

## **A guide to the use of distance sampling to estimate abundance of Karner blue butterflies**

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This guide is intended to describe the use of distance sampling as a method for evaluating the abundance of Karner blue butterflies at a location. Other methods for evaluating abundance exist, including mark-release-recapture and index counts derived from Pollard-Yates surveys, for example. Although this guide is not intended to be a detailed comparison of the pros and cons of each type of method, there are important preliminary considerations to think about before selecting any method for evaluating the abundance of Karner blue butterflies. These include:

(1) How will monitoring data be used to make management decisions?

- For the Karner blue butterfly, methods for monitoring adults that yield an absolute population estimate, rather than an index, can help managers assess the risk of Karner blue extinction at a site because the actual population density or abundance is being estimated. Distance sampling produces an absolute density estimate on a given day and therefore may be useful in this regard.
- Theoretically, distance sampling, mark-release-recapture, or index methods can be used to assess population trends through time, and these trends can be examined to assess the relationships among habitat change, climate, and population change. However, the ratio of the absolute population size, such as can be estimated by distance sampling, to a population index can vary from day to day. An index represents an unknown fraction of the total population and this fraction may be different on different days. Thus, it is not correct to assume that distance sampling and index counts are producing the same results concerning population trends.
- The Karner blue butterfly produces two broods of adults between May and August. Occasionally, adults from the end of the first brood may survive until the start of the second brood but generally little overlap between broods occurs. Analysis of population trends through time are probably best based on estimates of the total number of butterflies present throughout a single brood, rather than population estimates from one or two days within a brood. Aaron Ellingson (personal communication) has shown for the Uncompaghre Fritillary that peak numbers are not consistent predictors of brood size. Nowicki et al. (2005) found the same for several species of European butterflies and concluded, *“Taking everything into account it appears that the assumption of a constant proportion of individuals occurring at peak population is unlikely to be ever met in butterfly populations.”*
- Because large confidence intervals can exist around brood estimates, and because Karner blue brood sizes can vary substantially from year to year, it is important to determine before embarking on a monitoring program whether population trend in time can be separated from within-year variation in estimation and between-year variability in actual population size. This is one reason why it is important to

consult with a statistician when establishing a monitoring program and during the course of the program. Given some preliminary or typical data, a statistician can perform a power analysis that will help address issues such as whether you are likely to be able to discern population trends given background variation.

(2) Are we monitoring the correct thing?

- The relative importance of different life stages in determining Karner blue population size is not well established at present. However, recent work by Steve Fuller may significantly improve our assessment of how production and survival of different life stages (eggs, larvae, pupae) contribute to the overall adult population size (Fuller 2008). One should ask whether monitoring adults, as is typically done with distance sampling, will meet the objectives of your program. For instance, should one be monitoring egg or larval density and survivorship instead?
- Are the habitat monitoring units going to meet your program's needs? Rich King (2003), for example, has pointed out that the ability of monitoring data to comment on the effectiveness of habitat management is often compromised by the lack of proper experimental design comparing populations before and after management actions while maintaining proper control sites in which the management action does not occur.

Objectives of a distance sampling program

- If monitoring adults meets your program's needs, distance sampling can yield more reliable estimates of population abundance than typical index methods (Brown and Boyce 1998). Both distance sampling and index methods count butterflies along transects. Distance sampling adjusts these counts for differences in detectability of butterflies at different distances from the transect line while index methods make no such adjustment. Because of this adjustment, the end product of a distance sampling survey is an estimate of the actual density of butterflies along the transects. The end product of the index survey is a count that is assumed to be proportional to the butterfly abundance present in the surveyed area. However, we rarely know what that proportion is and whether that proportion remains nearly constant from day to day, year to year, and observer to observer. Therefore, if your monitoring program meets the assumptions of distance sampling, distance sampling can produce an estimate of actual butterfly density rather than an index whose relationship to actual abundance is not well defined.

Challenges in implementing a distance sampling program include the following.

- **How does a distance sampling program proceed?** A distance sampling program for the Karner blue butterfly might proceed by (1) delimiting the site for

which you want to estimate abundance, (2) establishing line transects through the site, (3) walking the transects and recording distances from the transect line to observed Karner blues, (4) using program Distance (Buckland et al. 2001, Thomas et al. 2004) to convert the recorded distances to an estimate of density in the site, and (5) multiplying the density estimate by the area of the site to yield an estimate of the population across the site for the day of the survey.

- **Ensuring that assumptions of distance sampling are met.** These assumptions include observing all butterflies that occur directly on the transect line and accurately measuring distances from the transect line to butterflies observed away from the line. Accurately determining which distance category an observed butterfly occurs in relative to the transect line (e.g., a butterfly was observed within 0.5 to 1.0 m from the transect line) is generally sufficient for producing a reliable estimate of density using the distance sampling protocol.
- **Many factors can affect the proportion of the population observed during a survey.** For example, time of day or temperature at the time of survey can affect results (Harker and Shreeve 2008) and should be standardized to the extent possible.
- **Survey design is a challenge for any type of population assessment survey.** A distance sampling program for the Karner blue butterfly might proceed by first delimiting the site for which you want to estimate abundance, as noted above. Often a tradeoff occurs in defining your site. In scenario 1, the border of a site is drawn, and transects are randomly placed throughout the site, without regard to the suitability of areas for the Karner blue. For example, you might be interested in knowing how many Karner blues occur in your entire park and you could then simply define the boundary of your survey site as the boundary of your park. Areas of non-habitat, wetlands, or parking lots, for example, may be surveyed and the number of Karner blues observed per hour might be relatively low. However, the area of the site is accurately known when it comes time to multiplying area by density to yield Karner blue abundance across the entire site. In scenario 2, one identifies “good” habitat and places transects only within the “good” habitat and estimates density only for “good” habitat. More butterflies are likely to be observed per hour surveying. However, this method assumes that one can accurately identify and map “good” habitat. When we multiply density by area to estimate total abundance, we are really estimating total abundance within “good” habitat. If the Karner blue only occurs in “good” habitat then this estimate will be of the entire population. If the Karner blue occurs in marginal habitat as well as “good” habitat, the total abundance estimate will be off but the error might be small enough that the added efficiency, and increased Karner blue observations, resulting from surveying from just the “good” habitat, might make this a worthwhile tradeoff. The tradeoffs between scenario 1 & 2, and within scenario 2, are indicative of difficulties in defining sites and effectively placing transects. You might consider using a stratified sampling design, where transects would be placed in low, medium, and high quality habitats to obtain a more comprehensive

population count. Different distance sampling analyses might be carried out for each habitat type and the population estimates from each habitat type summed for the total estimate across the entire location. Good statistical advice is very important at this stage. Strategic placement of transects can help to reduce the sampling effort. Information a statistician will need to help you effectively layout transects may include (1) accurate maps of possible survey sites that include information on habitat variation across the sites, (2) preliminary survey results that might inform the statistician of where butterflies occur, (3) a rough estimate of how many butterflies you might expect to encounter along a given survey route, and (4) an explanation of what you hope to accomplish from your surveys.

- **Distance sampling produces a population or density estimate for a single day but we are often interested in estimating the total number of butterflies present in an entire brood.** Therefore, we will often repeat the distance sampling survey on several days during a brood to produce population estimates for several days within the brood.
  - If total brood abundance is desired, these daily abundances must be converted into an estimate of total abundance for the brood. This conversion must take into account the fact that some of the butterflies present on a given day will be adults that newly emerged on that day, some will have survived from a previous day, and some butterflies present on the previous day will not have survived to the current day. Therefore, if you simply add abundances from two days, you will be double counting some adults. While methods for estimating total population size are readily available for vertebrate populations in which the entire population is often present on a given day, determining total size of butterfly populations is more challenging when using methods, such as distance sampling, in which individual butterflies are not marked. This is because butterflies like the Karner blue have short life spans, so the entire brood is not present at a given moment in time and the population estimate on a single day will not equal the total number of butterflies present in a brood. While methods for estimating butterfly brood size using mark-release-recapture are available (Gall 1985), a standard method for converting daily abundances from distance sampling, or similar methods, into a brood estimate with associated error has not yet been published. However, some possible approaches have been published or suggested. If a method is developed, it will likely apply information about survivorship to the data on daily abundances. Survivorship will allow us to estimate how many butterflies present on a given day were present on a previous day. Estimates of daily survivorship have been made at several Karner blue locations from mark-release-recapture studies. Although it is preferable to establish survivorship at each specific location, if this is not feasible, the estimates from these other locations can be used as an approximation, if survivorship rates are to be applied in the brood estimation process. Another, currently available method for converting daily abundance estimates into brood estimates is through use of INCA (Insect Count Analyzer) software

(<http://www.urbanwildlands.org/INCA/>) (Longcore et al. 2003). At other sites (e.g., Necedah NWR), abundances from surveys separated by seven days are added together to produce a brood estimate. Data are likely available for comparisons of these different methods for estimating brood size for the Karner blue. However, the comparisons have not yet been done.

- **Advantages and disadvantages of distance sampling.** Density estimation using distance sampling helps overcome biases in detection that are not accounted for by typical index methods. Nonetheless, distance sampling does not avoid all biases in density estimation. Bart *et al.* (2004) noted that distance sampling can have problems with incomplete coverage of an area (e.g., butterflies might be hidden by a hill), butterflies fleeing from the observer, inaccurate distance measurement, sampling unrepresentative habitats, insufficient butterfly detections to accurately estimate density, and observer-to-observer variation in detection of butterflies. In fact, these sources of error might be more problematic when applied to birds than relatively weak fliers such as the Karner blue that are measured within short distances of transects.
- **Seek statistical advice in setting up a sampling program.** Good statistical advice is especially important in survey design (i.e. where should we place transects, how many transects do we need to achieve a given level of confidence in the population estimate). It also is useful for the actual density calculations and when considering how to convert daily population estimates to brood size estimates. Brian Underwood, of the US Geological Survey, has recently developed a model, implemented through an Excel spreadsheet, for estimating the length of transects likely necessary for achieving a given level of error (amount of variation) around a daily population estimate when using distance sampling. The greater the length of transects surveyed at a site, the more accurate the density estimation is likely to be. Brian Underwood's model can help you understand the tradeoff between survey effort and estimation error.
- **Can results from distance sampling surveys be applied to different sites?** The distance sampling calculation involves estimating an "effective strip width" (*esw*). Knowing the *esw* for a transect allows one to convert counts from that transect to an estimated density. Buckland et al. (2001, p. 53) define *esw* as follows, "*The parameter  $\mu$  is often termed the effective strip width, or more strictly, the effective strip half-width; if all objects were detected out to a distance  $\mu$  on either side of the transect, and none beyond, then the expected number of objects detected would be the same as for the actual survey*". Although collecting the information on distances from transects to butterflies and then doing the distance calculations adds relatively little time to the process of counting butterflies and processing the results, there is still a question of whether a "universal" *esw* might exist for the Karner blue, obviating the need to estimate *esw* for particular sites. Here is a summary of *esw* at several Karner blue monitoring sites across its range. (N.B. These data belong to their respective researchers, so please do not disseminate without their approval). From these studies, there is about a 6-fold range of

estimated *esw*, suggesting that producing an *esw* estimate specific to your site is a good idea and that obtaining new *esw* in each survey year or brood might also be warranted.

Site	Subsite	Esw	Esw coefficient of variation (unless otherwise noted)	Source
Necedah NWR	East Rynearson	1.63	19.38	(Brown and Boyce 1998)
	North Rynearson	1.36	10.44	(Brown and Boyce 1998)
	South Rynearson	1.41	9.86	(Brown and Boyce 1998)
	East Sprague	1.24	6.46	(Brown and Boyce 1998)
	Goose Pool	1.54	8.39	(Brown and Boyce 1998)
Allegan, Flat River, Muskegon SGA, Michigan		1.12	1.07-1.18 CI	John Lerg
Glacial Lake Albany Recovery Unit	Apollo Drive North	0.93	7.07	Kirstin Breisch
	Apollo Drive Restoration	0.89	9.06	Kirstin Breisch
	Colebrook Christmas Tree Farm	1.17	9.54	Kirstin Breisch
	Crossgates Hill	0.83	10.62	Kirstin Breisch
	Crossgates PROW	0.98	13.04	Kirstin Breisch
	Curry Road	1.10	11.30	Kirstin Breisch
	Edie Road Restoration	0.98	13.04	Kirstin Breisch
	Edie Road Sandpit	0.99	15.00	Kirstin Breisch
	Forst and Bick	0.74	21.46	Kirstin Breisch
	Johnson's Junkyard	0.79	15.79	Kirstin Breisch
	Route 45 Sandpit	0.58	15.98	Kirstin Breisch
	Saratoga Airport	1.58	11.61	Kirstin Breisch
Oceana Co. MI,		3.05	18.15	Jim Dunn

Muskegon Recovery Unit, July 2005				
		3.12	14.45	Jim Dunn
		2.51	11.89	Jim Dunn
		1.98	9.81	Jim Dunn
		3.66	8.08	Jim Dunn
		1.99	9.73	Jim Dunn
		2.45	8.74	Jim Dunn
		1.81	7.61	Jim Dunn
		1.94	5.12	Jim Dunn
		2.74	3.75	Jim Dunn
Manistee N.F. 2005	Pooled across sites	1.87	(1.68-2.08) 95% ci	Sarah Mayhew

## IMPLEMENTING A DISTANCE SAMPLING PROGRAM

The following is a guide to setting up a distance sampling program written for Indiana Dunes National Lakeshore. Some of this information will be specific to Indiana Dunes – survey design, for example, and will need to be modified for other sites. This guide describes data entry using palmtop computers. You may chose to use paper forms or other computer systems and data forms in the field.

At Indiana Dunes, adult Karner blue butterflies (*Lycaeides melissa samuelis*) were sampled along twenty-five 100-m-long transects. For each butterfly observed, the perpendicular distance to the transect line was recorded. An index of nectar plant abundance was also recorded for each transect line. Note again that the specifics of transect placement, transect length, and transect numbers will likely be different at your particular site.

I. Identification of Karner blue butterfly adults.

The wingspan of a Karner blue butterfly adult (both wings open) is about the diameter of a quarter (1 inch).

Topside of male Karner blue. Note the solid bright violet blue color and black band and white fringes on margins of wings:



Underside of Karner blue (both sexes). Note the grayish fawn color and continuous band of orange crescents with metallic spots along the margins of both fore and hind wings:



Topside of female Karner blue. Note that the color is darker than that of the male. The female is dull violet to bright purplish blue near the body and light to dark gray-brown closer to the wing margins. A band of orange crescents occurs along the margin of the hind wing; faint orange crescents (or spots) may also be present near the margins of the fore wing:



#### OTHER SIMILAR BUTTERFLIES:

There are two other small blue butterflies that occur at Indiana Dunes in the same habitat as the Karner blue, often at the same time – the eastern tailed-blue (*Everes comyntas*) and the spring azure (*Celastrina ladon*). The “spring azure” is a widespread “species” that probably actually represents six or more species continent-wide. Having binoculars might help identify butterflies without having to chase them.

Another similar butterfly that occurs within the range of the Karner blue is the silvery blue (*Glaucopsyche lygdamus*). The silvery blue has one brood a year with the adult flight period (in the Karner blue range) generally occurring during mid May - late June (Glassberg 1999).

Because the topsides of the wings of the species discussed here have blue coloration similar to the Karner blue, the key to identification of the Karner blue is to inspect the undersides of the wings and confirm the presence of a continuous band of orange crescents along the wing margins. Only the Karner blue (of the species described here) has a continuous band of orange crescents on the underside of its wings.

Underside of spring azure. Note the lack of orange crescents along the wing margins:



Topside of male spring azure. Note more elongated forewings.



Topside of female spring azure. Note lack of lack of orange crescents.



Underside of eastern tailed-blue. Note “tails” on hind wings and fewer orange crescents than Karner blue:



Topside of male eastern tailed-blue. Note “tails” on hind wings.



Topside of female eastern tailed-blue. Females can range from darker blue to dark gray to brown-colored. Note “tails” and only two orange crescents on hind wings as opposed to several for female Karner blue:



Topside of male silvery blue. Note lack of orange crescents:  
(female similar but w/ blue shading to brown closer to the wing margins)



Underside of silvery blue (both sexes). Note lack of orange crescents:



## II. The transects.

Each 100-meter-long transect is marked with 11 flags – a green flag to mark the start of the transect (0 meters), 9 white flags marking each 10 meter interval (10, 20, 30, 40, 50, 60, 70, 80, 90 meters), and a red flag to mark the end of the transect (100 meters). The twenty-five transects are labeled as IM01, IM02, IM03...IM25 (IM = Inland Marsh, where these sample surveys were conducted). Each flag is labeled with the transect number and the distance along the transect. For example, IM13-20 is the 20 meter point along transect IM13.

Questions of where to place transects, how many transects to use, how long the transects should, and how far apart they should be placed are briefly discussed under “Challenges in implementing a distance sampling program” above. As noted there, Brian Underwood has recently (Spring 2008) developed a method for estimating the length of transect likely needed to yield population estimates with a certain level of accuracy. This estimation method will be tested for the first time in 2008. The amount of variability in butterfly counts from transect to transect plays an important role in determining the error (standard deviation or coefficient of variation) of the population estimate. Therefore, determining transect layout – number, length, placement of transects - may be an iterative process in which initial layouts may be established based on variability among transects recorded in preliminary trials or at other sites. The initial layout may be modified (total length of transects increased or decreased, for example) once site specific data on inter-transect variability in butterfly counts are gathered. Generally, the same transects can be used from year to year. However, as new habitat patches becomes available due to management or as patches are no longer used by Karner blues because of succession, for example, it is important to consider whether the transects in place are providing a population estimate for the area you are interested in evaluating. If not, the transect layout might need to be modified from that previously used.

III. Measuring lupine abundance along the transect. (NOTE: You may, or may not, decide to measure lupine or nectar plant abundance at you site. The following are two quick, simple measures that can be collected as surveys are done, with minimal survey disruption).

Once each year, lupine abundance along the transect was measured. The end of May is a good time for this because the plants are large, live, often in flower, and conspicuous. For each 5-meter-long interval along the transect (e.g., 0-5 meters, 5-10 meters, 10-15 meters...) the areal coverage of lupine is estimated within 1 meter (approximately one arms length) of each side of the 5-meter interval. Area is estimated into one of three categories:

- 0: no lupine present
- 1: < 50% areal cover of lupine
- 2: > 50% areal cover of lupine

For each 100-meter transect, 40 such estimates will be recorded – twenty 5-meter intervals (0-5, 5-10, 10-15, 15-20, 20-25, 25-30, ..., 95-100) on each of two sides of the transect line. Record the estimates as follows:

Interval	Cover LEFT	Cover RIGHT
0-5		
5-10		
...95-100		

#### IV. Measuring nectar plant abundance

During each survey of the transect line, abundance of nectar plants should be measured. At each 10-meter point (0, 10, 20, 30...100 – eleven points in total), stop and imagine a 1-meter-diameter circle (measure your arm length as a guide), broken into four equal sectors, centered on the transect line. For each of the four sectors, note whether there are any plants in flower. For each point, you will then have a number, from 0-4, representing the number of sectors with plants in flower. Obviously you can modify this protocol to include only a subset of plants in flower or to subcategorize plants by some grouping such as color or family. Record the estimates as follows:

Point	Number of sectors with plants in flower
0	
10	
...100	

This method can also be used to assess lupine abundance, as an alternative to the lupine assessment method described in III. above. If you do use the method described in IV for measuring lupine abundance, you might consider measuring abundance of all lupine, not just lupine in flower, because lupine is not a Karner blue nectar plant and we are interested in the amount of lupine leaves present, not in the amount of flowering lupine present.

#### V. Counting butterflies along the transect line

The most important part of this survey is to observe all, or nearly all, butterflies that occur on or near the transect line and to record accurately the perpendicular distance from the transect line to butterflies observed when walking the transect. One of the major assumptions of distance sampling is that all butterflies occurring on, or very near, the transect line are observed. If this is not accomplished, and if the degree to which butterflies on the transect line are missed is known, a correction factor can be applied in the analysis software to estimate density more accurately. The correction factor equals the actual number of butterflies present in the first interval (< 0.5 m from the transect line at Indiana Dunes) divided by the number in that interval counted by the observer, assuming a single observer is used. Although careful surveying by a single trained observer should identify most butterflies in the first interval, you can employ measures that will test or correct for any deviations from perfect detection on or near the transect line. One method would be to use two observers per transect rather than one. If two observers are used, the observer walking behind the lead observer could record observations made by the lead observer and could

further note butterflies occurring within 0.5 meters on either side of the transect (at Indiana Dunes) but not seen by the lead observer. Differences in counts, within 0.5 m of the transect line, between the total noted by the two observers compared to the total noted by the lead observer can be used to determine the correction factor for the distance sampling calculations. For consistency, it would probably be preferable to keep using two observers through time if this option is selected. Therefore, the added expense, and possible decrease in number of transects sampled, should be carefully considered before using two observers since a well-trained observer should be able to find most butterflies within the first interval (< 0.5 m) from the transect line. An alternative to using two observers would be to undertake a series of test surveys along transects through different habitat conditions. For example, you might send out your intended observer to transects in very open and shrubby habitats, if those conditions represent the extremes you encounter at your site. That observer could be followed by another observer, as described above, and differences in counts between the two observers recorded to use in calculating a correction factor for each habitat type. Several following observers might even be used to determine how many butterflies were missed within 0.5 m of the transect line by the lead observer. This correction factor calculation might be repeated over several sets of transects and seasons and under different weather conditions, until you feel confident that a reliable correction factor has been arrived at for particular habitat and weather conditions. This correction factor could be used in subsequent surveys in each habitat type. If you have several lead observers across your site, correction factors could be calculated for each.

## VI. Walking the transect line

Each observer should walk along the transect line at about the same pace. To achieve this, set out from the 0 point and walk about one pace every second. You can use “One thousand one, one thousand two, one thousand three...” to pace yourself. You will stop at each 10-meter point on the transect line to estimate nectar plant abundance. You can use this stop to spend about 10 seconds to scan ahead on the line for butterflies. Throughout the transect walk we will be looking for butterflies ahead of the observer.

## VII. When you see a butterfly

We will be following a protocol for counting butterflies established by Aaron Ellingson, in his study of the Uncompaghre fritillary. Here is a synopsis of his protocol taken from a personal communication from Aaron.

- (1) Stationary (perched) butterflies. Butterflies initially encountered while stationary are recorded at the location at which they are first observed. These individuals will often be detected when they flush, and it is to the location from where they flushed that you should record distance.
- (2) Flying butterflies. As you walk the transect line, imagine a vertical plane perpendicular to the transect line and about 0.5 m (about an arm's length) in front of you. Count butterflies only when they pass through that plane. Record distance perpendicular to the transect line to the point at which the butterfly passes through this plane.

- (3) To decrease double-counting of butterflies, try to keep track of butterflies you have already observed and try to walk at a pace that will tend to keep counted butterflies in back of you.
- (4) If you need to leave the transect line to confirm identification of a butterfly, you may do so. However, distances should be recorded from the transect line, not from where you catch up with the butterfly. Once identification is completed, resume the survey from the point at which you left the transect line. It might be handy to carry a marker (for example, a flag) that you can drop at the point at which you left the transect, so you can resume counting at the proper point.

In other words, if you INITIALLY SPOT a butterfly sitting on a plant, you will record the perpendicular distance from the transect line to that plant. If you INITIALLY SPOT a butterfly flushing (flying away) from a position where it had been perched, you will record the distance to that perch. If you INITIALLY SPOT a butterfly when it's flying without knowing that it just flushed from a particular location, then continue walking and if that butterfly passes through a plane perpendicular to the transect line, and about one arm's length in front of you, then record the perpendicular distance from the transect line to the point where the butterfly intersects the plane in front of you.

#### VIII. Recording distances

Once you have observed a butterfly, you'll need to record the PERPENDICULAR DISTANCE from the transect line to the observation point (see VII above). To do this, you will need to mark the observation point, mentally or physically, and you'll need to know exactly where the transect line is located. The transect line is marked every 10 meters. As you stop at each 10-meter point (see VI above), you should line yourself up to the next 10-meter point and walk that line. However, what is really important in defining the line is that it represents the actual path that the observer is taking. Therefore, at any one moment, the transect line should be defined as the straight line between the observer and the next 10-meter point. If you keep heading toward that next point, that line will define the transect line. This is important because most observations will occur ahead of you, so you will need to know where the line is. As you walk the line, you should try and avoid stepping on lupine plants, either by stepping over plants or by making short circumnavigations around plants that do not greatly change the total length of the transect. If you take a step or two to one side of the planned line, it is okay as long as the added distance is not too great and as long as you immediately line yourself up to the next 10-meter point from your new path.

So, now you know where the transect line occurs and you know where you've seen a butterfly. Each observer will be given a 3-meter-long piece of PVC pipe marked off at 0.5, 1, 1.5, 2.25, and 3 m. Use this pole to record the PERPENDICULAR distance from the transect line to the butterfly. Distances will be recorded in the following intervals:

0-0.5 m, 0.5-1 m, 1-1.5 m, 1.5-2.25 m, 2.25-3.0 m, 3.0-4.0 m, 4.0-5.0 m

which correspond to the following interval lengths

(0.5) (0.5) (0.5) (0.75) (0.75) (1.0) (1.0)

The recorded distance represents the END of the interval. Thus, a recorded distance of 0.5 m means that the butterfly was observed between 0 – 0.5 meters from the transect line. If a butterfly occurs at 1.3 m, you would record it as 1.5 meters; in other words, that butterfly occurred between 1.0 – 1.5 meters from the transect line. If the butterfly is observed at 2 m from the transect line, you would record it as 2.25 m, which corresponds to the interval 1.5-2.25 m. Because the cumulative interval distances (up to 5 m) are obviously longer than the measuring rod, it will be necessary to measure longer distances (> the length of the rod) in two sections. Extend the rod to its maximum length from the center line. Note where the end of the rod is, walk to that point then complete the measurement by taking a second measurement. If desired, you can tally any butterflies seen at a distance > 5 m, but distances will be recorded only for butterflies up to 5 m from the transect line.

#### IX. Leaving the transect line

The observer may occasionally have to leave the transect line to identify a butterfly or to measure a distance. The observer should carry a few flags – one to mark the spot where he or she left the transect and one to mark the point of observation, if necessary. After the observer has finished the distance measurement or gotten the identification straight, remove the marker flags.

Do NOT record butterflies you observe while away from the transect. Once you return to the transect, record any butterflies you observe. This might include butterflies that you observed away from the transect line.

During surveys, we strive to avoid counting the same butterfly more than once. When you leave the transect, you might have a concern about losing track of butterflies that you had already counted. Here's what Aaron Ellingson had to say about this concern (taken from the Distance sampling listserv (<http://www.ruwpa.st-and.ac.uk/distance/>):

*"The issue of "double-counting" may need some further examination. In general you are right, double-counting butterflies on the same transect is generally to be avoided. However, double-counting between transects is NOT a problem at all. It seems to be that double-counting after a suspension of sampling that takes you off the transect is more similar to a new transect and that double-counting is not a problem. To ramble on a bit more, there are two issues with double-counting: 1.) independence of detection distances, and 2) obtaining an approximately instantaneous count (snapshot). The way that butterflies get double-counted is by moving (and changing the distance at which they are detected). Unless they are consistently moving toward or away from the center line I think the re-detections are safely considered independent and 1) is not an issue. The snapshot count obviously is not possible and the best we can do is to keep walking and use the perpendicular plane protocol I describe. Basically, if we assume that butterfly movement is random relative to the transects, then the number of butterflies that leave the transect before the observer gets to*

*them is, on average, the same as the number that arrive after the count started. So our "moving window" count is a good approximation of a snapshot count. The same reasoning could apply to the butterflies recounted after an ID suspension. There would be some butterflies that were counted before the suspension and then redetected afterward. There would also be some that would have been detected if the count had not been suspended that are now behind the observer. On average these should be equal. And it won't apply to many butterflies anyway. So I would say this is not a large concern."*

#### X. Clusters of butterflies

Distance from the transect line should be recorded separately for each butterfly. However, butterflies can occur in groups or clusters (especially when mating or nectaring), and it would be helpful to have this information available. For our purposes, butterflies can be considered to be in a cluster if they are within 0.5 m of each other (that's not the same as being within the same 0.5 m distance interval). You should measure your arm to be able to estimate when butterflies are within that radius of each other. When you record the distance for a butterfly, there will also be a column on the data sheet indicating whether the butterfly was within a cluster. For the first cluster you observe on a transect on a day, put the letter "A" in that column for each butterfly within that cluster. For the second cluster you observe on a transect on a day, put the letter "B" in that column for each butterfly within that cluster. Continue on with "C", "D", etc. for other clusters for that particular transect on that particular day. Alternatively, you may choose to circle the butterflies in clusters on the data sheet.

#### XI. How often should you sample your sites?

As noted above, single counts are often poorly correlated with total brood size. In other words, it is not likely that Karner blue counts on a single day will represent the same proportion of the total brood size in different years, even if that single day always represents the "peak abundance" of butterflies. If you are trying to estimate brood size, the more days you sample during the brood, the more accurate your estimate is likely to be. So how much is enough? Consider the following. Imagine that surveying the population every day at a site. Graph the number of butterflies observed versus the date, producing a curve. That curve may be regular or irregular in shape. The brood size will be some fraction of the total area under that curve. If you count butterflies less often than everyday, your goal should be to approximate the shape of the everyday curve when you connect the points on the graph of the counts that you actually made. Another way of stating your goal is that the area under the curve derived from less than every day surveying should be as close as possible to the curve derived from everyday surveying. Thus, you want to put more of your effort into surveys when the most butterflies are out. Although in some years your population may emerge as a nice "bell-shaped" curve, in other years the weather may cause emergence to be irregular with multiple "peaks." Surveying at least 4 or 5 days per week is likely to catch most of that variation. If that level of surveying is not feasible, performing at least some frequent surveys will acquaint you with the level of variability you might expect. It is important to define what parameter you want to calculate at the end of your surveys. Ideally, it might be the total

brood size. Methods for calculating the total brood size from daily population estimates are discussed above.

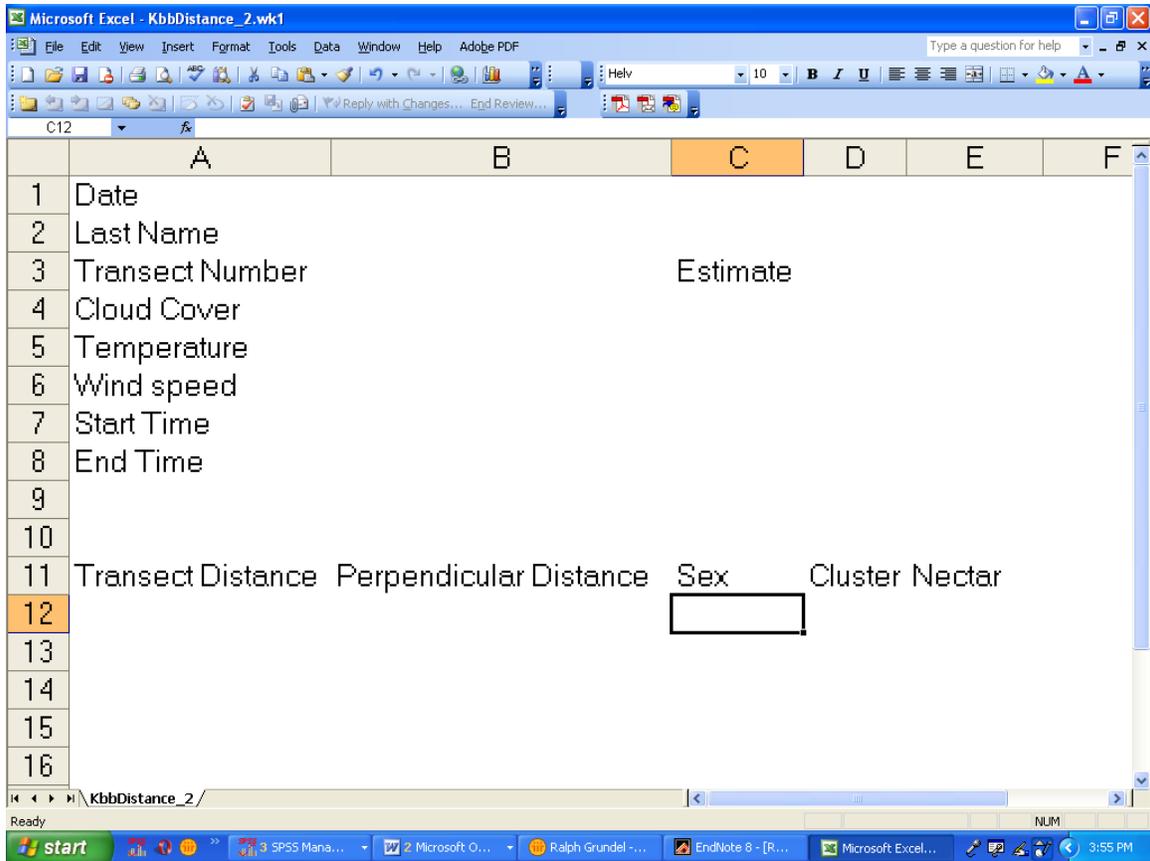
## XII. Environmental parameters

Wind speed, temperature, and cloud cover should be recorded using a digital meter for wind speed and temperature and a densitometer for cloud cover. If sufficient numbers of these devices are available, each observer should have them and record this information on their data sheet. If not, at least one observer who is out throughout the sampling day should have these devices and record the information for each transect he or she surveys. In this manner, a wind speed, temperature, and cloud cover reading will be available for all transects surveyed within a day at approximately the time of day a transect was surveyed. For cloud cover, haze should not be counted as cloud cover.

XIII. Data Entry (NOTE: This section reflects the data recording devices, palmtop computers, used at Indiana Dunes. Data sheets reflecting the specific data you collect and the specific method you use should be created for your specific situation).

Data will be preferably entered into a data sheet on a palmtop computer. If insufficient number of palmtops is available, data might be recorded on paper forms. Each surveyor should carry several copies of the paper forms as a backup in case of palmtop failure. Each observer should also carry spare, charged batteries for the palmtop. Each observer should also know how to use the palmtop and the spreadsheet application (Lotus 123 or Excel).

A copy of the data sheet is shown below:



The variables to be entered are as follows:

**DATE:** entered as mm/dd/yy – 06/02/05 would be June 2, 2005. If using Lotus 123 on the HP palmtop the date should be preceded by an apostrophe – i.e., '06/02/05. The apostrophe will not appear on the screen even though it has been entered.

**LAST NAME:** Last name of surveyor

**TRANSECT NUMBER:** Enter the transect number (i.e., IM01, IM02...IM25)

For the variables Cloud Cover, Temperature, and Wind Speed, data will be entered into one of two columns. If data are estimated (see below), enter the data into the second column (under Estimate). If the appropriate meter is available, enter the data into the first column.

**CLOUD COVER:** Exact: Enter number of densiometer squares covered (maximum 96) by non-hazy clouds. Estimate: Estimate, to nearest 10%, the percentage of sky covered by non-hazy clouds.

**TEMPERATURE:** Enter temperature in degrees Fahrenheit. If no thermometer is available, leave blank. If only Celsius thermometer is available, enter the temperature in the first column and enter "C" in the second (Estimate) column.

**WIND SPEED:** Exact: Enter wind speed in miles per hour if meter is available. Estimate: If meter is not available, use the following scale and enter data into Estimate column: (0) < 1mph (Smoke rises vertically), (1) 1-3 mph (Wind direction shown by smoke drift), (2) 4-7 mph (Wind felt on face; leaves rustle), (3) 8-12 mph (Leaves, small twigs in constant motion, light flag extended), (4) 13-18 mph (Raises dust and loose paper, small branches are moved), (5) 19-24 mph (Small trees in leaf sway, crested wavelets on inland waters)

For times you must enter an apostrophe (see above) if using Lotus 123.

**START TIME:** Survey start time (hr:min – in military time – e.g., 3:14 PM = 15:14)

**END TIME:** Survey end time (hr:min – in military time – e.g., 3:36 PM = 15:36)

**TRANSECT DISTANCE:** Approximate distance (meters) along the transect line where distance to the butterfly is measured from. Remember, white flags are placed out every 10 m. A value of 33 means that the perpendicular distance to the butterfly was measured from approximately 33 m on the transect line.

**PERPENDICULAR DISTANCE:** This is the MOST IMPORTANT thing you'll record on this survey – the PERPENDICULAR distance from the transect line to the butterfly. Recorded as the end of the interval – for instance, a value of 2.25 means that the butterfly was recorded between 1.5-2.25 m from the transect line. As another example, if a butterfly occurs at 1.2 m it will be recorded as 1.5 – i.e. within the 1.0 – 1.5 interval.

**SEX:** If the sex of the butterfly is known enter: M for Male; F for Female.

**CLUSTER:** Cluster letter (see X. above).

**NECTAR:** Nectar abundance on a scale of 0-4 (see IV. above). This is recorded every 10 m. An entry for NECTAR will NOT have a butterfly distance recorded – that line will only have entries for TRANSECT DISTANCE and NECTAR.

Each line from line 12 in the data sheet down will enter information either on the distance to a single butterfly or a measurement of nectar abundance at a 10-meter point.

Remember to go back to the top of the data file at the end of the transect to fill in END TIME.

#### XIV. Naming data files

Data from each transect should be saved in its own file. **IMPORTANT:** Files should be named according to the following convention. File names will be 8 characters long consisting of the letter K, month (1 number) day (2 numbers) year (2 numbers) transect number (2 numbers – omit the IM). For example, if you survey transect IM07 on June 2, 2005 the file those data will be saved in will be named: K6020507. The program will enter

the appropriate extension (.wk1 for Lotus, .xls for Excel, etc.). 6 = June, 02 = 2 (date), 05 = 2005, 07 = IM07. (FYI: Month codes: 5 = May, 6 = June, 7 = July, 8 = August)

#### XV. Conditions for surveys

Distance sampling depends on observing butterflies. Therefore, surveys should not be conducted during periods when butterflies might not be actively moving or basking. Surveys should be avoided during periods of heavy overcast and cool, rainy, or windy conditions. Counts should be conducted during sunny conditions, temperatures > 65° F, winds < 10 mph, without rain or drizzle. Surveys can start after 10 AM.

#### XVI. Equipment for surveyors:

3-meter measuring pole (a meter stick might be substituted in scrubby areas). Use material that is lightweight but does not bend too much - ¾ inch PVC pipe, for example.

Paper data forms

Palmtop computer

Spare batteries (AA)

Densimeter

Wind/temperature meter or wind gauge and thermometer

Pencil

Transect map

GPS with transect coordinates entered

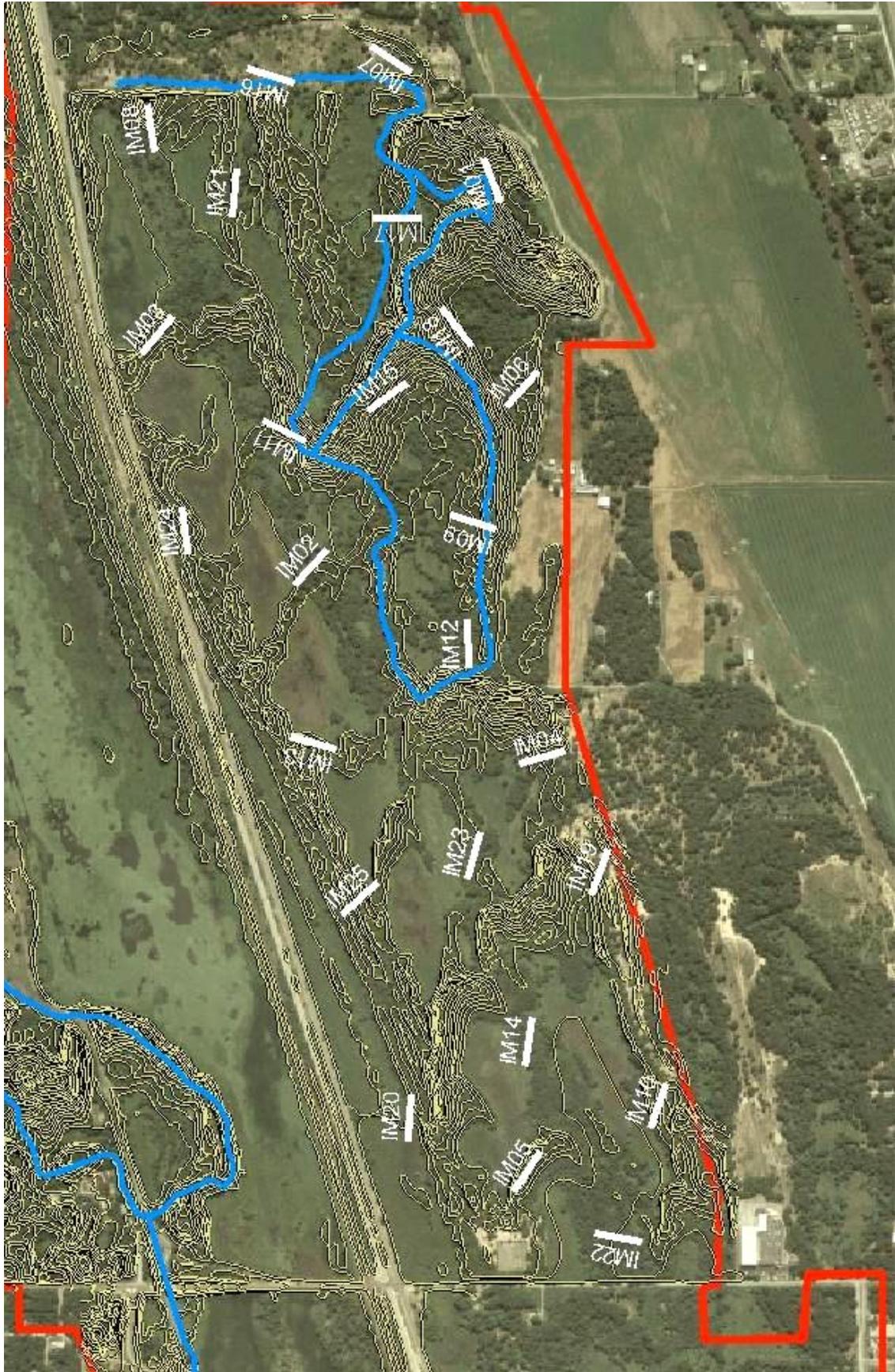
~5 flags (preferably not green, white, or red) plus flag bag if desired

Close focusing binoculars (help to identify butterflies)

Instructions

#### XVII. Transect map

Red lines are park unit boundaries. Blue lines are trails. (Note: to see this map you may need to set your “View” in Microsoft Word to “Reading Layout”.



The actual density calculation procedures using program Distance are not discussed here. Refer to the program Distance website (<http://www.ruwpa.st-and.ac.uk/distance/>) to obtain Distance and a guide to using the software. Refer to Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2001. *Introduction to distance sampling*. Oxford University Press, Oxford, UK. for an introduction to the theory and use of distance sampling.

## XVIII. Sampling other life stages

As noted above, before embarking on a long-term monitoring program, it is important to consider which life stage(s) are most important to monitor. This guide has described a method for determining adult density. Estimating egg or larval densities or abundances will likely require different methods because, given the difficulty in seeing eggs or larvae from a distance, distance sampling is not likely to be useful for monitoring eggs or larvae.

For eggs or larvae, an alternative protocol might be to divide your area of interest into a grid, where each square grid cell is small enough that it can be completely surveyed for either eggs or larvae or both, but large enough so that it would encompass at least the average distance between lupine stems. A subset of grid cells would be selected at random for survey. Each grid cell selected would be completely searched for eggs or larvae. A complete search might literally include the entire surface area of the ground and all the plants with stems in the grid cell or could be limited to particular substrates – for example, all lupines and all substrates within a certain surrounding distance. As a possible guide, Grundel et al (1998) found that Karner blues deposited eggs directly on lupines in the first brood but generally placed eggs near, but not on lupines, in the second brood. The average distance away from the lupines that eggs were placed was 47 mm during the second brood. For consistency, it might be preferable that egg or larval searches were conducted in the same manner in both broods even though eggs are often placed on somewhat different substrates in the two broods. Resulting counts would be reported as number per square meter or expanded to the whole area as a population estimate. If appropriate, you could make rules about which cells to survey, such as 100% of those cells that contain at least one lupine stem will be searched, and 10% of those cells that contain no lupine stems will be searched. You would have to make this rule before you started sampling, based on your expectation of the cells with lupine having more eggs or larvae than those without. This could be considered two-stage sampling or, if you know the identity of all the cells in the population, it could be considered further stratification. Given the ephemeral nature of the life stages, you might limit sampling to a very short time frame, one in which you can be certain of limited recruitment, mortality, or changes in life stage. One issue that you might have to address is that of detectability, which is currently undocumented. What is the probability of finding an egg or a larva in a grid cell, given that one is there? If detectability is less than 100%, that may not be an issue in terms of an index of relative abundance of eggs or larvae, but if detectability is variable that can cause problems for the determination of absolute densities.

Lane and Andow (2003) used a method similar to that outlined above for sampling Karner blue eggs. They studied egg distributions using random placement of transects and random sampling of host plants within defined subhabitats at each study site during peak egg laying periods for both broods. They randomly selected 100 lupine stems (50 reproductive/50 non-reproductive) for the first brood and 100 10 x 10 cm plots centered on the base of a lupine stem (again 50/50) for the second brood, in each subhabitat, at each study site. Each stem or plot was searched for

eggs. In the first brood, eggs were counted on the selected lupine stems. In the second brood, eggs were counted on lupine stems as well as on any other dead or living vegetation within the 100 cm<sup>2</sup> plot.

#### LITERATURE CITED

- Bart, J., S. Droege, P. Geissler, B. Peterjohn, and C. J. Ralph. 2004. Density estimation in wildlife surveys. *Wildlife Society Bulletin* **32**:1242-1247.
- Brown, J. A., and M. S. Boyce. 1998. Line transect sampling of Karner blue butterflies (*Lycaeides melissa samuelis*). *Environmental and Ecological Statistics* **5**:81-91.
- Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2001. *Introduction to Distance Sampling*. Oxford University Press, Oxford, UK.
- Fuller, S. G. 2008. Population Dynamics of the Endangered Karner Blue Butterfly (*Lycaeides melissa samuelis* Nabokov). Ph.D. dissertation. State University of New York College of Environmental Science and Forestry, Syracuse, New York.
- Gall, L. F. 1985. Measuring the size of Lepidopteran Populations. *Journal of Research on the Lepidoptera* **24**:97-116.
- Grundel, R., N. B. Pavlovic, and C. L. Sulzman. 1998. Habitat use by the endangered Karner blue butterfly in oak woodlands: the influence of canopy cover. *Biological Conservation* **85**:47-53.
- Harker, R., and T. G. Shreeve. 2008. How accurate are single site transect data for monitoring butterfly trends? Spatial and temporal issues identified in monitoring *Lasiommata megera*. *Journal of Insect Conservation* **12**:125-133.
- King, R. S. 2003. Habitat management for the Karner blue butterfly (*Lycaeides melissa samuelis*): evaluating the short-term consequences. *Ecological Restoration* **21**:101-106.
- Lane, C. P., and D. A. Andow. 2003. Oak savanna subhabitat variation and the population biology of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae). *Annals of the Entomological Society of America* **96**:799-809.
- Longcore, T., R. Mattoni, C. Zonneveld, and J. Bruggeman. 2003. Insect count analyzer: a tool to assess responses of butterflies to habitat restoration. *Ecological Restoration* **21**:60-61.
- Nowicki, P., A. Richter, U. Glinka, A. Holzschuh, U. Toelke, K. Henle, M. Woyciechowski, and J. Settele. 2005. Less input same output: simplified approach for population size assessment in Lepidoptera. *Population Ecology* **47**:203-212.
- Thomas, L., J. L. Laake, S. Strindberg, F. F. C. Marques, S. T. Buckland, D. L. Borchers, D. R. Anderson, K. P. Burnham, S. L. Hedley, J. H. Pollard, and J. R. B. Bishop. 2004. *User's Guide: Distance 4.1. Release 2*. Research Unit for Wildlife Population Assessment, University of St. Andrews, St. Andrews, UK.

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