

Monitoring methods for solitary bee species using bee bowls in North America

Gretchen LeBuhn, Sam Droege and Marta Carboni.

San Francisco State University and USGS-BRD

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The concern about loss of pollinators is driven largely by the perception that there has been or will soon be large declines in their populations. Unfortunately, while such declines may have occurred in the past and may be occurring now there are no large-scale systematic surveys of pollinators in North America from which estimates of those changes can be derived. Consequently, the study of population declines is currently characterized by conjecture and alluring anecdote.

While it is certainly appropriate and warranted to raise the issue of pollinator decline if the indirect evidence is strong and compelling as it appears to be, it is also important to begin laying the groundwork for the collection of more empirical measures of change. An appropriately designed and statistically justifiable monitoring program or set of monitoring programs would greatly help build the case for the conservation of this group of animals.

This paper provides a summary of the sampling issues we have tackled over the past few years while investigating the best ways to track changes in bee populations on this continent. In this paper, we compare the few data sets available to calculate inter-year variability of counts generated by different bee survey techniques (malaise traps, nest blocks, bee bowls (also called bowl traps), scent traps, and netting), discuss the impacts of different modifications of bee bowls (size, color, pattern, timing, and distance) on capture rates of bees, and outline a standardized protocol for sampling bees.

I. Background/Problem statement:

The value of bees is most often thought of in terms of products (wax, honey and royal jelly) generated by managed hives of the European honeybee, *Apis mellifera*. But undoubtedly

the most important service provided by the combined efforts of native and managed bees is their pollination of flowering plants. The value of these pollination services has been estimated to be 112 billion dollars a year (Kearns et al. 1998). Because of their high dependence on nectar, pollen, and oil from flower resources for feeding and larval food, bees exhibit among the highest floral visitation rates in the insect world, making them the single most important group of pollinators (Neff and Simpson 1993). *Apis mellifera* is only one of an estimated 25,000-30,000 species (exceeding the number of fish species on the planet) of obligate flower visiting bees (Kearns et al. 1998).

Non-*Apis* native bees play an especially important role in natural ecosystems and are among the most critical and effective pollinators of native plants. In temperate climates, large portions of the flora in many communities, including many woody and herbaceous members of the Caesalpinoideae and Papilionoideae, Bignoniaceae, Lamiaceae and Scrophulariaceae, are exclusively dependent on bees for pollination. In xeric areas and in Mediterranean scrub communities, bees are present in particularly high diversity and are the principal pollinators, contributing to the preservation and reproduction of the natural vegetation, which prevents erosion and provides the cover and food for native wildlife (Neff and Simpson, 1993; Michener, 2000). Further, because it may limit plant reproductive success, competition for pollinators may play an important role in plant population dynamics and in structuring plant communities (Neff and Simpson 1993). Because of their effectiveness as pollinators, bees are a prime example of "keystone mutualists", being essential for the maintenance of ecosystem integrity and of angiosperm diversity (LaSalle and Gould 1993). It is therefore clear that the conservation of native habitat is closely tied to the preservation of bees.

More directly related to the human pocketbook is the role bees play in the pollination of cultivated plants. About 30% of human food is derived from bee-pollinated plants (O'Toole 1993). The annual value of pollination for crop systems was estimated to be around 20 billion dollars in the United States only, and to approach \$ 200 billion in the whole world (Kearns et al. 1998 and sources therein; Southwick and Southwick 1992). Although honey bees are generally recognized as the most important pollinators of the major US agricultural crops, solitary bees play roles in this respect that are often as or even more significant (Allen-Wardell et al. 1998). Relying too heavily on honeybees for pollination of crops is a great risk for growers, which face catastrophic reductions of seed set in the case of epidemics and other sudden decreases in the availability of honeybee colonies (O'Toole, 1993). Moreover non-*Apis* bees are better pollinators for some specific crops. Honeybees, for example, are not effective pollinators of those angiosperms with poricidal anthers, such as Ericaceae (blueberries and cranberries) or Solanaceae (potatoes, eggplants and tomatoes), which require "buzz pollination". Therefore, only native pollinators can be relied upon in the cultivation of these crops (Michener, 2000; Neff and Simpson, 1993; O'Toole, 1993). Native bees are more effective pollinators for other crops including alfalfa, an important forage crop and cover crop contributing to soil fertility, and many orchard crops (O'Toole, 1993). Kremen et al. (2002) were able to show that the native bee community was able to provide an equivalent pollinator service to that of managed honeybees for watermelon on farms in the Central Valley in California. A conservative estimate placed the yearly economic value to US crops attributed to pollinators other than honeybees at \$ 6.7 billion (Kearns et al. 1998).

----Most of the introductory material above can be removed, if there is an overview of the pollinator decline situation elsewhere.

In the last decade or so, evidence has been mounting for a general decline of pollinator diversity and abundance in North America and worldwide. Managed and feral honeybee colonies in the United States dropped by 25% since 1990 (Allen-Wardell et al. 1998). One of the principal causes of the decrease of honeybee populations has been the introduction of mites such as *Varroa*, which parasitize honeybees. The decline in *A. mellifera* populations has made wild bees even more valuable pollinators of agricultural crops than in the past (Michener, 2000). Comparable declines are suspected but not proven for other invertebrate pollinators. Unfortunately, while such declines may have occurred in the past and may be occurring now there are no large-scale systematic surveys of pollinators from which estimates of those changes can be derived. Consequently, the current study of population declines has been characterized by conjecture and alluring anecdote.

The natural reaction to declines in bee populations, and the attendant economic and natural penalties that would follow, would be to define a set of management and conservation strategies to reverse those declines. Unfortunately, because there are no specific data on which species are declining, rates of decline, the localities where declines are the worst, and the physical features or activities most associated with these declines, management and conservation are unlikely to occur no matter the severity of loss.

Clearly there is a great need to quickly initiate the monitoring of native bee populations worldwide as information on population change in bees will take years to detect given the

natural variability of their populations. However, a monitoring program must employ a set of counting techniques and, unfortunately, there has been no consensus as to what techniques to use and how they should be deployed within a monitoring effort. Past localized efforts have used: bee bowls or bowl traps (O'Toole 1998), trap nests (Frankie et al. 1998), Malaise traps (Owen 1978), chemical baits (Roubik and Ackerman 1978), visual inspections (Kremen et al. 2002), and numerous faunal surveys using hand nets. Each of these techniques is biased to favor the capture or detection of some species over others and depending upon how the technique is used the data from these surveys will be corrupted by the impacts of changes in observers, habitats, weather, time-of-year, time-of-day and equipment.

For a standard protocol to have real value, the methods for collecting bees need to be tested, statistically evaluated, and standardized. An informative and effective standard monitoring program should have low cost, low variance, and minimal bias for the targeted species. It is highly desirable that sampling techniques yield low variance, since this is associated with needing a lower sample size to detect a given rate of change. On the other hand cost must also be factored in. A technique may exhibit a very low variance and collect a great abundance and wide diversity of species (for example malaise traps), but if the cost of deployment is prohibitive, a less efficient but more economically feasible technique (for example bee bowls) may be better suited.

II. Objectives/Purpose of the Activities:

In this paper, we compare the variability of data generated by different bee counting techniques (malaise traps, nest blocks, bee bowls, scent traps, and netting); discuss our favored

sampling methodology, bee bowls, in greater depth by examining the effects of size, color, pattern, timing, and distance on capture rates of bees; and suggest a two standardized protocols for sampling bees.

III. Details of the case study and the approach taken:

Variation in counts of bees among survey techniques.

Natural and methodological variation in the numbers of bees caught or observed determines how readily a change in population status may be detected. If counts fluctuate widely across years then it will be difficult to detect changes in populations and, conversely, if fluctuations are low then change can be more readily detected. Likewise, to detect a change of a given size sampling effort must increase with increased variability. We were able to discover only a few instances of published or unpublished multi-year counts suitable for measuring such variability. Brief descriptions of those data sets follow.

Data from California were taken from a paper by Frankie, et al. (1998). The authors collected information on populations from six sites in 1990-1992 using nine sets of wooden trap nests, each set consisting of 12 individual blocks of wood with one of three different diameter holes drilled into them. Nests were placed on living trees and picked up and replaced every three weeks throughout the nesting season. The yearly population index used was the frequency of occupied nests at a site for each species.

James Cane provided data from 2 sites in Georgia from a 9-year study (1985-1997) of visitations to flowering blueberry bushes by 4 species of bees (Cane and Payne 1993). The yearly population size index used was the number of bees visually detected per bush. Data

taken during 2 hard freezes were removed from the analyses because a monitoring effort would have not collected data during that period.

David Roubik provided unpublished data from Panama from periodic counts taken in lowland tropical forest spanning 1979-1999 (Roubik and Ackerman 1987). Counts of all species and individuals of Euglossine bees were taken from 3 chemical baits counted every 15 minutes over a 4-hour period. Three yearly population indices were derived from the monthly counts taken in October, May, and February. Eleven, 15, and 16 years of data were available and 21, 22, and 26 species were used in the analyses respectively. Species were included if there were 5 or more years with non-zero data. A square root of the count + 0.5 transformation was found to increase the normality of the data and was used on all the counts.

Frank Parker provided unpublished data from Utah. Hole-nesting hymenoptera were sampled using Elderberry (*Sambucus* sp.) stems that had 4 holes drilled into the stem walls and one in the top using 2 diameter sizes. Two hundred stems were placed in the ground along a transect at each of 13 sites in Logan Canyon and nearby locations. Seven years of data were collected (1973-1979) at all sites. The yearly population index for a species was the number of occupied holes for a species at either one individual site or pooled across all sites.

Data on bumblebees from malaise traps in Leicester England came from a paper by D.F.Owen (1978). Malaise traps were run continuously in a garden in England from 1972-1977. The yearly population index was the total number of bees of each species captured per year.

The coefficient of variation (CV) was used to compare the variability of counts across species and methodologies. The CV was calculated by dividing the population standard deviation by the mean. The CV permits comparisons of variability among counts having different means by removing the relationship between the mean and standard deviation. For

counts of greater than 5 years the effects of trends over time were removed from the counts using simple linear regression. The standard error of the regression was then used in place of the sample standard deviation in the calculations of the CV. CVs were transformed ($\ln(\text{CV}+0.01)$) prior to analysis to normalize the data. All analyses in this paper were performed using Systat 7.0 (SPSS 1997). Significance was set at $P < 0.05$.

Results. The CVs for the 123 species ranged from 0.079 to 4.939 averaging 0.545. Mean CVs for California, Georgia, Panama, Utah (pooled across all sites), Utah (average per site), and England were 0.657, 1.441, 0.387, 0.519, 1.084, and 0.345 respectively. ANOVA of transformed CVs demonstrated a significant difference in CVs among datasets ($F=17.574$, d.f. =5,117, $P = 0.000$). Bonferroni pairwise comparisons indicated that 10 of 15 comparisons among sites had significantly different means.

The standard deviation or standard error for counts of bees ranged from only 8% of the mean to over 500%. A textbook rule-of-thumb is that CVs of greater than 50% are likely to yield poor representations of the population mean. With an overall mean CV of 0.545 roughly half of the estimates would be characterized as poor, indicating that the variability of the counts would make tracking changes in counts difficult or at least inefficient for a number of species under some approaches taken by the authors of these particular studies.

While CVs over 0.5 are not optimal they do not necessarily mean that effective monitoring is precluded. Such variable data indicates that the number of samples necessary to achieve a set monitoring goal must be greatly increased. For example, in simulations presented in Gibbs et al. (1998) CVs of 0.50 required 9, 20, and 200 samples to detect 50%, 25%, or 10% population changes respectively in populations over 10 years (for a given set of statistical monitoring objectives). CVs of 0.25 (characteristic of counts of plants) required only 4, 8, and 50 samples

but if CVs jump to 1.00 (characteristic of butterfly counts and *Drosophilid* flies) the number of samples required rises to 50, 150, and >500 to do the same job.

Individual sites (200 elderberry stems) in the Logan Utah study and the Georgia study of counts of bees on blueberry bushes suffered from high degrees of count variability. As can be seen from the Logan Canyon data, pooling data across sites, while taming much of this relative variability increases the number of sites and samples or traps that have to be used to achieve a given degree of sampling precision, thus increasing the cost of the program.

Note that the examples above are for a single set of sampling goals and that by changing the statistical precision, the number of time you repeat counts within a year, or the number of years over which you would like to be able to detect trends, you can greatly influence the numbers of samples required to detect those changes. Our investigations of CVs must be interpreted with a great deal of caution. Changes to the approaches and methodologies (e.g. greater sampling duration, elimination of counts taken in bad weather, more trapping effort, etc.) used in each of the studies presented here could greatly influence the calculated CVs. What is important is that workers should expect that what ever their choice of survey methodology, it is more likely than not to require a great deal of resources to overcome the variability in these counts.

In addition to the potential differences in count variability expressed by each survey technique, these methods have biases, logistical constraints, and budgetary costs that must be weighed. Malaise traps have the advantage of capturing a broad spectrum of species, and trapping constantly over an entire season. They have the disadvantages of being relatively expensive to purchase, vulnerable to vandalism, require regular checking throughout the year, have limited flexibility in where they can be deployed, and involve processing many specimens.

Counts at baits have the advantage of being portable, low cost, and relatively easy to implement cover an extensive area. They have the disadvantage of being restricted to only a subset of tropical bees.

Trap nests located in wood blocks and in elderberry stems both had moderate amounts of variability associated with their counts when pooled across several sites. Materials cost little for this family of techniques, sampling can cover broad geographic regions (the bees and wasps essentially sample themselves, decreasing observer biases); and they provide unique information regarding parasitism, fecundity, and within-nest mortality. The disadvantages are that these techniques are restricted to the subset of hymenoptera that are attracted to nesting in those nesting substrates, it requires the manufacturing of the trap nests, and requires large numbers of sampling units before variances are reasonable (e.g., 2600 stems in the case of Utah). Additionally, most specimens within nests have to be reared in the lab for identification, a time consuming process.

While we were unable to obtain a data set containing multiple years of bee bowl (also known as bowl or pan trap) data appropriate to investigate count variability, this survey technique appears to have a number of advantages. Bee bowls are typically simple plastic bowls or soufflé cups filled with water (figure 1), to which a small quantity of detergent has been added, and occasionally a preservative (usually a little formalin) if trap time is more than a day. These sampling units are traditionally used to sample arthropods; most commonly: aphids, flies and other agricultural pests (Southwood 2000). In more recent years, bee bowls have been used for a greater variety of purposes and organisms, including sampling pollinators such as bees and wasps (Leong & Thorp, 1999; Aizen & Feinsinger, 1994). Insects captured are strained from the water, cleaned, dried, and pinned for identification. Large numbers of traps can be speedily

deployed using a wide variety of configurations to match the purpose of a study. Untrained technicians and volunteers run these surveys and observer effects are minimal. Most of the common species North American bees are caught in bee bowls with the apparent exception of members of the genus *Colletes*. Each bee species is likely to have a different probability of capture and capture may further vary with bee bowl placement in relationship to surrounding vegetation and extent of nearby flowering plants.

Bee bowl studies

After testing and investigating these survey techniques, the inexpensive materials, diminished observer effects, ease of sampling, and the reasonably representative captures of species using bee bowls made their use in a monitoring effort very attractive. In our efforts to establish surveys of North American bees we decided to further investigate characteristics of bee bowl such as color, size, pattern, duration of sampling, and distance among bee bowls in order to refine the technique.

General Approach. Bee bowls capture bees using bowls or cups filled with soapy water. The soap decreases surface tension causing even small insects to sink rather than alighting on the surface film and then leaving. In our studies we used 5-10 milliliters of Dawn dishwashing liquid added to a 3.78 liter jug of water. That soapy water was then supplied to bee bowls placed in the field. Casual observation indicated that once a bee flew into the bee bowl it generally stop struggling after 30 seconds under water but could often partially revive even after several hours, though apparently never to regain full health. Unless otherwise specified, bee bowls were placed in transects on the ground 5m apart within a uniform habitat. Transects or arrays of bee

bowls were left out in the field for either 24 hours or set out prior to 9:30a.m. and then picked up after 3:00p.m.

What follows is a summary of a series of tests we ran to determine the best means of capturing bees. In almost all cases our measure of superiority was to determine which treatments caught the most bees.

Color of Bee bowl

Within an individual trial there were 5 bee bowls of each color treatment. Colors were alternated in a fixed pattern within a transect. The bulk of the data were collected in Maryland in between Baltimore and Washington, however, some trials were run in Texas, Colorado, Maine, Pennsylvania, North Carolina, and Tennessee. Data were collected from April until September 2002. Twelve ounce plastic Solo® brand salad bowls were used. Solo's standard yellow, white, blue, and light blue bowls were chosen for testing. Earlier trials has shown bowls in non-flower colors such as black, green, gray, and transparent did not catch bees nor did bowls in red, a color which bees are not capable of detecting. The spectral characteristics of these bee bowls have not been measured.

These tested colors closely matched the primary color types with the exception of Solo's choice for blue which most people would describe as a dark blue. Matching ultraviolet (UV) colors for these colors were created by spraying or painting white Solo® bowls with paint supplied by Nocturn Ultraviolet Visual Effects (A unit of Xenotech-Strong International, North Hollywood, CA, USA 91605). Thirty-seven trials were run.

Analysis of variance suggests that there is a significant difference among colors (d.f. =7, 281, F-value=2.32, p-value=0.026). Tukey's pairwise comparisons were performed to investigate

which of these pairs of colors differed significantly. UV colors (other than white UV, which, interestingly, caught fewer bees than its non-UV counterpart) caught significantly more bees than many of the non-UV colors. In descending order of mean number of bees caught per bowl, the bowls were Blue UV, yellow UV, Pale blue UV, Pale Blue, White, White UV, Dark Blue, and Yellow. Also, in a bit of statistical irony, the non-UV yellow had the lowest mean despite it being the bee bowl of choice of previous workers.

Size of Bee bowl

Within each trial there were five bee bowls of each bowl size. Bee bowl sizes were alternated regularly within a transect. The bulk of the data were collected in Maryland in between Baltimore and Washington, however, some trials were run in Texas, Colorado, Maine, Pennsylvania, North Carolina, and Tennessee. The data were collected from April until September 2002.

The following plastic Solo® bowls and soufflé© cups were used: 12 and 6 oz. bowls and 4, 3.25, 2, 1, 0.7 oz. soufflé cups. All were painted with yellow UV paint. Twenty-two trials were run.

The effect of size of bee bowl was tested using a one-way ANOVA. The surprising result was that no difference in numbers of bees captured was found among the sizes (d.f. =6,147, F-value=0.20, p-value=0.977). In fact, the two smallest soufflé cups (about the size of a dentist's spit cup) had the highest means (9.0 (13.4 s.d.) and 11.0 (19.0s.d.)). If these results were standardized by the amount of water held in each cup the differences would be quite dramatic.

Pattern of paint on bee bowl

Within each trial there were 10 bee bowls of each pattern. Both Dark Blue and White 12 oz. Solo® Bowls were painted. Dark Blue bee bowls were painted with fluorescent white Nocturn brand ultraviolet fluorescent balance white paint and the white bee bowls painted with that same brand's sky blue fluorescent paint. The following painted patterns were used: entire inside of bee bowl and rim (f), just the rim (r), the rim and the sloping side of the interior of the bee bowl (h), and stripes from the rim to the center, but not including the center (s). In each trial a plain bee bowl with no paint was also used (p). Bee bowls of each pattern were used in a transect with bee bowls spaced 4 m apart in a repeated progression of bee bowl patterns. Six trials were run.

A 2-way ANOVA was used to look at the effects of bee bowls and patterns. No significant differences were detected in either bee bowl color or pattern.

Length of sampling period

Within each trial there were 10 bee bowls for each sampling period. Treatments were alternated regularly within a transect. Data were collected in Glen Canyon Park, San Francisco, CA, USA from March until May 2003. We used 6 ounce Solo® bowls painted with Krylon brand fluorescent yellow throughout the experiment.

Transects were set out by 8:00 a.m. and left out for either 10 hours or 24 hours. Data were analyzed using paired t-test. Six trials were run.

A t-test was used to look at the effects of the length of sampling on the number of bees caught per bee bowl. No significant differences were detected between the 10 hour and 24 hour treatments (d.f. =5, $t=-0.698$, $p\text{-value} = 0.26$).

Distance effect and the effect radius of individual bee bowls

Within each trial there 2 bee bowls. The data were collected in Glen Canyon Park, San Francisco, CA, USA. Treatments were placed randomly in the park. The data were collected from March until May 2003. We used 6 oz Solo® bowls painted with Krylon brand fluorescent yellow throughout the experiment.

We set out two bee bowls in each trial, those bee bowls were either touching each other or spaced 5 or 10 meters apart. We analyzed these data as a one-way ANOVA and did a post-hoc comparison of means. Twelve trials were run.

There was a significant effect of distance on the abundance of bees caught in the pairs of bee bowls (d.f. =2, 34 $F=2.80$, $p\text{-value}=0.076$). Bee bowls that were next to each other caught significantly fewer bees than either the 5m or 10 m pairs. There was no difference between the 5 and 10 m pairs. This suggests that when bee bowls are adjacent they compete for the available bees.

IV. Analysis:

To assess the status and trends of the world's pollinator diversity and to identify management practices, technologies and policies that promote the positive and mitigate the negative impacts of agriculture on pollinator diversity, and enhance productivity and the capacity to sustain livelihoods, we must have an appropriately designed and statistically justifiable monitoring program or set of monitoring programs. Using the results outlined above, we, in collaboration with many others, have developed two approaches to surveying populations native bees. One concentrates on obtaining detailed estimates from a single one hectare plot and the other uses bee bowls to survey larger landscapes. Both have simple equipment costs, most of which are available locally, with the exception of the paint. These protocols along with detailed

results of our studies of methods and information on sampling bee populations are available online (<http://online.sfsu.edu/~beeplot>).

The protocols are summarized below. We suggest anyone wishing to implement these protocols get the full method at the website listed above.

SUMMARY OF THE ONE HECTARE PLOT PROTOCOL FOR NORTH AMERICA

The one hectare plot protocol is usable by scientists of many interests, as well as amateurs and school groups. Data generated using this protocol will be useful for 1) conservation planning and strategy development 2) local, regional and national assessments of species richness; and 3) evaluations of population trends in different habitat types (e.g. agricultural versus wilderness sites). This collaborative effort will allow us to begin to detect differences in species richness and population trends among biomes, ecoregions, habitats, communities, and plots.

We are certain that use of the protocol will expand rapidly because of widespread interest in the fate of pollinators. This simple sampling protocol is designed to contribute to a database that will be available for comparisons across any geographic scale (local, within ecosystem or worldwide). The end results will be a rapid expansion of identified bees in collections, basic information on bee biodiversity and patterns of species richness from throughout the world, information necessary to conserve these important pollinators.

Location of plot. Since we are interested in habitat specific associations, it is preferable to locate plots within a single distinctive habitat type. To minimize captures of bees from other habitat types, plots should be located at least 5 m from the edge of adjacent habitat types.

Size of plot. Plots should be 1 hectare in area. If possible, the plot should be 100 m in length and 100m in width. If this is not possible due to the configuration of the patch, the plot dimensions may be modified but should still maintain an area of 1 hectare.

Survey periods. Plots should be sampled every 2-4 weeks from while plants are in flower (in North America, mid-March to the end of October). The starting and ending dates should be determined by the first and last major flight periods of bees at the particular site. Samples should only be taken on warm, calm, sunny days. If logistics restrict sampling to only one part of the flowering season (e.g., Spring: Mar 1-Jul 1 or Fall: Jul 1-Nov 1), a minimum of 4 samples must be made per plot.

Bee bowls should be placed in a plot prior to 9:00 am and picked up from the plot between 3:00 pm and 5:00 pm. Netting should be done for a total of one hour the morning (9 am-12 pm) and one hour in the afternoon (12 pm-3 pm).

Placement of bee bowls. Within the plot, two 50 m transects should be established. Fifteen bee bowls (described below) should be placed on each transect at 10 m intervals. Bee bowl colors should be randomly assigned each time the bee bowls are placed out. If there is not sufficient area to have a single linear transect, the transect may be broken into parts spaced as widely as possible.

Type of bee bowl. Bee bowls should be small, white Solo® brand stock number PB6-0099 (6 oz (177.4 ml)) or the smaller 3.25 oz (88.7 ml), soufflé cup white Solo® brand stock number P325W-0001. (Solo® at 800-367-2877). 1/3 of the bee bowls should be painted fluorescent blue, 1/3 should be painted fluorescent yellow and 1/3 should be left White. We have developed a standardized paint formula which is available from Sam Droege at USGS-

Patuxent. Alternatively, use Krylon fluorescent spray paints (1-800-4Krylon). Bowls should be filled with a solution made up of 1 Tsp (15 ml) per gallon (3.78 L) of Dawn brand blue soap.

Bowls should be placed on level ground.

Aerial Netting. Two collectors should aerial net each one-hectare plot for two half hour periods, 1 in the morning (9-12) and again in the afternoon (12-3) because bees differ in their diurnal activity patterns. The collectors should divide the patch up ahead of time so that sampling effort is spread over the entire plot and switch halves halfway through collecting. Collections by two collectors have the advantage of reducing collector bias and allowing for quantification of any bias. A collector should spend no more than 5 minutes at any particular patch of plants. Once the whole area has been sampled, a collector may return to rewarding patches. Bees should be collected on plants, on the ground, and in the air (patrolling males and both sexes of parasitic bees often spend little time on flowers).

For each bee netted the identity of the host plant should be recorded. If identification of plants to the species is difficult or time-consuming, identification to the genus level would still be useful, as specialist bees generally use several members of a genus rather than a single species.

Identification of specimens. Accurate identifications of collections are essential for comparisons across sites. Without a great deal of prior experience identification to the species level will impossible without access to a strong reference collection. Arrangements should be made **PRIOR TO THE START OF SAMPLING** as to who will identify your bees. By the end of 2005 the second author hopes to have a complete set of taxonomically current keys to the bee species in the East available at www.discoverlife.org. For some regions, Dr. Terry Griswold of

the USDA lab in Logan, Utah, USA is willing to do identifications if contacted prior to sampling. Since identifications will take a considerable amount of time and effort, we suggest that funds be allocated for identification and whenever possible, those with major involvement in identifications be included in authorship.

Data management. Data should be entered into a relational database (e.g. Access, Biota...). A crucial element in developing this collaborative effort is the creation of a central repository for both the data and specimens as well as mechanisms for providing data to agencies and researchers for analysis. We are currently developing a central repository and information will be provided on the website when it becomes available.

For those who will implement this protocol, a full description of the method is found at <http://online.sfsu.edu/~beeplot>. We also established a listserver that you can join by sending a blank message to the following email address: join-2beeornot2bee@rana.er.usgs.gov. Past email conversations on bee monitoring can be read at the web site for the above listserv (<http://rana.er.usgs.gov>).

SUMMARY OF THE LANDSCAPE LEVEL PROTOCOL FOR NORTH AMERICA

To fully assess whether populations of pollinators have declined one must develop statistically meaningful measures of change that can be applied over such landscapes as national parks, counties, states, and large physiographic provinces. Through our pilot studies we feel that at least in North America bee bowls have characteristics that lend themselves to such an assessment. They are inexpensive, sample sizes accumulate quickly (our record is 700 bowls set

out in an 8-hour day by a single individual), can be used by untrained volunteers and technicians, and capture a reasonably representative cross-section of the bee fauna.

Sample Frame. We know that surveys using bee bowl are undoubtedly biased by the differences in capture rates across species and habitats. We also know that counts used in any insect survey are likely to be highly variable. Consequently, to obtain an adequate portrait of bee populations one is likely to need a large number of samples spaced across a large number of sites. Since the resources required to set such a large number of samples is likely to be limited, surveys may be unaffordable if run every year. Thus, the best strategy is to run large surveys 5-20 years apart.

We believe that the best approach to constructing a landscape survey of native bees using bee bowls would be to place a grid over the survey region and take repeated samples from within each grid cell. This design has a number of analysis and reporting advantages. Establishing a grid over the area helps even out the coverage so that the landscape is more uniformly covered than if points were randomly placed. The multiple survey locations within each grid cell permit the calculation of an unbiased estimate of the probability that a grid cell has the species (McKenzie et al. 2002). In addition to the estimated number of cells having the species, such data also permit the production of estimates of relative abundance and frequency of species occurrence across points, maps of the locations of species' captures, estimates of species richness using unbiased estimators, a list of species, and calculations of association between these measures and their surrounding habitats.

Sample Size. Using a grid approach it is necessary to determine what grid size to use, how many points within a grid cell to place, and how many bowls to place at an individual point. Grid cell size is largely arbitrary, it can be set by the sponsors based on the level of detail

they want to achieve, the amount of money they have, and other factors. For example, if money is tight then a large grid with few cells can be employed to cover a broad region. Results from such a survey will consequently provide few details about where populations are changing as local patterns may be obscured. Additionally, some changes during subsequent surveys would remain undetected due to low sampling effort. However, as most geographic regions lack even a species list this approach would be a good first start and efforts in the future can be more detailed if money permits without penalty. At times it will also be convenient to use existing grids created by manufacturers of maps and atlases. These will diminish the effort needed to create, assign, and duplicate cell maps for participants.

Appropriate numbers of sample points needed within a cell is a bit trickier to determine as there are no pilot data currently available. Seat of the pants experience indicates that 20-50 points within a cell would be a useful target. Numbers of bowls per point has been estimated using two unpublished western U.S. data sets provided by (Robert L. Minckley and Claire Kremen). After randomly pooling individual bowl data from these data sets, it was found in both cases that the error term from a species richness estimator stabilized around 15 bowls. Consequently, until more data alter this pattern we suggest that each site use 5 bowls each of white, fluorescent blue, and fluorescent yellow. Bowl size is not critical, but the 3.25 oz. Bowls provide the greatest convenience in terms of water use and speed of deployment.

Sample placement. Pure random or systematic sampling is difficult to impossible to achieve in most landscapes. Access to private lands is often precluded and swamps, cliffs, and other landscapes features can remain inaccessible. Thus each survey will have to qualify its results as being a native bee survey of accessible landscape points rather than a native bee survey of the total landscape. A detailed description of how points were chosen must be

provided to document the procedure for those repeating your survey 100 years from now. In landscapes with a great deal of private land, such surveys are usually a combination of roadway edges and accessible public/private lands. Surveyors can be asked to allocate bowls to each habitat in proportion to that habitat's availability in a cell, using whatever lands they have access to. Another approach is to have observers survey predetermined spots along secondary public roads.

Usually sampling points should not be clustered any closer than 1km apart if the grid cell is large so that sampling doesn't over-represent small regions of a cell that may be particularly convenient to survey. Surveys can also eliminate samples from locations that do not get any bees, such as forests after leaf-out. Note that urban, suburban, and commercial habitats all contain bees, often a surprising number of them and should always be part of a sampling strategy. While the above may not represent the ideal, in most cases it does generate a repeatable and roughly representative regional portrait of the bee community.

Note that often there are special spots in a cell that warrant more attention than others. Often this is the location of a rare or uncommon plant community or any special soil type which may harbor rare bees. Such sites are often missed or not covered in detail when surveys are created as outlined above. However, such sites can be covered as intensely as participants wish using netting, malaise traps, etc. as long as the results are reported separately from the main survey. This will provide important records of species in need of protection but won't compromise the integrity of the overall sampling design, where the practical objective is to detect changes in the common species of bees.

Bowl placement within a site. Bowls should be spaced 5m apart and the colors alternated. Usually bowls are placed in a rough transect. The transect need not be absolutely

straight but can bend to avoid inappropriate sampling locations such as water and heavily shaded locations. Bowls should be run only when significant rain or heavy cloud cover is not predicted as well as when temperatures are above 50F. Bowls can either be set out before 9 a.m. and picked up after 5:00 p.m. (****note this is different than the one hectare plot protocol) or run for any 24-hour time period. Trials have indicated that the results are comparable.

V. Conclusions.

Using the results of the studies outlined above, we, along with a number of colleagues have developed and tested a simple and inexpensive set of sampling protocols (<http://online.sfsu.edu/~beeplot>). While these survey techniques were developed in North America, entomologists have used bee bowls throughout the world. From those experiences it appears that bee bowls work in almost any open environment where flowers occur at or near ground-level while bees coming to canopy trees appear to be unwilling to come to those same bowls. Prior to beginning any sampling effort using bee bowls outside of North America it would be best to check their performance via a series of pilot tests.

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Figure1. Shape of 1 oz soufflé cup used as bowl trap.