT(σ^2/q)
Upper CI for σ^2 = (q-1)* σ^2/(CHIINV(1- α /2, q-1))
Lower CI for σ^2 = (q-1)* σ^2/(CHIINV(α /2, q-1))

Because μ and σ^2 can be estimated from limited transitions, estimates presented without confidence intervals should be viewed skeptically. Confidence intervals describe the amount of trust that can be placed in the parameter estimates. Reporting confidence intervals allows others to understand the estimates of μ and σ^2 in the context of the rest of the data. The above equations are pre-entered into the count-based PVA template provided with this manual.

Probability Density Function (PDF) and Cumulative Distribution Function (CDF)

Once μ and σ^2 have been estimated, the Probability Density Function (PDF) and the Cumulative Distribution Function (CDF) can be calculated. The PDF estimates the probability that quasi-extinction will occur during a specific, small duration. When plotted against time, the PDF (Figure 2.6a) is like a histogram of predicted quasi-extinction times (Morris and Doak 2002). The CDF (Figure 2.6b) predicts the probability that a population will reach its quasi-extinction threshold at some time in the future. According to Morris and Doak (2002), a CDF is the single most useful metric of a population's extinction risk. Using this function may also be useful to make qualitative assessments about a particular population even without multiple years of population count data available. However, one should be careful when using only a few years of data, as it can skew interpretations, and using 5-10+ years of data is still best.

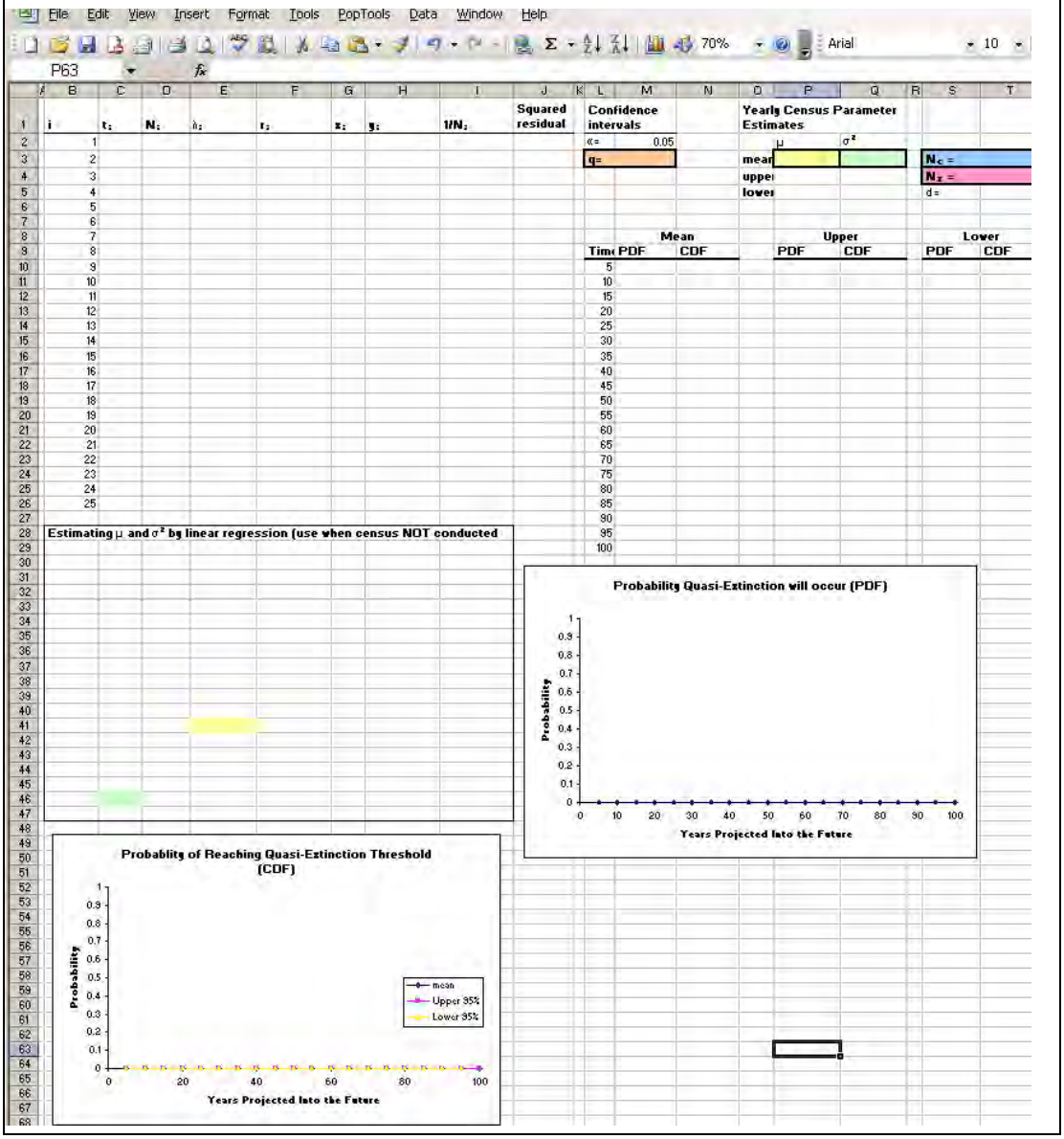
Figure 2.6: Probability Density Function (a) and Cumulative Distribution Function (b) plotted against time into the future to visualize extinction risk. (Data taken from Plants of Concern, Chicago Botanic Garden, unpublished)



Analysis in Excel

This section provides help in navigating the template for using the count-based PVA in the Microsoft Excel file, 'Template for Count Based PVA', which has been provided in this document. All equations and labels are pre-populated (Figure 2.7). Table 2.1 is a summary of each variable, its definition, and where it can be found in the Excel template. The first step in conducting the analysis is to enter the census data into the template; including the year each census was completed (t_i) and the corresponding population count (N_i). Once these data are entered, the values for λ_i, r_i, x_i, and y_i will be automatically calculated. The other variables that need to be entered are the most recent population count (N_c), the quasi-extinction threshold determined for the particular population or species (N_x), and the number of censuses completed (q).

Figure 2.7. Screenshot showing the layout of the Excel template for a count-based PVA provided along with this manual.



If the data have been collected annually, the μ and σ^2 values will be automatically calculated from the census data entered, however you must adjust the formulas based on the number of years of data included in the analysis. Double click on the box with the formula for each variable (μ or σ^2) and extend or contract the boxes that appear so they encompass the data you wish to include. **Please note:** If the data have not been collected annually, the tool to run a regression in order to estimate μ and σ^2 can be found in Excel (File: 'Template for Count Based PVA') under Tools > Data Analysis. The output of this regression can be placed in the box provided by setting the output value to cell B29. The values of the slope of the regression (cell E41) and residual mean squares (cell C46) must be copied and pasted into the highlighted boxes for μ and σ^2, respectively, replacing the automatically calculated values. The PDF of the population (see equation, Table 2.1) is plotted against time in the first graph in the template. Once the CDF is calculated (see equation, Table 2.1), it can be plotted against time to provide a visual representation of future extinction risk for a given population.

Table 2.1: Variables used in Count-Based PVA example along with equations/descriptions and the cells where each variable can be found in the template provided.

Variable	Equation / Description	Cell(s) in Excel Template
i	Census number	Column B
t_i	Year the census was completed	Column C
N_i	Population count	Column D
λ_i	N_{i+1}/N_i	Column E
r_i	$\ln(\lambda_i)$	Column F
μ	Mean(r_i); slope of y_i on x_i regression	P2
σ^2	Variance(r_i); residual mean of regression	Q2
α	Desired confidence interval	M2
q	Number of transitions or the number of counts taken minus 1	M3
x_i	$\sqrt{(t_{i+1} - t_i)}$	Column G
y_i	x_i/r_i	Column H
N_c	Most recent population count	T3
N_x	Quasi-Extinction Threshold	T4
d	$\ln(N_c)-\ln(N_x)$	T5
PDF*	$(d/(\sqrt{2\pi*\sigma^2*t^3}))*EXP(-((d+\mu*t)^2)/(2\sigma^2*t))$	M10-M29
CDF*	$NORMDIST((-d-\mu*t)/\sqrt{\sigma^2*t}))+$ $NORMDIST((-d+\mu*t)/\sqrt{(\sigma^2*t)})*(EXP(-2\mu d/\sigma^2))$	N10-N29

*These functions described using Excel functions and terminology

Measures of Extinction Risk

Estimates of extinction risk are more often used by animal ecologists than plant ecologists (Crone et al, 2011). This is because plant census numbers are often strongly affected by environmental and stochastic factors not taken into account in a standard count-based PVA. In particular, plants at different life history stages will have differing probabilities of detection, and individuals in some species may be dormant during the census period, but still actively contributing to population growth and stability. Therefore, calculations of extinction risks should always be viewed as relative measures of population performance and longevity (Crone et al. 2011). The following measures of risk should not be thought of as absolute, and they should be used more in the vein of a comparative measure across populations or species through time, or among treatments or habitats within a species.

A variety of parameters that describe and summarize population viability can be derived from the CDF. These measures include the **ultimate probability of extinction,** the **probability of extinction over a certain time horizon,** and the **mean, median** and **modal extinction time** for the population. While the mean and modal extinction times are often the easiest to calculate, they are the least dependable estimates of extinction risk (Morris and Doak 2002). The **mean time to extinction** almost always provides an overestimate of the stability of a population due to skews in the distribution of extinction times from environmental stochasticity. The **modal extinction time** does not take into account the full distribution of estimated extinction times and tends to produce a time to extinction that underestimates the stability of a population. For these reasons, calculating these estimates is not discussed here, but more information about these measures can be found in Dennis et al. (1991) and Morris and Doak (2002). The **median time to extinction**, calculated $(N_x/N_c)^{[(2\mu)/(q-1)*(\sigma^2/q)]}$, is reported as the time where the probability of quasi-extinction reaches 0.5. This measure, along with the **probability of ultimate extinction**, is considered to be more meaningful than either the mean or modal measures because they are less likely to be skewed (Morris and Doak 2002).

The most useful measure of extinction risk is the **probability of extinction over a certain time horizon**. This measure can be easily determined from the CDF, and it has the added benefit of allowing the investigator to incorporate biological and/or management significance into the extinction risk estimate. Deciding on a reasonable timeline for which to calculate the extinction probability should depend not only on species biology, but also environmental variables like future population threats and management practices, and practical variables like funding deadlines and changes in administration (Morris and Doak 2002). Discussion of this measure of viability emphasizes the time-dependent nature of a count-based PVA and its relationship to the practicalities of management decisions and population or species biology. Morris and Doak (2002) also suggest presenting the entire CDF, along with confidence estimates, highlighting certain time horizons that correspond to relevant biological or management milestones.

Assumptions of a count-based PVA

This method for conducting a PVA assumes the following are true. By testing for violations of these assumptions, it is often possible to determine whether the actual times to extinction are likely to be shorter or longer than the estimated extinction value calculated from the PVA (Morris and Doak 2002). Any violations of the following assumptions should be taken into account when interpreting estimates of extinction risk..

1. There is no observation error
    - A well-trained field staff with a well-organized census method (described in Section II; Level 1B Monitoring) will reduce human error (double counting individuals, incorrect species identification, sampling variation, etc.)
    - Conduct the census at the same time of year or phenological phase and with the same search intensity to ensure a consistent fraction of the population is included each year
    - Avoid using this method when population area variables (surrounding vegetation very dense, very large population area) or species-specific detection variables (species is small or cryptic, a substantial dormancy at one time) may interfere
    - Estimates of the magnitude of observation error can be calculated by repeated sampling (Chapter 5, Morris and Doak 2002) and "ground-truthing" (Gibbs 2000) and incorporated into the model

2. Mean population growth rate is density-independent
    - Regress r_i (rate of population increase) vs. N_i (population count) and look for trends. A positive slope at low N_i or a negative slope at high N_i indicates density dependence
    - Density dependence has a complex effect on extinction risk, and can be added into a count-based PVA with appropriate data. More information on this topic can be found in Chapter 3 of Morris et al. (1999) and Chapter 4 of Morris and Doak (2002)

3. Demographic stochasticity is unimportant
    - Regress squared residuals vs. $1/N_i$ to look for a pattern
    - If demographic stochasticity were important, the squared residuals would increase linearly with $1/N_i$
    - The best way to account for demographic stochasticity is to set the quasi-extinction threshold high enough to make the assumption negligible. This number will depend on the life history characteristics of the focal species.
    - As a general guideline, populations of 100 total individuals or 20 individuals in the most important life stages can be considered safe to ignore demographic stochasticity (Morris and Doak 2002)
    - If demographic stochasticity must be included in the model, methods on how to do so when appropriate data are available can be found in Chapter 4 of Morris and Doak (2002)

4. The population has experienced no catastrophes or bonanzas
   - Catastrophes and bonanzas are identified by unusually high or low values of $\boldsymbol{r}$
   - To test for significant differences, rerun the regression analysis on $\boldsymbol{x_i}$ and $\boldsymbol{y_i}$ using the 'standardized residuals' option
   - Compare the standardized residuals to a t-distribution using $\boldsymbol{q}$-2 degrees of freedom (=TDIST(ABS(std. residual), # of observations – 2, 2)); p-values of less than 0.05 should be investigated to see if they correspond to biological anomalies

5. Neither μ nor σ^2 change over time
   - Trends in μ
     - Run a regression of r on time
     - A significant positive or negative slope indicates a temporal trend in μ
   - Trends in σ^2
     - Run a regression of r on time
     - A significant positive or negative slope indicates a temporal trend in σ^2
   - If trends are detected in either parameter, two options are available to take these trends into account
     - Use the most current estimates if it is believed they are now stable
     - Simulate on a computer the effects of the trend, assuming the past rate and pattern of change will continue into the future (Morris and Doak 2002)

6. There is no environmental autocorrelation
   - Determine if environmental conditions correlate from one inter-census interval to the next by looking at population growth rates
   - Create a new variable $\mathbf{r_{i+1}}$ and plot vs. $\boldsymbol{r_i}$
   - Run the correlation tool on the r_i variable found under Data Analysis
   - Optional: If you have PopTools installed in Excel, you can instead run the Autocorrelation tool from that menu on the r_i values (PopTools>Extra Stats>Autocorrelation)
   - A significant *positive* autocorrelation (a good year is likely to be followed by another good year, or a bad year followed by another bad year) in the population growth rate indicates an increase in the extinction risk
   - A significant *negative* autocorrelation (a good year is likely to be followed by a bad year and vice versa) indicates a reduced extinction risk
   - Methods to incorporate environmental autocorrelation into a count-based PVA model are described in Chapter 4 of Morris and Doak (2002)

SECTION III: Level 2 Monitoring

Level 2 Monitoring – Survey/Trend Monitoring

Number of People Required: 2-4
Estimated Field Days/Year Required: 1-2

Level 2 monitoring is most appropriate to use when looking for trends in a population or species. Level 2 monitoring is especially helpful when looking to evaluate management actions (Menges and Gordon 1996). These trends or responses to management can be changes in numbers, density, and/or spatial extent, and they can help to elucidate the short and long term effects of management actions such as prescribed burns, mowing, etc. Level 2 monitoring generally looks at the population as a whole, without focusing on a specific age or stage class.

Depending on the monitoring question/objective of the study, three types of measurements can be used to monitor population trends: frequency, density, and cover. When looking for changes in numbers of individuals, frequency is the most appropriate variable to measure, while changes in spatial extent are better monitored using either density or cover. The Level 2 flow chart in our initial report (Tienes et al. 2010, p. 29) provides a more detailed guide in deciding which survey techniques are best suited for different species and monitoring objectives.

Frequency

Definition[**]: The percentage of possible plots within a sample area occupied by the target species.

When to use frequency

Frequency is the best measure to use when the goal of the monitoring project is to assess changes in numbers in a population. Measuring frequency is also appropriate for any species or situation where a change in spatial extent of the population is of interest, such as monitoring in an invasion front. This measure is especially helpful for species with rhizomatous growth, because there is no need to define an individual or consistent counting unit. Frequency measures can be done fairly quickly (especially for easy to spot species) and without much training. Another advantage of frequency measures is that they are a fairly stable measure throughout the growing season. This expands the time window in which data can be collected and still remain comparable to other populations and/or years.

[**] Definitions for frequency, density, and cover taken from Elzinga et al. (1998)

Cautions/Problems

Both the spatial distribution and the density of a population affect measures of frequency, making changes in frequency difficult to interpret biologically. Frequency is also more difficult than density or cover for many people to visualize for a site, making the results somewhat complicated to describe to land managers or other interested parties (Elzinga et al. 1998).

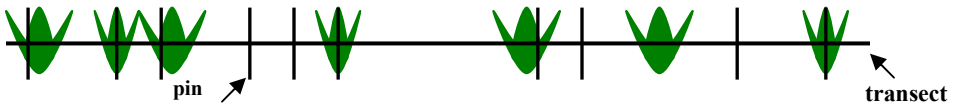
Data collection methods

There are three major methods of collecting frequency data, and the best method to use will depend on the particular species and site of interest. If the species of interest is common, then a point-intercept method of measuring frequency is usually the most appropriate. However, if you are monitoring a rare species, either a line- or plot-based method will be more appropriate. It is best to use a line-intercept method if the surrounding vegetation is sparse and with species with large basal areas like trees or large shrubs. If the surrounding vegetation is dense and you are monitoring a species with a smaller basal area, like most herbaceous species, a plot-based method will be more appropriate.

Point-intercept

The point-intercept frequency method consists of first placing multiple transects across the population of interest (for more information on transects see Section I). A rod or pin is then lowered at randomly or systematically located points along each transect (Figure 3.1). The frequency is calculated by the number of times the rod contacts a plant of interest out of the total number of points along each transect. When using this type of measure it is very important to specify whether the base or canopy needs to be intersected in order for the point to count. It is often better to use the shoots/leaves for frequency measure. Point-intercept monitoring is most often used with common species because it does not adequately sample populations with very small frequencies.

Figure 3.1: Diagram of point-intercept sampling (side view). The number of pins contacting a plant divided by the total number of pins lowered equals the percent frequency or cover.

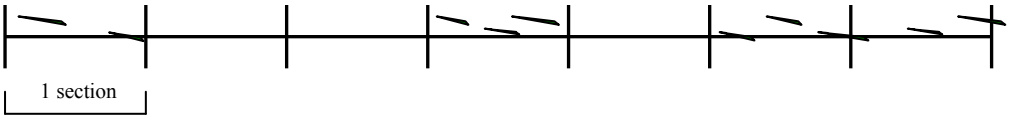


Line-intercept

The line-intercept frequency method uses transect lines separated into segments of equal lengths (Figure 3.2). Sections with plants observed crossing the transect line are recorded. The number of segments occupied divided by the total number of segments gives the percent frequency of that transect. Line-intercept sampling is very similar to

quadrat sampling using long, thin plots, or a Daubenmire transect (Daubenmire 1959), where plots are placed systematically along a transect and monitored.

Figure 3.2: Diagram of line-intercept sampling for frequency (top view). Each line perpendicular to the transect creates a new section, with the number of occupied sections divided by the total number of sections equaling the percent frequency of the transect.

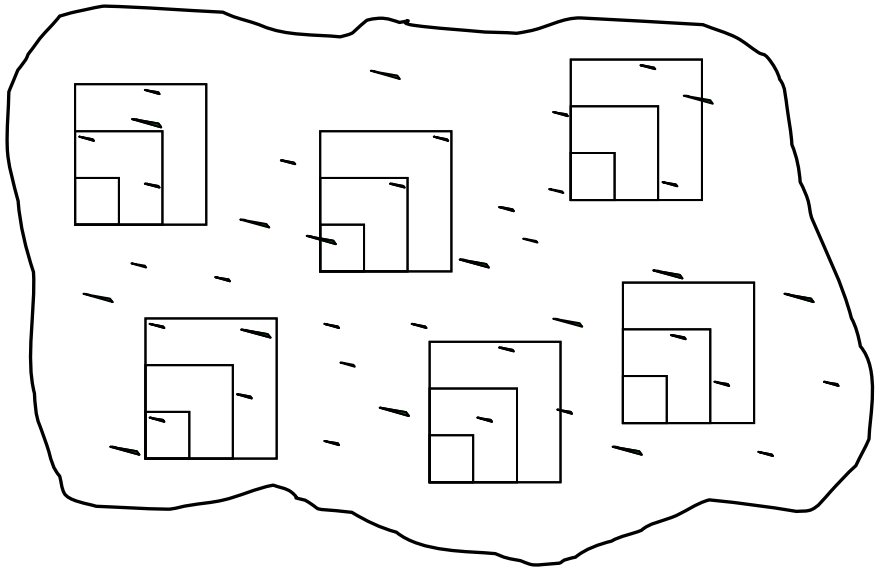


Plots / Nested Frequency

Plot based measures are the most commonly used technique when monitoring frequency. Plot size is the most important factor to consider when measuring frequency in a plot-based monitoring study. Heywood and DeBacker (2007) present three recommendations for designing a plot-based frequency monitoring study in order to maximize statistical efficiency.

- First, when determining the plot size, the size should produce a mean frequency between 30% and 70% (Elzinga et al. 1998). Plot sizes allowing for this initial frequency will provide enough sensitivity to detect frequency changes in either direction over time. Using a nested design initially is an efficient way to determine the best size for the particular species and population being monitored (Figure 3.3). This technique involves placing sets of larger plots with increasingly smaller plots nested within them throughout the population. A plant counted in a smaller plot is then automatically included in the larger plots, but not vice versa.
- Secondly, the number and arrangement of the plots should be dispersed over as large of an area as possible in order to minimize spatial structure among sites and increase the power of a nested ANOVA.
- Finally, study sites, but not individual plots, should be marked and re-sampled over time. When monitoring frequency in fixed sites, there is no statistical advantage to measuring the exact same plots each year, and they can be difficult to find and exactly relocate (Heywood and DeBacker 2007, personal observation). In addition, returning to the exact same plots each year can also cause damage to surrounding vegetation and should be avoided in sensitive habitats.

Figure 3.3: Diagram of a population with a nested-plot setup to measure frequency. Each larger square plot has two smaller square plots nested inside.



Density

Definition: The number of census units (individual plants, ramets, etc.) per unit area.

When to use density

Density is the best measure to use when results of monitoring are to be compared across study sites or personnel who may be using different sampling strategies such as differing plot sizes or shapes. It is also very useful when trying to determine the effects of a management or other impacts on recruitment of new individuals into a population as well as mortality rates. It is important to define a consistent census unit when measuring density. The census unit does not have to be a genetic individual, but it must be clearly recognizable and consistently defined. Because density is a per area measure, it allows for easy comparison between sites and years. This measure is theoretically independent of plot size or shape, however, making boundary decisions (Box 2) may remove some of this independence.

Cautions/Problems

Estimates of density can be affected by the choice of plot size and shape because of the variability of boundary decisions (see Box 2). Density measures are also insensitive to changes in vigor or reproductive output. Therefore, it is an unreliable measure to use when plants respond to stress with reduced biomass or cover, rather than individual

mortality. Density can also be a difficult method to use with annual plants, whose numbers tend to fluctuate more wildly from year to year, making it difficult to draw conclusions from the data (Elzinga et al. 1998).

Data collection methods

The most appropriate method used to monitor and calculate density in a population is generally plot-based. When dealing with a smaller population, or one that has a clumped distribution (common in rare plants), density plots or quadrats should be used. However, when dealing with a very large population that is randomly distributed (usually trees or large shrubs) a line-intercept or distance method will be more appropriate. In this case, however, density is estimated as a function of frequency of individuals per unit area sampled.

Depending on the monitoring question being asked, having the density of each stage class of plant can sometimes be very helpful. For example, when population density is stable, knowing that there was an increase in the ratio of seedlings versus reproductive adults provides a better estimate of population status than just knowing that the density of individuals remained the same from census to census. Collecting these data require more time and effort than standard density counts. Therefore, of the value of density numbers in your data set should be measured against the additional resources required to collect these data.

Quadrats

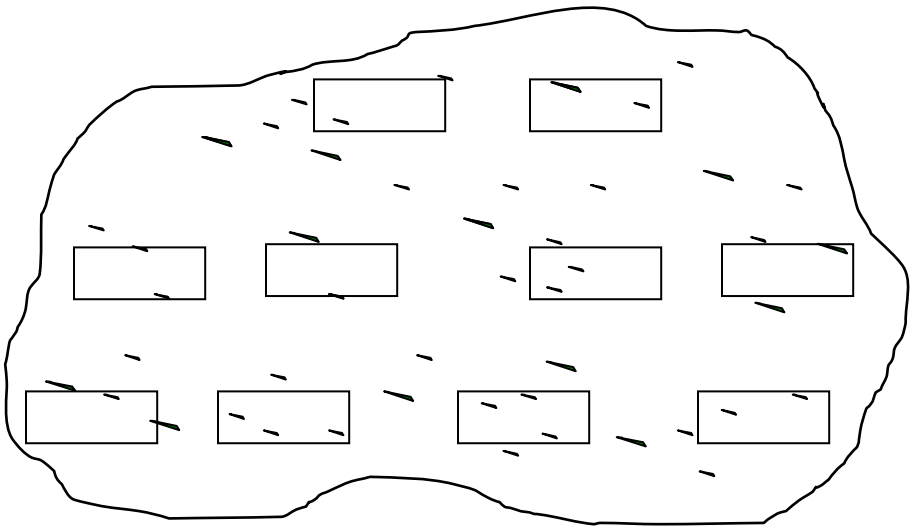
The main factor to consider when designing a monitoring scheme based on density quadrats is the size and shape of the quadrats to be used. This will not only affect the accuracy of density measures, but will also determine the efficiency of the study. It has been shown that the most efficient shape for a density plot is usually a rectangle (Elzinga et al. 1998). This is because rectangular quadrats tend to increase within-quadrat variation in density versus between-quadrat variation when placed along a gradient. Because they capture more variation within each quadrat, fewer rectangular quadrats are needed compared to square quadrats to achieve the same precision (Carah et al. 2008, Clapham 1932, Stockdale and Wright 1996, Elzinga et al. 1998, 2001, Salzer and Willoughby 2004). Rectangular quadrats also reduce the number of zero counts in populations with clumped distributions, especially if the length of the quadrat is longer than the mean distance between plant clumps. Finally, a longer, narrower quadrat allows for easier tracking of plants that have already been counted, while large square quadrats can be very difficult to track (see Figure 3.4 for an example with rectangular quadrats).

Additional points of consideration for density quadrats:

- Do not to make quadrats so large that they are impractical to search in a manageable amount of time, or that you have to stand inside them while collecting data.

- A very large quadrat also increases the number of boundary decisions that must be made (see Box 2), which can add to the time needed to sample each quadrat, as well as decrease the accuracy of the density measure.
- Weigh the importance of decreased travel and setup time as well as searching and counting time when determining the size quadrats to use.
- When monitoring large plants it is appropriate to sample a very large area, while monitoring small or hidden plants makes it imperative to minimize the area needing to be searched and sampled.
- Quadrats should be oriented to capture the majority of variation within quadrats rather than between quadrats.
- Increased plant density calls for smaller quadrats, while decreased plant density will require larger quadrats.
- A procedure to help determine an appropriate quadrat size and shape can be found in Appendix 17 of Elzinga et al. (1998).

Figure 3.4: Diagram of a population with systematically located rectangular density quadrats.

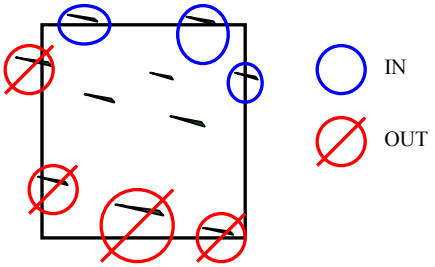


Box 2: Boundary Decisions

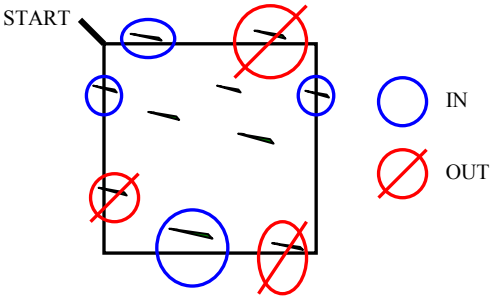
When conducting monitoring using plots or quadrats, one of the most important things to consider is deciding which plants should be counted as inside the plot and which should be considered outside the plot. The most important part of making boundary decisions is to be consistent over the course of the monitoring study. The rules for deciding which plants are in or out should be made very clear so they can be followed consistently for each plot and into the future. This is especially true of long term monitoring projects where multiple investigators will likely be collecting data.

While it is usually easy to decipher whether plants with small, thin stems are in or out of a plot, plants with large stems or trunks and matted plants can be much more difficult. When dealing with these types of plants, it is necessary to decide on a set of rules to determine whether or not to include certain plants in the count so as not to over- or underestimate the population's density or frequency. First decide if it makes more sense to base boundary decisions on canopy or basal area. This will depend on the structure of the species you are monitoring. After that decision is made, there are two main ways to deal with boundary decisions when conducting survey monitoring (Elzinga et al. 1998):

1. Count all plants touching the boundary 'in' along two adjacent sides of the plot and 'out' along the other two adjacent sides. Make sure to specify which sides and use those consistently across plots.



2. Count plants that touch the boundary as alternating 'in' then 'out' all the way around the plot edge, starting at the same corner in each plot. This can be difficult to keep track of for larger plots.



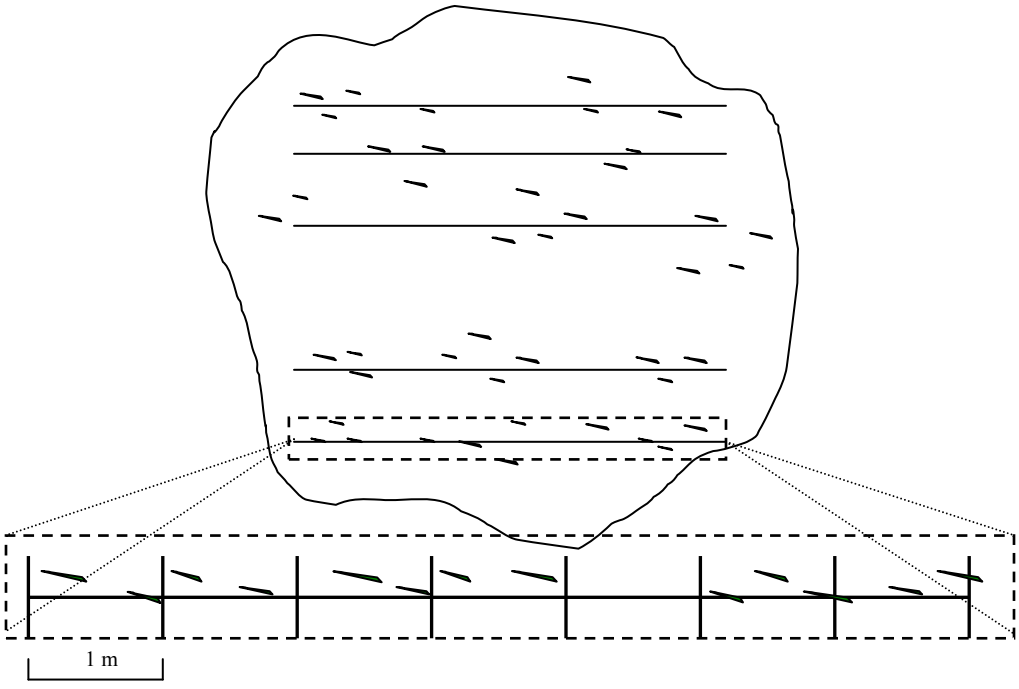
There are also a number of methods that should be **avoided** when making boundary decisions (Elzinga et al. 1998):

1. Count all plants 'in' that are more than 50% inside the plot. This should be avoided because it increases observer bias to determine what is 50% in.
2. Count all plants 'in' that touch the border. This strategy should be avoided because it will overestimate plant density.
3. Count only plants that are completely inside the border as 'in.' This strategy should be avoided because it will underestimate plant density.

Line-intercept

When measuring density with the line-intercept method, the first step is to place transects across the population (see Section I). Each transect should be long enough to cross over most of the variability in the population. Then each transect should be divided into equal intervals, and the number of individuals that contact the transect in each interval are counted (Figure 3.5). The intervals along the transect can be treated like quadrats, with boundary decisions being made at the edge of each interval.

Figure 3.5: Diagram showing transects used for line-intercept monitoring. Each transect (insert) is divided into equal segments, and the number of plants in each segment are counted.



Cover

Definition: The vertical projection of vegetation from the ground as viewed from above. There are two types: basal, the area where the plant intersects the ground and aerial, the vegetation covering the ground surface above the ground.

When to use cover

Cover is the best measure to use when the goal of the monitoring project is to assess changes in the relative abundances and spatial extent of plants or populations. Cover is also the measure most directly related to biomass, making it the best measure to use when interested in tracking changes in vigor. Species with a well defined canopy like

matted herbs or shrubs are the easiest to study using cover measurements, but these methods are applicable for nearly all plants.

Cautions/Problems

The major drawback of measuring cover is that it changes substantially over the course of a growing season. This makes it very important to collect data in a very short time frame to compare multiple populations. It is also important to take data at the same growing stage each year when planning to establish a long term data set. Additionally, because cover measures can be affected by both changes in density and changes in vigor, it can be difficult to tease out changes in density from those caused by differences in fluctuating environmental conditions from year to year. Collecting additional information on the population and environmental conditions each year can help to tease out the causes of cover increases or declines seen in monitoring.

Data collection methods

There are a number of methods that can be used to measure the cover of a species. The technique used should be decided upon based on the species being monitored and the monitoring objectives. Cover quadrats are best to use for shorter vegetation (<1m tall), but when dealing with a tall species (>1m tall), a line- or point-intercept technique will often be more appropriate.

Quadrats

When determining size and shape of cover quadrats, the same factors should be considered as described for the use of density quadrats. In addition to size and shape, when monitoring with cover quadrats it is important to determine whether the plots should be permanent or temporary. Monumenting or permanently marking a set of permanent plots, will take more time to establish, but will make the plots easier to relocate. Monumenting plots can involve using stakes or posts, marking trees, and/or referencing landmarks. A full discussion on best practices for monumenting plots can be found in Chapter 8 of Elzinga et al. (1998). Three important factors should be considered when deciding whether to use permanent or temporary plots:

1. Plant Morphology – Thin leaved and/or single-stemmed species cause more problems than matted species because the likelihood of intersecting the same plant on future measurements is reduced.
2. Field conditions – Replacement of permanent transects or plots can be complicated in areas that are difficult to travel through.
3. Sampling unit – Transects and quadrats are reasonable to use and relocate, while points are much more difficult.

When estimating cover using quadrats it is important to employ a consistent system of defining cover class among investigators, sites, and years. Cover values are notoriously variable among evaluators. Thus, it is recommended to use a cover class system, rather

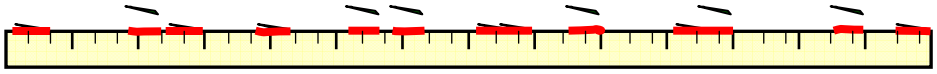
than recording individual percent cover when monitoring cover in quadrats. Cover classes not only have the advantage of producing higher precision, more reliable data, but they also give you data that are statistically easier to analyze. A cover class system with narrow ranges at each end and more broad ranges in the middle, like a Daubenmire cover class scheme (Daubenmire 1959; McCune and Grace 2002; 1 = 0-5%, 2 = 6-25%, 3= 26-50%, 4 = 51-75%, 5 = 76-95%, 6 = 96-100%), or the Arcsine Square Root cover class scheme (Muir and McCune 1987; McCune and Grace 2002; 1-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-99% and 99-100%) are the most appropriate schemes for providing good transformations of proportion data like percent cover (McCune and Grace 2002).

There are a number of ways to help alleviate observer bias and increase the accuracy of monitoring data when using quadrats. Having well trained monitors with templates that represent certain cover class values in the field will greatly decrease bias and differences between observers. Additionally, if a group of monitors with different experience levels are working on the same project, measuring cover in the first few quadrats as a group will help to increase consistency across the entire group. If possible, conducting a full pilot study to estimate observer variability and to test the ability to mark and relocate permanent plots will help to increase accuracy and efficiency of a plot-based cover monitoring design. Despite these drawbacks, cover quadrats are often used because data can be collected relatively quickly and easily. Additionally, cover quadrats are more effective for monitoring very rare species than point or line-intercept methods (Meese and Tomich 1992; Dethier et al. 1993; Elzinga et al. 1998).

Line-intercept

When measuring cover with the line-intercept method, the first step is to extend a measuring tape across the transects placed in the population (see Section I for transect placement). Then the intercept distance is recorded for each area where the species canopy crosses the line (overlapping canopies are not counted twice, Figure 3.6). The total length of the canopy cover measured divided by the transect length will then give you a percent cover. This method is particularly useful when monitoring trees.

Figure 3.6: Diagram of a transect for a line-intercept cover measure. A tape measure is stretched across the transect in order to simplify the canopy measurements of each plant.



There are a few down sides to monitoring cover using line-intercepts. There can be observer bias if your sighting line is not perpendicular to the ground. This can be somewhat mitigated by using a plumb line or optical sighting device. The wind can also lessen the accuracy of length measurements, and windy days should be avoided for data

collection when possible. Additionally, gaps in canopy cover can be troublesome. It is important to clearly document rules on when a gap should be considered, and when it should be ignored. One common method is to ignore all gaps smaller than one centimeter, while stopping and starting cover estimates for any larger gaps.

Point-intercept

The point-intercept method is the most objective of the three cover methods, eliminating bias caused by cover estimates and decisions on gap measures. To conduct a point-intercept cover measure, one or more transects must be run across the population (see Section I). Then a pin is placed at multiple random points along each transect and presence/absence of a plant at that spot is recorded (Figure 3.1). The number of times a plant is encountered, divided by the total number of points sampled, will then provide a measure of percent cover.

Point-intercept monitoring works best for plants with higher levels of cover, because low cover values and small changes in cover values are difficult to adequately sample and detect with this method. When using point-intercept monitoring, the main source of bias comes from the pin or rod used to sample the plants. The diameter of the pin used should be kept consistent during the entire monitoring study to avoid these problems.

Data Analysis

When dealing with frequency, density and cover, calculating summary statistics such as averages, totals or proportions is often the first step in investigating the data. Graphing these values with error bars calculated from confidence intervals often provides a good visual representation of the monitored population over time. The data analysis of a survey monitoring program will be strongly driven by the particular monitoring question being asked. The main driver in determining an analysis technique is whether the monitoring is being done to track status and trends or whether to investigate the effects of different management actions.

When the purpose of a Level 2 monitoring program is to follow trends in a population over time (after a disturbance, etc.) the general form of analysis to use is a regression. Regressions are the most appropriate form of analysis to use when dealing with only continuous variables like time, plant size, etc. Regressions can be run in most statistical software, including Microsoft Excel and R. More information about regressions can be found in Chapter 8 of Crawley (2007). On the other hand, when the objective of a Level 2 monitoring program is to investigate the effects of certain management actions, or to compare two different sites or populations, an ANOVA is the most appropriate data analysis approach. An ANOVA allows for categorical variables (burned vs. not burned, mowed vs. not mowed, etc.) to be incorporated into the analysis. More information on these types of analyses can be found in Chapter 11 of Elzinga et al. (1998). Another form of analysis that can be helpful in these situations is a mixed effects model. These models account for difference sources of non-independence, for example, sampling the same plots year after year. More information on these models and how they can be used

can be found in Crawley (2007). Because of the wide-ranging nature of survey monitoring objectives, it is not feasible to go into detail about every possible experimental setup or data analysis strategy here. While each situation is different, the above references should serve to lead you to more detailed information on the analyses that may work best for your particular situation.

SECTON IV: Level 3 Monitoring

Level 3 Monitoring – Demographic Monitoring

Number of People Required: 2-4
Estimated Field Days/Year Required: 2-6 (depending on species and number of sites)

Level 3 demographic monitoring is the only level of monitoring that provides the power to answer questions about specific ages or life stages of a population in addition to making predictions about population viability in the future. Demographic monitoring can be used to predict trends in population numbers or a response to specific management actions. A well-defined monitoring objective with a specific question should be developed to justify the use of demographic monitoring. When done correctly, demographic monitoring requires 3-5 years of data before any substantial predictions of future population growth can be made, though longer durations are preferred because they provide more robust predictive assessments and power. Jäkäläniemi et al. (2013) tested how well demographic data collected over four years projected the fate and growth of populations after eleven years. This study found that the models correctly predicted survival in 91% of the populations and abundance increase or decrease in 65% of the populations.

Although few years of data are needed to make a prediction, demographic monitoring is the most time and resource intensive level of monitoring. Marking and following individual plants requires a great deal of time, effort, and planning; and it must be done regularly and consistently to obtain useful data. If the time and resources are not available to conduct demographic monitoring on a consistent basis, resulting data will be inconsistent and lack statistical significance. The work done by Jäkäläniemi et al. (2013) indicates that the inclusion of data on habitat changes and dynamics over time can increase the accuracy of assessments of population viability. Additionally, knowledge of, or access to, mathematical and statistical expertise is necessary to take full advantage of the work and analyze the data collected from a demographic monitoring program. In most cases, the investment in time and resources needed to complete an informative demographic study are not warranted, and a Level 1 or 1.5 monitoring study should be considered instead.

General thoughts on designing a demographic monitoring program

Demographic monitoring involves following individual plants through multiple transitions, or changes in age or life stage classes from year to year. A transition can either be movement from one age/stage class to another (growth) or stasis within the same age/stage class (survival). Before undertaking a full-scale demographic monitoring project, it is best to conduct a pilot study if at all possible. Elzinga et al. (1998) suggest that a two-year pilot study is necessary for two reasons. First, a pilot study allows the testing of field methods during the first season to ensure that individuals are easily located, tagged, measured, and, most importantly, relocated the

following year. Second, after the first transition, the variability of the estimated parameters can be evaluated and a comparison of sampling effort to elasticity matrices can be made. Elasticity matrices contain values that are a measure of the sensitivity of the population growth rate to changes in each transition probability (Elzinga et al. 1998). High elasticity values represent more important transitions to focus on, and should be the stages given the greatest sampling effort (Elzinga et al. 1998). After the pilot study, time and resource commitments should be re-evaluated in order to ensure the demographic study is providing results worth the effort. A pilot study can often be undertaken in conjunction with a Level 1 population census or other low intensity monitoring to make it less time and resource intensive.

Challenges

Some life forms make it very difficult to follow individuals from year to year in the way needed to complete an informative demographic study. Annuals, geophytes (plants with underground storage organs like tubers or corms) and plants with long dormant phases are difficult to study because their hidden phases are difficult or impossible to measure. The same is true for species with long-lived seed banks, the dynamics about which very little is typically known. Plants with clonal growth or asexual reproduction are also problematic for a demographic study. In these species, it is very difficult to determine exactly what should constitute an "individual," making measurements and transitions difficult to quantify. Careful counting of the number of stems or rosettes, or very clearly defined assumptions regarding the study definition of an individual may help alleviate some of these difficulties.

Ongoing management activities can also pose a challenge when designing any monitoring program. For example, one challenge commonly seen on Forest Service lands is grazing. In many cases, grazing will take place unevenly across a population. In these cases, it is important to coordinate the timing of monitoring each year with the grazing schedule. In addition, following plots in both grazed and ungrazed areas when possible will increase the information that can be gained from your monitoring program. Along with grazing, similar considerations will have to be made when burning is taking place in the area of a focus population.

Data Collection

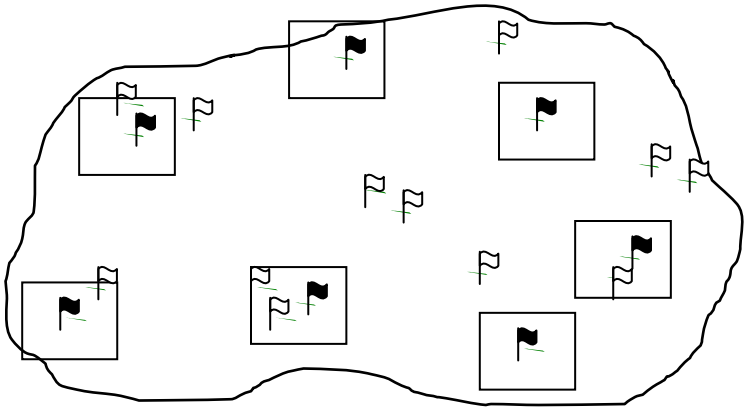
Sampling

The defining feature of demographic monitoring is following specific individuals over multiple (often yearly) transitions. To accomplish this, plants must be marked and labeled to keep track of each individual over the course of the study. This should be done in a way to be able to easily relocate plants each year, but should not be so flashy as to attract animals or vandals. A backup system of mapping each plant is also beneficial as field markers can often go missing. If possible this can be done with an accurate GPS unit or using a coordinate system made from taking measurements from permanent stakes located throughout the population.

The most common method of sampling for a demographic monitoring study is to set up a number of randomly placed quadrats throughout the study area, and mark and follow all plants inside each quadrat. The size of the quadrats will be related to the size of plants you are studying. Smaller plants require smaller quadrats, potentially a meter squared or less, while larger plants may need very large quadrats, 10m squared or more. Transects may be easier to use for larger plants, such as trees, because of the requirement for such large quadrat sizes. A setup with clearly marked permanent plots makes plots as well as individual plants easier to find from year to year. More information on setting up quadrats and transects can be found in Section I of this report.

When dealing with certain population distributions, it can be very difficult to get a truly random sample of the population and certain strategies can be used to aid in this effort. For example, when individuals or clumps in a population are diffusely distributed over a large area, it is helpful to place a numbered pin flag at each individual, and then randomly select a subsample from these numbered flags. Each randomly selected individual then defines either the center or corner of a plot (Figure 4.1).

Figure 4.1. Diagram showing a random selection (black flags) of diffusely distributed plants in a population used as the central points for monitoring quadrats.



Collecting measurements and counts

When conducting a demographic monitoring study, a standardized data collection sheet including individual plant tag labels and counts or measurements to be taken is very important to keep the study consistent over the years of data collection. In addition to information previously provided on designing a data sheet (Section I), in a demographic study, detailed descriptions of exactly how plants are measured, including what parts of the plant were measured, what was used to do the measuring, and when plants were measured, as well as a "Notes" column for additional observations, should be included for use by future data collectors. Inclusion of annotated photos and/or diagrams with these descriptions can also be helpful as a visual reference, especially for small plots undergoing extreme changes due to management. To quantify reproductive value, it is important to record fruit and seed production of individuals. Some life stages, such as

seedlings, may only be rarely encountered; therefore, care should be taken to conduct searches aimed at identifying them.

Size and Stage Classes

When designing a demographic monitoring study, data should be collected on all biologically relevant classes of plant (seedling, juvenile, adult, reproductive, post-reproductive, etc.). When planning to use a continuous size measurement to create discrete size or stage categories for use in matrix analysis and PVAs, it is important to collect size data in a consistent and easily repeatable way. The size measurements and the protocol for collecting them will have to be tailored specifically for each species.

When determining which demographic variables to measure, choose size and reproductive variables that correlate to survivorship and fecundity. Demographic, or structured, population models reflect differences in performance of differently sized individuals in the population by breaking the population up into classes of individuals, attempting to accurately reflect the fate(s) of individuals in each class. For example, a small rosette is more likely to die than a large one, and a large reproductive plant will produce more seeds, on average, than a small one. For these reasons, it is important to determine early in the study if size reflects the biology more accurately than stage.

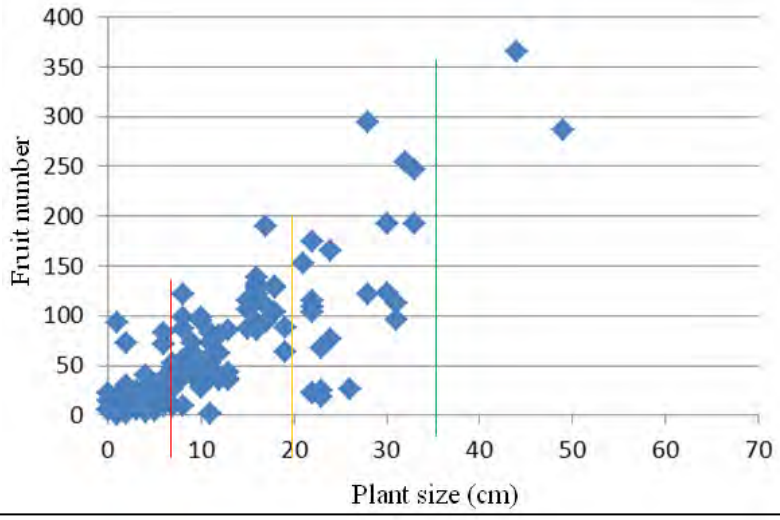
Often this is not known at the start of a project, making it important to measure a large number of variables (stem diameter, stem height, leaf area, number of leaves, fruit number, etc.) over the course of the first 1-2 years of the study, and then determine if size classes or stage classes will best reflect reality. Some species naturally fit into stage-based models, as their life history supports this, particularly monocarpic, rosette-forming species such as *Cirsium pitcheri.* Individual plants of this species are easily assigned to one of four stage-classes, based on leaf morphology: (1) seedling (both cotyledons are present); (2) juvenile (at least one true leaf has formed); (3) vegetative (leaves are all pinnatifid); and (4) reproductive (flowers are present) (e.g. McEachern, 1992).

For other species, size classes or stages are not easily recognizable in the field. In these cases it is necessary to collect data on size, as well as survivorship, growth and other transitions into a new life history stage, such as onset of reproductive maturity, and then determine if size classes or stage classes, or even a mix of both, will be used to develop the projection model. Generally, this will require data collection over two field seasons, as well as subsequent data analysis, to determine which parameters will create a model that best reflects the biology of that species and population. For this reason, project setup and data collection increases the time commitment during the first year of a demographic monitoring program, perhaps as much as two to three times greater than in subsequent years of the study. This should be taken into consideration both when designing a study, and when determining time and resources required for future years of the study. After the initial data have been collected, correlation or regression analyses can be done to investigate importance and determine what data should continue to be collected in following years.

Variables that have a strong correlation with survivorship and fecundity are the most important to continue measuring. However, there may be substitute or proxy variables that offer enough predictive power and that are easier and faster to obtain in the field. When this is the case, these variables should be the ones collected. For example, *Viola conspersa*, the Dog Violet, has been studied by the authors for several years. A variety of size measures were collected the first year, including number of reproductive stems, number of basal rosettes, number of leaves, number of cleistogamous and chasmogamous flowers, and average leaf area. Regression analysis determined that counts of basal rosettes, leaves, and reproductive stems accurately predicted the total leaf area. Moreover, these counts were highly correlated to fruit production across the season. In subsequent years, therefore, only the rosette, leaf, and reproductive stem count data were collected, reducing field time considerably.

After the first year or two of data collection, the data can then be subdivided into size or stage classes. Detailed information on highly regarded ways to divide a population into size classes can be found in Vandermeer (1978) and Morris and Doak (2002). In the example provided here, we have used the method outlined on page 196, paragraph 2 in Morris and Doak (2002) to assign size classes to *Lespedeza leptostachya.* We estimated that the slope of an imaginary regression line (size versus fruit/seed number) would increase at the boundary of the size classes, and subsequent analysis supported these size classes (Figure 4.2). There is a six-fold increase in seed/fruit production between Size Class 2 (8.5cm-10.0cm) and Size Class 3 (20.1cm-35.0cm), and a three fold increase between Size Class 3 and Size Class 4 (>35.1cm). Individuals in the smallest size class, Size Class 1, do not produce seed/fruit. This size class was determined by analyzing size and survivorship data, which revealed that seedlings grow to a maximum of 8.5cm in the year in which they emerge. Although some same-class individuals represent individuals that do not grow, as well as those that may regress from a larger size class, we determined it was appropriate to pool individuals in Size Class 1 because none of them produced seed/fruit.

Figure 4.2. Regression of fruit number on plant size, showing the determination of size classes.

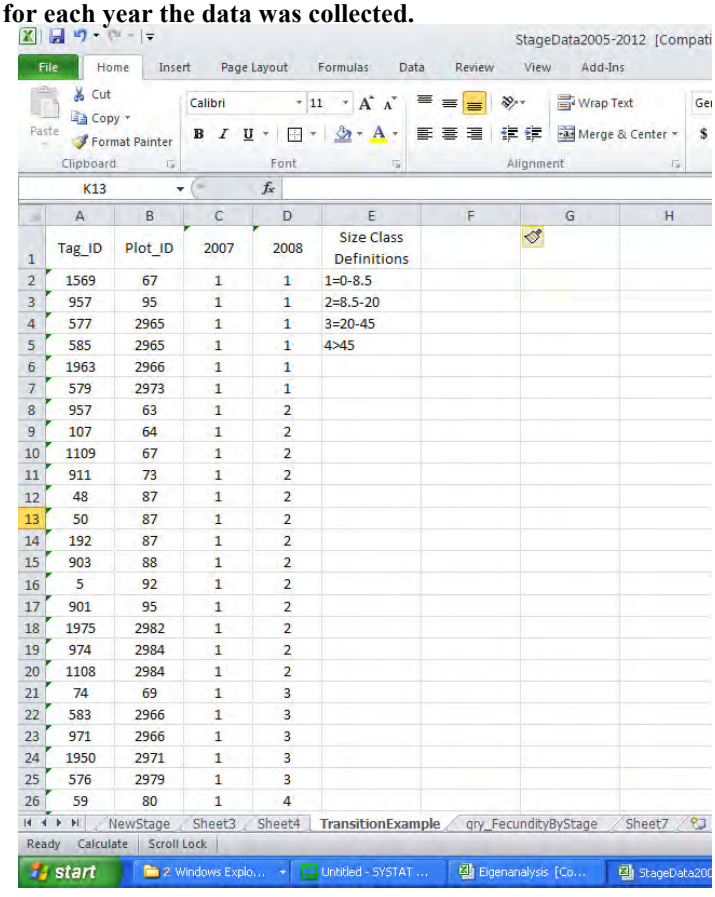


The use of size or stage classes will depend on species life history and plant morphology. It is very important that all life stages of the species be consistently recognizable in the field, and some models will contain both size and stage classes. For example, a model may incorporate a "seed" stage, and have the balance of the life cycle represented as size classes. The number of classes used to build a demographic model must equal the complete life-cycle, and often drawing a diagram can be very helpful in ensuring this. It is important to capture every size or stage class each year, therefore counts and measurements will sometimes have to be taken more than once a year. In *Lespedeza leptostachya*, for example, seedlings emerge in late spring or early summer, but cannot be easily distinguished from surviving individuals from the previous year when censused in late August. Cotyledons or cotyledon scars are only easily seen early in the field season, before the hypocotyl has expanded. Therefore it is important to census this species in both early and late summer to ensure the inclusion of both seedlings and fruiting individuals.

Data Analysis

Data should be organized into an array of size class/stage values which represent a change in size or state between one year and the next, as well as survivorship between years (Figure 4.3). All figures in this section use data from a demographic study of *Lespedeza leptostachya* at Nachusa Grasslands in Franklin Grove, IL (Vitt et al.).

Figure 4.3. Sample data prepared for analysis by organizing data into an array of size class values for each year the data was collected.



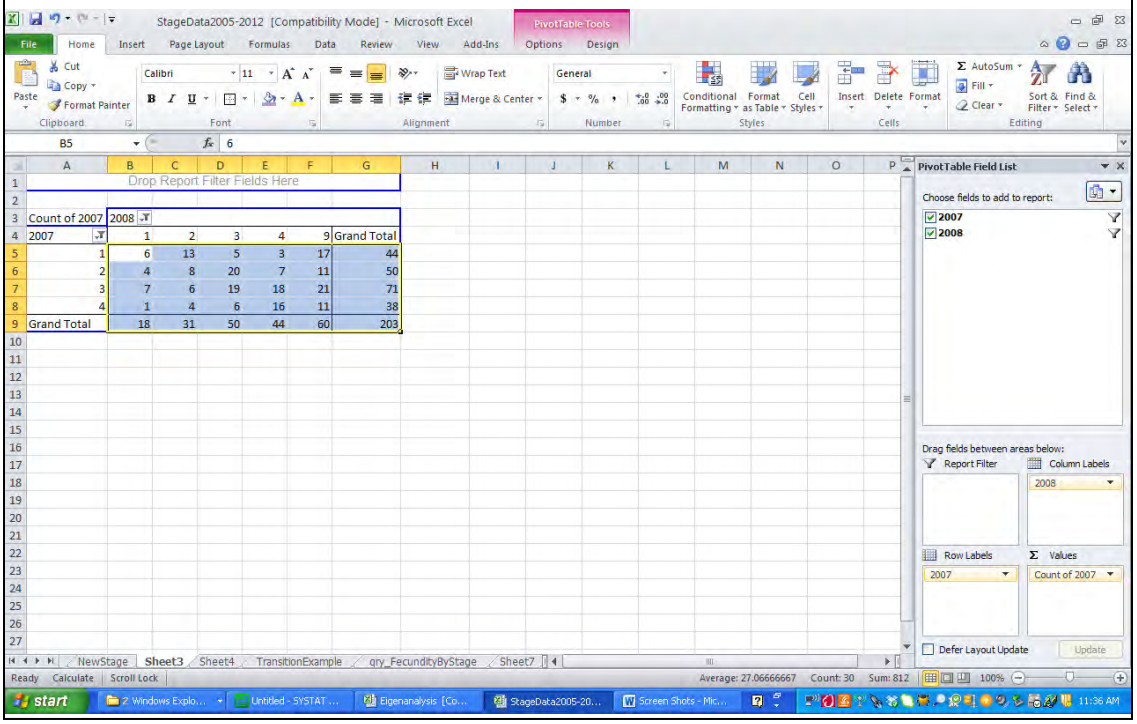
	A	B	C	D	E
1	Tag_ID	Plot_ID	2007	2008	Size Class Definitions
2	1569	67	1	1	1=0-8.5
3	957	95	1	1	2=8.5-20
4	577	2965	1	1	3=20-45
5	585	2965	1	1	4>45
6	1963	2966	1	1	
7	579	2973	1	1	
8	957	63	1	2	
9	107	64	1	2	
10	1109	67	1	2	
11	911	73	1	2	
12	48	87	1	2	
13	50	87	1	2	
14	192	87	1	2	
15	903	88	1	2	
16	5	92	1	2	
17	901	95	1	2	
18	1975	2982	1	2	
19	974	2984	1	2	
20	1108	2984	1	2	
21	74	69	1	3	
22	583	2966	1	3	
23	971	2966	1	3	
24	1950	2971	1	3	
25	576	2979	1	3	
26	59	80	1	4	

To begin a matrix analysis in Excel with these data, the first step is to use the Pivot Table function to create a transition array. Pivot Table is found under the “Insert Tab” in Excel 2010 and under the “Data Tab” in Excel 2003.

- Select 'Microsoft Office Excel list or database' from the first section and 'Pivot Table' from the second; Click Next.
- For the data range, highlight the two columns of data, including the headers; Click Next.
- Decide where you want to place the table; Click Finish.
- Drag 'Year 1' from the Pivot Table Field list to "Drop Row Fields Here."
- Drag 'Year 2' from the Pivot Table Field list to "Drop Column Fields Here."
- Drag 'Year 2' from the Pivot Table Field list to "Drop Data Items Here."

The transitions should then appear in the matrix as shown in Figure 4.4. These values will then be used to calculate transition probabilities in order to complete the rest of the analysis as described in Box 3 and the section *Using PopTools*.

Figure 4.4. Screen shot of a Pivot Table created in Microsoft Excel 2010 with size class data for *Lespedeza leptostachya* from 2007 and 2008.



The transition array created in Pivot Table can then be used to calculate transition probabilities, survival probabilities, and fecundities as described in Box 3.

Box 3. Demography analysis overview

Creating a transition matrix

TO stage class (t+1) \ FROM stage class (t)	1	2	3	4
1	0	F_2	F_3	F_4
2	G_1	P_2	0	0
3	0	G_2	P_3	0
4	0	0	G_3	P_4

F = fecundity
P = Survival probability
G = Growth; transitional probability

Calculate transition probabilities, survival probabilities, and reproductive coefficients

The transition matrix represents all of the contributions that each stage or size class makes to every other stage or size class during one time interval transition. Each transition (m_{ij}) from time 1(j) to time 2(i) is calculated with the equation $m_{ij} = (n_{ij}/n_j)$, where n_{ij} is the number of individuals in class *j* at time t that moved to class *i* in time t+1 (Bierzychudek 1982). An example of these calculations can be seen in Figure 4.5.

Figure 4.5. Calculation of transition probabilities from the transition array created in Pivot Table.

	To: 2008	1	2	3	4	Total
From: 2007	1	6	4	7	1	18
	2	13	8	6	4	31
	3	5	20	19	6	50
	4	3	7	18	16	44
	Dead	17	11	21	11	60
	Total	44	50	71	38	203

To calculate transition probabilities:

mij = (nij/nj)

nij is the number of individuals in class j at time t that moved to class i in time t+1
transition from class 1 to class 1 = 6/44 or 0.136

Survival probabilities are calculated as the number of individuals in the size class in the Year 1 that are still alive in Year 2, regardless of what class they occupy in Year 2. Transition elements should sum to the survivorship probabilities as shown in Figure 4.6. Final matrix elements are then the product of the transitional probabilities and the survivorship probabilities (Figure 4.7).

Box 3 (Continued)

Figure 4.6. Calculation of survival probability from 2007 to 2008 for all size classes of *Lespedeza leptostachya*.

	To: 2008	1	2	3	4
From: 2007	1	0.136364	0.08	0.098592	0.026316
	2	0.295455	0.16	0.084507	0.105263
	3	0.113636	0.4	0.267606	0.157895
	4	0.068182	0.14	0.253521	0.421053
		0.613636	0.78	0.704225	0.710526

Survival Probability between 2007 and 2008

Size 1	0.613636	Equals the number of individuals in the size class in 2007,
Size 2	0.78	divided by the number that are still alive in 200,
Size 3	0.704225	regardless of size class
Size 4	0.710526	

Notice that the transition elements sum to the survivorship probabilities

Figure 4.7. Final transition matrix elements calculated by multiplying the transition probabilities by the survivorship probabilities for each size class.

	1	2	3	4
1	0.08367769	0.0624	0.069431	0.018698
2	0.18130165	0.1248	0.059512	0.074792
3	0.0697314	0.312	0.188455	0.112188
4	0.04183884	0.1092	0.178536	0.299169

Matrix elements are the product of the transition probabilities and the survival probabilities. Multiply the size-specific survivorship by the transition probability. For example, for Size Class 1 the transition probability equals 0.1363, which is then multiplied by the size-specific survivorship probablity of 0.613636 to obtain 0.08367769

Sexual reproduction (fecundity, see Figure 4.8; average number of seeds or seedlings per female in each stage or size class plus germination rates) and vegetative propagation should both be included in the matrix. It is important to understand the seed class and have an idea of how long they remain in the soil. If a seed germinates within the same year it is shed, no seed category is needed, only a seedling category.

Figure 4.8. Calculations showing the fecundity for each size class over each of 3 years in the study.

Fecundity Average Seed Production Per Year

	Year 1	Year 2	Year 3
1			
2	5.75	1.5	5.5
3	20.5714286	11.14286	29.875
4	91.6375	60.6087	93.66667

We have three annual estimates of fecundity
Using Year 3 estimates, the percent of seeds produced across each of the reproductive size classes are:

Size 2	Size 3	Size 4
0.042647	0.231068	0.72628572

1	2	3	4
0	0.38382031	2.079608	6.536571

9 Seedlings survive to August census, on average, so the proportion of seedlings produced by each size class is shown to the left

Figure 4.9. This is a screen shot of the completed transition matrix for Lespedeza leptostachya including transitional probabilities, survival probabilities, and fecundities.

	1	2	3	4
1	0.08367769	0.44622	2.149039	6.55527
2	0.18130165	0.1248	0.059512	0.074792
3	0.0697314	0.312	0.188455	0.112188
4	0.04183884	0.1092	0.178536	0.299169

This is the completed matrix, with the fecundity estimates added to the product of the transition probabilities and survivorship probablities to the elements in the first row

Using PopTools

PopTools is an add-in for PC versions of Microsoft Excel that can analyze matrix population models. PopTools can be downloaded for free from the PopTools website at: http://www.poptools.org/download/. Once a transition matrix has been created, using PopTools to analyze and project the population into the future is very simple. Many demographic studies hope to model populations into the future where the environment is different or even fluctuating. If this is the case, a stochastic model is more appropriate for your data, and can be run in PopTools using the Numerical Projection macro under Simulation Tools in the PopTools menu. However, deterministic population growth rates are more precise in cases of high variance and with less than 5 years of data. Because these conditions are present in the majority of demographic studies, (Crone et al. 2011), a deterministic model is often the more appropriate model to use.

If you have not already created a life-cycle diagram, one should be produced as the first step to a successful model. These diagrams are helpful in checking that the values in the transition matrix are accurate and reasonable. Color coding the matrix elements can also help in this regard (Figure 4.10a and 4.10b).

Figure 4.10a. Screen shot of the PopTools menu showing where to find life cycle diagram prompt in the Poptools menu

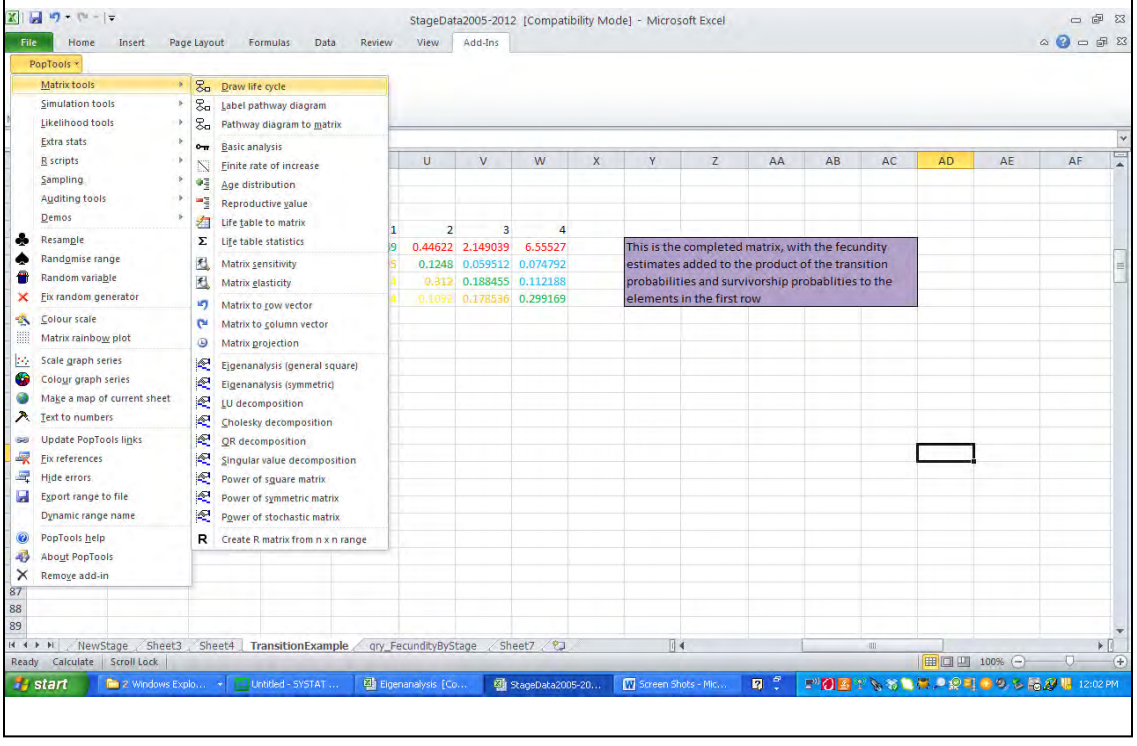
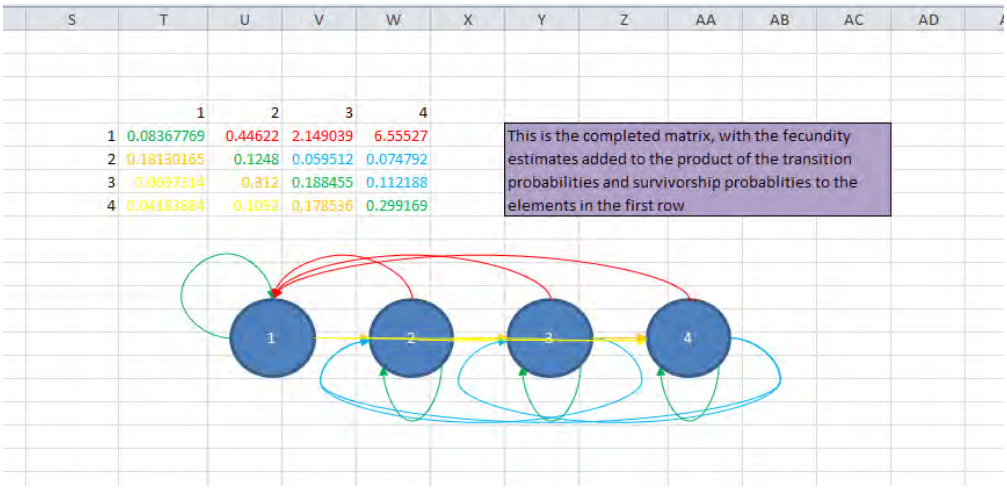


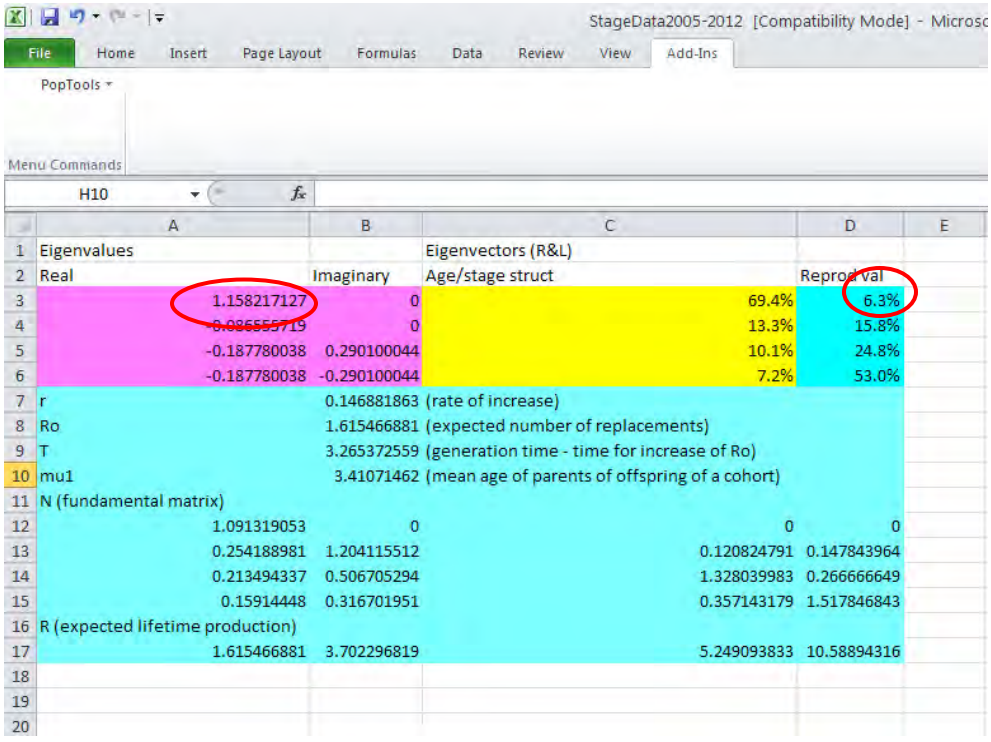
Figure 4.10b The output of the life cycle diagram once it is created. In this example, the red arrows indicate reproduction via seed into size class 1 as well as size reversion from each of the other size classes. The blue arrows indicate size reversion into every class except 1.



To run a basic matrix analysis, go to PopTools > Matrix tools > Basic analysis

- In the dialog box that pops up, click in the 'Projection matrix' field and then highlight the transition matrix.
- Click in the 'Output' field and enter an empty cell for the output to be pasted.
- An annotated screenshot of the basic analysis output in shown in Figure 4.11.
- R_0 = Net reproductive rate; the mean number of offspring by which a newborn individual will be replaced by the end of its life, and thus the rate by which the population increases from one generation to the next.
- T = Generation time; the time required for the population to increase by a factor of R_0; $T = (\log R_0)/(\log \lambda_1)$.
- Dominant eigenvalue, rate of intrinsic population growth = λ.
- Finite rate of increase = r.
- Stable age/stage structure: Proportions of individuals in each class are stable even as total population density grows or declines.
- Reproductive value: The relative contribution to future population growth an individual currently in a particular class is expected to make over its lifetime. It takes into account the number of offspring an individual might produce in each of the classes it passes through in the future, the likelihood of that individual reaching those classes, the time required to do so, and the population growth rate, λ.

Figure 4.11. Screen shot of an annotated example of the output of a basic matrix analysis in PopTools. The leading eigenvalue is 1.15, indicating approximately a 15% rate of growth in population size between 2007 and 2008. We can also see the basic size or stage structure of the population (the yellow block) and the percentage of reproduction that comes from each stage (bright blue). Again, because we have no "Seed" stage in the model we ran, it looks as if Size Class 1 produces offspring, rather than just individuals which stay in the same size category.



	A	B	C	D	E
1	Eigenvalues		Eigenvectors (R&L)		
2	Real	Imaginary	Age/stage struct	Reprod val	
3	1.158217127	0	69.4%	6.3%	
4	-0.086555719	0	13.3%	15.8%	
5	-0.187780038	0.290100044	10.1%	24.8%	
6	-0.187780038	-0.290100044	7.2%	53.0%	
7	r	0.146881863	(rate of increase)		
8	Ro	1.615466881	(expected number of replacements)		
9	T	3.265372559	(generation time - time for increase of Ro)		
10	mu1	3.41071462	(mean age of parents of offspring of a cohort)		
11	N (fundamental matrix)				
12	1.091319053	0	0	0	
13	0.254188981	1.204115512	0.120824791	0.147843964	
14	0.213494337	0.506705294	1.328039983	0.266666649	
15	0.15914448	0.316701951	0.357143179	1.517846843	
16	R (expected lifetime production)				
17	1.615466881	3.702296819	5.249093833	10.58894316	
18					
19					
20					

Box 4. Matrix Algebra terms

This is a very brief overview of the basics of matrix algebra, more detailed information on this topic can be found in Caswell (2001). These definitions are helpful in understanding the terminology of matrix algebra.

Matrix – A rectangular array of symbols (number, variables, functions, etc.)
Entry/Element – One of the symbols a matrix contains
Dimensions – The number of rows and columns in a matrix. A matrix with *m* rows and *n* columns is of dimension (*m* x *n*)
Column vector – A matrix of dimension (*m* x 1)
Row vector – A matrix of dimension (1 x *n*)
Scalar – An ordinary number, or a (1 x 1) matrix
Eigenvector – A vector (x) with the property that matrix (A) multiplication is equivalent to scalar multiplication, so that $Ax = \lambda x$ for some scalar λ.
Eigenvalue – The scalar λ used in relation to an eigenvector

Additional Analyses/Statistics

Calculating sensitivities and elasticities of matrix elements

Both sensitivity and elasticity analyses are forms of a perturbation analysis. This type of analysis considers what would happen to some dependent variable if one or more of the independent variables are changed. A sensitivity analysis is conducted by slightly perturbing each matrix element (transition or fecundity) around its "central" value, then recalculating the population parameters (λ) and recording the per unit change. The result is reported as the amount by which the population parameter changes for each unit change in the matrix element (with all other transition probabilities and fecundities held at their central values).

Sensitivity analysis determines which ages or life stages of a species will cause the biggest changes in population persistence if altered (for example see Figure 4.12). This can be used for predicting future changes in vital rates and quantifying the effects of past changes in response to natural or management factors that affect particular life stages.

To calculate sensitivity in PopTools:

- PopTools > Matrix tools > Matrix sensitivity
- Highlight transition matrix
- Click Go

Figure 4.12. This array represents a sensitivity analysis, and indicates that seed germination, growth and survivorship into the seedling stage, or Size Class 1, is the most important element in the matrix. Growth from Size Class 1 to Size Class 4, though it happens with a low probability, also has a very strong effect on population growth rate. These two vital rates, therefore, have the largest effect on population growth and stability. Managing the population for these two stages will have the best chance of stabilizing and/or increasing the population.

	Seed	1	2	3	4
Seed	0	0	0.003235	0.002514	0.001804
1	3.757503	0.259417	0.051909	0.040342	0.028945
2	0	0.635429	0.12715	0.098817	0.0709
3	0	0.971907	0.194479	0.151143	0.108444
4	0	2.044821	0.409171	0.317994	0.228159

Sensitivity analyses can be difficult to interpret because the demographic variables or transition probabilities and fecundities are often measured in different units. Calculating elasticity in addition to sensitivity can help to alleviate this problem, because elasticity measures are proportional changes. Elasticity values measure the sensitivity of population growth rates to transition probabilities. These measures are scaled so that the

sum of all of the elasticities adds up to one, making it easy to compare the values across all life history variables.

To calculate elasticity in PopTools:

- PopTools > Matrix tools > Matrix elasticity
- Highlight transition matrix
- Click Go

The output is a matrix of elasticity values that correspond to the stages and transitions of the transition matrix (for example see Figure 4.13). Increased elasticity values equal an increased effect of changes in that transition on the population's vital rates (λ). These measures can often be more useful to people like land managers than the actual population parameter estimates themselves.

Figure 4.13. This Elasticity Analysis, which scales for the different measures used to estimate the vital rates of different life stages and size classes, confirms that seed germination and seedling survivorship is very important in terms of its effect on the population growth rate. Additionally, we can see that seed production by individuals in the largest size class is also very important. We shall need to determine in future analysis whether or not these two vital rates are affected by the same management activities.

	T	U	V	W	X	Y	Z
		Seed	1	2	3	4	
	Seed	0	0	0.015901	0.06718	0.151051	
	1	0.234132	0.019402	0.002895	0.002504	0.000484	
	2	0	0.102971	0.014183	0.005256	0.00474	
	3	0	0.060576	0.054234	0.025459	0.010874	
	4	0	0.076468	0.039937	0.050745	0.06101	

Population projection matrix

A projection matrix predicts population size years into the future based on current population size and the transition matrix calculated from demographic data. This is done by simple matrix algebra where each successive year is projected by replacing the previous population numbers with the ones newly calculated. While this can be done with a simple calculator, the Matrix Projection function in PopTools can be used to complete a projection for multiple years in one step. A projection model describes what would happen in the future if the measured conditions remain constant. It is important to not that these models provide little predictive power in most situations, because the environmental and stochastic conditions that occurred during the measurement period are unlikely to remain consistent into the future.

Steps for Matrix Projection:

- Go to PopTools > Matrix tools > Matrix projection.
- Click once in the field labeled 'Matrix' and highlight all cells in your transition matrix.
- Click in the field labeled 'State Vector' and highlight cells containing current population numbers.
- The number of iterations chosen equals how many times the matrix will be multiplied by the population vectors (Use the number of years in the future you are interested in).
- Click in the field for 'Output' and select a cell for the output values.
- Click Go (see example output in Figure 4.14).

Figure 4.14. Sample output of a matrix projection in Microsoft Excel using the add-in PopTools.

Time	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
0	492	175	20	14	7	10
1	707.31	147.31	30.93	10.13	10.9	18.26
2	960.6501	148.8514	41.834	10.975	12.6939	24.2972
3	1272.957	170.9816	54.50275	12.94921	15.26529	30.11714
4	1667.24	210.4571	70.42883	15.805	18.70836	36.84103
5	2170.836	267.2596	90.96428	19.70665	23.23562	45.22934
6	2817.619	343.6835	117.5893	24.90148	29.18003	56.03033
7	3650.557	443.9625	152.1031	31.72596	36.96415	70.09536
8	4724.719	574.2571	196.7872	40.62861	47.1231	88.4544
9	6111.01	742.9028	254.5786	52.19761	60.34257	112.3949
10	7900.898	960.8736	329.2737	67.19815	77.50619	143.554
11	10212.47	1242.46	425.7845	86.62218	99.75507	184.0318
12	13198.24	1606.186	550.4641	111.7535	128.564	236.5332
13	17055.23	2076.018	711.5274	144.2524	165.8391	304.5492
14	22037.95	2682.935	919.5928	186.2652	214.0443	392.5887
15	28475.22	3466.975	1188.383	240.5656	276.364	506.4779
16	36791.85	4479.867	1535.629	310.7385	356.9134	653.7455
17	47536.68	5788.448	1984.243	401.4156	461.0104	844.1204
18	61418.84	7479.075	2563.828	518.5821	595.5266	1090.174
19	79354.47	9663.315	3312.63	669.971	769.3406	1408.152
20	102527.3	12485.32	4280.064	865.5739	993.9247	1819.044
21	132466.5	16131.32	5529.976	1118.3	1284.101	2349.972
22	171148.2	20841.95	7144.855	1444.83	1659.022	3035.977
23	221125	26928.07	9231.274	1866.712	2143.429	3922.336
24	285695.3	34791.37	11926.93	2411.789	2769.295	5067.548
25	369120.6	44950.78	15409.72	3116.036	3577.923	6547.195

SECTION V: Conclusions and Glossary

Conclusions

There are many methods of rare plant monitoring, and it is important to choose the method that will provide the most relevant data while using the least amount of time and resources. The techniques described in this manual span all levels of monitoring from lightly to heavily time/resource intensive. We highlighted some of the most popular techniques to provide consistent and informative data on the population being monitored. Once the monitoring method has been determined, details should be well-documented so the method will be used consistently over time; even with changes in personnel.

Because raw data from a monitoring program do not provide useful information, it is important to summarize and analyze your data. In general, the more data available, the more robust these analyses can be. If more than simple descriptive statistics are to be employed, careful consideration should be given to the planning of the sampling design to pair sampling methods with statistical reliability. This should include seeking the advice of a statistician for appropriate advice. Information from analyzing monitoring data can help to inform land managers on the most appropriate management actions and on the status of rare plants. These analyses are also needed to estimate and predict extinction risk and population viability into the future. Analyzing your data and documenting the results will not only help to ensure your agency is aware of the status of populations, but can help to educate others.

This guidance manual does not include a description of either Integral Projection Models (IPM) or Bayesian statistics, as these are outside the scope of this project. However, Bayesian statistical methods provide an alternate way to analyze data that is likely to be more appropriate to conservation biology problems than traditional statistical methods, and IPMs have been demonstrated to be particularly useful when sample sizes are small, as is often the case for rare species. These techniques should be investigated and considered the future direction of monitoring data analysis.

Glossary

Allee effects – A positive correlation between population size or density and the mean individual fitness of a population or species.

Census unit – A consistently recognizable unit used when density monitoring or conducting a population census. This can be either and individual plant, a ramet, or some other consistent unit.

Count-based PVA – An analysis using data (numbers of plants) from a series of censuses used to predict the total number of individuals in a single population some time in the future.

Cumulative Distribution Function (CDF) – The probability that a population will have hit the quasi-extinction threshold at or before a given future time.

Demographic stochasticity – The temporal variation in population growth resulting from chance variation in the actual fates of different individuals within a year. The magnitude of demographic stochasticity is strongly dependent on population size.

Elasticity – the proportional change in the population growth rate (λ) in response to a proportional change in any element of the transition matrix.

Fecundity – the potential reproductive capacity of an individual.

Genet – A group of genetically identical individuals.

Mean extinction time – A viability metric drawn from the cumulative distribution function (CDF) that shows the average time of extinction.

Median extinction time – A viability metric drawn from the cumulative distribution function (CDF) that shows the time at which half of the possible paths the population might follow have gone extinct.

Modal extinction time – A viability metric drawn from the cumulative distribution function (CDF) that shows the most-likely instant at which extinction will occur.

Probability Density Function (PDF) – The probability density at a certain time (t) is proportional to the probability that quasi-extinction occurs in a small interval of time centered on that time (t).

Quadrat – An area marked off to be used as a sampling unit in which to collect monitoring data. They can be rectangular, square or circular.

Quasi-extinction threshold (N_x) – The minimum number of individuals below which the population is likely to become extinct.

Ramet – One member of a genetically identical group of plants that often appears as an individual plant.
Sensitivity – the rate of change of the population growth rate (λ) with respect to a change in any element of the transition matrix.

Stage class – A division of plants in a population based on age or size to use in demographic matrix modeling. These divisions should be biologically relevant, consistently recognizable, and complete the life cycle of the plant.

Transect – A line run across a population used to sample plants while monitoring.

Transition matrix – A matrix of the probabilities of each transition from one age or stage class to another occurring.

Ultimate probability of extinction – A viability metric drawn from the cumulative distribution function (CDF) of the probability of a population ever going extinct.

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Appendix A: Sampling and Data Analysis Resources

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Appendix B: Case studies

Example 1 – Level 1 Monitoring – *Lespedeza leptostachya* population census

Investigators contributing to this study:
Alona Banai, Northwestern University
Robert T. Bittner, Cornell Plantations
Bill Kleiman, The Nature Conservancy IL
Tiffany Knight, Washington University
Michelle R. Schutzenhofer, McKendree University
Pati Vitt, Chicago Botanic Garden

Introduction

Lespedeza leptostachya, commonly known as prairie bush clover, is a Federally Threatened herbaceous perennial legume, and is endemic to Minnesota (55 populations), Iowa (29 populations), Illinois (16 populations), and Wisconsin (27 populations). Nachusa Grasslands supports the largest population of *Lespedeza leptostachya* in Illinois.

Lespedeza leptostachya occurs in remnant gravel hill prairie at Nachusa Grasslands and, like other species in the genus, it reproduces via seed produced by both cleistogamous and chasmogamous flowers; however vegetative reproduction is also known to occur. Plants reach maturity after six to nine years and may live up to ten years (Sather 1989). A single plant can flower up to four years with low mortality rates (USFWS 1988), and reproductive adults produce up to 560 pods per plant but average about 235 pods.

Seeds are borne singly in pods, reach maturity in October, and are produced primarily from cleistogamous flowers (about 75%). A greenhouse study, conducted at the University of Kentucky, revealed that most *L. leptostachya* seeds germinated in the first growing season and the *L. leptostachya* seed bank longevity lasted only 3 years (Baskin and Baskin 1998). Seed bank and germination studies are underway at Nachusa Grasslands.

Adaptive management of existing populations, coupled with monitoring of the responses to that management, is a critical tool to assess the viability of the species, and controlled burns and grass specific herbicide treatment is being conducted at Nachusa Grasslands (Chicago Botanic Garden 2007).

Data Collection / Monitoring

Lespedeza leptostachya was discovered in Dixon, Illinois in 1981. At that time, there were two subpopulations with a total of 125 plants. In 1986 The Nature Conservancy acquired the first parcel of land that was to become the Nachusa Grasslands Preserve. They eliminated grazing on the preserve in 1986, a standard practice throughout the range of *L. leptostachya,* when a parcel was brought into protection. In order to collect baseline data on the populations after grazing was suspended, a complete population

count was undertaken using a patterned search. The count was performed along a transect of a known sub-population. Groups of 7-10 people walked the length of the site about 1 meter apart, and all plants encountered were recorded and categorized as either sterile, subadult, or adult (See Figure 2.1, Section II). These additional enhanced count data on stage classes were taken in order for us to have the ability to perform a count-based PVA. Very small plants and juveniles are not encountered with this method, and as a result, the true populations could be at least twice as large as the census populations in well-managed sites.

Data Analysis: count-based PVA

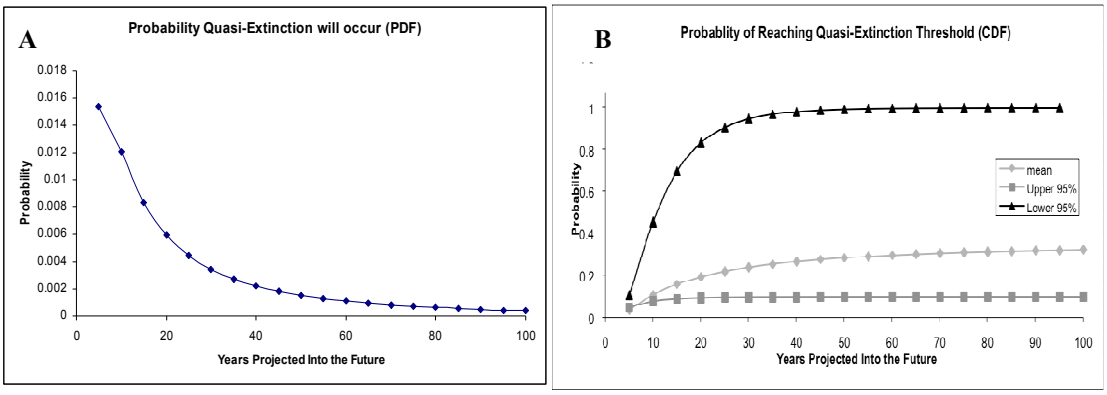
To determine the likelihood of extinction at the site, an analysis of the census counts over a 20-year period was conducted. Visually assessing the trend in this sub-population leads to two conclusions: 1) the sub-population appears to be increasing over time; and 2) the sub-population appears to experience both bonanzas and catastrophes. Although we might expect to see a pattern of "boom and bust" cycles in an annual species, this is not generally true for long-lived perennials. The apparent pattern may simply be an artifact of sampling error. However, it may also indicate a population that is unstable, which may therefore be more susceptible to stochastic environmental events.

To assess if the population has an increased risk of extinction, a count-based method of population viability analysis was undertaken to determine the extinction probability of this species at this site, given a particular extinction threshold. We chose a threshold of 15 individuals of *L. leptostachya*. That is, if the population ever declines to 15 plants the population is considered to be demographically extinct. This threshold, along with the year and count data collected, were then entered into an Excel template in order to calculate the Population Density Function (PDF) and Cumulative Distribution Function (CDF) for the population.

Results and discussion

By entering the year and count data into the Excel template, the values for μ and σ^2 were calculated to be 0.07376 and 0.32017 respectively. These values were used to calculate the PDF and CDF of the population every 5 years for 100 years into the future. The values of these functions, along with the 95% confidence intervals of the CDF, are plotted against time in Figures B and C below. Because the value of μ is positive, the ultimate probability of extinction will be less than 1, and can be calculated in Excel with the following formula: =IF(μ<0, 1, (N_x/N_c)^(2*μ/((q-1)*(σ^2/q))). The probability of ultimate extinction for this population is calculated as 0.9845, or over 98%. However, looking at the graph for the mean CDF, the population looks like it will be relatively stable for the next 100 years, with only a 30% chance of it going extinct before 100 years.

Figures A and B: Graphs of the PDF (A) and CDF (B) plotted against time for the *L. leptostachya* population in Nachusa Grasslands, with census data from 1982-2004.



Conclusions

We conclude from these results that while the population at Nachusa Grasslands is viable over the long-term, adaptive management of the population might decrease the risk of extinction. Given a correlation with local population decline and the cessation of grazing, periodic grazing might be used to increase growth rates and lower the probability of extinction of prairie bush-clover, given its susceptibility to competition. Current burning regimes should also be modified to include grazing, as only burning tends to increase competition. Literature also indicates the species is threatened by lack of genetic diversity within and among populations (Cole and Biesboer, 1992). A plan for interbreeding would, then, allow for more genetic exchange.

If this population did go extinct in 100 years, it could possibly be re-introduced from another site. Finally, further development of sampling techniques to include juvenile individuals would also useful for making more accurate estimates of population size and probability of extinction.

Example 2 – Level 1 Monitoring – *Oenothera harringtonii* population census

Investigators contributing to this study:
Krissa Skogen, Chicago Botanic Garden

Overview of species and study site

This project focuses on *Oenothera harringtonii*, an annual endemic to the short grass prairie of the middle Arkansas River Valley of southeastern Colorado. The flowers of *Oenothera harringtonii* open soon after sunset and are pollinated primarily by two hawkmoth species, *Manduca quinquemaculata* and *Hyles lineata*. Hawkmoths have been documented to travel up to 20 miles in just one night, and may therefore contribute significantly to long-distance gene flow among populations. These moths feed on the nectar of *Oenothera* flowers, which they locate by the strong fragrance produced by the flowers. The autecology of *O. harringtonii* and its pollinators should allow for a comprehensive evaluation of range-wide geographic divergence in floral traits, pollinator-mediated selection through measurements of lifetime fitness, and an understanding of the role of gene flow in constraining divergence. Furthermore, major fluctuations in population size are believed to occur in response to winter and spring precipitation and may have strong impacts on persistence of individual populations.

The middle Arkansas River Valley of southeastern Colorado is known for the Niobrara Formation, a unique geologic feature, and high levels of rarity and endemism, supporting 12 endemic, 30 state-, and 20 globally-imperiled plant species. The combination of rare, imperiled and endemic plants and the intense development pressures (residential, commercial, military, mining and recreational) have made the region a primary focus of conservation efforts in Colorado. However, little is known of the biology of these threatened plants, particularly with regard to their pollination ecology, limiting the ability of land managers to determine appropriate conservation and management. Due to the threats imposed, *O. harringtonii* is considered vulnerable to extinction both globally (G3) and in the state (S3) and is a target species for The Nature Conservancy's Central Short grass Prairie Ecoregional Assessment.

Oenothera harringtonii life history

Oenothera harringtonii is an annual or biennial herb with a basal rosette and 1-5 flowering stems. Individuals often occur in small populations that grow at altitudes between 1,400m and 2,000m. Individual plants can be somewhat cryptic, especially in moderate to heavy vegetation cover (Ladyman 2005). Plants flower from late-April through June, with 5-10 flowers per stem open each day. Individuals are self incompatible (gametophytic) obligate outcrossers with no vegetative reproduction. Fruits mature in July with each stem having 6-20 capsules. There are 60 to 100 reddish-brown seeds per capsule, and seeds are gravity dispersed.

Threats to *Oenothera harringtonii*

Oenothera harringtonii is found in an increasingly fragmented landscape due to anthropogenic influences like urbanization, resource extraction, and recreation. Little is known of the impacts that fragmentation may have on this species and on the community of pollinators upon which it relies for reproduction and long-term

population persistence. Studying populations in both fragmented/developed and unfragmented areas will allow us to determine the extent to which habitat fragmentation may negatively impacting both hawkmoth populations as well as populations of *O. harringtonii*. Results of this work will also have direct relevance to management of *O. harringtonii* populations, which are state-imperiled.
Because *Oenothera harringtonii* is an annual, it is likely to respond rapidly to changes in habitat quality and can serve as an indicator species for other endangered plants in this region.

Data Collection / Monitoring
Numerous populations of *O. harringtonii* in southeastern Colorado have been censused since 2008, to monitor population change over time and to inform PVA analyses to assess long-term viability of populations. Complete population censuses are conducted at the end of each flowering season using a patterned search. At each site, the team locates and flags the margins of the population. Parallel transects approximately 2 meters apart are walked by a team of monitors from one edge of the population to the opposite edge. As each transect is walked, each plant (reproductive and vegetative/rosette) is flagged. Then each person turns around and walks back over the same transect, picking up the flags and counting each plant as they go (see Figure 2.2, Section II). Separate counts are recorded for vegetative and reproductive individuals.

Data Analysis: count-based PVA
A count-based method of population viability analysis was undertaken to determine the extinction probability of *O. harringtonii* at each site, given a particular quasi-extinction threshold. We chose a threshold of 20 individuals of *O. harringtonii*. That is, if the population ever declines to 20 plants at a site, the population is considered to be demographically extinct. This threshold, along with the year and count data collected, were then entered into an Excel template in order to calculate the Population Density Function (PDF) and Cumulative Distribution Function (CDF) for each of four different populations.

At the time of publication of this manual, there were only four years of population census data to analyze, and this is not enough data on which to base a reliable count-based PVA (six to ten or more years of data are preferred). However, this research is ongoing, and additional years of data will be added in the future to increase the accuracy of the PVA. This example serves to illustrate the structure of the analysis, and to begin to qualitatively investigate basic trends in the persistence of each population.

Results and discussion
By entering the year and count data into the Excel template created, the values for μ and σ^2 were calculated for each site and are listed in Table 1, along with the calculated probabilities of ultimate extinction, calculated with the following Excel formula: $=IF(\mu<0, 1, (N_x/N_c)^{(2*\mu/((q-1)*(\sigma^2/q)))}$. These values of μ and σ^2 were used to calculate the PDF and CDF of each population every 5 years for 100 years into the future. The values of these functions, along with the 95% confidence intervals of the CDF, are plotted against time in Figures A through D below. While the predictive

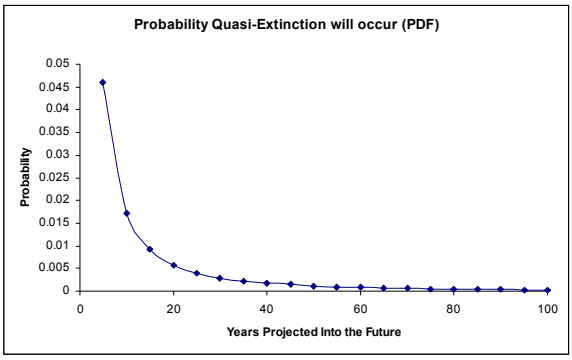
ability of these particular PVAs is not weak to the inclusion of just four years of data, the results can be qualitatively compared. While the Florence and David's Canyon populations are predicted to go extinct quickly, the Pueblo West and Monson populations appear to be viable for a longer period of time. It may be informative to keep note of differences between these two sets of populations to determine if these trends hold up after more years of data have been added to the analysis.

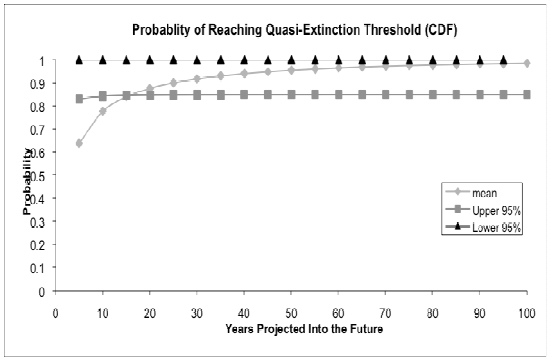
Table A: Values of μ and σ^2 calculated for four different populations of *Oenothera harringtonii* in southeastern Colorado based on population censuses conducted from 2008-2011.

Population	μ	σ^2	Probability of ultimate extinction
Florence	-0.16015	1.53421	1
Pueblo West	0.50964	2.70868	0.21146
David's Canyon	-1.11691	0.83185	1
Monson	0.96896	2.51042	0.13762

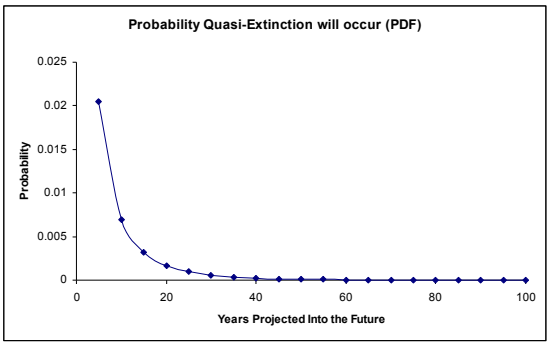
Figures A-D: Graphs of the PDF and CDF plotted against time for four *O. harringtonii* populations in southeastern Colorado (A: Florence, B: Pueblo West, C: David's Canyon, D: Monson) using complete population census data from 2008-2011.

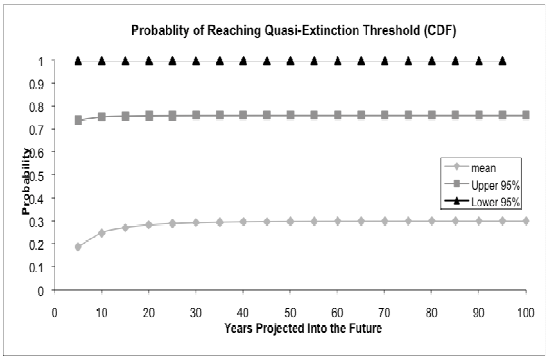
A: Florence



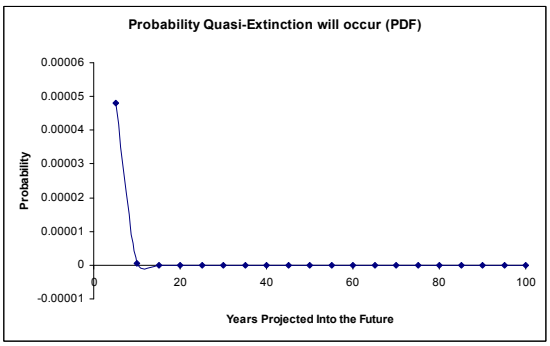


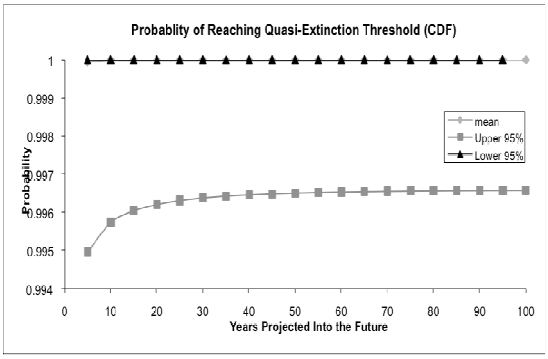
B: Pueblo West



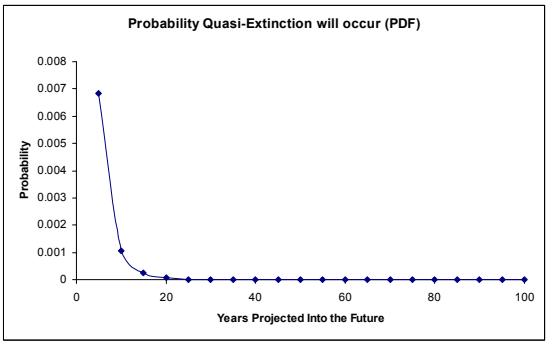


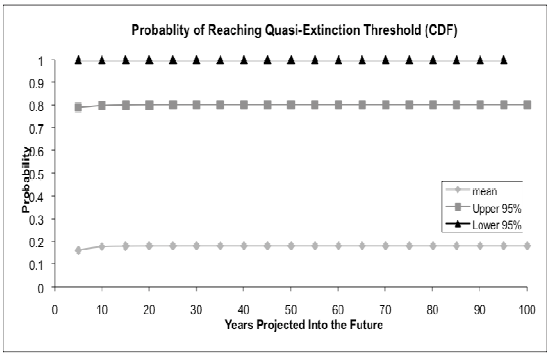
C: David's Canyon





D: Monson





Conclusions

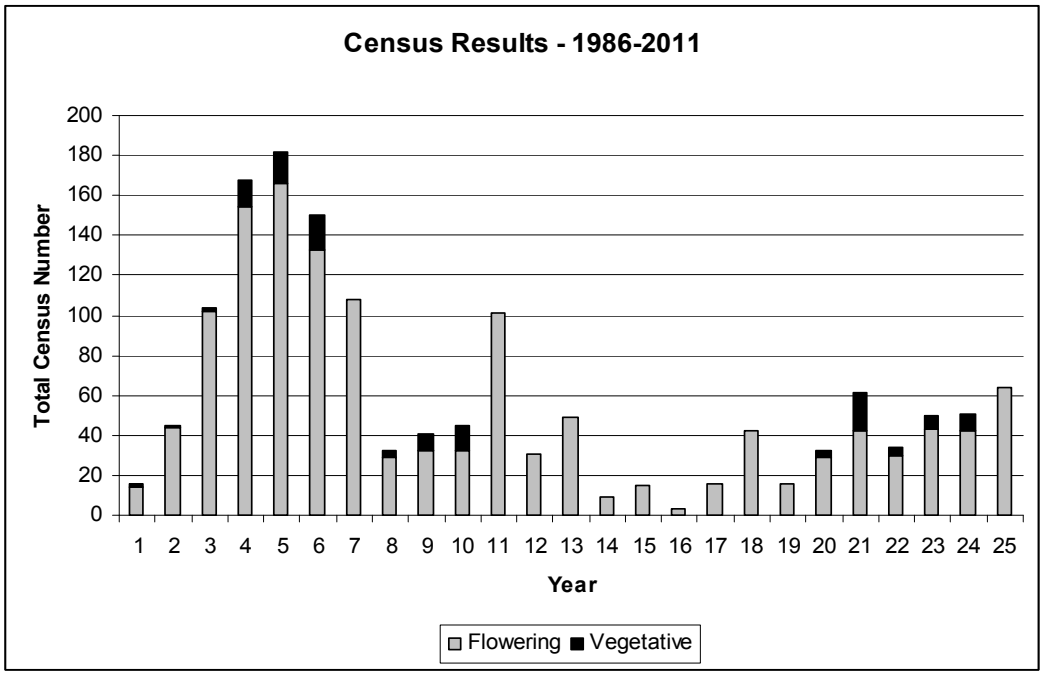
Based on these results from just four census years, two populations, Florence and Pueblo West, appear to be more stable than the David's Canyon and Monson populations based on the probabilities for ultimate extinction and CDF values. An additional 6-10 years of data will contribute to a more robust analysis and will provide more reliable estimates of each population's viability.

Example 3 – Level 1.5 Monitoring – *Lespedeza leptostachya* grazing study with enhanced count data

Population Census with enhanced count data

A population census of the rare plant, *Lespedeza leptostachya*, has been undertaken at Nachusa Grasslands in Dixon, IL since 1986, as described in Example 1. The standing stage distribution within a population can tell you much about the viability of a population, regardless of whether or not there are management activities being performed at the site. As with most count-based data, consistency is a necessity in obtaining useful data. For example, Figure A shows data from a long-term census with stage counts of one sub-population of *L. leptostachya*.

Figure A. Results of data collected from an enhanced count (Level 1.5) population census of *Lespedeza leptostachya* over 25 years at Nachusa Grasslands. Bars show proportions of the population that are flowering (gray) or vegetative (black).



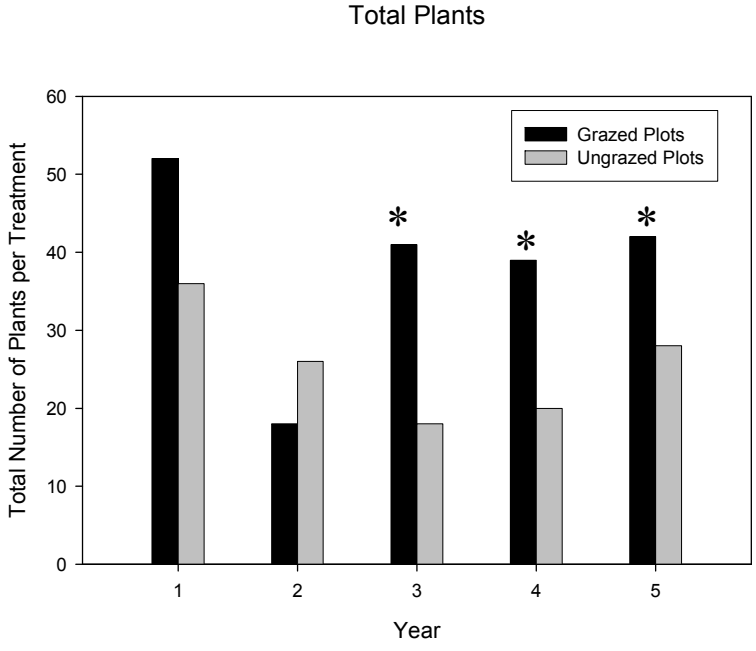
The population clearly fluctuates over time, and, without a count-based PVA or another projection method, the population appears to be in decline. The assumption that this species is a fairly long-lived perennial makes the decline from year to year seem particularly troublesome for the population. However, the demographic matrix model presented in Example 4 reveals that this species has a generation time of approximately 4.5 years. Given this insight, the population might instead be oscillating over time, with the count in the 25th year showing the beginning of an upward trend. Only further long-term monitoring will provide the data necessary to distinguish between these two scenarios.

One way to increase the information of a population census is to either simultaneously record observations of stage distributions during a walking census (as done above), or to take random subsamples before the full census when it is difficult to distinguish juveniles from the background vegetation. The graph in Figure A illustrates this issue, as the running proportion of vegetative individuals does not reveal a clear pattern. This is mostly due to the cryptic nature and low detectability of vegetative plants in this species. The lack of a pattern suggests either a failure to count the stage across all years, or the inability of naïve participants to detect vegetative plants amongst background vegetation. Smaller, vegetative plants are also more difficult to detect in years in which the habitat has not been burned, as they are hidden by the standing dead biomass. Therefore, the smallest stages or size classes will likely be underestimated unless a formal sub-sampling scheme for these plants is utilized.

Grazing study

In addition to the annual census, a study was conducted beginning in 2000 to investigate the effects of grazing on juvenile recruitment in *L. leptostachya*. In this study, 30 permanent plots were established, but marked individuals were not followed. Half of the plots were grazed, while half were left ungrazed to serve as a control. Looking at the count and stage class data collected in this design, there was only enough statistical power to determine that grazed plots had a significantly greater number of plants than ungrazed plots. We also determined that the number of juveniles present approached significance (p-value 0.06) in the grazed versus the ungrazed plots. Had we initiated our study with more than 30 plots, we might have had enough statistical power using stage counts alone to have clearly indicated a treatment effect.

Figure B. Results of data collected from an enhanced count (Level 1.5) population census of *Lespedeza leptostachya* in plots grazed and left ungrazed at Nachusa Grasslands. Asterisks indicate statistically significant differences p= 0.05.

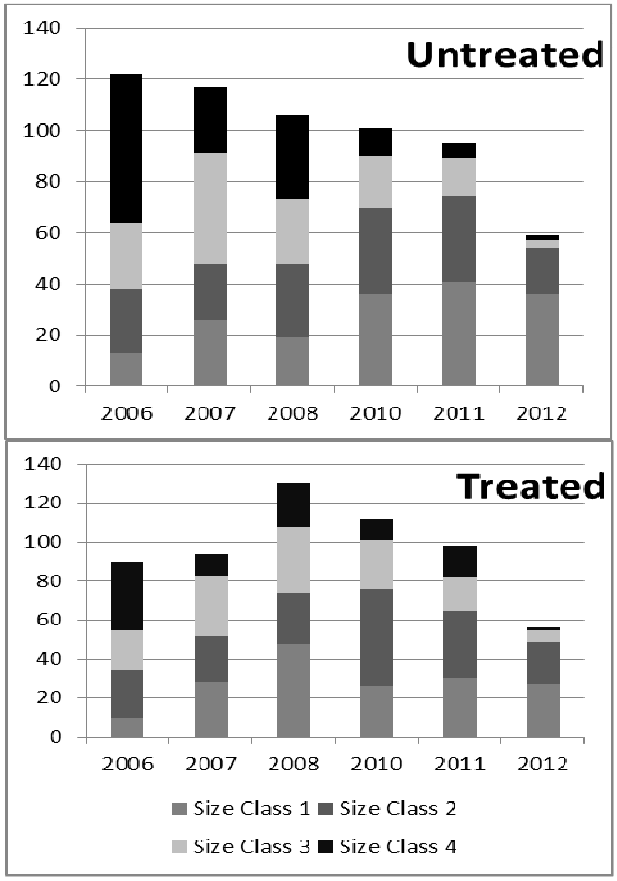


Simulated Grazing

In 2004, we increased the number of plots, and as grazing cattle on-site proved to be difficult to implement, particularly because of a lack of available water, a grass-specific herbicide was applied to 22 out of 58 study plots in 2006, 2007 and again in 2008 to simulate grazing. Herbicide was re-applied each spring, and census and stage class data were collected annually in each plot. Data collection was missed in 2009, and there was a severe season-long drought in 2012, which affected the number of plants seen that year.

While the untreated plots initially had a greater number of plants, it is clear from the count data that the population size is trending downward. Additionally, the number of large reproductive individuals is decreasing as well, indicating mortality among Size Class 4 plants. We see essentially the reverse trend in the treated plots. In the years following treatment, the standing count of individuals increases, with a clear increase in the number in the smallest size classes. As the time-since-treatment accrues, the population size begins to diminish (Figure C).

Figure C. Number of plants in each stage class each year in plots treated with herbicide and untreated plots from 2006 to 2012.



In this example, we can clearly determine that the treatment applied has the desired effect, both in terms of increasing the standing count of the population, and increasing the proportion of seedlings recruiting into the population. If this is the monitoring goal, a standing census count, coupled with recorded data on the stage distribution, allows us to confirm that we have obtained our management objective in the most efficient manner. However, the graph does not tell us whether the short-term trends we see in response to management are effective over time. It also does not allow us to address the question of whether the population is actually growing or just oscillating naturally over time. If we have done a reasonable job of randomizing our treatments, this should not be the case. Given the two graphs above, however, it is reasonable to question if, by chance, we are seeing natural periodic increases and decreases in the standing counts that happen to be occurring at different phases. To address this possibility, it is necessary to undertake a full demographic analysis, and calculate the intrinsic rate of population growth to compare across treatments. This analysis is covered in some detail in Example 4.

Example 4 - Level 3 Monitoring: *Lespedeza leptostachya* demography

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Introduction and Data Collection

In 2006, we initiated a study on *Lespedeza leptostachya* to determine if simulated grazing could be used as a management tool to increase population growth rates. We placed 58 meter-square plots centered on randomly selected plants. All plants within each plot were marked with metal tags and mapped. We randomly assigned half of the plots to a grass-specific herbicide treatment, to simulate grazing and reduce grass competition. We hypothesized that this would increase seedling production and therefore increase the overall population growth rate. We conducted annual surveys in late August to census the plots and took measurements on plant size and seed set. We closely examined plots for seedlings and other new plants. We also recorded observations of plot-level plant diversity and cover, and measure the average height of the dominant grass, *Schyzachyrium scoparium*.

Data Analysis and Results

We collected data annually before and after years in which treatment was applied, totaling six transitions over the course of the study period. We built two models for the study site, one for plots that received treatment, and one for plots that served as the control. Matrix elements for which we had multiple years of data were averaged across years, creating a single matrix for each treatment type. We have four years of seed counts, and, as there were no significant differences in seed production between treated and untreated plots, we averaged fecundity elements as well. We observed seedlings only rarely during the study, and a Chi Square analysis early in the treatment period indicated that there was a significant difference between treated and untreated plots. In 2010, however, one control plot experienced a pulse in seeding emergence, only two of which survived into the next year. As the total number of seedlings observed was low, we used the average number produced across years to parameterize models for both treatment levels.

The leading Eigenvalues (λ) for treated and untreated plots were 1.182306 and 0.988357, respectively, indicating a strong treatment effect on population growth rates. Sensitivity analysis revealed similar patterns between treated and untreated plots. Seedling establishment (seed germination, growth, and survivorship into the seedling

stage Size Class 1) is the most important element in the matrix. Growth from Size Class 1 to Size Classes 3 and 4, also has a very strong effect on population growth rate, although such transitions occur with low probability. Elasticity analysis confirmed that seed germination and seedling survivorship is very important in terms of its effect on the population growth rate. Seed production by individuals in the largest size class is also very important, as was the transition of Size Class 1 individuals into Size Class 4, according to the elasticity analysis. Adult survivorship does not appear to be an important factor in the population dynamics of this species at this site, and individuals appear to be much shorter-lived, on average, that previously understood. Generation times for treated and untreated plots, 4.579 and 4.510 respectively, were similar, though slightly longer for treated plots.

Conclusions

When we began the study, we hypothesized that decreasing grass competition and cover would significantly increase the probability of seedling growth and survivorship, and this appears to be the case. Continuing management activities, including prescribed fire and grazing to decrease both living and dead grass cover are indicated. Indeed, as a result of the cumulative monitoring on this species, the Site Stewards at Nachusa Grasslands have determined that including bison in the community may provide the most effective and efficient management for this species.