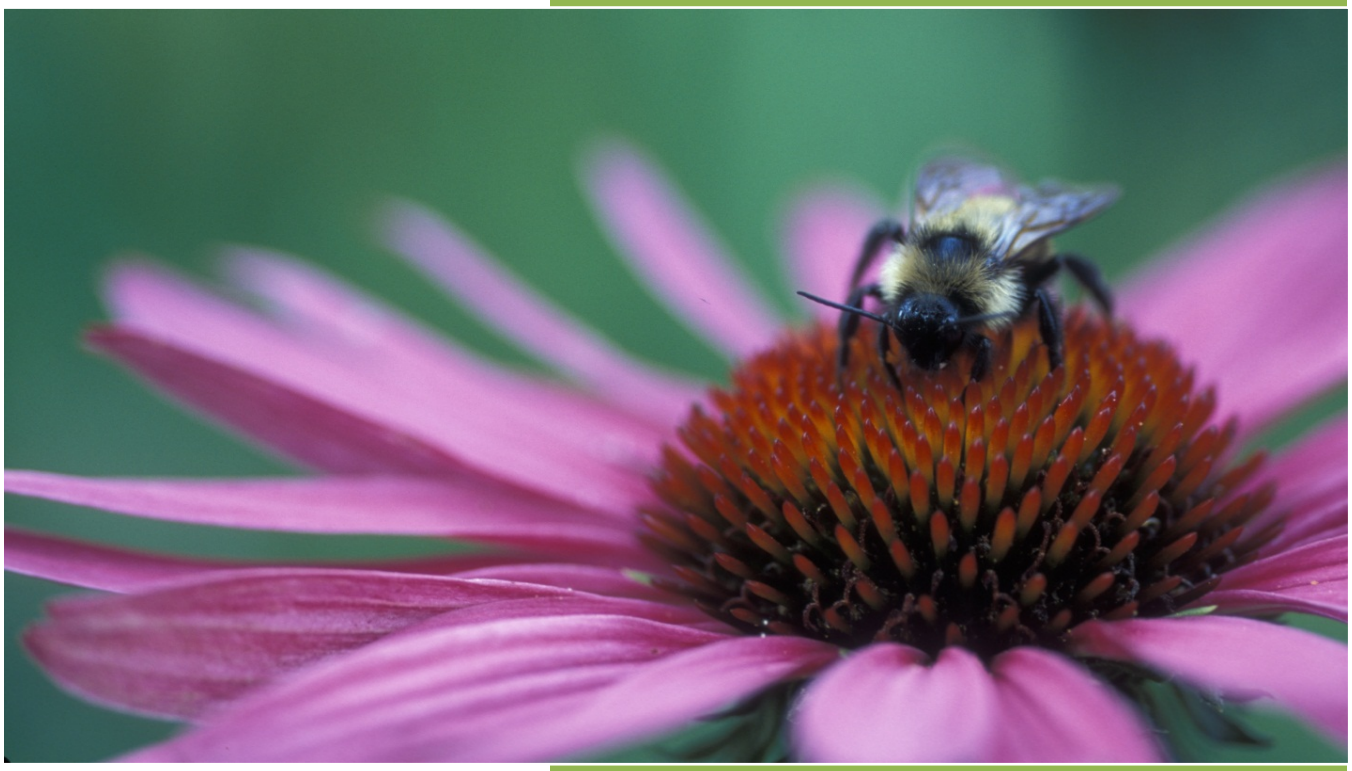
U.S. Fish and Wildlife Service
U.S. Department of the Interior

National Wildlife Refuge System

National Protocol Framework for the Inventory and Monitoring of Bees



SAM DROEGE
JOSEPH ENGLER
ELIZABETH SELLERS
LEE O'BRIEN

VERSION 2.0

ON THE COVER
Bumble bee (*Bombus* sp.) foraging on purple cone flower (*Echinacea purpurea*)
Photograph by: Ryan Hagerty

NWRS Survey Protocol Signature Page

Protocol Title: National Protocol Framework for the Inventory and Monitoring of Bees

Version[1] : 2.0

Station Name: National	**Authors and Affiliations** Sam Droege, USGS Patuxent Wildlife Research Center Joseph Engler, U.S. Fish & Wildlife Service, Region 1 Elizabeth Sellers, USGS, Eco-Science Synthesis Lee O'Brien, U.S. Fish & Wildlife Service, I&M

Approvals

Action	Signature/Name	Date
Prepared By:	Sam Droege, USGS; Joe Engler, USFWS; Elizabeth Sellers, USGS; and Lee O'Brien, USFWS, NWRS, I&M	
Submitted By:	Lee O'Brien, USFWS, NWRS, I&M Lee O'Brien	4/19/2017
Regional I&M[4] Approval:	N/A	
National I&M[5] Approval:	Jana Newman, USFWS , NWRS, I&M Jana Newman	9/19/2017

Version[1]	Date	Author	Change Made	Reason for Change

[1] Version is a decimal number with the number left of decimal place indicating the number of times this protocol has been approved (e.g., first approved version is 1.0.; prior to first approval all versions are 0.*x*; after first approval, all minor changes are indicated as version 1. *x* until the second approval and signature, which establishes version 2.0, and so on).

[4] Signature by Regional I&M Coordinator signifies approval of a protocol framework to be used at multiple USFWS stations within a Region.

[5] Signature by National I&M Coordinator signifies approval of a protocol used at multiple USFWS stations from two or more Regions.

Survey Protocol Summary

This national protocol framework is a standardized tool for the inventory and monitoring of the approximately 4,200 species of native and non-native bee species that may be found within the National Wildlife Refuge System (NWRS) administered by the U.S. Fish and Wildlife Service (USFWS). However, this protocol framework may also be used by other organizations and individuals to monitor bees in any given habitat or location. Our goal is to provide USFWS land managers (Service lands include national wildlife refuges, national fish hatcheries, wetland management districts, conservation areas, leased lands, etc.) with techniques for developing an initial baseline inventory of what bee species are present on their lands and to provide an inexpensive, simple technique for monitoring bees continuously and for monitoring and evaluating long-term population trends and management impacts. The latter long-term monitoring technique requires a minimal time burden for the individual station, yet can provide a good statistical sample of changing populations that can be investigated at the station, regional, and national levels within the USFWS' jurisdiction, and compared to other sites within the United States and Canada. This protocol framework was developed in cooperation with the United States Geological Survey (USGS), the USFWS, and a worldwide network of bee researchers who have investigated the techniques and methods for capturing bees and tracking population changes. The protocol framework evolved from field and lab-based investigations at the USGS Bee Inventory and Monitoring Laboratory at the Patuxent Wildlife Research Center in Beltsville, Maryland starting in 2002 and was refined by a large number of USFWS, academic, and state groups. It includes a Protocol Introduction and a set of 9 Standard Operating Procedures or SOPs and adheres to national standards of protocol content and organization. The Protocol Narrative describes the history and need for the protocol framework and summarizes the basic elements of objectives, sampling design, field methods, training, data management, analysis, and reporting. The SOPs provide more detail and specific instructions for implementing the protocol framework. We welcome use of this protocol framework by our partners, as appropriate for their bee inventory and monitoring objectives.

Suggested citation:
Droege, S, JD Engler, E Sellers and LE O'Brien. 2017. U.S. National Protocol Framework for the Inventory and Monitoring of Bees, Version 2.0. Inventory and Monitoring, National Wildlife Refuge System, U.S. Fish and Wildlife Service, Fort Collins, Colorado.

This protocol is available from the USFWS Service Catalog (ServCat):
https://ecos.fws.gov/ServCat/Reference/Profile/74109

Acknowledgments

The authors acknowledge the numerous and generous contributions of countless researchers, students, and volunteer citizen scientists throughout the U.S. and other countries to the testing and refinement of the techniques and tools described in this protocol; specifically Jim Labonte, Dave Smith (USFWS), Tracy Zarrillo (Connecticut Agricultural Experiment Station), and Zak Gezon (Rocky Mountain Biological Laboratory and Dartmouth College). We also acknowledge John Ascher (National University of Singapore), Harold Ikerd (USDA Agricultural Research Service), Gretchen LeBuhn (San Francisco State University, Department of Biology), Jack Neff (Central Texas Melittological Institute), and Karen Wright (University of New Mexico) for their valuable additions to the “Stereo or Dissecting Microscope” section.

Contents

List of Figures

List of Tables

Narrative

Element 1: Introduction

Background

Why do we need a protocol for conducting surveys of bees?

The National Wildlife Refuge System (NWRS) is directed by policy to maintain and restore the biological integrity, diversity, and environmental health of lands under its jurisdiction (601 FW 3). By definition, this includes the variety of all living organisms, the habitats within which they occur, as well as the maintenance and/or restoration of these populations and their associated habitats in an ecologically functional condition.

Bees are recognized as an integral part of virtually all ecosystems on earth and are essential for the long-term persistence of most flowering plant species via their pollination of trees, shrubs, and forbs. As pollinators, bees represent an essential link between flowering plants and the food and shelter they provide to humans and wildlife. Though generally overlooked during NWRS station management activities (NWRS stations are land units managed by the USFWS such as national wildlife refuges, national fish hatcheries, wetland management districts, conservation areas, leased lands, etc.), bees provide a critical, yet often unrecognized function in almost every aspect of wildlife and habitat management and restoration – including providing pollination services for the reproduction and survival of plants and the non-pollinating nectivores, herbivores, and frugivores that depend on them for forage; and as a forage source themselves for insectivores such as the bee and wasp specialist scarlet tanager (*Piranga olivacea*) (Robinson 1996; Skutch 1989) and tyrant flycatchers (Tyrannidae) (Craig and Williams 1998). Because of the specialized nature of bees and their preferred or sole pollen sources, there is a direct link between healthy and biodiverse bee populations and a healthy and biodiverse plant population (Kearns and Inouye 1997, Mandelik et al. 2012a and 2012b, Kevan, 1999, Russell et al. 2005). As human dominated landscapes become more and more utilitarian, fragmented, invaded by non-native species, and otherwise disturbed and simplified, their capability to hold and maintain uncommon, sensitive or narrow niche native plant and bee habitats declines, making restoration and maintenance of native habitats on NWRS stations and other types of protected areas increasingly valuable as repositories of regional biodiversity. Therefore, it is vital that NWRS stations maintain healthy and diverse plant and native bee communities within their managed and unmanaged landscapes.

Standardized protocols are important for inventory and monitoring (I&M) activities related to wildlife and plants. Bees, in particular, are easily overlooked by standard visual encounter types of surveys, as most are unidentifiable when observed in the field. Therefore, passive capture methods are the preferred means of creating repeatable surveys for bees (LeBuhn et al. 2013). Although, for those who need non-lethal sampling, *SOP 7: Netting and Non-lethal Techniques* provides an overview of available options and considerations. Depending on objectives, a properly-developed standard protocol allows comparable and unbiased sampling to occur across both spatial and temporal scales using different observers/collectors and assists in documentation of surveys and their results, thus providing defensible data and repeatable surveys that can be directed toward specific objectives.

Currently, most national wildlife refuges, other federal and state land management agencies, and local agencies, in North America are using some form of colored bowl, pan trap, or cup trapping system to survey bees in conjunction with the more traditional netting and/or use of malaise and vane traps. These are deployed in a variety of different ways to meet the individual objectives of a site. Our purpose in writing this protocol framework is to attempt to: 1) create and standardize techniques for bee I&M programs across the continent, and 2) assess the status of and changes in the bee fauna at a local to regional scale, specifically its genera/species composition, relative abundance, species richness, and distribution.

Lethal vs non-lethal techniques

In general, this manual describes survey methods that are lethal to the captured bees. The mortality caused by inventory and long term monitoring programs have not been shown to impact subsequent year bee populations. Gezon et al. (2015) found that the standardized method for sampling bees, with specimens from 132 morphospecies, did not affect bee communities in terms of abundance, rarefied richness, evenness, or functional group composition. Their results indicate that the bee communities were robust to such sampling efforts, despite removing an average of 2,862 bees per season. Mortality from collecting bees is generally compensatory, where collection deaths replace deaths that would have occurred naturally, and does not impact the population size/viability of bees in subsequent years.

To obtain the most complete assessment of bee populations at a site, collecting bees is recommended. Most species of bees cannot be identified in the field with binoculars or by photographs because identification characteristics are microscopic. Identifying bees on the wing is very difficult as bees are constantly moving and seldom provide sufficient opportunity to view and identify them. Variable skill levels of observers also make confidence in identification and comparisons between observers or surveys impossible.

The study of bees is still in the rudimentary stages compared to that of vertebrates and other insect groups such as butterflies. Without collecting specimens, we won't get vital information on native bees to be able to practice successful bee conservation and management. Failure to document a species' occurrence, by foregoing collecting, could jeopardize targeted conservation in areas that are able to support rare species.

There are circumstances, however, where non-lethal survey methods may be warranted. If bees are listed as threatened or endangered (T&E) and are known to occur in an area, there may be restrictions on "taking" these bees. Consult with regional USFWS Ecological Services (ES) staff and the ES Handbook on Incidental Take (https://www.fws.gov/endangered/laws-policies/policy-final-hcp-handbook.html) for guidance in such cases.

Non-lethal methods may also be used when there is a concern for impacting populations of rare species or vulnerable life stages. One situation to be aware of is that bumblebee queens forage in the spring until their first brood hatches and appear to be very susceptible to capture in blue vane traps. These traps should probably be avoided, or at least minimized, during times when queens fly in the spring. At other times these traps can provide information on bees that other techniques do not. Analyses of different trapping methods have shown that large numbers of non-bumblebees are also attracted to these traps, so caution using blue vane traps is warranted and capture rates should be monitored (Geroff et al. 2014; Kimoto et al. 2012; Ptasznik 2015).

The rusty patched bumblebee (*Bombus affinis*) has been listed as an endangered species. The USFWS maintains a webpage that gives guidance on doing surveys for *B. affinis*, and in areas where they may occur: https://www.fws.gov/midwest/Endangered/insects/rpbb/guidance.html. This page has maps of where *B. affinis* may occur and a downloadable survey protocol that discusses survey methods, including hand netting and photographing bees, similar to what is described in *SOP 7: Netting and Non-lethal Techniques*.

Considerations for using lethal and non-lethal techniques

1. Many more places and more specimens can efficiently be sampled with traps, and trapping is more likely to detect rare/uncommon species.
2. Trapping techniques are more easily replicated, and can provide statistically valid comparisons among sites and over time, providing information needed for monitoring and conservation actions.
3. Most species of bees cannot be identified in the field with binoculars or photographs because identification characters are microscopic and the bees are highly mobile.
4. Mortality from collecting bees is generally compensatory, where collection deaths replace deaths that would have occurred naturally, and does not impact the population size/viability of bees in subsequent years.
5. Most field bees collected in summer and fall are not reproductive, but early spring trapping should be avoided, when capturing bumblebee queens may be a concern.
6. Most solitary bees produce offspring throughout the summer season. So, only a portion of a captured reproductive female's potential progeny output will be affected by the female being captured part way through the summer.
7. Males provide only sperm, they do not help with nest construction or provisioning, thus their captures have little impact on the next generation, assuming population size of the species collected is not extremely small.
8. Taxonomy of bees is still evolving and many new bee species are being discovered and collected. Specimens provide the opportunity to upgrade identifications and allow for additional studies of morphology, taxonomy and DNA in the future.
9. Not collecting bees will mean that many species in a region will go unrecorded and consequently, conservation planning and the identification of species of conservation concern, will be limited.
10. Bowl traps (the primary survey technique for many) capture relatively few bumble bees and could be used in all locations, except possibly at locations with known populations of T&E bees.
11. Blue vane traps can capture large numbers of bees, including bumble bees, and should be used with caution. This method should be avoided during the times when queens fly in the spring. These traps do provide information that other techniques do not, so small numbers of these traps may be used at other times of the year, if captures are monitored.
12. When working near known populations of T&E species, non-lethal techniques provide the most reasonable means of monitoring the health of the existing population of bees during those species' flight times.
13. Non-lethal identification techniques, such as photography, can provide vouchers for the occurrence of species in an area.
14. Field observations of highly visible bees, such as some bumblebee species, by experts and advanced amateurs using binoculars or net and release techniques, can be an effective way to survey some species.

15. Net collecting could be used in any location, but at sites with T&E species, training and care need to be taken that individuals are not harmed.
16. Photographic and observation techniques are greatly affected by the skill of observers; therefore observations cannot be reliably compared between observers.

See *SOP 7: Netting and Non-lethal Techniques* for information on non-lethal survey techniques.

What other organizations are interested in bee surveys?

A number of multi-agency working groups are now focusing attention on bee inventories, conservation planning, and implementation and monitoring in North America. However, efforts to develop regional or national standardized I&M programs have not occurred until recently, and the programs are still being refined. These working groups are complemented by I&M programs, research, and educational programs administered by a wide-range of governmental agencies, universities, museums, non-governmental organizations, and private citizens. These diverse entities, each with their own objectives and expertise, contribute extensively to the knowledge-base and needs assessments for bee populations across the continent and beyond. Some of the agencies and groups working on bee I&M programs directly applicable to USFWS I&M efforts include:

The US Geological Survey Bee Inventory and Monitoring Lab (BIML) in Beltsville, Maryland is developing and testing a nationwide method to inventory and monitor bee population trends on public lands, in conjunction with universities and other researchers. This protocol is based upon that effort. The BIML is also assisting on the development of on-line identification resources and mapping tools, such as those available on Discover Life (www.Discoverlife.org). The BIML is also currently assisting NWRS stations with bee I&M development and specimen identification.

The US Department of Agriculture, Agricultural Research Service's Bee Biology and Systematics Laboratory at Utah State University in Logan, Utah works on a variety of agricultural-related bee issues and specializes in native bee I&M and bee taxonomy. The laboratory is also assisting NWRS stations with specimen identifications.

University-based scientists conduct a wide variety of research on pollinators. While many programs focus on the pollination of agricultural crops with non-native species such as honey bees (*Apis mellifera*), many research programs delve into native bee populations from both an agricultural and a natural lands perspective. Graduate programs and related research include topics such as disease, taxonomy, DNA barcoding, bee ecology, and outreach and education. These universities work closely with the Federal and State agencies and assist with development of methods and taxonomic refinements directly related to field-based I&M.

As the concern for pollinating species grows due to environmental degradation, climate change, increases in non-native and invasive species occurrences, and invertebrate health issues, many private organizations are emerging as galvanizing forces for community action and education. These organizations promote public awareness from local to international levels, help influence pollinator-based legislation, provide educational materials and tools, and assist with grass-roots, on-the-ground projects. The Xerces Society for Invertebrate Conservation (www.xerces.org) and the North American Pollinator Protection Campaign (www.nappc.org) administered by the Pollinator Partnership (www.pollinator.org) have been instrumental in refining USFWS-wide

approaches to pollinator conservation. Other organizations are important to individual NWRS stations on local and regional scales. Due to the increased knowledge of and concern for bee populations world-wide, natural lands (both managed and un-managed) are viewed as providing critical source populations and refugia for maintaining many bee species populations. Given that many managers of these natural lands have limited knowledge of their bee fauna and the needs of these species on their lands, many federal land management agencies, state, tribal and local governmental agencies, and private land management groups that protect and manage natural lands have initiated small-scale inventories of the bees on their lands.

All of the entities mentioned previously are seeking to improve the quality and efficiency of native bee conservation, and many individuals from these groups and organizations provide guidance and expertise regarding I&M efforts. However, there is no coordination through a larger network or agencies on these surveys and, while local results are important, the inconsistency in methodology does not always allow comparisons of data across sites or across temporal scales. This diminishes the usefulness of the data for monitoring long-term trends in bee populations at local, regional, and continental scales. This protocol framework is a first step in bridging that gap by establishing consistent standards that are to be utilized on NWRS stations nationwide.

Objectives

The USFWS established a national pollinator working group in 2007 and in 2008 added representatives from each region of the USFWS. This working group has the responsibility within the USFWS for pollinator conservation and education. Additional subgroups have been formed from the national group and within the NWRS I&M Initiative to define common pollinator management, I&M needs across NWRS stations, as well as to integrate these needs at regional and national levels. Defining management objectives for an I&M program is often the most difficult step in the process. A range of management objectives must be considered when developing a standardized survey protocol designed for use in a wide variety of situations.

This protocol framework is written so that a user can develop a site-specific protocol to collect data on the bee fauna present at a given station using standardized and therefore repeatable I&M methods. These data include occurrence and identification of most of the bee species present on a station, their relative abundance within the confines of the protocol/methods, species richness of the bee fauna, and basic phenology (period of adult activity) (LeBuhn et al. 2002; Williams et al. 2001). Application of this protocol framework should result in comparable data when applied across habitats and/or stations and provide the baseline information needed for developing subsequent management objectives. These data can also be used to monitor bee populations over time, or in an adaptive management framework, to see what the results of land management actions (e.g., vegetation manipulations) may have on bee populations.

The techniques outlined in the SOPs are also applicable for a variety of I&M situations, but the implementation of specific I&M programs will require clear management objectives and an evaluation of sampling sizes and input from statisticians to ensure that the resulting information is going to meet the needs of managers.

This protocol framework attempts to balance statistical rigor with practical application. It is currently beyond the scope of this protocol framework to define a regional or continental

sampling design using glycol traps; however BIML is developing national standards for this endeavor. It is the intent of this protocol framework to provide general information for conducting repeatable baseline bee inventories, while providing the groundwork for development of more comprehensive monitoring programs.

Who will use this protocol framework?

This protocol framework will be used by station staff or their affiliates for surveying bees on NWRS stations throughout the United States and its Territories and can be used by others that want to use a standardized protocol so their data can be pooled. The basic survey protocol is adaptable to many situations where there is a need to determine in a standardized fashion the type, faunal composition and number of bees occurring in an area and can help inform and address the development of local and regional studies and management actions at various spatial and temporal scales.

A standardized protocol framework will streamline I&M plans and help ensure that data can be shared among stations and partners. In addition, the data will have value for future meta-analyses designed to assess regional or national status and trends of bee populations or examine bee-habitat relationships at large spatial scales.

Element 2: Sampling Design

Defining station-specific objectives and selecting an appropriate sampling design is an essential step in developing a quality bee I&M program. Identifying current and future capabilities and constraints is also important during the selection process in order to develop and maintain a station-specific protocol that can be conducted in a manner that meets objectives.

Sample design: inventory versus monitoring

The Survey Coordinator for a single or group of stations (see *Element 6: Personnel Requirements and Training*) will ensure that a sampling design is developed (see *SOP 1: Sampling Design*) before the field season begins. They should consult with the Regional Pollinator Coordinator, Regional I&M Coordinator, or a statistician to verify that the sampling design will meet the defined management and I&M objectives. These persons may assist the Survey Coordinator in selecting the sampling design, providing database and other options, such as GIS data layers needed for sampling.

Sampling designs for bee surveys vary depending on management, inventory or monitoring objective, size, diversity and accessibility of habitats on a station, geographic location, prevailing climatic conditions, and availability of staff, equipment, and other resources. Clarifying the management objectives is the most important and the most difficult part of designing an I&M program; it is a step that is often overlooked or avoided entirely (Johnson 2000). Once the management objective is clear, the sampling design can then be developed.

A management objective for bee conservation can be relatively simple, such as determining the bee species (or more typically a subset of species) that occur in a specific place, such as within a specific habitat or on the entire station. This inventory gives a snapshot of the species' occurring at a site during a discrete timeframe. It needs to be recognized that no single technique is capable of catching all bee species. Deploying multiple techniques over time (years) will increase

captures, but a complete catalog of bee species is unlikely given the often unknown variation in bee life cycles, ecological preferences and tolerances, influences of natural and unnatural conditions on population dynamics, and the taxonomic uncertainty of some bee species. A complete bee species list is generally un-necessary for land managers. Understanding the requirements of the most common genera or species and documenting rare species may be all that is needed to evaluate and improve management actions on a given site.

A more typical objective of a bee I&M program is to draw conclusions about the magnitude and direction of change in bee population size through time. Monitoring of a discrete segment of a bee community over space and time can provide data more useful to managers such as effects of management actions, anthropomorphic influences or climate change. Each of these monitoring questions will require different sampling designs and range from a site-specific to a regional or continental scale.

Design considerations

There is a range of methods, equipment, and nomenclature that abound within the bee I&M community (Table 1); some of these accomplish the same purpose but collectively may cause confusion in their variations. For the sake of simplicity, clarity, and consistency this framework adopts the following conventions:

Pan traps (2 types) = Bee Bowls (3.25 oz. cups) or (Propylene) Glycol Traps (12 oz. cups)

Bee bowls are used for short-duration (24 hours or less) sampling events. Traps are set out, usually in linear transects for 1-2 days, once every two weeks throughout the active flight season. They incorporate 3 color combinations to attract bees and are filled with soapy water to capture bees. Use of these traps is further defined in *SOP 3: Setting Up Bee Bowls for Short-Term Sampling Periods*. Glycol traps are used when traps are deployed either in linear transects or in circular arrays for longer time frames (1 week or more to a full season); they also use the same 3 color scheme. Their larger size allows more bee captures over time and the propylene glycol (environmentally-safe anti-freeze) generally resists evaporation, except in extreme weather conditions. The propylene glycol helps preserve specimens while they are in the traps; after removal from the trap, specimens should be stored in the freezer. Use of these traps is further defined in *SOP 4: Setting Up Glycol Traps for Long-Term Sampling Periods.*

In very broad terms, small bees (e.g. sweat bees, mason bees, 5 to 14 mm long) are sampled well in pan traps, but larger bees (e.g. bumble bees, carpenter bees, 15 to 25 mm long) often need to be collected with sweep nets to create the most complete inventory. Individual bee mobility, activity periods, trap color, trap height, and placement all influence the ability to capture certain bee species (Cane et al. 2000, Toler et al. 2005, Roulston et al. 2007, Tuell and Isaacs 2009, Droege et al. 2010, Gollan et al. 2011). Pan trapping will provide the most repeatable way of surveying bees across years, personnel and sites, while for a more complete inventory, netting or malaise traps can be added (Campbell and Hanula 2007).

Pan trapping is a method for surveying rather than censusing bees in an area, which is essentially impossible to do with these insects. Thus, the raw number of bees captured in pan traps is not a measure of true species composition or density, but some reflection of it. For trend analysis, if the raw counts of bees represent a constant proportion of true population size, it may be possible

to use unadjusted counts as an index of the population. However, if the chance of observing a bee is not constant through time or among habitats – that is, if there is heterogeneity in the detection probability – then the raw counts will not be an unbiased index of population size.

There is currently disagreement in the scientific literature about the degree to which detection heterogeneity influences conclusions about trends, with some authors being adamantly opposed to the use of unadjusted counts for any conclusions and other authors being unconvinced that accounting for detection heterogeneity improves the estimate of trend (Johnson 2008, Nichols et al. 2009).

Despite the controversy, there is a growing body of literature that indicates raw counts of individual animals need to be adjusted for detection probability to accurately estimate densities (Nichols and Conroy 1996, Pollock et al. 2002). While it may be some time before there is scientific consensus on this issue, there currently exist few alternative methods for evaluating and incorporating detection probabilities into the analysis of any bee capturing technique.

If the monitoring is undertaken primarily to inform future management decisions or actions, an adaptive management framework is needed to place the monitoring in its proper context. The adaptive management framework will ensure that the monitoring information is really needed, properly designed, and will be useful (Nichols and Williams 2006, Williams et al. 2007). The design of an adaptive management project usually involves expert consultants and partners outside the USFWS and one or more workshops to achieve agreement about objectives, alternative management actions, and how to evaluate success. This process can take 6-12 months or longer; however, careful thought and planning in the beginning can save years of wasted time and effort conducting monitoring that fails to inform management.

Table 1. Key Considerations for a Bee Survey, with Rationale

CONSIDERATIONS	STRATEGY	RATIONALE
Trap liquid	Propylene glycol	Propylene glycol has an extremely low rate of evaporation and thus the traps can potentially maintain their liquid component longer - for one to several weeks without replenishment (depending on the local climate aridity) when at full concentration. Propylene glycol [unlike ethylene glycol] is completely non-toxic to humans and animals and is used in many prepared foods. It is also a preservative, and thus insect specimens do not rot when the proportion of propylene glycol to water is high.
Trap liquid	50:50 Propylene glycol and soapy water	This combination will generally last for one week in arid climates. This mixture provides propylene glycol as a preservative and conserves its use by including soapy water.
Trap liquid	Soapy water	Pan traps can be filled to a ¾" depth with soapy water as the trapping liquid. Soap (dish washing liquid) decreases the surface tension of water, is cheap, readily available/obtainable and kills the bees quickly. Without the soap, bees would simply alight on the water, supported by the surface tension and fly off again. Note that in dry and windy locations water can evaporate from small pan traps in only a few hours.

CONSIDERATIONS	STRATEGY	RATIONALE
Trap collections for propylene glycol	Every one or two weeks	Because propylene glycol keeps specimens preserved and evaporates only slowly, trap collections can occur every one or two weeks. However, trap collections occurring on a weekly basis are more useful for detecting week to week changes and decrease the probability that disastrous events such as loss of bowls or trap liquids will corrupt the value of the data. Collecting trap residues every two weeks also decreases the impact on the station staff involved in the program and on the amount of handling time required to collect and process the specimens.
Number of traps in a glycol trap array	9	Based on experiences with the U.S. Forest Service throughout the U.S. and Puerto Rico, reasonable numbers of bee specimens are caught using configurations of nine traps. Nine glycol traps are used in an array (three each of fluorescent blue, fluorescent yellow, and white). Nine traps represent a balance in the number of traps needed to be tended, the number and amount of space needed to deploy traps, the cost of filling the traps with propylene glycol, and the number of specimens to be processed.
Number of traps in a bee bowl transect using soapy water	24	Data from BIML indicate the number of traps used at their sites for estimating species richness varied, but that a minimum of 24 bowls (eight of each of the three colors – fluorescent blue, fluorescent yellow, and white) should be adequate (Shapiro et al. 2014)
Distance between traps	5 m apart	Droege *et al.* (2010) found that a distance of 3 to 5 m apart is sufficient to decrease competition among bowls for bees.
Time of year traps are deployed	Early spring until after the first hard frost in temperate locations	Different species of bees have different requirements for pollen and actively fly during different seasons often for only brief 1 to 2 month periods of time. Thus, to sample the entire fauna of bees, traps need to be deployed throughout the local bee activity period OR flight period.
Continuity of trapping for glycol traps	Continuous	Continuous trap operation alleviates the need to sample only on good weather days and creates much less of a burden on station staff and volunteers involved because trap collection can occur on a fixed date and time. Additionally, phenological changes in bee populations' flying dates within a year are unpredictable, and having traps continuously capturing bees accommodates shifts from year-to-year in bee activity. Potential drawbacks are having too many bees to ID, losing a lot of sampling data if traps are lost, and potentially oversampling small sites.
Continuity of trapping for bee bowl transects with soapy water	1 day or 24 hours (every 2 weeks)	Bee bowls with soapy water can be set out early in the morning or just after sunrise before most bees are active and collected just prior to sunset. Transects of bee bowls can be set out and collected daily, one day per week or every two weeks depending on sampling objectives and logistical limitations.
Location of traps	Weather stations, maintenance areas, or areas of management importance	If the objective is to detect or monitor long-term population changes in bees and there are only one or a few suitable locations on a NWRS station, a weather station, maintenance area, or other place of convenience would be the best location to deploy traps for long-term stability of the survey. These sites additionally, are maintained, have roads and other conveniences associated with them that permit NWRS station staff and volunteers to access them quickly and easily. If the objectives are to assess management effects, or determine habitat associations, then sites appropriate for these objectives should be selected.

Sampling units, sample frame, and target universe

A sampling unit is the defined element at which data is collected and analyzed. The sampling unit can be an individual pan trap or a collection of traps (e.g., transect or an array) or a discrete netting event. In some instances, a single trap color or multiple combined transects (all colors) may be the designated sampling unit. Sampling units differ spatially in their zone of influence (capture rate) which is an important consideration for determining which sampling unit fits project objectives. The placement and type of sampling unit in relation to habitat, geographic and manmade features can influence the size of the sampling unit's zone of influence. Broadly speaking, a trap or sampling unit's zone of influence is the area within which it is visible, detectable, and able to entice or trap bees. For example, a tree canopy foraging bee is likely to fall outside of a ground-based pan trap or handheld net's zone of influence – it is physically or otherwise beyond the 'reach' of the trap or sampling unit. A 50 m linear transect of pan traps may have a broader zone of influence/attract insects from further away than the same transect running along a large lake or forested verge where it might be hidden from view to insects foraging on the other side and unlikely to cross the verge or the lake. A lake-side trap or sampling unit's zone of influence may be limited to the water's edge on at least one side. Sampling units also differ temporally based on the sampling method (e.g. short versus long-term pan trap sampling versus hand netting). Long-term sampling will capture more uncommon to rare species, provide better relative abundance data, and provide more useful phenology information. A single sample consists of multiple bee specimens collected from a single sampling unit (e.g. a transect) at the end of a sampling event (e.g., all bees from all traps in a single short term transect at the end of a day (short-term) or from an array that is sampled once every 2 weeks).

It is also important to understand the difference between long and short term traps and sampling events versus long or short term surveys or studies. For the purposes of this protocol, a short-term trap is one that can only be left out in the field for a few days before needing to be replenished/replaced; while a long-term trap is one that can be left out in the field for at least two weeks or more. Having said that, a trap's effectiveness and therefore its 'term' will decline over time spent in the field and be impacted by trap liquid evaporation rates, overflow due to rain, how many other insects are already in the trap, and how likely the trap is to get knocked over by other wildlife/events. The duration of a sampling event is also largely defined by the type of trap(s) used therefore how often the trap or sampling unit is checked, refreshed or replenished.

The duration of a survey or study is usually determined by criteria such as the type of research being conducted and the expected outcomes, funding, and availability of personnel and other resources. A long term survey or study may be carried out over a period of years but consist of numerous short term sampling events or a mix of long and short term sampling events. A transect or sampling site may be permanently established for 20 years or more, but the duration of sampling events or how long traps are left out might only be 24 hours, repeated once every 2 weeks during the active/flying season of the target species. Similarly a short term survey or study may only be carried out over a single season or six months to one year but consist of both long term (e.g. 1 month) and short term (e.g. 1 day) sampling events. For example, a nature preserve currently carries out a year-long survey (Feb-Nov), consisting of a combination of short term (30 mins – for handnets; 24 hours – for pan traps) and long term (2 weeks – for vane traps) sampling events. But it has been established as a 'long term survey' because it is and will be repeated once every 5 years, indefinitely, to detect longer term patterns across decades.

Hand netting events also result in sample units, each consisting of multiple bee specimens collected during a specified time period and/or from a target sampling location such as a flowering plant, nesting location, or other substrate that bees are collected from.

The sample frame is the spatial area to which the sample is associated. This can be a discrete measured sampling area, a specific habitat patch or coarsely applied to an entire NWRS station. Due to a multitude of factors (e.g., weather, vegetation cover, species dispersal or foraging distances, trap visibility), ascribing a distinct zone of influence (e.g., X bees per X square meters) for a trap or set of traps is virtually impossible. Therefore, objectives and data analyses need to take into account these limitations.

The target universe refers to the bee-specific attributes that a protocol aims to sample. This may include all or specific bee species using a specific habitat type or certain flowering plants, showing certain phenological patterns, using certain nesting substrates, etc.

Recent work on the ability of trapping systems for native bees to adequately detect changes over time indicate that at the species level, a monitoring program will have to aggregate information across multiple sites. At the genus level, however, richness or total numbers of bees at a station should have adequate statistical power to detect changes at the level of an individual site (LeBuhn et al. 2013).

Sample selection and size

Sampling method varies depending on a station's management objectives and multiple logistical and biological constraints. Station size, habitat complexity, and staffing constraints may all dictate the when, where, and what sampling units are selected. A typical sampling unit on a station is either a transect of 24 bee bowls (using 8 each of blue, yellow, and white bowls) or a circular array of 9 glycol traps (3 of each of the 3 colors) with the bee bowls or glycol traps placed 3-5 meters apart. This is sufficient to collect a reasonable subset of the bees for a basic inventory program for an individual location, and may be augmented by netting for larger bees. Multiple transects within the same area or habitat should be placed at least 100 meters apart.

A sampling unit should be placed where it can be easily deployed and monitored. An easily accessible area that is representative of the area can work as a sampling site assuming the site is relatively open and has a variety of vegetation, structure, and substrates, and is representative of the habitat(s) on the refuge or land area in which bee information is needed and management objectives questions can be answered. Depending on objectives, this basic sampling scheme can be modified to sample specific habitats, and increase the rates and number of species captured.

Because objectives vary, there is no set standard for the number of sampling units per unit area. The number of sampling units, spatial configurations, and locations are determined during development of the station-specific protocol. Additional consideration must be given to study design when objectives are to compare and contrast monitoring efforts across spatial and temporal scales.

Survey timing and schedule

A survey, consisting of one or more short or long duration sampling periods, can be as short as one month or a specific plant species' flowering period, or as long as the period between the last

spring frost and the first fall frost in temperate regions during a calendar year. It might be a good idea to do a pilot study to determine bee seasonality and test site-specific protocols prior to developing a final protocol. In the western U.S., bees are active from just prior to the time that willow trees (*Salix* spp.) are blooming until the first hard frost in temperate locations; other early-season indicator plants occur depending on the region of the country. It may be necessary to consult with a botanist or plant ecologist to identify appropriate early-season and late-season indicator plant species or earliest and latest sampling period for your location. In southern Florida and truly tropical areas flowering plants can support bees even in January. If a station's objective is to monitor shifts in phenology, scheduling sampling events one week or so early would be advantageous. Many of the early blooming plants are small, inconspicuous ground-hugging species taking advantage of comparably warmer soil temperatures than the surrounding air. Thus areas that seem to be relatively devoid of flowering plants may actually be supporting early emerging bees. Many bee species have a strong seasonal component due to their preferences for gathering pollen from certain genera of plants. Different bee species are emerging throughout the year and often have only a five week or so window in which they are active and available for collecting as flying adults (Linsley, 1958). Consequently, if you are interested in a complete survey of all the bee species in an area, you will need to set out bee bowls every 2-3 weeks, or operate glycol trap arrays continuously.

Bees captured in continuously running glycol trap arrays are collected and processed at a minimum of once every two weeks throughout the bee flying season. However, glycol traps may need to be inspected more frequently to maintain fluid levels in arid climates. This pattern of collection may allow refined analyses using the tools of occupancy analysis (MacKenzie et al. 2005) and provide an alternative way to estimate detection probabilities.

The sampling period for bees will be defined by the Survey Coordinator (see *Element 6: Personnel Requirements and Training*) to coincide with when bees first come out and until the bees are no longer flying or foraging or become uncommon (often this occurs within several weeks prior to the first hard frost in temperate locations). It may be necessary to consult with a botanist or plant ecologist to identify appropriate early-season and late-season indicator plant species or earliest and latest sampling period for your location. The time required for sampling depends on how many traps are set, in how many groups, and how far apart transects or arrays of traps are from each other and the objectives of the survey. The logistics of accommodating other day-to-day activities and whether a special trip needs to be taken to set out and/or collect the traps need to be considered during the sampling design.

The Survey Coordinator will schedule sampling dates and organize logistics before the start of each field season. One advantage of the continuously running glycol trapping technique is that collection of trap samples is independent of the weather during the time of sample collection and thus the collection can be scheduled completely for an entire year. Bees captured in these arrays are collected and processed at a minimum of once every two weeks throughout the bee flying season. This pattern of collection may allow refined analyses using the tools of occupancy analysis (MacKenzie et al. 2005) and provide an alternative way to estimate detection probabilities.

Sources of error

As mentioned previously, pan traps are just one method of sampling bees and no single method has the capability of capturing representatives of all bee genera and/or species within the area of interest. Pan traps are considered the mainstay of many I&M programs due to their ease of deployment and operation, low cost*****, and their ability to capture a relatively high proportion of the bee fauna. There are many potential sources of error when collecting bees and therefore the interpretation of results must recognize these inherent short-comings.

***Note:** the cost of collecting bees may be low, but accurately identifying bees may be expensive.

Because of the difficulties of studying free ranging bees and obtaining accurate density estimates, at this point, we cannot say that captures of bees in a set of colored bee bowls is truly representative of the bee community, because we do not have an independent and unbiased assessment of any bee community for comparison. However, based on observations, it is safe to say that pan traps are not collecting all bee species in direct relationship to the population size. It is likely that factors such as pollen availability, surrounding vegetation composition and phenological status or phenophase, and pan trap color and size preferences influence the number and species of bees collected in pan traps. As with I&M of many species, results can also be affected by weather, observer bias (not following protocol), sampling effort, annual variations in life cycles, regional trends, misidentifications, and erroneous analyses. As station objectives and expectations become more specific and complex, increasing sources of error need to be taken into account.

Element 3: Field Methods and Sample Processing

Establishment of sampling units

The same sampling locations are generally maintained from year to year when a monitoring program is established. Inventories may be conducted in new locations depending on the station objectives. To establish accurate locations, a GPS (Global Positioning System) unit or Geographic Information System (GIS) can be used to determine the center of each transect or array of traps. Latitude and longitude or Universal Transverse Mercator (UTM) coordinates are a standard requirement for labeling at most museums and other institutions that archive bees (and other specimens); center or centroid coordinates for sampling locations serve this purpose. Make sure to record the geodetic datum (and the UTM zone, if using UTMs) when recording coordinates. For transects, some indication of the start and end points are helpful and may be recorded as coordinates or a compass direction and distance. Transects do not necessarily need to follow a straight line, as habitat conditions (excessive shading, terrain) may dictate a meandering transect; therefore a written description may be appropriate should transects need to be re-established. When possible, end points or center points should be permanently marked with a fencepost or other visible marker. This allows subsequent site revisits to collect vegetation or other site-specific information, should the need arise.

Not all sites will allow permanent markers to be placed due to terrain, animal activity, or management activities. Sites within managed fields may need to be mowed, hayed, or grazed. Cattle and elk are extremely fond of pulling out flagging, and rubbing fence posts. They also can upset pan traps, spilling the contents and thereby reducing sample size. Sites with large ungulates may require some type of fencing or deterrent to inhibit disturbance.

Thick grass, especially non-native species, can form near impenetrable barriers to movements of small bees and conceal bee bowls and glycol cups. Consider elevating traps where quickly-growing vegetation may impact bee movements and describe any sampling adjustments in the data sheets.

Equipment

The station will need to acquire a minimal amount of field equipment depending on the final station sampling design and the number of individuals participating. A station may also need to invest in lab equipment if the station intends to process samples and/or identify and house specimens. The station needs to have a computer to enter (digitize) and/or archive electronic data, space for storage/display of specimens, and space for storage for field collecting equipment. A 4-wheel drive vehicle, boat, or even a helicopter may be required to access survey points. *SOP 2: Equipment and Preparation* includes detailed information on what equipment and preparations must be made prior to conducting short or long-term surveys.

Data collection procedures (field, lab)

SOPs 3, 4 and 5 describe how to setup and collect samples through the use of bee bowls with soapy water for short-term surveys, arrays of glycol traps for long-term surveys, and using hand nets. Data collection should include the collecting or general documentation of site-specific information, such as weather, plant phenology (e.g. plants observed in leaf or flowering stage [buds, peak flowering…], fruit, strong fragrance) in the immediate area (e.g. at or within sight of the transect/trap position), signs of trap disturbance, trap damage, loss of fluid, or other issues. If resources permit, plant phenology may be collected within the immediate area and within the foraging distance for the largest bee collected in samples. Bee body size is correlated with foraging distance, however species-specific foraging distances for native bees have been studied for only a limited number of species (e.g. Gathmann and Tscharntke 2002; Greenleaf et al. 2007; Jha and Kremen 2012; Osborne et al. 1999; Wolf et al. 2008; Zurbuchen et al. 2010a, 2010b). It needs to be noted if traps are missing or disturbed, and thus rendered ineffective, as these traps must be discounted during data analyses where trapping effort is considered. *SOP 5: Storing, Shipping and Pinning Collected Bee Specimens* outlines standard lab procedures for handling and preparing samples for storage and/or shipment to a third party.

Processing of collected materials

Before the field season begins, the Survey Coordinator should identify and develop an agreement with an authorized collection facility such as a museum, university, or other research organization to receive and archive any field specimens as necessary (per policy: 701 FW 5). This agreement needs to identify whether the station or the third party will be processing specimens prior to identification. If threatened or endangered species (including other invertebrates) might be collected, contact the Regional Ecological Services Program prior to the sampling period for handling procedures and permit requirements. If collection is to occur within a candidate or listed species' range or within critical habitat for the species, an ESA Section 7 consultation is required prior to collection. For discussion of non-lethal sampling methods see *SOP 7: Netting and Non-lethal Techniques*. For this framework, processing refers to preparing, pinning, and labeling specimens for identification. In some instances, stations may contract out processing and/or identification services to a third party. Volunteers may also help with processing specimens. *SOP 5: Storing, Shipping and Pinning Collected Bee Specimens* contains detailed information on how to prepare bees for shipping, identification, and archiving.

End-of-season procedures

Once the survey season is complete, the field site(s) needs to be cleaned up, flagging and other equipment removed. Permanent transect or array markers should be used if feasible and secured in place for the off-season. All traps should be removed, cleaned and repainted if needed, and stored dry. Glycol cup holders should also be removed and stored to increase their longevity.

Element 4: Data Management and Analysis

Bee identification

Decisions regarding bee identification should be made when the survey's objectives are defined. Bee identifications can be time-consuming and costly, therefore it is imperative to understand how various identification issues may or may not achieve the desired objective. There are approximately 4,000 bee species known to occur in North America north of Mexico, many of which are difficult to identify to species level. It should not be expected that all specimens can be identified to the species-level. In all cases, the minimal taxonomic level for identifications should be the genus level. Projects that have a general management focus or an educational (e.g. young student participation) purpose may not need identifications beyond the morphospecies level. If for example, the management focus is to conserve or manage or detect the presence of bee pollinators at a site; or the educational focus is to involve young students, then it is sufficient to identify specimens as bumble bees, large carpenter bees, sweat bees and honey bees for example – in order to answer high level questions such as, "Are bees present?" or "What kinds of bees are present?" without becoming bogged down in species-level identification. Conversely, projects that aim to construct species lists or attempt to determine the presence of rare or uncommonly collected/detected species may attempt to identify all specimens to species. Identification expectations need to be a key consideration during objective setting and study design.

Arrangements should be made before the survey for how the station plans to identify and record bee species. The station usually will ship bee specimens to a university, a museum, a research organization, or a suitable contractor for identification (per policy: 701 FW 5 Collections, Donations, and Disposals - www.fws.gov/policy/701fw5.html). These entities generally have a preference for how samples should be handled prior to receiving them, as this may influence the identification procedures, as well as their time-investment and cost. If threatened or endangered species might be collected, contact the Regional Ecological Services Program prior to the sampling period, for handling procedures, permits, consultation, and reporting requirements. Stations may also attempt to identify some or all specimens themselves. For in-house identifications, stations should have someone on staff that has attended a bee identification course or has other suitable training or experience. For those stations that prefer to conduct the identifications themselves, see *SOP 6: Identifying Collected Bees* for information on how to identify bee species. If NWRS stations plan to identify specimens on their own, it would be advisable to have an expert confirm the identification of subsamples of each species. Regional or local species lists may be available from sources such as Discover Life – www.discoverlife.org or USGS Biodiversity Information Serving Our Nation (BISON) – bison.usgs.ornl.gov. Local species lists may be available from sources such as Discover Life – www.discoverlife.org or USGS Biodiversity Information Serving Our Nation (BISON) – bison.usgs.ornl.gov.

Data entry, verification and editing

Data entry and management can vary depending on whether a station chooses to establish their own database or use an existing database. There is a stand-alone MS Access database that accompanies this survey protocol (in ServCat[1]) that matches the attached field data sheets, and which the USDA Bee Biology and Systematics Laboratory in Logan, UT has agreed to populate with ID information (if the metadata for each survey is filled in). They will upload the bee observations to the USDA national bee database and return the Access database to the station. Contact the lab before starting any surveys to confirm these arrangements. The USGS Native Bee Inventory and Monitoring Lab at the Patuxent Wildlife research Center may make similar arrangements. Contact the lab to check. Museums, universities and research organizations have their own systems and will prefer to upload all bee data and identifications into that system. In most cases, these organizations should be willing to share their database and/or export the data to a station database. For additional information on data entry and management and for example data collection sheets see *SOP 8: Data Collection and Management.*

Data security and archiving

The Survey Coordinator will archive raw survey data, field notes, and photographs in compliance with relevant USFWS data standards (www.fws.gov/stand) and pursuant to the USFWS Policy on Service Information and Technology Architecture (270 FW 1; www.fws.gov/policy/270fw1.html) and the USFWS Policy on Electronic Records (282 FW 4; www.fws.gov/policy/282fw4.html). Details of archiving are described in *SOP 8: Data Collection and Management.*

For safekeeping, the Survey Coordinator will store one hard copy of data and associated materials at the station and one in a secure location off the station. This includes both raw and summarized survey data, as well as associated maps, photographs, and field notes. As data are digitized, do the same with electronic files. Field notes should be retained even after data are digitized.

At the termination of a survey, or every 3 to 5 years, archive the protocol and any amendments, associated metadata, maps, photographs, field notes, data sheets, electronic data files, a record of the locations (or dispositions) of archived specimens, together with interim and final reports, as a package. This package should be duplicated and stored in two places, one at the coordinating station and one in a secure location off the station (e.g. the Regional Office, another station, or other location that is reasonably accessible to station staff). Reports and site-specific protocols should also be stored in the USFWS Service Catalog (ServCat).

The Survey Coordinator will archive field specimens determined to have archival value on the station in appropriately designed facilities for storing specimens, or transfer them to authorized

[1] ServCat is a Web application available to Fish and Wildlife Service employees that compiles documents and organizes data, such as reports, surveys, databases, geospatial data and images.

collection facilities such as museums or universities, following any permit requirements (per policy: 701 FW 5). If threatened or endangered species might be collected, contact the Regional Ecological Services Program before sampling begins, for handling/permitting procedures. Recommendations on minimum requirements for appropriate specimen storage facilities can be obtained by consulting museum or university curatorial staff. At minimum, if specimens not kept in an appropriate storage facility they should be frozen at -20C for 3 days every 6 months to minimize pests.

The location (or disposition) of archived specimens should also be documented and included in the archived package generated at the end of a survey or every 3-5 years.

Analysis methods

The data analysis has three main functions: (1) Provide basic summaries of the data, intended for use in quality control and annual reporting; (2) Analyze occurrence, abundance, annual variations, or long-term trends for species and changes in the composition of bee communities over time; and (3) Use in measuring differences among management options and techniques. See *SOP 8: Data Collection and Management* for detailed instructions. More advanced analyses, such as evaluating associations between bee taxa, plant communities and soil types, will need to be conducted under the supervision of the Survey Coordinator or contracted to someone deemed competent to perform the analysis.

Element 5: Reporting

Objectives and methods

Once you have determined that surveying bees is necessary to inform management decisions and the attributes that will be surveyed, it is important to think about exactly what information about the bees needs to be communicated. What types of tables, graphs or charts do you want to be able to generate from the data? Do you simply want a list of species occurring at your station? Do you want to quantify trends in bee populations? Do you want to explain habitat relationships of certain bees? Do you want to know if the entire bee community at your station has changed (possibly as a result of management), or are you mainly interested in 2-3 key species? If you know how you will analyze the data and produce your summary information, the sampling design will be easier to determine.

Once you have answered the questions above, you can define your target population. This protocol framework is designed to inventory or monitor the majority of common species of bees living in the study area.

Implications and application

The Survey Coordinator (see *Element 6: Personnel Requirements and Training)* should meet annually with the Project Leader (see also *Element 6*) to discuss how the survey results can be or are being used to improve types and timing of management practices that benefit the bee species present. These practices may include controlled burns, road/trail construction and maintenance, mowing or other vegetation trimming, restoration plantings/plant selection, pesticide/herbicide applications, livestock (cattle, hive bees) rotation/management, etc. It may also be determined that the survey needs to be improved to include less, more or different data in order to better inform management practices.

Reporting schedule
The Survey Coordinator produces Annual Reports and Synthesis Reports (every 3-5 years) and submits them to the USFWS Project Leader at the station. The USFWS encourages the Survey Coordinator to see that significant findings are published in peer-reviewed media (U.S. Fish & Wildlife Service 2007) and share their reports with the USFWS Pollinator Work Group through their Regional Pollinator Coordinator (see *Element 6*).

Report archiving
Reports should be archived in the USFWS Service Catalog (ServCat).

Element 6: Personnel Requirements and Training

The size of a station, difficulty of terrain and other factors will influence the number and skillsets of personnel required for conducting surveys of bees.

Native bees can be difficult to identify to the genus and/or species level. No one can successfully identify bees (even to genus) without having received adequate training, or without working with other people or groups that are experienced in bee identification. At minimum bees will need to be sent away for verification if identifications were made by inexperienced staff/biologists. The USGS Native Bee Inventory and Monitoring Lab and USDA ARS Logan Bee Lab are two useful sources for validating bee specimen identifications completed by refuge staff/biologists. Local museums and/or universities may also be able to offer some assistance/expertise with bee specimen identification/verification. It may be required to pay for these services, so investigate options before beginning a bee survey.

The protocol for tending the pan traps is extremely simple and can be performed by a volunteer or seasonal technician as long as they follow the straightforward protocol for handling specimens and tending the amount of fluid (soapy water or glycol) in the traps.

The Survey Coordinator and all field crew members will review this entire protocol, including all of the SOPs before the field season. The equipment and supplies listed in *SOP 2: Equipment and Preparation* should be organized and made ready for the field season, and copies of the field data forms or the Access database loaded on a portable device (*SOP 8: Data Collection and Management*) should be available.

Roles and responsibilities

Regional I&M Coordinator
USFWS Regional I&M Coordinators, among other duties, provide scientific support to refuges within their Regions and coordinate I&M needs with LCCs, JVs, and other partners.

Regional Pollinator Coordinator
USFWS Regional Pollinator Coordinators identify and encourage opportunities for the USFWS to promote pollinator conservation and education. They compile and share information on pollinator-related accomplishments and identify opportunities for the USFWS and the North American Pollinator Protection Campaign (NAPPC), and/or NAPPC partners, to work together on projects that are mutually beneficial.

National Pollinator Coordinator
The USFWS National Pollinator Coordinator chairs the USFWS Pollinator Work Group and is their liaison to the NAPPC.

The Survey Coordinator
The Survey Coordinator is the USFWS employee that oversees and coordinates the implementation of the survey protocol at a station or group of stations. The Survey Coordinator leads surveys and ensures that survey data are managed, analyzed, reported, and archived properly; provides biological expertise and oversight of the survey, including assisting in the identification and categorization of habitats and target plant and bee species for sampling at the station; advises on station-specific implementation of the SOPs impacted by site-specific environmental considerations such as weather, temperature, terrain, substrates, other wildlife etc.; works with the Project Leader to coordinate I&M activities across or with other adjacent stations in the NWRS; and responds to enquiries by the public or Field Crew Volunteers in regards to sampling techniques, specimen collection and handling, etc.

Project Leader (Refuge Manager)
The Project Leader ensures USFWS policy compliance and helps develop, implement and revise the station's Inventory and Monitoring Plan (IMP), including any bee surveys.

Refuge Biologist
The Refuge Biologist leads refuge staff in selecting surveys and preparing IMPs; participates in developing the site-specific survey protocol; ensures surveys follow an approved protocol; and often serves as the Survey Coordinator.

Field Crew Volunteer Coordinator
If the station will be employing volunteer labor to conduct a survey, then we strongly advise the appointment of a Field Crew Volunteer Coordinator if one does not already exist for the station. The responsibilities of this role include assisting the Survey Coordinator in identifying, recruiting, and documenting appropriately skilled and available volunteers, assigning responsibilities and tasks to them, and coordinating their participation, training, and contributions to the project. The Field Crew Volunteer Coordinator may also prepare and implement training for Volunteers on the methods and techniques described in the SOPs and coordinate periodic opportunities to check and review Volunteer's work. The Field Crew Volunteer Coordinator may also coordinate some activities as regular group events such as specimen preparation workshops. A good Field Crew Volunteer Coordinator should be reliable and organized, while also having good 'people skills' and teaching skills. They should be able to work well with and lead people from a wide range of age groups and abilities; be able to recognize when team members might need assistance or additional/refresher training; and know when questions or issues should be elevated to the Refuge Biologist or other higher level staff member.

Field Crew
Field Crew will be responsible for learning the methods and techniques in the SOPs including trap and hand net operation, transect set up and take down, sample/specimen collection, sample/specimen preparation, data collection, and possibly specimen preparation and data entry.

In the case of hand net sampling, and general implementation of the survey, two volunteers or personnel are better than one in terms of safety, and shared tasks – especially when operating in remote locations. Two crew members may also be desirable with setup, sampling, and/or take down of pan trap transects. In the case of pan traps, the field crew can share tasks between each other and also help to monitor each other's implementation of the SOPs.

The field crew can be assigned to operate more than one transect or array. If pan trap transects and arrays are within a half-mile distance from each other, on foot, a single able-bodied individual or team of two can reasonably set up (put out or place traps), collect samples from, document, and retrieve up to three 150 meter pan trap transects, each consisting of up to 30 bee bowls, in approximately one hour. And depending on distances, multiple arrays of 9 glycol traps could be set up and serviced in an hour.

Good field crew should be physically capable of carrying, placing or using, and retrieving trapping equipment and of traversing the terrain on foot or using vehicles to reach sampling locations. They should also be able to write and record field data clearly in provided field data sheets and/or electronic devices for data recording and entry (e.g., mobile phones, GPS units). Some degree of good hand-eye coordination is required for collecting and filtering insect specimens from traps in the field and especially if field crew will also be involved in pinning specimens. Crews involved in pinning and/or identifying specimens may also need to be able to use stereo or dissecting microscopes or have good to very good eyesight and steady hands.

Regional Data Manager

The Regional Data Manager is responsible for ensuring scientific integrity by establishing quality assurance standards for data collection and management; ensuring that I&M data are conserved and archived; and assisting Refuge System staff with digitizing, managing, and analyzing data and reporting survey results. The Regional Data Manager is also responsible for preparing or tailoring, distributing, retrieving, and storing forms and software applications (either hard copy or electronic) for field data collection, digitization, and databasing or storage as applicable. The Regional Data Manager should work with the Survey Coordinator and refuge staff conducting the survey where appropriate to review the quality of raw and digitized data throughout the survey period and make corrections or adjustments where needed. Training on use of field data collection forms, electronic devices, field notebooks, and software for data digitization should be offered at the beginning of the survey season and also at least once half-way through the season as a refresher. Initially the emphasis will be on quality field data collection, but as the volume of field data increases, it will be necessary to also prioritize instruction in data digitization and databasing. The Data Manager may also be responsible for coordinating in-house or third party data digitizers (see *SOP 8: Data Collection and Management*).

Qualifications

Vehicle Drivers

Station staff with the appropriate safety, operator, and defensive driver training will be needed if government vehicles, boats, helicopters or other specialized vehicles are required for accessing sampling sites.

Biologists

Stations should employ or arrange access to one or more Biologists capable of assisting in identifying bee and plant species observed at or collected from the sampling location(s). These personnel may be sourced from within the USFWS or from universities, museums and other professional organizations. As mentioned, the USGS BIML and USDA ARS labs are providing support for specimen identification.

Training

Training will be required at the beginning of the season, before sampling begins and may be offered again half-way through or later in the season if needed.

Staff involved in bee I&M activities should receive training in all aspects of the project at some broad level of detail and more detailed training for tasks specific to their role(s). For example, all staff should have a basic understanding of the full path and processing procedures for data and specimens from field collection, through the lab, and to final reporting and archiving. This basic understanding of all aspects of the project often serves to inform and reinforce the reasons for correctly following certain procedures that might not otherwise seem very important.

Staff involved in these activities should receive initial training on site/transect selection and documentation; pan trap and transect or array set up; specimen collection from pan traps and via hand net; specimen preparation; data review and entry (both field-based and electronic); all aspects of documentation and data handling (e.g. documenting transect location and composition, sampling events, samples, individual specimens, etc.), and specimen preparation, storage and stewardship if these latter tasks will not be conducted by a third party.

Note: We recommend that those who know they are truly allergic to bee stings and bee venom should not participate in any aspects of these surveys that involve actually handling bee specimens directly. However, some individuals may have an allergic reaction to bee stings or venom at any time even with no prior allergic response. First aid training and availability of an on-site EpiPen is advisable.

Staff entering data into a spreadsheet or database should receive necessary training in the software being used, before data entry begins. Use of global positioning systems, mobile devices, collection of geographic coordinates and datums, and mapping transect locations or sampling sites will also require some basic training to ensure accurate readings. Basic training in plant identification and local species, and provision of any supplemental materials such as identification guides and handbooks may assist field staff in identifying plants of interest either while at sampling sites or from specimens collected for later identification.

Element 7: Operational Requirements

Budget

The Survey Coordinator will develop a budget for implementing this protocol framework (Table 2). Field costs will vary depending upon logistics. Travel costs include travel to field sampling sites, lodging, and per diem. Startup costs for equipment include the purchase of equipment and supplies, as well as maintenance and or replacement of equipment shared among multiple projects (e.g. GPS units, cameras).

Budget estimates should include funding to process and identify specimens, and enter or digitize, review for quality, and analyze data, if time or capacity does not reside in-house.

Table 2. Example Budget Estimating the Annual Costs of Implementing the Survey at a Site (actual costs will vary by location, effort, etc. and some costs may not be incurred in all years of a multi-year effort).

ESTIMATED TIME/FINANCIAL COSTS	**PER SITE**
Collecting trap contents (specimens)	5 hr
Preparing log	1 hr
Fabrication of glycol traps (usable for many years)	2 hr
Deployment of glycol traps	1 hr
Deployment of pan traps	30 min.
Preparation for mailing specimens	1 hr
Data entry	TBD
Reporting	TBD
TOTAL	**10 hr**
Local costs	
3.25 oz plastic cups or equivalent (short-term surveys)	
12 oz. plastic cups or equivalent (long-term surveys)	$2.00 each
Paint	$10.50 per 2 oz bottle / $36.50 per 16 oz jar*
Pre-painted pan traps (bee bowls)	$0.15 each
9 glycol trap holders (can be made from scrap material for free in most cases)	$15.00
Propylene glycol	$50.00 (~$12.50 per gallon for 4 gallons)
Mailing unprocessed specimens (Fedex, UPS or USPS*)	$5.00 per unprocessed specimen package
Cardboard sample boxes for mailing pinned specimens	
Assorted insect pins and label paper	$50 per 500 pins (inc. shipping costs)
Sweep nets, killing jars and killing agents	
Mailing processed (pinned) specimens (USPS)	$15.00 per for 5 12" square boxes
Whirl-Paks® or Ziploc® bags (for holding specimens)	$3.00 per pack of 40 (Ziploc) or 100 (Whirl-Pak)
Disposable paint strainers, (non-disposable) shrimp (aquarium) nets, tea strainers	$1.50 each
TOTAL	**$76.00**
Specimen identification costs will vary as to how many specimens and whether identification is done to species or genus and if specimens are put on pins and returned as a collection.	$100-$1500 or $80 per hour (@1 specimen per minute)

** Price estimate according to Guerra Paint and Pigment (Phone 212-529-0628 or order online at www.guerrapaint.com/tandc.html)*

Staff time

The field work can be implemented by a single observer (but a two-person team may be preferable) e.g. setting up 3-6 150m transects of 24-30 pan traps in a day for short-term monitoring, depending on terrain and distance between transects; or 6 or more arrays of glycol traps for long-term monitoring (again, depending on terrain and distance between arrays). Note that short-term monitoring transects need to be set up early in the morning and retrieved shortly before sunset. So for short-term sampling period transects only the early morning hours can be used to set them up and the hour or two at most before sunset is available to retrieve them. Multiple transects within the same area or habitat should be placed at least 100 meters apart. An individual on a survey team may also be responsible for more than one role.

Coordination

Consideration should be given with respect to other station activities, especially those that directly impact habitat condition, wildlife behavior, and access to sites such as controlled burns, herbicide or pesticide applications, grazing, mowing, logging, flooding, tidal changes, trail maintenance etc. These activities can directly and negatively impact sampling success.

Element 8: References

Abou-Shaara, H. F. 2014. The foraging behaviour of honey bees, *Apis mellifera*: a review. *Veterinarni Medicina* 59(1):1-10. Available from: http://vri.cz/docs/vetmed/59-1-1.pdf

Aiello, A., Domínguez Núñez, E. and H. P. Stockwell. 2010. Nothing is Perfect: Biodegradable packing material as food and transportation for a museum pest, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). *The Coleopterists Bulletin* 64(3):256-257. DOI: http://dx.doi.org/10.1649/0010-065X-64.3.256.12

Ascher, J. S. and J. Pickering. 2015. Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Available from: http://www.discoverlife.org/mp/20q?guide=Apoidea_species

Campbell, J. W. and J. L. Hanula. 2007. Efficiency of Malaise traps and colored pan traps for collecting flower visiting insects form three forested ecosystems. *Journal of Insect Conservation* 11:399-408. DOI: http://dx.doi.org/10.1007/s10841-006-9055-4

Cane. J. H., Minckley, R. L., and L. J. Kervin. 2000. Sampling bees (Hymenoptera:Apiformes) for pollinator community studies: pitfalls of pan-trapping. *Journal of the Kansas Entomological Society* 73:225–231. Available from: http://www.jstor.org/stable/25085973

Craig, D. and P. L. Williams. 1998. Willow Flycatcher (*Empidonax traillii*). In The Riparian Bird Conservation Plan: a strategy for reversing the decline of riparian-associated birds in California. California Partners in Flight. Available from: http://www.prbo.org/calpif/htmldocs/riparian_v-2.html

Droege, S. 2015. The very handy manual: How to catch and identify bees and manage a collection. United States Geological Survey. Available from: ftp://ftpext.usgs.gov/pub/er/md/laurel/Droege/Handy Bee Manual.pdf

Droege, S., Tepedino, V. J., Lebuhn, G., Link, W., Minckley, R. L., Chen, Q., and C. Conrad. 2010. Spatial patterns of bee captures in North American bowl trapping surveys. *Insect Conservation and Diversity* 3:15-23. DOI: http://dx.doi.org/10.1111/j.1752-4598.2009.00074.x
Engler, J. 2012. 2012 Pollinator Monitoring Program, U.S. Fish and Wildlife Service, Region 1. U.S. Fish and Wildlife Service.

Gathmann, A. and T. 2002. Foraging ranges of solitary bees. *Journal of Animal Ecology* 71(5): 757–764. DOI: http://dx.doi.org/10.1046/j.1365-2656.2002.00641.x

Geroff, R. K., Gibbs, J., and K. McCravy. 2014. Assessing bee (Hymenoptera: Apoidea) diversity of an Illinois restored tallgrass prairie: methodology and conservatoin considerations. Journal of Insect Conservation 18(951):964. DOI: http://dx.doi.org/10.1007/s10841-014-9703-z

Gollan, J. R., Ashcroft, M. B., and M. Batley. 2011. Comparison of yellow and white pan traps in surveys of bee fauna in New South Wales, Australia (Hymenoptera:Apoidea:Anthophila). *Australian Journal of Entomology* 50:174-178. DOI: http://dx.doi.org/10.1111/j.1440-6055.2010.00797.x

Gonçalves, R. B., and P. S. Oliveira. 2013. Preliminary results of bowl trapping bees (Hymenoptera, Apoidea) in a southern Brazil forest fragment. *Journal of Insect Biodiversity* 1(2):1-9. Available from: http://insectbiodiversity.org/index.php/jib/article/view/3

Greenleaf, S. S., Williams, N. M., Winfree, R., and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153(3): 589-596. DOI: http://dx.doi.org/10.1007/s00442-007-0752-9

Hunter, G. 2006. Curation of Insect Specimens. National Park Service Conserve O Gram, September 2006, Number 11(8), pp 8. NPS Park Museum Management Program, 1849 C Street NW (2265), Washington, DC 20240. Available from: http://www.cr.nps.gov/museum/publications/conserveogram/11-08.pdf

Integrated Taxonomic Information System (ITIS). http://www.itis.gov

Jha, S. and C. Kremen. 2012. Resource diversity and landscape-level homogeneity drive native bee foraging. Proceedings of the National Academy of Sciences of the United States of America 110(2):555-558. DOI: http://dx.doi.org/10.1073/pnas.1208682110

Johnson, D. H. 2008. In defense of indices: The case of bird surveys. *The Journal of Wildlife Management* 72(4):857-868. Available from: http://www.jstor.org/stable/25097626

Johnson, D. H. 2000. Statistical considerations in monitoring birds over large areas. Strategies of bird conservation: the Partners in Flight planning process (R. Bonney, D. N. Pashley, R. J. Cooper, and L. J. Niles, Eds.). U.S.D.A. Forest Service Proceedings RMRS - P - 16. Available from: http://www.fs.fed.us/rm/pubs/rmrs_p016/rmrs_p016_115_120.pdf

Joshi, N. K., Leslie, T., Rajotte, E. G., Kammerer, M. A., Otieno, M., and D. J. Biddinger. 2015. Comparative Trapping Efficiency to Characterize Bee Abundance, Diversity, and Community Composition in Apple Orchards. *Annals of the Entomological Society of America* 108(5):785-799. DOI: http://dx.doi.org/10.1093/aesa/sav057

Kearns, C. A., and D. W. Inouye. 1997. Pollinators, flowering plants, and conservation biology. *BioScience* 47:297–307. Available from: http://www.jstor.org/stable/1313191

Kevan, P. G. 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agriculture, Ecosystems & Environment* 74:373–393. DOI: http://dx.doi.org/10.1016/S0167-8809(99)00044-4

Kimoto, C., Debano, S. J., Thorp, R., Rao, S., and W. P. Stephen. 2012. Investigating temporal patterns of a native bee community in a remnant north American bunchgrass prairie using blue vane traps. Journal of Insect Science 12(108):1-23. DOI: http://dx.doi.org/10.1673/031.012.10801

Kirk, W. D. 1984. Ecologically selective coloured traps. *Ecological Entomology,* 9, 35-41. DOI: http://dx.doi.org/10.1111/j.1365-2311.1984.tb00696.x

LeBuhn, G. 2002. The Bee Inventory Plot. Available from: http://online.sfsu.edu/beeplot/

LeBuhn, G., Droege, S., Conner, E.F., Gemmill-Herren, B., Potts, S.G., Minckley, R.L., Griswold, T.L., Jean, R., Kula, E., Roubik, D.W., Cane, J.H., Wetherill, K., Frankie, G., and F. Parker. 2012. Detecting insect pollinator declines on regional and global scales. *Conservation Biology 27*(1):113-120. DOI: http://dx.doi.org/10.1111/j.1523-1739.2012.01962.x

LeBuhn, G., Griswold, T., Minckley, R. L., Droege, S., Roulston, T. H., Cane, J. H., Parker, F., Buchmann, S. L., Tepedino, V., Williams, N. M., Kremen, C. and O. Messenger. 2003. A standardized method for monitoring bee populations – The Bee Inventory (BI) Plot. Available from: http://online.sfsu.edu/beeplot/pdfs/Bee Plot 2003.pdf

Leong, J. M. and R. W. Thorp. 1999. Colour-coded sampling: the pan trap colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. *Ecological Entomology* 24(3): 329-335. DOI: http://dx.doi.org/10.1046/j.1365-2311.1999.00196.x

Linsley, E. G. 1958. The Ecology of Solitary Bees. *Hilgardia* 27:543-599. DOI: http://dx.doi.org/10.3733/hilg.v27n19p543

MacKenzie, D., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey, L. L., and J. E. Hines. 2005. Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence. Elsevier/Academic Press, Burlington, MA.

Mandelik, Y., Winfree, R., Neeson, T., and C. Kremen. 2012a. Complementary habitat use by wild bees in agro-natural landscapes. *Ecological Applications* 22(5):1535-1546. DOI: http://dx.doi.org/10.1890/11-1299.1
Mandelik, Y., Dayan, T., Chikatunov, V., and V. Kravchenko. 2012b. The relative performance of taxonomic vs. environmental indicators for local biodiversity assessment: A comparative study. *Ecological Indicators* 15(1):171-180. DOI: http://dx.doi.org/10.1016/j.ecolind.2011.09.033

Mitchell, T.B. (1960) Bees of the eastern United States. I. Technical bulletin (North Carolina Agricultural Experiment Station), 141, 1-538. [Introduction, Andrenidae, Colletidae, Halictidae, Mellitidae]. Available from: http://insectmuseum.org/easternBees.php

Mitchell, T.B. (1962) Bees of the eastern United States. II. Technical bulletin (North Carolina Agricultural Experiment Station), 152, 1-557. [Megachilidae, Anthophoridae, Apidae s.s.]. Available from: http://insectmuseum.org/easternBees.php

Nichols, J. D., Thomas, L., and P. B. Conn. 2009. Inferences About Landbird Abundance from Count Data: Recent Advances and Future Directions, pp 201-235. In: Thomson, D. L., Cooch, E. V., and M. J. Conroy, (Eds.), Modeling Demographic Processes In Marked Populations, Environmental and Ecological Statistics Volume 3, Springer Science+Business Media, LLC 2009. DOI: http://dx.doi.org/10.1007/978-0-387-78151-8_9

Nichols, J. D., and M. J. Conroy. 1996. Estimation of Species Richness, In: Measuring and Monitoring Biological Diversity, Standard Methods for Mammals, Wilson, D. E., Cole, F. R., Nicholes, J. D., Rudran, R. and M. Foster (eds.), Smithsonian Institution Press, Washington, D.C., pp. 226-234.

Nichols, J. D. and B. K. Williams. 2006. Monitoring for conservation. *Trends in Ecology and Evolution* 21:668- 673. DOI: http://dx.doi.org/10.1016/j.tree.2006.08.007

Osborne, J. L., Clark, S. J., Morris, R. J., Williams, I. H., Riley, J. R., Smith, A. D., Reynolds, D. R., and A. S. Edwards. 1999. A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *Journal of Applied Ecology* 36(4):519-533. DOI: http://dx.doi.org/10.1046/j.1365-2664.1999.00428.x

Packer, L., Genaro, J.A., and C.S. Sheffield. 2007. The Bee Genera of Eastern Canada. *Canadian Journal of Arthropod Identification* No. 3, 25 September 2007. Available from: http://www.biology.ualberta.ca/bsc/ejournal/pgs03/pgs_03.html
DOI: http://dx.doi.org/10.3752/cjai.2007.03

Pollock, K. H., Nichols, J. D., Simons, T. R., Farnsworth, G. L., Bailey, L. L., and J. R. Sauer. 2002. Large scale wildlife monitoring studies: statistical methods for design and analysis. *Environmetrics* 13:1-15. DOI: http://dx.doi.org/10.1002/env.514

Ptasznik, A. 2015. A comparison of pan trap and blue vane sampling methods for determining bee diversity. Undergraduate Honors Theses. 856. Ecology and Evolutionary Biology Department. University of Colorado, Boulder. http://scholar.colorado.edu/honr_theses/856

Robinson, W. D. 1996. Summer tanager (*Piranga rubra*). *In:* A. Poole and F. Gill (eds.), The birds of North America, No. 248, A. The Academy of Natural Sciences, Philadelphia, Pennsylvania, and The American Ornithologists' Union, Washington, D.C.

Roulston, T. H., Smith, S. A. and A. L. Brewster. 2007. A comparison of pan traps and intensive net sampling techniques for documenting a bee (Hymenoptera: Apiformes) Fauna. *Journal of the Kansas Entomological Society* 80:179–181. DOI: http://www.jstor.org/stable/25086376

Russell, K., Ikerd, H., and S. Droege. 2005. The potential conservation value of unmowed power-line strips for native bees. *Biological Conservation* 124:133–148. DOI: http://dx.doi.org/10.1016/j.biocon.2005.01.022

Schauff, M. E. (Ed.). 2001. Collecting and preserving insects and mites: techniques and tools. Update and modified WWW version of: G. C. Steyskal, W. L. Murphy, and E. H. Hoover (eds.).

1986. Insects and mites: techniques for collection and preservation. Agricultural Research Service, USDA, Miscellaneous Publication 1443:1-103. Available from: http://www.ars.usda.gov/SP2UserFiles/ad_hoc/12754100CollectingandPreservingInsectsandMites/collpres.pdf

Shapiro, L.H., V. J. Tepedino, and R.L. Minckley. 2014. Bowling for bees: optimal sample number for "bee bowl" sampling transects. *Journal of Insect Conservation* 18: 1105-1113.

Skutch, A. 1989. Life of the tanager. Comstock Publishing Association, Cornell University Press, Ithaca, New York.

Stephen, W. P., Bohart, G. E., and P. F. Torchio. 1969. The Biology and external morphology of bees with a synopsis of the genera of northwestern America. Agricultural Experiment Station, Oregon State University, Corvallis. Available from: https://catalog.extension.oregonstate.edu/sites/catalog.extension.oregonstate.edu/files/project/pdf/sp001.pdf

Toler, T.R., Evans, E.W., and V. J. Tepedino. 2005. Pan-trapping for bees (Hymenoptera: Apiformes) in Utah's west desert: the importance of color diversity. *Pan Pacific Entomology* 81(3/4):103-113

Tuell, J. K., and R. Isaacs. 2009. Elevated pan traps to monitor bees in flowering crop canopies. *Entomologia Experimentalis et Applicata* 131:93-98. DOI: http://dx.doi.org/10.1111/j.1570-7458.2009.00826.x
USNVC, 2015. U.S. National Vegetation Classification. Available from: http://usnvc.org/

Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S. G., Roberts, S. P. M., Szentgyörgyi, H., Tscheulin, T., Vaissière, B. E., Woyciechowski, M., Biesmeijer, J. C., Kunin, W. E., Settele, J., and I. Steffan-Dewenter. 2008. Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs* 78:653-671. DOI: http://dx.doi.org/10.1890/07-1292.1

Wilson, J. S., T. Griswold, and O. J. Messinger. 2008. Sampling Bee Communities (Hyme¬noptera: Apiformes) in a Desert Landscape: Are Pan Traps Sufficient? *Journal of the Kansas Entomological Society* 81(3):288–300. http://www.jstor.org/stable/25086445
Williams, N. M., McKinley, R. L., and F. A. Silveira. 2001. Variation in Native Bee Faunas and its Implications for Detecting Community Changes. *Conservation Ecology* 5(1): 7. Available from: http://www.ecologyandsociety.org/vol5/iss1/art7/

Williams, B. K., Szaro, R. C., and C. D. Shapiro. 2007. Adaptive management: the U.S. Department of the Interior technical guide. Adaptive Management Working Group, U.S. Department of the Interior, Washington, D.C. 72 pp. Available from: http://www.doi.gov/initiatives/AdaptiveManagement/documents.html

Wilson, D. 2009. Net Spoon. *The Volunteer Monitor* 20(2):11. Available from: http://water.epa.gov/type/rsl/monitoring/upload/2009_10_16_monitoring_volunteer_newsletter_volmon20no2.pdf

Wilson, J. S., Griswold, T. and O. J. Messinger. 2008. Sampling bee communities (Hymenoptera: Apiformes) in a desert landscape: Are pan traps sufficient? *Journal of the Kansas Entomological Society* 81, 288-300. DOI: http://dx.doi.org/10.2317/JKES-802.06.1

Wolf, S. and R. F. A. Moritz. 2008. Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). *Apidologie* 39:419-427. DOI: http://dx.doi.org/10.1051/apido:2008020
Zurbuchen, A., Cheesman, S., Klaiber, J., Müller, A., Hein, S., and S. Dorn. 2010a. Long foraging distances impose high costs on offspring production in solitary bees. *Journal of Animal Ecology* 79(3): 674–681. DOI: http://dx.doi.org/ 10.1111/j.1365-2656.2010.01675.x

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., and S. Dorn. 2010b. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biological Conservation* 143(3):669-676. DOI: http://dx.doi.org/10.1016/j.biocon.2009.12.003

Bee Monitoring Discussion List and Announcements
If you are interested in bee monitoring or identification issues, you might want to sign up for the Bee Monitoring listserv. It is a good way to alert you to interesting developments.

Email Sam Droege (sdroege@usgs.gov) to sign up.

Archives can be read at:
https://groups.yahoo.com/neo/groups/beemonitoring/conversations/messages

Standard Operating Procedure 1: Sampling Design

In order to effectively complete a survey of bee species occurring in any given area, we recommend incorporating a combination of sampling methods (described in SOPs 2-4) consisting of the use of pan trap transects supplemented with hand net specimen collection.

If the objective is to determine what species occur on a station, Survey Coordinators should decide whether they have the necessary resources for surveying the entire station or not. If resources will permit a station-wide survey, the sampling area can be stratified by habitats (representative of the environmental conditions for each habitat) for sampling. Survey Coordinators should also consult with I&M Coordinators and partners to see if there are any ongoing, or planned, bee surveys in their area that they might be able to cooperate with and possibly pool resources and analysis in a larger scale effort.

If Survey Coordinators attempt to sample the habitats represented on the station, habitats may be identified and categorized based on the National Vegetation Classification Standard (USNVC 2015) and/or in regard to structure and composition e.g. pine forest, meadow, early successional field, willow thicket, etc. (see *SOP 9: Associating Vegetation Classes with Survey Locations*). Some habitats may be targeted for sampling due to the presence of plants or nesting substrates known to be associated with or attractive to specific bee species or broader taxonomic groups of bees.

At its simplest level, a station can collect some subset of its bee fauna with minimal resources by setting up a pan trap transect or a circular array in any open habitat at a readily accessible site on the station. The habitat at the sampling location should preferably be representative of the area of interest rather than of unnatural landscaping or gardens often associated with headquarters or visitor centers.

Depending on objectives, terrain, and accessibility, multiple transects or arrays can be placed in a given habitat or area of interest. But multiple transects within the same area or habitat should be placed at least 100 meters apart to avoid overlapping their zone of influence (see Pg.8) and trapping bias given that solitary bee foraging distances can be 250m to 1500m from their nest sites depending on the species (up to 3km for hive/honey bees (*Apis mellifera*) (Abou-Shaara 2014; Zurbuchen et al. 2010a, 2010b). Transects placed closer than 100 meters apart would each benefit from (be biased) and therefore potentially attract more bees because of the closeness of the neighboring transects or compete with each other because they both fall within the foraging range of the same individuals. When you need to make an inference beyond the particular sites of data collection (e.g., inference about an entire habitat type on a refuge), the arrangement and number of transects should be based on a more formal design and may need to be developed with a statistician.

Survey Schedule

A single survey can consist of one or more sampling or collection periods (events). A survey can be as short as a single trapping event or as long as the period between the last frost in the spring and the first frost in the fall in temperate locations. The Survey Coordinator and station staff

should determine the duration of their survey and choose whether to schedule short or long collection periods and use methods according to their monitoring goals and objectives.

For short-duration (e.g. 24 hours or less) sampling periods (*SOP 3: Setting Up Bee Bowls for Short Sampling Periods*), pan traps should be set up and collected on the same day (e.g. being set out at dawn or before 9 am on day 1 and collected at dusk or after 5pm on day 1) or within 24 hours (e.g. being set out at a specific time on day 1 and collected at the same time on day 2). The trapping duration should strive to provide operable traps during the entire active bee flight cycle in a 24 hour period. Pan traps should be set once every two weeks, weather permitting, during the active flight season for bees in the target area. In temperate climates, the active flight season generally equates to the timeframe between the willow bloom in the spring and 2 weeks before the first frost in the fall. In southern climates, the active flight season may extend almost year-round. It may be necessary to consult with a botanist or plant ecologist to identify appropriate early-season and late-season indicator plant species or earliest and latest sampling period for your location.

Pan trap transects should consist of 30, 3.25 oz pan traps positioned approximately 5 meters apart along a 150 m transect that bisects or is positioned in or towards the center of a habitat or targeted sampling area. The pan traps should consist of 10 white (unpainted)*****, 10 painted on the inside surface in fluorescent blue non-toxic paint, and 10 painted on the inside surface in fluorescent yellow (see Table 2. Example Budget - Paint) and set out in an alternating color sequence.

***Note:** that the recommended Dixie or Solo cups are usually white in color on the inside surface to begin with.

For long-term sampling periods (*SOP 4: Setting Up Glycol Traps for Long-Term Sampling Periods*), sampling periods run continuously from spring through fall (approximately March-October depending on local climate) deploying a transect of nine 12 oz cups. The array uses 3 cups of 3 different colors on the inside surface, white (unpainted), painted fluorescent blue or fluorescent yellow, with samples being collected at a maximum of once every 2-weeks.

Hand netting is used to supplement pan trap samples which do not effectively target all bee genera. For example, larger bees or those in the family Colletidae (Droege, unpublished data) are often under-represented in pan traps, though this may vary between regions of the country, at different elevations, or due to local conditions (such as availability of food resources or proximity to nesting sites). Hand netting however is difficult to standardize because of the differing abilities of people to use hand nets and detect/see bees (some are better/more experienced than others and may catch more bees or more large bees than small bees for example); and therefore is not a repeatable and reliable technique for most purposes, other than a basic inventory (see *SOP 7: Netting and Non-lethal Techniques*).

Standard Operating Procedure 2: Equipment and Preparation

Field Sampling Equipment

Note: Many of the pieces of equipment described in this SOP are illustrated in photos and diagrams in The Very Handy Bee Manual (Droege 2012).

Pan traps for short sampling periods

White 3.25 oz plastic cups or bee bowls can be used for short (24 hours or less) sampling periods. Solo brand 3.25 oz white cups (model number: p325w-0007) work well. Solo distributors can be located by calling 1-800-FOR-CUPS. The Solo product line catalog is online at www.solocups.com. Purchase enough for 30 cups (10 of each color after painting) per transect.

Keep 1/3 of the pan traps unpainted – white on the inside surface. Paint another 1/3 of the pan traps on the inside surface with fluorescent blue paint. Paint another 1/3 of the pan traps on the inside surface with fluorescent yellow paint. See the 'Paint' section below for more information about paints.

Pan traps for long sampling periods

Twelve ounce (12 oz) stadium cups or inexpensive plastic beer cups can be used for what are referred to in this document as "glycol traps" for long (e.g. two weeks or less) sampling periods and can be purchased in bulk from a variety of sources. Stadium cups are very sturdy and are likely to hold up for more than one season of sampling, whereas other cups may need to be replaced at least once during the field season.

Keep 1/3 of the pan traps unpainted – white on the inside surface. Paint another 1/3 of the pan traps on the inside surface with fluorescent blue paint. Paint another 1/3 of the pan traps on the inside surface with fluorescent yellow paint. See the 'Paint' section below for more information about paints.

Poke or create 4-5 small pin-holes spaced evenly around and a few millimeters below the rim of each cup to allow rain/excess liquid to drain away without overflowing and causing specimens to be lost. If the holes are pin-holes, very small bee specimens should not be lost through them.

Pan trap stands

Pan trap stands can be fashioned from commercially available wire plant-stays that often include a perfectly sized loop at the top (for holding a pan trap or cup by its lip) and are available from home hardware and gardening stores; or from pieces of PVC pipe or plastic electrical conduit attached to a stake. Twelve ounce plastic stadium cups will fit in a stand made from one-half inch PVC pipe or plastic electrical conduit with a hoop cut from a plastic culvert pipe or PVC pipe depending on the size of the cup. The hoop is attached to the PVC pipe with a bolt and lock nut or with a self-tapping screw. The pipe can be slipped over a piece of rebar set into the ground if the ground is rocky, or is simply pounded into the ground if it is not.

If the pipe is white it should be painted a neutral (non-bee attracting) color like grey or black so that the only thing attracting bees is the cup or pan trap itself. The top of the pipe should also be plugged to prevent bees and other organisms from crawling down inside and becoming trapped.

There is a vast collection of research available on the influence and effectiveness of different colored traps and other equipment (e.g. flagging tape) used to conduct wildlife and field-based sampling. This is also true for bees and colored pan traps (e.g. Campbell and Hanula 2007; Gollan and Ascroft 2010; Gonçalves and Oliveira 2013; Josh et al. 2015). Stations may choose to use a single pan trap color in order to target a particular species or group of species known to be attracted to that color, or because they don't want to go to the trouble of maintaining/painting anything other than white pan traps. But an assessment of the effectiveness of different colored traps in a survey can be incorporated into the study design.

Paint

For both short and long-term surveys, the inside surfaces of pan traps and cups are either left white or painted fluorescent blue or fluorescent yellow. To standardize the color used, we recommend ordering paint from Guerra Paint and Pigment (Phone 212-529-0628 or order online at: www.guerrapaint.com/tandc.html) located in the Eastern U.S., and specify Silica Flat, Yellow Fluorescent, and Blue Fluorescent pigments.

You can also purchase pre-painted fluorescent blue or yellow and unpainted white 3.25 ounce soufflé cups from New Horizons Support Services (Phone 301-249-0206 or order online at: www.nhssi.org). Email your queries to Cynthia Swift-King (cking@nhssi.org) or call the number listed above.

The paint can be applied in bulk using a sprayer or for smaller volumes, using simple paint brushes. In both cases try to ensure even coverage of the paint all over the inside surface of the traps. A primer intended for plastic made be needed to get the paint to adhere. Yellow paint usually needs to be repainted once during the season because it fades in the sun more readily than the fluorescent blue paint. Blue paint also has a tendency to peel and may need to be repainted in hot areas such as in Arizona, USA.

Detergent (short sampling duration)

Detergent or dishwashing liquid acts as a surfactant, breaking the surface tension of the water in pan traps used for short (e.g. 24 hours or less) sampling periods. We recommending standardizing by using Dawn Original Scent (blue colored) dishwashing liquid, which is available from most grocery stores in 9oz or larger bottles. Laundry soaps have been tested and do work, but contain so many fragrance chemicals that we fear that changes in formulation could easily affect the capture rate. Some researchers prefer to add a drop of dishwashing liquid to each pan trap but we recommend adding a big squirt of dishwashing liquid directly to a gallon jug of water ahead of time, enough to give the water a very light blue tinge.

Propylene glycol (long sampling duration)

Propylene glycol can be used instead of water in pan traps used for long (e.g. 2 weeks or less) sampling periods and is available for purchase from veterinarian supply houses (mostly online), bulk apothecary (online), automotive and recreational vehicle (RV) centers, swimming pool supply stores, livestock supply stores, and heating and cooling supply stores. Be sure to add a

large squirt of Dawn Original Scent (blue colored) dishwashing liquid to each gallon and gently mix before use. Note that dishwashing liquid is important in this mix or bees will simply land on the propylene glycol's surface tension and fly off.

Heating and cooling suppliers have glycol with a few additives, usually this glycol is only available with a blue dye, but is usually not diluted with water (which evaporates). RV and swimming pool propylene glycol is usually dyed blue or red. The red color can be eliminated by adding a tablespoon or two of household bleach and shaking (The red color may attract non-target organisms – such as hummingbirds). In a couple of hours it will be completely clear. The blue color does not go away even with bleach, but does not affect capture rates. RV and swimming pool propylene glycol is usually diluted heavily with water and thus should either not be diluted again or the water evaporated prior to use by airing the glycol in open buckets in a shed or in a covered area outside. Propylene glycol from heating and cooling supply stores (usually blue) should be full strength/undiluted. Veterinarians use food grade undiluted propylene glycol that is readily available online. It is more expensive, but would be the best option, as it would be the most benign formulation and have less environmental impact if spilled or ingested. You can also order large drums of propylene glycol directly with no added colorants. One common supply company for the basic material is Comstar (www.comstarproducts.com).

Note that outside of fall and winter (and in areas that do not have cold-weather) stores may not stock antifreeze related propylene glycol material and will need to special order it. So, you may need to allow sufficient time for the store to order the product and get it into your hands ahead of your survey season.

CAUTION: Propylene glycol is a safe alternative to regular antifreeze (ethylene glycol). Ethylene glycol is toxic to mammals while propylene glycol is not and is used in prepared foods, skin cleansers, and baby wipes.

To date most people have found propylene glycol not to be of interest to mammals per se (with the possible exception of bears), but anything left outside for long periods of time may eventually attract attention and be chewed on or knocked over.

Trapping liquid container/dispenser

Propylene glycol can often be dispensed directly from the gallon sized container it was purchased in – assuming no additional treatment is necessary (other than adding at least a squirt of blue Dawn dishwashing liquid). Heavy plastic, large handled plastic bottles such as AriZona® Green Tea gallon bottles can also be rinsed and repurposed for soapy water or propylene glycol preparation and dispensing into traps or cups in the field, be sure to label the container. Determine how much liquid will be needed to fill all pan traps or glycol cups before going to the field. You may need about 1 gallon of soapy water to fill 1-2 transects consisting of 30, 3.25 oz pan traps. One 9-glycol trap array will require 108 ounces of propylene glycol.

Trap liquid disposal

Unused soapy water can be disposed of by pouring down the drain. Used soapy water from pan traps can be disposed of by pouring out onto the ground at the time of specimen collection. However, care should be taken to pour soapy water out some distance away from the banks of

streams or other wetland habitats to avoid contamination and negative environmental impacts especially for aquatic and amphibian species.

Unused propylene glycol can be repurposed for vehicle or in building cooling systems. Otherwise it can be diluted and poured down the drain/sewer but not if you are on a septic system. Consult your local hazardous waste disposal regulatory authority.

Global position system (GPS) and/or maps

A GPS and/or maps should be used to determine the latitude and longitude of either the beginning and end, or center (centroid) of a line transect of short-term survey pan traps or a circular array of long-term survey cups. Google and other online map viewers can also be useful for determining latitude and longitude points for survey sites. Always document in the field data the geodetic datum used and whether or not latitude and longitude coordinates represent a centroid or the end(s) of a transect or other sampling or geographic area.

Transect markers

Mark the beginning and end points and/or center of line transects and circular arrays so that the sites can be relocated for trap collection and if repeated sampling is planned. Wired flags or flagging/surveyor's tape can be used, but be mindful of placement of brightly colored flags or tape too close to traps as these may bias sampling by attracting or deterring bees. Avoid using tape that is the same color as the traps (e.g. white, blue, or yellow). Otherwise, wood stakes, rebar or rebar covered with a short capped piece of PVC pipe can also be used as long-term markers for the locations of survey sites. Site markers should be placed at the very beginning of the sampling season on or before the first sampling date.

Specimen filters

For washing/filtering bee specimens from pan traps, it is very important to choose a strainer with extremely fine mesh in order to catch the smallest of bees, some of which may only be 2-4mm. Brine shrimp nets and disposable paint filters (preferably white in color) work the best under most circumstances. Brine shrimp nets can be purchased from most pet stores. A brine shrimp net has finer mesh than a small aquarium net. Some collectors have recently shifted to using disposable cone shaped paint strainers used by commercial painters. The easiest way to find these paint strainers is to search the Internet for "disposable paint strainer" and look at the image results. These filters work well in that they can be taken out in the field, labeled directly in lead pencil (before you wet it), placed in a funnel (for support), and when finished straining, the specimens remain in the filter, which can then be folded, stapled and frozen in alcohol in Ziploc or Whirl-Pak® bags. Coffee filters are another alternative to paint strainers used by several researchers. Tea strainers with very fine mesh can also be found in specialty food stores. They are very durable and easy to use. Alternatively, Figure SOP 2.1 was clipped from the Environmental Protection Agency's Volunteer Monitoring Newsletter (Wilson 2009) and includes instructions for how to create a specialized specimen filter or 'net spoon', which should be handy for removing individual bee specimens from individual pan traps.

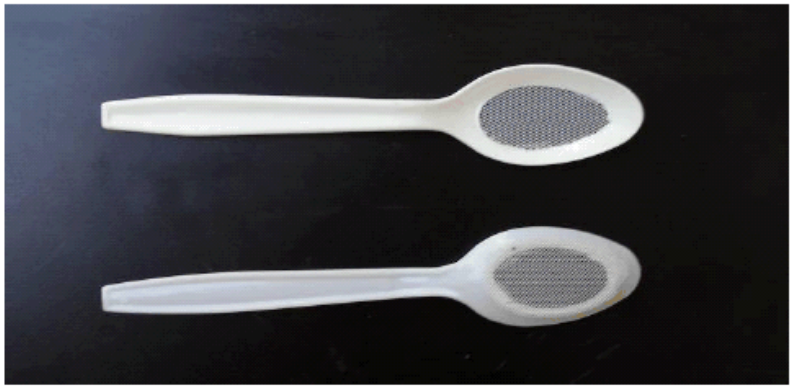
Net Spoon

by David Wilson

Sorting benthic macroinvertebrates from the tray with forceps is a pain, and often results in rather badly mauled specimens. With a net spoon, it's much easier to catch the critters and they are virtually never damaged.

To make a net spoon, use an electric grinder to grind off the bottom of a plastic picnic spoon, leaving only the rim. Be sure to wear safety glasses while grinding. Use waterproof cement to glue a small piece of fine mesh netting to the back of the rim. After the cement has dried, trim excess netting with small sharp scissors.

DAVID WILSON



David Wilson is a volunteer monitor with the Huron River Watershed Council in Ann Arbor, Michigan.

Figure SOP 2.1. "Net spoon" for removing bee specimens from traps/bowls (Wilson 2009).

Plastic spoons

Use a white plastic spoon to remove wet bee specimens from traps, shrimp nets or filters. These are easily obtained in boxes of 25 or more at most grocery stores.

Ziploc or Nasco Whirl-Pak bags

Ziploc or Nasco Whirl-Pak bags are used for short to mid-term (less than a year) storage of bee specimens immersed in alcohol or propylene glycol. Nasco Whirl-Pak bags are preferable because Ziploc bags tend to leak. Whirl-Pak bags are readily available from Nasco (www.enasco.com/Whirl-Pak) and a variety of online scientific equipment suppliers. Ziploc bags can be purchased in boxes of 25 or more from most grocery stores. Bag size will depend on your intended/expected sample size. In general 4 or 7 oz Whirl-Pak bags may be a good size/volume to start with for short-term samples; larger size/volume Whirl-Paks may be necessary for collecting samples from long-term traps. Sandwich or smaller sized Ziploc bags will also suffice but may be prone to incomplete seals and can be harder to open with wet fingers. Combine Whirl-Paks for each trap array with a binder clip before going into the field to save time and be assured that adequate and properly labelled Whirl-Paks are available at each site.

To transfer bees from the trap to the Whirl-Paks use an oil funnel purchased from an auto parts store. The bottom opening is wide enough to allow samples to be quickly poured into the Whirl-Pak without clogging the funnel or losing specimens.

Wet labels

Each bag of bee specimens should contain a tag or label inside listing the sampling site, date and other information written on paper or heavy card stock (i.e. index cards) with a dark lead pencil (do not use ink of any kind as it will dissolve in the preserving solution). Do not trust any kind of writing to stay on the outside of a bag as they inevitably get wet with alcohol or water and any ink-based writing will again dissolve or run, quickly becoming illegible. Figure SOP 2.2 gives an example and a blank printable label to be filled in using a dark lead pencil and inserted into bags with specimens while in the field and for storing specimens for 2 months or less. Formal labels like this can help ensure that station staff and volunteers include all of the required information on the label at the time the sample is collected (rather than later when memories fade). But less formal sample labels can also just be made out of a piece of paper torn out of a notebook (this assumes that the sample collector remembers to include all of the required information on the label). To save time in the field prepared labels can be placed beforehand in the appropriate Whirl-Paks.

BANSHEE REEKS NATURE PRESERVE (BRNP) **BEE INVENTORY** **Date**: SEPT, 10 2013 **Site #**: 1 **T1**: 8:50 am **T2**: 5:30 pm **Name**: Dave McCarthy **High Temp (ºF)**: 98 ºF (of the day) **Weather**: sunny, humid, light breeze, thunderstorm at 3:30pm **Plants**: Swamp milkweed, Mullein, Red clover (**write more on other side*) **# Bowls collected**: 29 / 30	**BANSHEE REEKS NATURE PRESERVE (BRNP)** **BEE INVENTORY** **Date**: ____, ___ 2013 **Site #**: _____ **T1**: __ : __ am **T2**: __ : __ pm **Name**: _____ **High Temp (ºF)**: ____ ºF (of the day) **Weather**: _____ _____ **Plants**: _____ _____ **# Bowls collected**: ___ / 30

Figure SOP 2.2. Sample pre-formatted 'wet label'.

To be filled in with DARK PENCIL and inserted into bags with bee specimens while in the field. (T1 = pan trap placement time; T2 = pan trap/sample collection time; Name = Name of Collector; Weather = ambient conditions on sampling day; Plants = plants in flower along the transect at the time of sample collection; # Bowls collected = # pan traps collected out of the total number originally placed. 'NET' can also be entered here to indicate a net-collected sample).

Field notebooks and data sheets

The same data recorded on the 'wet label' that's placed inside the bag with the specimens must be recorded (duplicated) in a field notebook or on separate data sheets. Field notebooks and/or data sheets provide a separate and original written record of the samples' data and sampling effort even after the specimens, their 'wet label', and their associated data have been processed, digitized, and/or distributed to other staff/locations for processing, storage or further study. Information recorded in field notebooks can help reconcile sampling effort/events with samples and associated data, helping to keep track of samples and their status. Field notebooks

(preferably with water-resistant paper such as Rite in the Rain®) can also be used to record additional notes about site conditions observed at the time of sampling and allow you to follow up with collectors in case there are questions. It is also advisable to photocopy field notes and keep them in a separate notebook or file. See *SOP 8: Data Collection and Management* for appropriate data sheets to use with this survey protocol.

Specimen (emersion) storage solutions

Propylene glycol, isopropyl, ethyl, or denatured alcohols are all appropriate for storing insects, but isopropyl alcohol should never be mixed with the other alcohols or it will cloud up. Pint bottles of ethyl alcohol, ethanol, or denatured alcohol (be aware that alcohol names are not consistent) can almost always be found at pharmacies or in grocery store toiletry aisles. If not readily available in the store, it is possible to have the pharmacy order what you want. Hardware stores carry gallon and pint size cans of denatured alcohol. Drug store denatured alcohol is easier to work with, as it is made with a smaller amount of methanol. Isopropyl alcohol is the least preferred as it hardens specimens more than the others and reduces their usefulness in DNA studies.

Often alcohol needs to be diluted to achieve the right percentage (70%). All hardware store alcohol should be considered to be 95% alcohol. Drug store alcohol can be close to 100%, but usually is something less. You will have to read the bottle's label to check. Note that most cheap dollar type stores sell isopropyl that is only 50% alcohol. To add confusion to the matter, drugstores often label the percent alcohol in terms of "proof." Proof is a simple doubling of the percentage. Therefore, 100 proof is 50% alcohol and 190 proof is 95% alcohol. To dilute from 100% alcohol to 70%, choose a convenient sized container, such as a pint bottle, then fill it ~70% full with alcohol and the rest with tap water. This measurement doesn't need to be exact. Use of above 90% alcohol for specimen storage is recommended for DNA preservation (if specimens will be used for DNA analyses) but it is not necessarily ideal for specimen processing. Specimen storage in EtOH of about70% is better for specimens that are to be processed within the short term (present to about 1 year). Specimens stored in 90% EtOH or higher concentrations can become brittle.

Individual Whirl-Paks of collected insects should be triple bagged in Ziploc bags and stored in a freezer until it is time to mail or process.

Insect nets

Almost any type of insect net will catch bees. However, bee collectors do have preferences. Most people now use aluminum rather than wood handled nets. Some prefer the flexible strap metal netting hoops for the net heads, as they work well when slapping nets against the ground to capture low flying or ground resting bees. Others prefer the more traditional solid wire hoops. Hoop size varies from about 12" to 18." The larger the hoop, the greater the area of capture, however larger hoops are more difficult to swing quickly due to air resistance and there is more netting to snag on branches.

Some manufacturers make a net that is very portable for travel or backpacking; these nets may be useful in sampling areas with rough terrain and accessibility issues that require collector's to backpack into sites. The pole disconnects into 3 small sections and the hoop can be folded into itself. Additional sections can be added to reach into out of the way places. Telescoping poles are

also available but must be treated with care or their locking mechanisms will jam. An inexpensive long pole can be rigged by attaching a net hoop to a section of bamboo with hose clamps. Aerial nets, rather than beating or sweep nets, are normally used. More critical than the structure of the net is how fine is the mesh of the aerial net beg. A fine mesh or muslin net bag rather than the traditional medium to large gauge aerial net bag can keep the smallest bees (such as those in the genus *Perdita*) from escaping.

Insect kill jars

Bees collected from hand nets are usually killed using an insect kill jar - a clear lidded container with a small amount of ether, ethyl acetate, alcohol, soapy water, or prepared cyanide inside.

CAUTION: Use extreme caution if using cyanide, wrap glass jars in tape to prevent breaking.

Bees are usually transferred directly from the inside surface of an insect net to the kill jar opening. So a clear container whose lid is easy to remove and replace works best. Any small clear container such as a plastic film canister, medium to large centrifuge bottle, pharmacy pill bottle, or specimen jar with a snap on or half to ¾ turn screw-on lid; or corked glass or plastic test tubes will work. Glass containers must be used if ethyl acetate is used as a killing agent; plastic containers will be damaged by this chemical. Clear containers allow you to confirm that you've successfully transferred bee specimens from the net to the container. Ethyl acetate which has historically been used in kill jars denatures the DNA from specimens and is therefore less desirable.

Lab Equipment for Specimen Preparation and Identification

Tools for washing specimens

A 4" diameter fine mesh metal tea strainer works best for rinsing and transferring bees from sample bags to washing jars. Brine shrimp nets also have sufficiently fine mesh for rinsing bees, but it is more difficult to transfer the specimens from the net into a jar because of the flexibility of the netting.

Glass pint or half-pint sized canning jars (e.g. Ball brand) with two-piece lids (a threaded part and a removable central metal disk) make excellent 'bee washing' jars. Cut out a piece of fiberglass window insect screen mesh with the same diameter as the lid and place it under the cap section that screws on to the jar. Note that you can buy loose fiberglass screen from the hardware store and cut it with scissors. The other, flat circular piece of the canning jar cap will be used to cover the exposed section of insect screen during bee washing.

Other clear plastic or glass containers with a lid, punctured to let air and soap bubbles out, will also work.

See *SOP 5: Storing, Shipping and Pinning Collected Bee Specimens* for more information about these specimen washing methods.

Tools for drying specimens

A small hand towel or paper towels will be needed for soaking up excess water during the washing and drying process. A folded cloth towel is more environmentally friendly than using a

lot of paper towels and provides padding for glass 'bee washing' jars. Note that paper towels can also be reused many times.

A handheld hair dryer can be used to dry specimens prior to pinning and can be obtained from a variety of online sources and from most drugstores. An air compressor can also be used for larger scale specimen processing operations. See *SOP 5: Storing, Shipping and Pinning Collected Bee Specimens* for more information about these specimen drying methods.

Autobeedryer: If you are involved in collecting and processing many specimens, you may want to invest in the creation of an autobeedryer. A slideshow and video that demonstrate how to make such a device can be seen at: www.slideshare.net/sdroege/how-to-create-an-autobeedryer and www.youtube.com/watch?v=935jlJep6go.

Stereo or dissecting microscope (stereoscope)

When using bowls or nets, it is easy to quickly amass a large collection of bee specimens. Unfortunately, unlike most butterflies, bees (even the bumble bees) need to be viewed under a stereo or dissecting microscope to see the small features that differentiate among the species. If you plan to attempt to ID specimens yourself, you will need a microscope. While even inexpensive microscopes and lights can be of some use, in the long run they lead to frustration. Inexpensive microscopes usually have poor optics, very low power, small fields of view, difficult to set or fixed heights, and their stands are usually lightweight and often designed in such a way that makes specimens difficult to manipulate.

Unfortunately, a good microscope is not cheap. Our experience is that an adequate microscope costs over $1000, and good ones run over $2000. That said, microscopes with even moderate care can be seen as a one-time investment. Additionally, because a good microscope has optics that can be adjusted and cleaned (unlike most inexpensive ones), it is usually safe to buy a used or reconditioned microscope from an online dealer (buying off of E-Bay or Craig's List is more risky as the seller has less of a reputation to risk). There are many reputable used microscope sites; we have purchased microscopes from several of them, and have never had a bad experience. In two cases, the purchased microscopes had a problem, and in both cases, they were repaired for free. Usually, used prices are about half the cost of new.

Good stereoscope brands to consider that we have experience with include Leica, Zeiss, Olympus, Wild, Wild-Heerbrug, Nikon, and Meiji. BIML can supply you with some model numbers from their collection, or you can send BIML links to the Web sites that are selling microscopes you are considering. BIML will be glad to give you their impressions. Of special consideration are the Bausch and Lomb StereoZoom series. These microscopes have been around for years, and often form the core of college biology and entomology department teaching labs. These are adequate to good scopes and we have about 5 in our lab. They are readily available used from $500 -$900 online. Their negatives include a view that is not as good as the better scopes and the zoom magnification is on the top, rather than on the side. Finally, be aware that many of these scopes only go up to 30X power with the standard 10X oculars, though higher powered models exist and higher power replacement oculars are readily available.

What follows is a list of Microscopes recommended by other Bee Researchers and amateurs. They range from high end to low in no particular order.

Zeiss Stemi DV4 - about $2000
Leica EZ 4 - $1150 to $820 (several people responded that they use this line)
Omano Stereoscope OM9949 - <$1,000
Bausch and Lomb Stereo Zoom 5 - $150 used (these are the standard college student scopes of the past)
Leica 2000 - $850
Leica S6E - $1100
Leica S8 APO - $3400
Wild M8 - $1500 used
Wild M3Z - $1500 used
Olympus SZX12
Olympus SZ60 zoom
Olympus SZ61 with an aftermarket ring-light. $2,000-$2,400 range
Meiji EMZ-5TR body - $2000 10 years ago
Leica MZ12.5 - $6,000 - 8,000
Olympus SZX16 - $6,000 - 8,000

Magnification power needs some mention here. Any adequate to good scope will have variable power settings. We have never seen any instance where the lowest magnification was an issue, but a useful scope should go up to about 60X power or higher, something that many good scopes do not achieve with the standard 10X ocular. If the scope does not go to that high a power, it is a simple matter to change the magnification by purchasing a higher power set of ocular pieces (these are the eyepieces that you look into). Oculars simply slide into tubes on top of the scope and are readily removed (as some of you who have turned a microscope upside down have found out). However, sometimes there is a set screw that needs to be released first. That said, replacement oculars, while almost always available for every model and brand, can be expensive to purchase. Magnification is determined by multiplying the magnification of the ocular lens (this number is listed usually on the side of each ocular piece, but sometimes is found on the top, and is most commonly 10X) by the zoom or magnification level which is listed on the zoom knob. Note that some manufacturers list the zoom levels multiplied out with the assumption that you are using 10X oculars.

Most higher-end microscopes come with a zoom magnification where all powers are available in any increment. In some scopes, powers are available only in steps. Scopes that have the magnification/zoom feature available on the sides of the scope in the form of a small knob are the easiest and quickest to use. The ones with the knob on top or located as a movable ring around the base of the scope head take more time to change. The magnification often needs to be changed several times when viewing and identifying a bee specimen.

Some microscopes come with a measuring reticule in one of the oculars, but most do not. A measuring reticule is a very small ruler etched into a piece of glass. These are useful for taking precise measurements or, more often the case, taking relative measurements. This piece of glass is inserted into the bottom side of one ocular. All or almost all oculars are built in a way that they can be taken apart for cleaning. Often there is a threaded tube inside the body of the ocular that holds the lenses in place. If taking one apart, be gentle as the threads can be delicate. Measuring reticules can be ordered online, or some microscope dealers will custom-make one for you.

Holding specimens and general microscope setup

Most people when viewing specimens under the microscope, place them on a piece of clay, foam, cork, or some sort of stand. The USGS BIML has found that it is far faster to view specimens when held in the hands of the observer, although be very careful not to drop and damage the specimen. To hold pinned specimens, pick up the head of the pin using the thumb and forefinger of your dominant hand. This allows you to easily spin the specimen around the axis of the pin. The point of the pin is then either lightly pressed against the middle or forefinger of the other hand, or held between the thumb and forefinger.

The final part of microscope setup is to adjust your chair or the table holding the microscope such that you do not have to bend or strain your body to look into the microscope.

Small hand tools

Tweezers can be handy for teasing apart tangled bees and for positioning them for pinning. A variety of styles and sizes of tweezers can be purchased from hobby, arts and craft, and sewing stores, and from the toiletry aisle of most grocery stores. Feather-weight forceps provide a very light pressure grip work well when handling specimens. They allow a firm but gentle grip that lessens the threat of crushing or breaking a specimen. Reverse clamping tweezers may apply slightly less pressure on specimens than regular tweezers and may be more ergonomic/cause less finger-fatigue as they only require pressure to open them rather than close them and keep them closed around a specimen. A small or fine paint brush is also handy for sorting bees and for picking up very small bees without crushing them or breaking legs. Dissecting needles can also be useful for teasing apart tangled bees or for more meticulous pinners who prefer to reposition or arrange the legs of specimens before they are completely dry and brittle.

Insect pins

Bees are usually pinned using pin sizes 1-3, with size 2 being the most common. Pin size 1 is prone to bending when pressed into traditional hardboard lined trays and boxes, but does nicely in foam units. Pin sizes below 1 should not be used as they are delicate, do not hold labels well, and end up bending if the specimen is moved or viewed often. Size 4 is generally too large for anything other than bumble bees. Large pins do provide a larger surface area for glue to adhere to if specimens are being mounted by that means. In humid environments, stainless steel pins should be used to prevent rusting. Student pins should be avoided as they are cheaply made; the tips bend and the balls or end-caps come off. Insect pins can be expensive. The cheapest way to purchase them is to order in bulk directly from Czechoslovakia, where apparently most are made. Some newer inexpensive (same price as European steel pins) stainless steel pins are now available from China that appear to be of high quality.

Some people prefer to keep their pins ready-at-hand in a purchased or home-made insect pin holder or magnetic wrist-wrap (e.g. Magnogrip Magnetic Wristband) while others prefer to simply poke a bunch of pins into their pinning board and select from them as needed. The original box the pins came in or an old film canister may also be useful for holding the pins while in use or for storing them.

Pinning block

For someone new to pinning, training is very important (see ‘Pinning Specimens’ later in this SOP) and use of a purchased insect pinning block is helpful to determine the correct height a

specimen or insect pinning point should be placed. With experience, one can use pieces of foam of the correct depth, or even adjust specimen height by eye, which will be the quickest. Insect pinning blocks can be purchased from most scientific or biological equipment suppliers such as BioQuip (www.bioquip.com) and Carolina Biological (www.carolina.com).

(Insect pinning) points

The use of points is traditional – especially for pinning very small insects or bees. Points are very small, acute triangles that can be purchased or cut from stiff paper using a special punch (a 'point punch'), which can be ordered from entomological supply houses such as BioQuip (www.bioquip.com/).

Specimen glue

Reversible or water soluble glues, such as Elmer's Glue Gel, white glues, tacky glue, clear nail polish, shellac, hide glue, and others can be used to glue smaller specimens to pins or points. The USGS Bee Inventory and Monitoring Lab (BIML) has used white, tacky glue in the past. This is a thick glue which sets within seconds. It allows the glued specimen to be set upright in a box immediately, without the danger of it falling off or sliding down the pin. From our limited investigations, Aleene's Original Tacky Glue in the gold bottle or archival paper glue appears to be the best gripping, tacky glue. If regular white or Elmer's glue is used it is advised to allow exposed glue to set for a few minutes to get tacky. Bees adhere better and quicker to pins if "tacky" glue is used rather than fresh glue.

BIML now uses glue gels when gluing smaller bees to pins. Glue gels have a longer work time, dry crystal clear and are easily reversible. Because the set-up time is longer than tacky glue, leave the pin laying down flat, resting on the specimen for at least 5-10 minutes prior to picking it up.

Parchment paper

Parchment paper can be purchased from the baking aisle of most grocery stores and is very helpful to have around when gluing bees. It is a silicone or wax impregnated paper that can withstand the heat of an oven. It provides a "non-stick, Teflon-like" substrate on which to work, because glue does not adhere well to it. Another nice thing about parchment paper is that dried specimens can be easily sorted and positioned on it. They will slide around without sticking, catching or breaking. Tip or pour dried specimens onto the paper and pull up the sides, causing the specimens to slide into the center. Once in the center, they can be arranged in a line which makes pinning even more rapid. At this point, you can pin the paper to the top of a large foam pinning board.

Pinning board

Pinning boards can make pinning insect specimens easier by providing a soft, springy surface that can save your fingers by accommodating multiple, repeated pin pricks without damaging pins (unlike tables and counter-tops). Expanded polyethylene foam (often referred to as Ethafoam) or cross-linked polyethylene foam (our preferred foam) is better than polystyrene foam (usually referred to as Styrofoam) for pinning purposes. Styrofoam is not supportive enough; both labels and specimens will bend too much when pinned upon Styrofoam. The foam can be cut to your preferred size and glued (using carpenter's glue) to a piece of cardboard or corrugated plastic board (like that used for roadside political signs) to create a longer lasting

pinning surface. You may even want to cut an oval-shaped hole as a handle in one side/end of your pinning board to make it easier to carry. To increase the durability of Ethafoam, glue it to a piece of plywood, which will form a sturdy pinning surface. To manufacture a pinning board, smear white or wood glue across both surfaces, rub together and then place another (unglued) board on top of the foam. Pile books or other heavy objects on that board to clamp the foam and board tightly together. Let dry overnight. It can then be used as is, or the edges can be trimmed with a saw for a nice and tidy look.

Specimen labels

Paper: In a good museum cabinet, specimens deteriorate only very slowly and can last for well over 100 years. That is not true of the paper used in making labels. Paper that is not archival or acid free gradually deteriorates. Fortunately, archival paper is readily available in office supply stores. A heavier weight paper is also important to use so that the label stands up to handling and the pinning process. A 35-65 pound acid-free/archival quality paper is good label stock.

Label Production: BIML uses a label generating program developed by Discover Life (www.discoverlife.org/label).

Dan Kjar has generalized the Discover Life labeling program so that it will print out on a laser printer. You can use his simple Web based form (bio2.elmira.edu/cgi-bin/fieldbiolabel.pl) to generate insect labels. Each label is unique based on the specimen number. Depending on how many labels you are making and your Internet speed, it will take a little time to build the label page. Fifty labels take about 1 minute to assemble. The system will be integrated into Discover Life soon and when that occurs Dan will announce that on his Web site.

Gretchen LeBuhn (San Francisco State University, Department of Biology; Director of the Great Sunflower Project - http://www.greatsunflower.org) has also developed a system for making labels in Word, which is explained beginning on page 12 of The Handy Bee Manual (ftp://ftpext.usgs.gov/pub/er/md/laurel/Droege/Handy Bee Manual.pdf).

Specimen boxes and storage

Pinned insect specimens must be stored in a clean, dry, pest-free environment and checked periodically for damage/deterioration. There are a variety of drawers, cabinets, and boxes available to hold specimens. The BIML prefers to use a simple cardboard specimen box (like a 12” x 12” pizza box blank) with a completely detachable lid, and an Ethafoam glued to the bottom for everything, except for housing our synoptic collections. These boxes are stackable, the date and location can be written on the outside in pencil and then erased when reused, are relatively inexpensive, and, unlike hinged lid boxes, are convenient to use in cramped spaces on a desk or worktable. Such boxes can be purchased from: www.uline.com/BL_8682/Pizza-Boxes?keywords=pizza.

Pinned specimens in boxes can be stored inside clear or white plastic kitchen trash bags and/or large Ziploc bags as long as each box contains a pest deterrent and is checked regularly for any signs of deterioration. Note that you should let the specimens dry out thoroughly after pinning (one month or so) before enclosing them in a plastic bag. For more permanent, longer term storage or display, there are numerous sources of museum quality cabinets, drawers and specimen boxes available from a variety of suppliers. Alana Taylor-Pindar has alerted us to an

inexpensive source for quality cabinets and drawers for your collection at: www.quebecinsectes.com/pages/pages_english/macrodontia_english.html in addition to www.bioquip.com.

Instructions for "How to Make a Pizza Insect Pinning Box" are also available beginning on page 36 of The Handy Bee Manual (ftp://ftpext.usgs.gov/pub/er/md/laurel/Droege/Handy Bee Manual.pdf).

Specimen care and pest control

Simple cardboard boxes are not pest proof. Mice, insects, and mold may damage specimen collections that are not properly protected and stored. Dermestid beetles are the primary pest of insect collections. Fortunately, infestations are usually small, perhaps seeing one beetle larvae in a box scattered here and there. An infected specimen is usually easy to spot, as small black droppings and shed skin are visible below the specimen. Control and prevention take place, according to the literature, by freezing the box at -20° C (~0° F) for 3 days, thawing for a day and then freezing for another 3. In a pinch, kitchen freezers appear to work too. Spring is a good time to freeze your entire collection, as that is when dermestids appear to be most active. An excellent means of keeping your collection pest free (particularly if using cardboard boxes) is to keep each box in a large Ziploc bag (2 gallon bags fit most boxes). Note that you should let the specimens dry out thoroughly after pinning (one month or so) before enclosing them in a plastic bag. Mothballs and pest strips can be effective, but carry some apparent health risks with long-term exposure. Mothballs can be purchased from hardware stores and some grocery stores and Hot-Shot No-Pest strips and other fumigants can be purchased from BioQuip (www.bioquip.com). Hot-Shot No-Pest strips can be cut into about 6 smaller pieces that can be easily wedged in between the foam base and side of specimen boxes by hand. But wear gloves and/or wash hands thoroughly after handling these and other fumigant products and do not store specimens treated with No-Pest strips in rooms regularly occupied by people or animals for periods longer than 2 to 3 hours.

In humid conditions (such as July and August in Maryland), unprotected specimens, particularly those just caught, can easily become moldy. Either store them in an air-conditioned space or put them in plastic bags or tightly closed bins that contain active desiccants. Specimen cabinets are recommended for long-term storage. A specimen curation plan should be developed to ensure long-term care of a collection if it is not being deposited in an existing facility such as a museum.

Standard Operating Procedure 3: Setting Up Bee Bowls for Short-Term Sampling Periods

Over the past decade, pan traps (Kirk 1984, Leong and Thorp 1999, Cane et al. 2000, Toler et al. 2005, Wilson et al. 2008) have become widely used to sample bees. Recently, Westphal et al. (2008) concluded that pan traps were the most efficient, unbiased, and cost-effective of six tested methods for sampling bee diversity. Pan traps are also apt tools for the sampling of bees as they are inexpensive, easily obtained/readily available, need not be deployed by someone with entomological training, and capture most of the bee species present in a community. In general, small bees are sampled well in pan traps, but larger bees often need to be netted.

Pan traps are small traditionally white, blue or yellow colored plastic bowls or cups that are filled with soapy water (water plus dish liquid). Bees are attracted to the colors, fly into the water, and drown. Originally meat trays (pan traps) and 12 oz salad bowls were used. Field experience and experiments have demonstrated that the size of pan traps is not necessarily correlated with capture rates (see online.sfsu.edu/~beeplot for several reports that document those results).

A pan trap is set when it is filled with soapy water or propylene glycol and left outside. The soap (dish liquid) decreases the surface tension of the water, permitting even small insects to sink beneath the surface. Most insects stop moving (drown) within 60 seconds of becoming immersed in the soapy water. However, if insects are removed from traps right away after being captured, some insects will begin to "wake-up". Thus we recommend that specimens either be stored and/or frozen in alcohol in sampling bags for 24 hours prior to processing.

The amount of soapy water in a pan trap does not affect the capture probability. However, in hot and arid climates, pan traps can dry out, if not completely filled with soapy water, or if the pan is too shallow. We suggest that people standardize the soap by using blue-colored Dawn Original Dishwashing liquid. It is readily available and fragrance free and appears to function as a surfactant similar to other brands. Be aware that citrus-scented detergents when mixed with water will decrease the bee catch compared to other detergents. The addition of salts, floral oils, sugars, honey, and other compounds to pan trap water has been found to either not affect capture rates or decrease them compared to pan traps using blue-colored Dawn Original dishwashing liquid (unpublished USGS BIML data). While some bee collectors add detergent directly to each trap, it is easiest to add a big squirt of dishwashing liquid directly to a gallon jug of water (such that the water has a slight blue tinge) and pour it from there into the traps. A gallon jug (e.g. such as a clear Arizona Green Tea jug) of water provides enough liquid to fill at least 30, 3.25 oz pan traps to a depth of 0.75 inches.

A mixture of propylene glycol and Dawn dishwashing liquid can also be used as a trapping medium and has the advantage of being a preservative, completely reusable, readily available, and has an extremely low evaporation rate. It has the disadvantage of being relatively expensive, compared to water. Note that propylene glycol is not the same as ethylene glycol, which is used in car antifreeze solutions. Propylene glycol is an edible liquid and is used in the food industry to make processed foods (like granola bars) chewy and moist.

While conducting experiments in Ontario, Matthew Somers found no significant difference in the number of bees captured between yellow pan traps filled with soapy water versus those filled with propylene glycol (pers. comm. unpublished results). Interestingly, he found that about 33% of the bees that landed in either fluid would escape, and that rate apparently varies with species. He also noted that a high proportion of insects were attracted to the bowls, but either only flew low over them or simply landed on the rim.

If you have problems with vertebrates being attracted to or disturbing your propylene glycol traps, add quinine sulfate (a common fish medication sold in pet supply stores) in the glycol traps to prevent animal disturbance. You can also pin down a dome of chicken wire over your traps to prevent things like raccoons and foxes from accessing or disturbing them.

CAUTION: Propylene glycol is a safe alternative to regular antifreeze (ethylene glycol), which is attractive to animals, but toxic.

Good pan trapping conditions

Sunny days are best when setting out pan traps for bees. Bee bowls can be left out for the middle part of the day (i.e. 9 AM to 5 PM) or for any 24 hour period (beware that water will evaporate from the bowls in hot/windy climates within 24 hours). The effect of temperature is often unclear, but catch appears to be reduced in the spring if day time temperatures are 50^{o}F, or below. In the fall, temperature seems to have less impact on pan trap capture rates. Pan traps catch fewer bees on completely overcast, very windy, or very foggy days, and almost never catch bees on rainy days.

Where to set a pan trap transect or array

The best places to put pan traps are exposed open settings where bees are likely to see them (e.g., fields, roadsides, grassy areas, barrens, scalds, sand). Avoid putting bee bowls in any heavy shade as few to no bees will come to those bowls. This is true of the shade under trees or the shade of thick grass or herbaceous vegetation. In North America, this also extends to deciduous woodlands prior to leaf out. Within any of these habitats, pan traps left under any dense vegetation (e.g., thick cool season grasses, leafy shrubs) will catch few bees. The general rule of thumb is to not place any traps in the shade. Open warm season grasslands often have good capture rates of bees if the grass canopy is not too thick. If you can easily see the pan trap, then bees can too. Flowers need not be apparent in an area in order for bee catches to be quite high in pan traps. However, the presence of a superabundant nectar and pollen source (e.g., creosote bush, mesquite, a field of blooming mustard) often appears to lead to low pan trap capture rates. All that said, it has been the experience of many that small openings, rabbit paths, trails, open tree canopies etc. can be places where you will find bees, so experiment even if the habitat is not completely open. Beware that placing pan traps on trails frequented by rabbits, deer and other wildlife can lead to destruction or loss of the pan trap, liquid, and specimens.

Pan traps seem to capture bees effectively in open habitats around the world (e.g., Fiji, Taiwan, Thailand, South Africa, Central America, and South America). The bycatch in pan traps can be very interesting, and include parasitic hymenoptera, sphecids, vespids, skippers, thrips, flys, leaf-hoppers, crickets, spiders, and other things that often come to flowers.

Most researchers put multiple pan traps out in strings or transects rather than as single traps. Capture rate per unit of trap field time is much higher this way. Once a location has been chosen in which to place pan traps, it takes relatively little additional time to place many pan traps as compared to just one, particularly when compared to the cost of traveling to a new place. An internal study available from Sam Droege (sdroege@usgs.gov) indicated that the variances for characterizing the species richness of a single site may level out around 15 to 30 bowls (Droege 2015). A minimum of 24 bowls (8 of each color) is recommended (Table 1).

Pan traps placed immediately adjacent to one another have been shown to have reduced individual per/trap capture rates. Studies in Maryland using three separate trapping webs in open fields showed a distance of 3 to 4 meters to be the threshold below which bowls competed with one another for capture. They did not compete above that level (e.g. at 5 meters distance from each other). Therefore a transect consisting of 30 bowls, spaced approximately 5 meters apart (as measured out by a person's stride) is effective. Without infringing on the 5 meter spacing rule, pan trap transects can also meander around vegetation and other obstacles and don't need to be laid out in a strictly straight line. Traps of different colors should be alternated along the transect such that no two traps of the same color are next to each other (e.g. blue, white, yellow, blue, white, yellow, and so on).

Elevated pan trap placement

In addition to ground placement, pan traps can also be set into stands to elevate them off the ground where terrain (e.g. shifting sand on dunes) or thick vegetation prevent stable positioning or obscure pan traps from sight. Traps can also be placed into stands for long term survey efforts. The importance of placing traps at the flower level has been demonstrated by Tuell and Issacs (2009) and by Wilson, Griswold and Messinger (2008).

Set the hoop or cup-holding loop high enough so the cup rests at ground or the desired level or if in the desert slightly off the ground so that the cup doesn't absorb the heat of the surface of the earth. If desired, a trap number can be written on the white plastic hoop.

You can see how to make these pan trap stands online at: youtube/x87CXM7mq54 and read a pilot report on using these stands in long-term monitoring at: ftp://ftpext.usgs.gov/pub/er/md/laurel/Droege/Draft USFS Glycol Report22711.docx

Collecting bee specimens from pan traps

Remove all moths, butterflies, skippers, slugs, and very large bodied non-hymenoptera (e.g., grasshoppers and crickets) from each pan trap first. These groups tend to contaminate the bee specimens with wing scales and bodily exudates when placed in alcohol. Be careful to check for any bees that might be attached before removing these larger insects from the sample. All the other insects are fine to leave in as they will be sorted in the lab. Following the removal of unwanted non-target organisms, the remaining bee specimens and soapy water can be poured out from the bowl into a (preferably white) brine shrimp net, sieve, disposable paint filter, or tea strainer (see *SOP 2: Equipment and Preparation*). Non-target organisms removed from pan traps may also be pinned and documented if the station or survey design requires this. This protocol however will not cover preparation of non-target specimens.

Pool all of the specimens from all of the pan traps in one transect or plot into an individual Ziploc or Whirl-Pak bag rather than keeping individual trap data separate. Handling time increases greatly when collecting and documenting separate samples from individual pan traps. If you have time you may wish to use a separate squeeze-bottle of alcohol to wash the soapy water from the specimens while they are in the brine shrimp net or filter before or as you transfer them to the Ziploc or Whirl-Pak bag. When using pan traps filled with propylene glycol, alcohol can be used to store the specimens.

Use a plastic spoon or your fingers to gather the specimens from the brine shrimp net and then transfer them to the Ziploc or Whirl-Pak bags. If you are concerned or prone to drop specimens when transferring them to the bag, try doing this over a clean light colored or white surface (a sheet of 8.5 x 11" paper or a handkerchief laid on the ground works well) in order to avoid losing tiny bees in the vegetation and dirt if you accidentally drop them.

Create a 'wet label' for each bag/sample of specimens and place it inside the bag with the specimens (see *SOP 2: Equipment and Preparation*). This label should stay with the specimens while in storage, through processing and up until individual specimen labels are applied to the pinned specimens. Pour a small amount of 70% alcohol or propylene glycol into the bag with the specimens, so that it only just covers the specimens. Then try to eliminate as much of the air from the bag as possible by squeezing the sides of the bag together with your fingers and pushing air pockets up and out of the bag opening. Roll the top of the Whirl-Pak bag down so that the specimens are covered with liquid but loosely gathered in the bottom of the bag and twist the wire ends together with multiple twists (DO NOT just fold them around the sides of the bag like you would with a bag of coffee beans). Fold the twist ends towards the inside of the loop they make when twisted together, so that the ends of the wires won't poke holes in other sample bags. Place multiple sample bags into a larger Ziploc bag with a piece of, or folded up paper towel (to keep leaks in check) for temporary storage in the field, and later storage in the refrigerator or freezer. If the specimens are not going to be processed or shipped immediately or within a few days of collection, they may be stored in this condition in the refrigerator or freezer.

Field operation efficiency tips

It is helpful to create or compile your pan traps into sets for each intended transect or sampling site the day before setting them out. An empty, divided seedling flat like those found holding plant seedlings at your local nursery can be used to hold the separate sets of pan traps quite nicely. If you only have 2-4 sets of pan traps, you can also use a cardboard Starbucks® 4-cup holder to hold each stack of pan traps. Wire flags (very useful for relocating your transects from a moving vehicle) can be set in an external pocket or umbrella holder of your backpack, or the passenger foot well of your vehicle for easy access. If driving between sites using a 4-door vehicle, it is fastest to keep the container of soapy water on the back seat or on the floor of the back seat behind the driver (with the lid on!). While getting out, drivers can grab a set of pan traps and a flag in their right hand, open the door with their left hand, get out of the car, turn and grab the jug through the back window and then move quickly to the sample site to put out the traps. Two people can also tag-team with the first person placing the traps on the ground or in elevated stands while the second person follows along behind, pouring the soapy water or propylene glycol into the traps. By collecting GPS points (at each end, or a centroid) for your transects as you put out bowls you can use the GOTO feature of your GPS unit to track back to your transect locations that evening or the next day. This is particularly useful when working in

an area with few landmarks. Be sure to indicate in your data collection (e.g. field note book or data sheets) the geodetic datum and whether or not you have recorded end-point or centroid GPS points for each of your transects/sampling sites.

Entering and exiting a vehicle many times a day while putting out pan traps can be hard on the human body. In particular, it is hard on the left leg as it levers you into and out of the car. That action can lead to some slow healing muscle strains. The best way to get in is to sit down on the seat first and then swing both legs over. Getting out is the reverse operation, swinging both legs out and then standing up.

Video Parts I and II on "Surveying Bees Using Bee Bowls: Part I" are available at: www.youtube.com/watch?v=3X3VH-2s-kU and www.youtube.com/watch?v=POQmHUVwFjw

Standard Operating Procedure 4: Setting Up Glycol Traps for Long-Term Sampling Periods

Readers of this SOP will also benefit from reading *SOP 3: Setting Up Bee Bowls for Short-Term Sampling Periods* as some methods for setting out pan traps, collecting and storing specimens from pan traps are the same or very similar.

Glycol traps have the following advantages: They catch bees continuously, thus circumventing problems of shifts in phenology from year to year; Once deployed they are easy to tend and the times for tending the traps can be scheduled rain or shine; The traps can be associated with weather stations where other devices are also tended regularly; and they provide a continuous record of bee species occurring in the area. Although they may require more effort and materials for installation, stations may choose to use glycol traps because they are less intensive in terms of personnel time required for monitoring them and tend to be more structurally stable and better able to withstand extended periods of time exposed to weather and wildlife. Smaller and non-glycol traps require less effort and materials for installation but need to be checked/refreshed more often because they are more susceptible to evaporation, rain, and disturbance by wildlife or livestock. So they may be better suited to sampling sites that are more frequently visited, more accessible, and less likely to be trampled by wildlife or livestock.

Basic design

Glycol traps (described in *SOP 2: Equipment and Preparation*) should be arranged into circular arrays consisting of 9 traps (3 each of 3 colors) for long term surveys and can be placed around a weather station, headquarters or other convenient sample site typical of regional conditions and habitats. Traps are filled with propylene glycol up to the level of the small overflow holes just under the rim of the cup. Specimens should be collected from glycol traps once every 2 weeks.

Setting up glycol traps for the first time

Glycol traps may be placed directly on the ground or in a trap stand or holder (see Figure SOP 4.1). An instructional YouTube video for how to deploy glycol traps for long term surveys can be viewed online at: youtu.be/z0DAY7bNOR4.

Elevated pan trap placement

In addition to ground placement, pan traps can also be set into stands to elevate them off the ground where terrain (e.g. shifting sand on dunes) or thick vegetation prevent stable positioning or obscure pan traps from sight. Traps can also be placed into stands for long term survey efforts.

Set the hoop or cup-holding loop high enough so the cup rests at ground or the desired level or if in the desert slightly off the ground so that the cup doesn't absorb the heat of the surface of the earth. If desired, a trap number can be written on the white plastic hoop.

Figure SOP 4.1. Elevated propylene glycol trap for long-term sampling.

You can see how to make these elevated trap stands online at: youtube/x87CXM7mq54 and read a pilot report on using elevated glycol traps for long-term monitoring online at: ftp://ftpext.usgs.gov/pub/er/md/laurel/Droege/Draft USFS Glycol Report 22711.docx

Height and location of pan traps

Traps need to be placed in the open, exposed to full sunlight and not overhung by trees or grass and forbs during the trapping season. Bees see things differently than we do and don't resolve things in the shade very well. Bottom line: Traps in shade = no bees, so do not put traps in the shade.

Glycol traps can be kept in grassy mown areas, but care should be taken not to let them get filled with grass clippings during mowing operations. If the traps are to be set up in an area such as a field of very close-growing tall grass that would completely obscure the trap when viewed from above, taller stands may be used to position the glycol traps higher off the ground but still (e.g. 15-20 cm) below the vegetation canopy (including grass canopies). The idea is to position the traps within the vegetation but at height where they will be visible to insects flying above (not necessarily above the vegetation canopy.

The height of the glycol traps should be positioned so that the bottom of the cup is just (e.g. 2.5 cm) above the level of the ground or underlying substrate (e.g. matted down grass) (note that this is lower than shown in Figure SOP 4.1).

As with 3.25 oz pan traps, glycol traps should also be spaced proximately 5 m apart in any configuration that is convenient. Keep in mind that these sites should be considered permanent throughout the season and perhaps into upcoming years, so think ahead about vegetation growing and other activities that might interfere with the glycol traps into the future.

Glycol traps and trap fluid

After the pan trap stands are positioned or pounded into the ground, place a trap in each stand (alternating the colors). Fill each trap up to the holes under the rim with diluted propylene glycol (50% water:50% propylene glycol) mixed with a squirt of blue Dawn Original Scent dishwashing detergent. Traps filled three quarters full with the 50% industrial grade propylene glycol (50% water:50% propylene glycol: one squirt of blue-colored Dawn Original dishwashing liquid) will easily last for a week. Check the traps once per week to ensure that they remain full of liquid and top off with the diluted propylene glycol mixture if necessary.

If you are using premixed propylene glycol used in RV antifreeze solutions then DO NOT dilute the mixture as it is already diluted. This premixed propylene glycol lasts for weeks in even dry conditions, however, some evaporation of the water component can be expected so once a week check and top off (with the same premixed propylene glycol) as needed (lower fluid levels decrease the catch).

Collecting bee specimens from glycol traps

Although glycol traps should be checked on a weekly basis to ensure that they do not dry out, bee specimens should be collected from glycol traps once every 2 weeks. Strain the sample and put the specimens and a 'wet label' into a Whirl-Pak, using the following procedure. Remove each cup in the array from the hoops or trap stands and pour their contents into a brine shrimp

net or a paper disposable paint strainer under which is another empty glycol trap cup that re-captures the strained propylene glycol that can be poured back into the now empty trap that is returned to the pan trap stand. Replenish the propylene glycol in all of the traps in the array so that they are filled to the top or to the drainage holes for continued operation (or if it has become cloudy or dirty, replace the liquid solution completely). Pool all specimens from a single array of glycol traps into one sample/brine shrimp net. Pouring the solution of multiple traps over the specimens collecting in the strainer will not damage them. Transfer the specimens from the shrimp net or strainer into the sample bag using your fingers or a white plastic spoon also works well. Fill in (using PENCIL) and insert a 'wet label' into the bag with the specimens and fill the bag with enough alcohol to cover the specimens. Clamp your fingers across the bag, just above the specimens - flattening the sides of the bag against each other, and slide your fingers up towards the bag opening to eliminate air bubbles before sealing the bag up.

Be sure to note any traps that have been disturbed, tipped over, dried out, or destroyed such that their contents are gone or unusable or they collected no specimens, and record this information (including exactly how many traps were affected) in your field notebook so that you can correct for different numbers of traps during any analyses.

See *SOP 3: Setting Up Bee Bowls for Short-Term Sampling Periods* for additional instructions.

Standard Operating Procedure 5: Storing, Shipping and Pinning Collected Bee Specimens

Storing immersed specimens

Specimens immersed in alcohol or propylene glycol in Ziploc or Whirl-Pak bags inside another plastic bag should be kept refrigerated in the short term (e.g. up to 2 months) or frozen if stored for longer periods prior to processing the specimens and/or mailing them to a processing center.

Bee specimens are usually stored at a station in liquids, either alcohol or propylene glycol. Note that while the liquids are preservatives, specimens should be stored in their bags in a freezer prior to shipping. It is true that if the alcohol and propylene glycol content is sufficient they do not require freezing, however, freezing slows down any evaporation that might occur (particularly a problem in alcohol immersed specimens), and it also removes any threat of improper dilutions of alcohol or propylene glycol resulting in specimen deterioration or rotting while waiting to be processed or shipped.

Due to both postal regulations associated with the shipping of alcohol and the difficulty in securing the liquid and the specimens in their bags the following procedure has been developed to prepare specimens for shipping.

Preparing immersed specimens for shipping/mailing

If the station is not preparing bees (pinning and labelling cleaned and dry specimens) before shipping them to be identified the following procedure should be followed. However, it should be noted that shipping un-processed samples increases the cost of having them identified at the particular lab they are being sent. This decreases the funding available to identify the bees. Stations should be encouraged to process samples so that funding goes to work that the station is actually unable to accomplish.

Immediately prior to shipping, drain the liquid while retaining the damp specimens in the bag. Remember that if the bees are stored in alcohol, the alcohol should be disposed of properly and not poured down the sink. Be careful when draining to not lose the smaller specimens. Use a brine shrimp net or tea strainer to catch any specimens that might accidentally come out with the drained liquid.

Strip the remaining air out of the bag with your fingers and if using a Whirl-Pak bag, roll the wire top down until you reach the bolus of specimens collected together in the bottom of the bag, at which point you will take the free ends of the wires and twist them together. That twisted section of wire should then be tucked in towards the bag to minimize the wire ends poking holes in other bags.

A video instructing how to prepare specimens for mailing is available at:
www.youtube.com/watch?v=POQmHUVwFjw

Once the bags are drained and prepared they should remain in the freezer until the actual shipping moment to minimize drying out and rotting. Nobody likes to work with partially rotted specimens! Prior to shipping, the bags of specimens should be placed inside another larger

Ziploc bag that also contains a paper towel to soak up any possible leaking liquids. That Ziploc bag should then be placed in another Ziploc bag just to be sure that any spilled liquids are contained. If there are only a few specimens to be mailed then the specimens can be placed in a padded envelope for shipping to save costs. If there are a large number they should go into an appropriately sized cardboard box and any open space not filled with specimens or specimen bags should be filled with packing material to minimize jostling.

Preparing specimens for pinning

Bees can be pinned directly from the killing jar or sample bag into boxes, or they can be washed first. If the bees in a killing jar are dry and not matted down, then pinning directly into a collecting box is best, as it preserves the pollen load for future analysis (which is not the case for bees capture in pan traps) and speeds up the entire process. However, if the bees are matted from too much moisture and regurgitate, wash and dry them using the methods described in this SOP. They will result in better looking, easier to identify specimens. If the pollen load is not going to be analyzed, then washing the specimens also has the advantage of eliminating the pollen from the scopal hairs and diminishing the "dustiness" of the specimens, making other morphological characters easier to see.

Washing specimens

Pinning bees directly from water, glycol, or alcohol usually results in matted hairs and altered colors, along with a good coating of pollen, scales, and other detritus picked up from the sample. Many bee species are identified by hair characteristics that cannot be examined or properly identified if the hairs are matted and stuck together. Concomitantly, the pin should be inserted accurately into the right hand side of the bee's scutum adjacent to the point where the wings are attached (between the tegula and the mid-line). The midline of the scutum often contains characters that are very useful in identification, which can be destroyed by a pin. The longer a specialist has to look at a poorly prepared specimen the less time and funding they have in identifying additional bees. Washing and processing bees using the process describe here will result in well-groomed specimens that can exceed the quality found in unwashed specimens pinned directly from the field.

Note that the best looking bees are those that are cleaned within 24 hours of capture. We recommend using one of two main approaches to wash bee specimens, using either a strainer or a 'bee washer' to accomplish the task. Both are explained below.

Strainer/'bee washing' jar method: Fill your specimen Whirl-Pak with water and then pour the water and specimens into a tea strainer and rinse them gently under warm running water. Tip the rinsed specimens into a 'bee washing' jar (see *SOP 2: Equipment and Preparation* for instructions on how to make a 'bee washing' jar). Add warm water and Dawn Original dishwashing liquid or conditioning shampoo (more if the specimens were stored in propylene glycol), replace the lid, screen and cap, and very vigorously shake the specimens around for at least 60 SECONDS or up to 4 minutes.

After 1 to 4 minutes, empty the water out through the insect screened lid of the washing jar (the insects will be kept inside the jar by the screen). Refill the jar again with warm water and pour it back out a couple of times until water running through/out of the jar runs clear of soap suds. Or

place the specimens back into the tea strainer and rinse them under warm tap water until no more suds are present. Use your hand to break the force of the water to protect the specimens. Rap off loose water and use a towel to blot out as much excess water on the bottom of the strainer or brine shrimp net or 'bee washing' jar as possible before beginning the drying process.

Whirl-Pak washing method: An alternative to using washing jars is using the original Whirl-Pak. Carefully drain the preservative from the Whirl-Pak, add a drop of soap and warm tap water. You can close the top of the Whirl-Pak in a clenched fist and shake the sample. After the allotted time pour the sample into a tea strainer. You can rinse the sample carefully under the tap until clean.

Magnetic stirrer washing method: Rather than cleaning bees by swirling them around in a jar by hand, you can use a magnetic stirrer, the same as those used in all chemical labs. A small magnet is turned inside a jar or cup by a magnetic plate. The water, dishwashing liquid and bees are swirled around as gently or quickly as you wish. It does the best job of removing pollen, nectar, and gunk on specimens, simply because you can leave it washing for quite a while.

A video that demonstrates how to wash bees can be seen at: www.youtube.com/watch?v=A2y-ind12Cc

Drying specimens

After washing, bee specimens can be dried to ensure separation of their wings and hair from their bodies. Without using one of the following drying methods, hair may remain matted down, obscuring identifying characters on the thorax and wings can stick to the bees' bodies making important wing venation characters difficult to use for identification. We recommend using one of following approaches to dry bee specimens.

Paper towel drying method: After washing, either squirt 95%+ alcohol onto the specimens, dip the strainer into a bowl of alcohol, or drop them into a jar of alcohol and blot again. Tip the specimens out onto a set of 3-6 paper towels and fold the paper towels over the specimens and roll them around gently with your finger, pencil, or tweezers and refold a few times to remove the bulk of the alcohol from the specimens. At this point you can fold corners of the paper towel up and shake the specimens around inside to further dry them. Stop shaking once their wings are no longer stuck together or folded up on themselves and all bee hair is nice and fluffy. Note that you will likely have to hold the corners AND the towel area between the corners in your fingers or the specimens will jump out while you are shaking them.

Note that after the specimens have been dipped in alcohol you can leave them lying on the paper towel for a bit (up to 45 minutes or so) before further fluffing, if you aren't in a hurry.

Proceed to pinning the specimens as normal.

Hair dryer method: We have found that you can obtain *beautifully coiffed* hair on even the longest-haired of bumble bees, if you spend the time shaking them around in a paper towel. Unfortunately, that drying method can take a while. Most people shake the specimens only until their wings unfold and then pin them, leaving the specimen less than presentable. We then have to identify bedraggled specimens which, in the worst cases, can lead to errors in identification

and always leads to a lessening of the aesthetic experience. That need not be as you can use a 'bee washing' jar and hair dryer and the procedure, or modification thereof, below to speed things up.

Follow the same procedure as listed under the strainer/'bee washing' jar section and the Paper Towel Drying section above. Tip or pour the specimens from the paper towel into the canning jar using a funnel. Or you may choose to skip the Paper Towel Drying step, leaving the bees in the 'bee washing' jar. Put the screened lid back on the 'bee washing' jar and make sure the screen is snug around the entire lid.

Turn the hair dryer on to a moderate to high heat setting. Heat is not always necessary, particularly if the specimens are rinsed in quick evaporating alcohol before drying. Be careful not to heat the specimens for too long or they may become too dry and brittle. Place the jar on its side on the folded towel and place the hair dryer pointing into the jar as close as possible, without causing the hair dryer to overheat and automatically shut off (usually about 1 inch). This can be hand held or set up in a wide variety of ways so that you don't need to hold the jar or the hair dryer. Note that an overheated hair dryer can be unplugged and cooled quickly in the freezer.

With the hair drying blowing through the screened opening, shake the specimens back and forth vigorously, tapping the sides of the jar on the towel periodically to dislodge the specimens if they stick to the glass. Specimens, when wet, are very flexible and tough, so they can take a moderate amount of bumping around. Once the specimens are all loose, shift the jar slightly downward so that the specimens slide towards the screen and whirl around in the dryer's wind; continue shaking the specimens.

Small short-haired specimens are dry once their wings are flexed away from their body and their hairs are not matted. Bumble bees and long-haired specimens take longer. Depending upon your hair dryer and your technique, this may take anywhere from 1.5 to 3 minutes.

Note that some have reported good success in creating extra fluffy bee specimens by adding small rolled up bits of paper towel in with the specimens during drying. While others have developed efficiencies such as placing the drying jar on its side in the top drawer of a desk and taping the hairdryer to the desk pointed into the jar; or by laying both the jar and the dryer on a towel on a counter. This frees the technician's hands for more bee processing and ensures that the bees are sufficiently dried. Do not leave a running hair dryer unattended.

The Very Handy Bee Guide also shows a self-standing bee dryer using a blow dryer held in a wooden frame. Bees are placed in a plastic tube (or piece of PVC pipe) with netting on both ends which is placed in a special holder. Use of this equipment works well in keeping bees moving around so they dry faster and can help with high volume specimen preparation. (ftp://ftpext.usgs.gov/pub/er/md/laurel/Droege/Handy Bee Manual.pdf).

Compressed air drying method: We have found that using compressed air results in the quickest drying of wet bee specimens. When using compressed air, be aware that there can be moisture in the air lines. Run the air wide open for a few seconds to get rid of any loose

moisture. Also be aware that at high pressure, compressed air can blow apart specimens, particularly their abdomens. Direct the air stream to the side of the jar and let it swirl the specimens around in a vortex (if the pressure is too high or they are bouncing violently around you can rip some abdomens off). Small specimens with short hair take less than 1 minute to dry using this method while bumble bees take about 2 minutes to have all the hair on their thorax fluff up.

Regardless of the drying method used, dried bees should have the appearance of a dry and alive bee before pinning and labelling. If hairs are still matted down the bee should be cleaned and dried again. However specimens will become increasingly ragged (especially the wings) if put through multiple washing and drying cycles.

If you pin specimens which have been stored in alcohol immediately after drying them, alcohol inside the specimen will leak out and ruin their coiffed hair. Putting them into a chlorocresol humidor for a week before pinning eliminates that problem. A humidor provides a controlled humidity environment that can be used to dry or preserve insect specimens in a pliable state for extended periods of time (6 months to 1 year or longer if moisture conditions are kept right). Chlorocresol (p-chloro-m-cresol or 4-chloro-3-m-ethylphenol) is an antifungal crystalline substance that can be used to prevent fungal growth on specimens while they are drying or being preserved in a humidor. Instructions for how to construct a chlorocresol humidor can be found on page 8 of the Handy Bee Manual (Droege 2015).

Pinning specimens

In addition to a foam pinning board, parchment or wax paper can be used as a sorting tool that can speed up the pinning process (see *SOP 2: Equipment and Preparation* for details about this equipment). Tip or pour dried specimens onto the paper and pull up the sides, causing the specimens to slide into the center. Once in the center, they can be positioned in a line which makes pinning even more rapid. At this point, you can pin the parchment paper to the top of a large foam board so that it won't move while you work through pinning your line of bees.

Each person develops his or her own process when pinning bees. Some pin under the microscope, which usually results in very accurate placement of the pin, but many pin by eye. One technique is to hold larger specimens between the thumb and forefinger of your subdominant hand with the pin ready in the other dominant hand. Use another finger from the hand holding the pin to help hold the specimen steady while inserting the pin accurately into the right hand side of the bee's scutum adjacent to the point where the wings are attached (between the tegula and the mid-line) (e.g. See Figure 19-D, Pg. 28 in Schauff 2001; and Figure 1, Pg. 4 in Stephen 1969). The midline of the scutum often contains characters that are very useful in identification, which can be destroyed by a pin. Most museums prefer that specimens be pinned on the right side. Others pin larger bees using a pair of forceps or tweezers, trapping the specimen on a foam pad.

Remember to leave enough room at the top of the pin so that the specimen can be safely picked up by the largest of fingers. Equally important, leave enough room at the bottom for two or more labels and room for the pin to go into the foam of a collection box.

If you pin specimens which have been stored in alcohol immediately after drying them, alcohol inside the specimen will leak out and ruin their coiffed hair. Putting them into a chlorocresol humidor for a week before pinning eliminates that problem. A humidor provides a controlled humidity environment that can be used to dry or preserve insect specimens in a pliable state for extended periods of time (6 months to 1 year or longer if moisture conditions are kept right). Chlorocresol (p-chloro-m-cresol or 4-chloro-3-m-ethylphenol) is an antifungal crystalline substance that can be used to prevent fungal growth on specimens while they are drying or being preserved in a humidor. Instructions for how to construct a chlorocresol humidor can be found on page 8 of the Handy Bee Manual (Droege 2015).

A video that demonstrates how to pin bees can be seen at:
www.youtube.com/watch?v=V2F8LBQV5L0

Pinning very small specimens

If specimens are too small to be pinned, they can be glued to an insect pinning (paper) point, glued to the side of a pin, or attached as minuten (insect pins without heads) double or staged[2] mounts. See *SOP 2: Equipment and Preparation* for detailed information about pins, glues and other equipment necessary for gluing insects.

Gluing to paper points: Place the pin through the base of the paper point. Elevate the point on the pin to the same height as a regular pinned specimen (pinning blocks can help elevate points to the same uniform height). Glue the small bee to the tip of the paper point, usually on the bee's right-hand side or underside.

Note: A paper point is just what it sounds like. A small 'point' of paper – purchased from entomological supply companies or punched out of a sheet using a specialized paper point-punching tool. Paper points can be used with or without minuten pins (pins without heads) or glue (as mentioned above) to mount very small insect specimens such as midges to insect pins that are too big to be inserted directly into the insect specimens.

Gluing directly to pins: When gluing a specimen directly to a pin, rather than to a point, the pin is glued to the specimen's right hand side or the underside between the thorax and abdomen. Again, most museums prefer that specimens be glued on the right side. Gluing specimens to the side of the pin has the advantage of speed, better prevention of glue hiding useful characters, and a specimen that is easier to view under the microscope. Its axis of rotation is minimized and the point is no longer there to hide the view or block the light. In addition, specimens that have been preserved in liquid will often leak fluid when a pin is inserted. This mats down the hair that you just took the effort in drying. This will not occur if the bee, regardless of size, is glued to a pin. Specimens should be glued to the pin at the same height as those that are traditionally pinned.

[2] When specimens are mounted using two forms of support – usually a smaller support which in turn is supported on a normal full-sized entomological pin, this is called staging.

Place glue on the pin first, at the height where you would normally pin a bee. Roll the bee specimen over so that its right side is facing upwards (or so that the specimen is lying on its left side) and then lay the pin with glue onto the specimen. Leave the glued bee lying down with the pin resting on top of the bee until the glue has had time to set. After the glue has set, press down lightly on the pointed tip of the pin with your finger. This will cause the end of the pin with the specimen on it to rise up, allowing you to grasp the top of the pin and move it into a collection box. A video that demonstrates how to glue a bee to a pin can be seen at: https://www.youtube.com/watch?v=9KfLCmYOKtA

General Videos on how to mount and work with insect collections are available at: nau.edu/Merriam-Powell/Biodiversity-Center/Museum-of-Arthropod-Biodiversity/Instructional-Videos

Labeling specimens

Following pinning, individual labels should be prepared for each batch of specimens. Labels are usually generated by whoever is processing and pinning the specimens. Labels can be generated using the Access database. *SOP 2: Equipment and Preparation* contains detailed information about paper selection and software applications for generating labels.

Unique specimen numbers are required for effective specimen and data management and for referencing and integrating digital data. Many a beginning student of bees has rued the day that they did not give their specimens unique numbers. Each batch or site should be assigned a unique site number and each specimen should be assigned a unique specimen number. On each specimen label, the specimen number and site number should be listed, as well as the country, state, county, latitude, longitude, date of collection, and collector. We suggest recording dates as in this example (15 SEPT, 2013) to avoid confusion with regard to international numerical date formats. Do not abbreviate the year to a 2 digit number. Capitalizing the abbreviated month name (e.g. SEPT) can improve readability and interpretation both on printed and hand-written specimen labels, 'wet labels', and field notes and data sheets. A small square data matrix included on each Discover Life generated label encodes the specimen number and permits the information of each pinned specimen to be scanned with a hand-held scanner directly into a database while the specimens remain in the box. These data matrices are included automatically in the free Discover Life labels (see *SOP 2: Equipment and Preparation*) or can be added using commercial software such as BarTender (www.seagullscientific.com/), or can be generated using the Access database.

Once printed, cutting out labels can be a time consuming aspect of any project. Speed up the process by cutting out rows of labels; placing them in their corresponding specimen box and then cut the individual labels apart with scissors. See: www.slideshare.net/sdroege/preparing-insect-labels-a-faster-way and youtube/HqxrkC6xe40 for more examples of these techniques. You may find it easier to use a surgical/dissection scalpel rather than scissors to cut out labels. Try combining a #4 handle with a #21 or #22 blade. This combination works well for cutting the strips and works really well when cutting the individual labels apart once they are in single strips.

Specimen labels are quickly added to specimen pins by laying them across a piece of Ethafoam or a pinning board (see *SOP 2: Equipment and Preparation*) that is the same thickness and

desired height of the label on the pin. Labels are oriented along the same axis as the specimen with the specimen's head over the left hand end of the label – the end you would normally begin reading from. Try to place the point of the pin through a section of the label that does not contain any printed information e.g. a space between words. Prior to putting labels on specimens, do a quick check to make sure the label information matches the row tag (if using the Discover Life labeling program).

Storing pinned specimens

After a batch of specimens is washed, dried and pinned, place them in a cardboard specimen box (see *SOP 2: Equipment and Preparation* for details about specimen boxes and storage equipment). At the upper left hand corner of the box, pin a tag with the date, place, site or batch number on it. This tag is usually the original 'wet label' (now dried out) that was placed in the bag with a batch of specimens when first captured (see *SOP 2: Equipment and Preparation* for details about 'wet labels'). Pin a line of specimens to the right of the tag, and continue adding specimens from top to bottom, and left to right, until complete. The next tag or 'wet label' belonging to each sample is placed immediately thereafter and so forth until the box is filled. In general, it helps if each box contains specimens from only one region or in some cases sample. Label the year across the top of the box, then the month, and then the locality, so that you can quickly pick out the box you want from a shelf of multiple boxes.

See *SOP 2: Equipment and Preparation* for more information about storing boxes of pinned specimens.

Protecting stored specimens from pests and humidity

This section is duplicated from *SOP 2: Equipment and Preparation* but is worth repeating. Simple cardboard boxes are not pest proof. Dermestid beetles are the primary pest of insect collections. Fortunately, infestations are usually small, perhaps seeing one beetle larvae in a box scattered here and there. An infected specimen is usually easy to spot, as small black droppings and shed skin are visible below the specimen. Control and prevention take place, according to the literature, by freezing the box at -20° C (~0° F) for 3 days, thawing for a day and then freezing for another 3. In a pinch, kitchen freezers appear to work too. Spring is a good time to freeze your entire collection, as that is when Dermestids appear to be most active. An excellent means of keeping your collection pest free (particularly if using cardboard boxes) is to keep each box in a large Ziploc bag. Note that you should let the specimens dry out thoroughly after pinning (one month or so) before enclosing them in a plastic bag. Mothballs and pest strips can be effective, but carry some apparent health risks with long-term exposure. Mothballs can be purchased from hardware stores and some grocery stores and Hot-Shot No-Pest strips and other fumigants can be purchased from BioQuip (www.bioquip.com). Hot-Shot No-Pest strips can be cut into about 6 smaller pieces that can be easily wedged in between the foam base and side of specimen boxes by hand. But wear gloves and/or wash hands thoroughly after handling these and other fumigant products and do not store specimens treated with No-Pest strips in rooms regularly occupied by people or animals for periods longer than 2-3 hours.

In humid conditions (such as July and August in Maryland), unprotected specimens, particularly those just caught, can turn into balls of mold. Either store them in an air-conditioned space or put them in plastic bags or tightly closed bins that contain active desiccants.

Preparing pinned specimens for shipping/mailing

Ensure that specimens are firmly pinned into the foam base of the box. Consider placing bracing pins on each side of heavy or long-bodied specimens to prevent them from rotating (cartwheeling) on their pins and damaging adjacent specimens. Unless the box in which the specimens are pinned is shallow enough so that the heads of the pins almost touch the lid, a piece of firm cardboard should be cut to fit snuggly inside the specimen box and lie on top of the specimen pins. Do not use foam for this layer as it can engulf the tops of the pins, damage the specimens, and cause problems when removed. Place either pinned specimens or empty pins in all four corners of the box to support the cardboard piece.

Note: You may wish to also pin loose cotton wadding in the corners of each specimen box so that if a specimen comes loose in transit, it will be trapped by the cotton and perhaps avoid further damage.

Note: Although it is good practice to fumigate boxes before shipping, do not leave loose fumigant in the box with the specimens nor any fumigant strips, or balls on pins in containers. They are especially prone to work loose and damage specimens.

Affix the piece of cardboard in place using two pieces of tape applied on opposite sides of the top of the cardboard in such a way as to form handles that can be used to safely remove the cardboard without upsetting the specimens below. Simply press one end of the tape to the piece of cardboard and then fold the other end of the tape back on itself so the sticky sides meet. If there is space between the top of the cardboard and the lid of the specimen box, put in some bubble wrap or packing peanuts, so that when the lid is closed it slightly compresses the piece of cardboard down onto the tops of the pins, keeping them in place during travel. Tape the lid of the specimen box closed.

Place one or more specimen boxes inside a box large enough to allow or leave at least 2 inches of free space around/on all sides of the boxes of specimens. You may need to fill the bottom 2 inches of your shipping box with Styrofoam packing peanuts, bubble wrap, or other light packing material first before you put your specimen boxes inside the shipping box.

Note: Avoid using starch-based biodegradable packing peanuts as these have been known to carry or attract Cigarette beetles and other museum pests, which may consume or damage specimens (Aiello et al. 2010).

Specimens may be successfully shipped this way via the U.S. Postal Service (USPS), United Parcel Service (UPS), and by Federal Express (FedEx). Specimens intended for genetic analyses should be shipped via FedEx to avoid them being irradiated by the USPS. For valuable specimens all companies provide tracking and confirmation of receipt services.

Additional detailed information on shipping specimens is provided by Schauff (2001) and Hunter (2006).

Standard Operating Procedure 6: Identifying Collected Bees

Because the identification of bee fauna is difficult, time-consuming, and requires assistance from university and agency experts, continued refinement of identifications is expected to take several years. However, considerable information can be acquired from easily identifiable genera, species, and/or morphospecies. If you need expertise for identifying bees, you should identify and develop an agreement with an authorized collection facility such as a laboratory, museum or university and ship the bees to them for identification and possibly archival storage. This should be done PRIOR to collecting any bees. For information on how to prepare bees for shipment, see *SOP 5: Storing, Shipping and Pinning Collected Bee Specimens*.

This section provides guidance for the use of the online Discover Life identification guides or keys (www.discoverlife.org/mp/20q?search=Apoidea#Identification). These instructions are designed for use with the guides to the genera and species of bees, however, these instructions will largely hold true for any of the non-bee guides also available at the site. Be sure to also see the section at the end regarding the use of already identified specimens. A set of identified specimens can be obtained at no charge from Sam Droege (sdroege@usgs.gov).

Hint 1: If you are just beginning to learn how to identify bees, we suggest that you look at the glossary of terms, vocabulary, identification tips, "Bee Body Part" figures (Pg. 63), and pronunciation materials in *The Very Handy Manual: How to Catch and Identify Bees and Manage a Collection* by Droege (2015).

Training

The American Museum of Natural History offers a highly recommended annual two-week intensive training course "The Bee Course", usually located at the Southwest Research Station in Portal, AZ for those wishing to learn how to identify bees to genus level. For more information visit research.amnh.org/iz/beecourse or contact Jerry Rozen, Curator, Division of Invertebrate Zoology - Invertebrate Zoology and Professor, Richard Gilder Graduate School (www.amnh.org/our-research/staff-directory/jerome-g.-rozen).

Classes for identifying bees (especially eastern bees) to species level are offered periodically by Sam Droege (sdroege@usgs.gov, USGS Patuxent Wildlife Research Center, Beltsville, MD) and others in the bee community subject to schedules, funding, demand, and resource availability, contact Sam for more information on upcoming classes.

Organizing specimens for identification

After the specimens are labeled and those labels have been double-checked against the data records, the specimens can be freely moved about for identification. Sort and identify only those specimens in a single box before moving on to the next box rather than trying to merge and identify all specimens of any given species across many boxes. If working with large groups of specimens associated with different projects, place colored pieces of paper under the locality label in each box that corresponds to a project so that projects can be tracked visually/at a glance. In this way, multiple projects in multiple states of completion can be tracked and are less likely to become confused with each other. A pin with a small brightly colored label-sized piece of paper – such as that cut off the end of a Post-it® Page Marker works well as a 'place-holder' for

any specimen you need to temporarily remove from a box while you're working on identifying it.

If technicians are identifying specimens they should, make a first pass through the box, looking quickly at each specimen under the microscope and removing those easily identified without using a guide. As bees are identified, they are taken out of the box and pinned to a separate box, or rearranged in the original box, if there is room. As new species are detected, a determination label is created (available as a modifiable Excel file from Sam Droege or with this protocol in ServCat) listing the genus and species name of the species. Pin the identification label to the board separately from the specimens (aka not on the same pin as a specimen), so that it can be easily viewed. All subsequent specimens of that genus/morpho-species/species are then placed to the right of the label. Keep specimens that cannot be immediately identified separate and return to identify them after the easy ones have been identified and removed using computer and paper identification guides.

Once the specimens have all been identified, return them to their original box. Place the specimens in the box in rows starting at the upper left corner, working from left to right, top to bottom, inserting labels at the beginning of each new group of species. Position female specimens so that their labels are vertical, and position male specimens so that their labels are horizontal. Positioning the sexes this way permits those who enter or digitize the data to quickly ascertain and check the sex based on the label and specimen's orientation rather than having to read the label. To assure accuracy, have a separate person double check data entry.

Identification guides and materials

The taxonomy of North American bees, like that of many insects, is quite fluid and subject to significant changes and updates on an ongoing basis. Until recently, most taxonomists and those tasked with identifying bees relied on dichotomous keys available in the published literature for species determinations. These historical references such as Mitchell's 1960 and 1962 two volume set on Bees in the Eastern United States are still very valuable reference books and sources for identification keys, illustrations, and species accounts. While now quite expensive to purchase via rare book dealers, Mitchell's set is now freely available online as a series of Portable Document Format (PDF) files at: insectmuseum.org/easternBees.php

Note however that Mitchell's taxonomy is out of date. All identifications made with this book should be cross-referenced against the lists of bees of North America (United States and Canada lists) available at www.discoverlife.org (Ascher and Pickering 2015) and within the polychotomous [3]bee identifications guides located at that same site. You can cross-reference scientific names by either going directly to one of the genera guides or by simply typing the species name into the search field on the Discover Life Web site home page.

[3] A polychotomous key is one in which the resolution can be reached through a variety of routes. The user is not required to make choices in a specific order.

As our ability to identify and distinguish one species from another improves over time, the scientific names of species' can also change as a result of new knowledge and understanding of a species. Not surprisingly, this regularly results in some species or specimens being identified or labeled with old scientific names that have been usurped by newer scientific names. If recognized by the international taxonomic community and authorities, the new scientific name is usually referred to as the 'valid' name for the species while older names (or synonyms) are referred to as 'invalid'. Fortunately, taxonomists maintain and map the relationships between old and new scientific names for us and publish them in peer reviewed scientific literature and online such as in the lists maintained at www.discoverlife.org or the Integrated Taxonomic Information System (ITIS, www.itis.gov). To learn more about the regulation of taxonomies for animal species visit the International Commission on Zoological Nomenclature (/iczn.org/); and for more information about the regulation of taxonomies for plant species visit the International Code of Botanical Nomenclature (www.iapt-taxon.org/nomen/main.php) maintained by the International Association for Plant Taxonomy.

Mike Arduser (Missouri Department of Conservation, Retired) has also been building identification keys to several genera of bees from the Midwest. Those can be viewed online at: www.pwrc.usgs.gov/nativebees/Keys.html

Laurence Packer's Lab has produced a guide to the bee Genera of Canada: www.biology.ualberta.ca/bsc/ejournal/pgs_03/pgs_03.html (Packer et al. 2007) and to the bee Families of the world: www.yorku.ca/bugsrus/BFoW/Images/Introduction/Introduction.html

And a pictorial catalog of the tribes of bees of the world is available online at: www.yorku.ca/bugsrus/bee_genera_of_the_world/Bee_Tribes.html

All of the Discover Life ID Nature Guides are located online at: www.discoverlife.org/mp/20q. However, the consolidated links to the bee guides and associated materials are online at: www.discoverlife.org/20/q?search=Apoidea. ID Nature Guides for bee species found west of the Mississippi river are under development.

A guide to bees of the Western United States is not currently available.

Using Discover Life ID Nature Guides to identify bees

Discover Life ID Nature Guides (Figure SOP 6.1) differ from traditional dichotomous keys in that characters that help differentiate species are evaluated and scored for all or almost all of the species. Think of it as a matrix, with species as rows and character states as columns. That matrix is employed by answering questions regarding the presence or absence of characters for a specimen. As questions are answered the list of possible species is narrowed until, in most cases, the list resolves to a single name. If NWRS station staff plan to identify specimens on their own, they should have subsamples reviewed by an expert to confirm the identification. Novices using the Discover Life ID Nature Guides to identify their bee specimens should have their determinations reviewed by an experienced entomologist who is familiar with bee identification. On the bee page at Discover Life there are a series of guides listed for Eastern North American bees (states and provinces east of the Mississippi River). Many of these guides have been expanded to include Western species and over the coming years we will expand all guides to

include the western states and provinces. Guides are constantly being updated with pictures, corrections and better wording.

Most guides deal with a single genus of bees. If there are a large number of species present, these guides are often divided into two guides, one for each sex, as characters useful for identifying species are often gender specific.

Hint 2: If you are unfamiliar with the bee genera we suggest that you start your identification process by using the guide to bee genera to divide your collection into genera.

The instructions that follow apply equally to the guide to bee genera or to any of the individual bee genera guides.

Each guide has questions on the right, a species list on the left, and navigation links across the top of the Discover Life Web page. The list of species and the list of questions interact with each other. Answering any question (in any order) narrows the list of candidate species, when any SEARCH button (search) is clicked. Similarly, one can flip the process, by clicking the SIMPLIFY button (simplify), and have the computer narrow the set of questions based on the species that remain on the list.

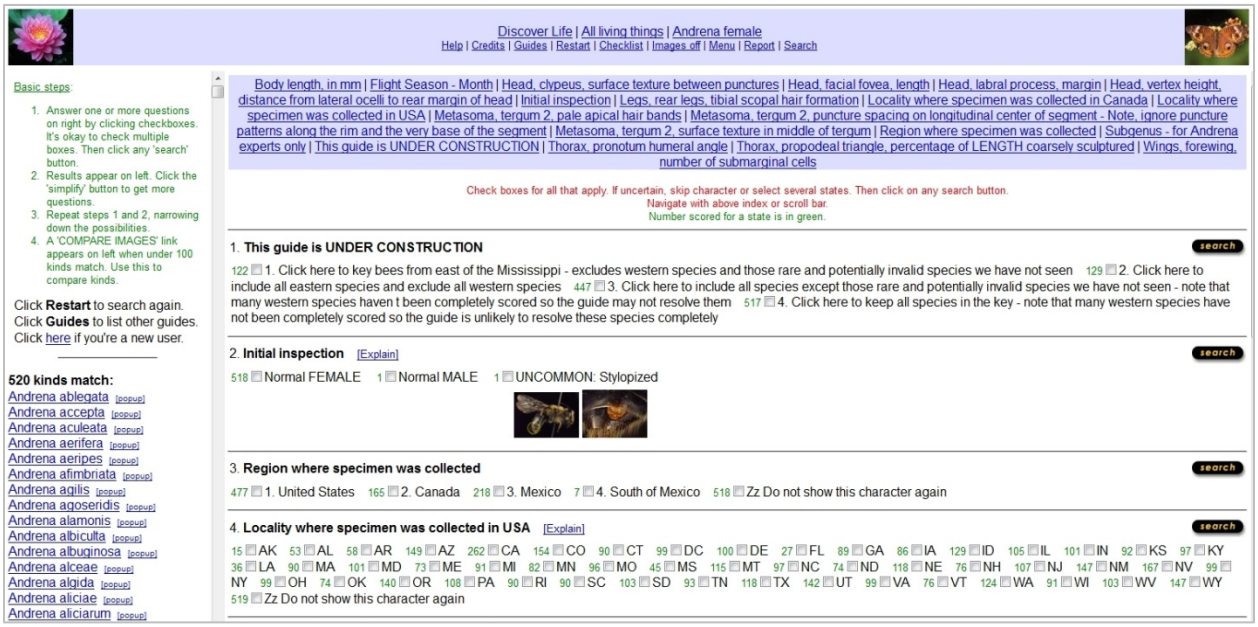


Figure SOP 6.1. Discover Life bee guide. www.discoverlife.org/mp/20q?search=Apoidea

Clicking on any pictures present within the guide will display an enlarged or other version of the picture. Many species names can also be clicked on to reveal species-specific pictures and often have associated text material on the natural history or identification of that species.

The initial page of a guide presents a subset of all the questions in the guide. These questions are both easiest to understand and most likely to separate out large numbers of species.

There is no need to answer the questions in the order presented.

At least initially, you will find that there are some questions that are clearer in your mind than others. These should be answered first.

Hint 3: Unlike a dichotomous key, you may answer ANY NUMBER of questions IN ANY ORDER. You do not need to answer all questions. Initially answer ONLY questions where you are sure about your answer.

When using the guides, leave questions you are unsure of blank! Don't guess! We also recommend that you spend more time reading and learning about the morphological characters in the questions before choosing your answer, or simply skipping the question.

Not all characters will have been scored for all species. If both sexes are present in a guide then characters that only apply to one sex will obviously not be scored for the other sex. Similarly, if we have been unable to obtain a specimen of a rare species, we may not be able to score some characteristics from the available literature. The consequence of this is that any species that has not been scored for a particular question will remain on the list of possible candidate species, regardless of whether it actually has that character or not, simply because it cannot be eliminated from the list of possibilities.

Hint 4: While using a guide, there are two types of species that remain on the list. 1. Those species that have the characters you have indicated; 2. Those species that have not been scored for some or all of the characters you chose in your answer. The second type of species will stay in the list simply because we do not have enough information about its characters to eliminate it.

Hint 5: For many characters you are given three or more choices of character states. If you are not sure which of the character states your specimen's character fits into don't hesitate to click on all possible correct combinations rather than trying to narrow it to the one that best fits.

At any point you can press any of the SEARCH buttons (

) that are located on the right throughout the main page. Doing so will update the species list on the left based on the characters you have chosen.

At any point you can also click on the SIMPLIFY button (

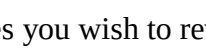
) that appears in the left hand column above the species list after your first search. Doing so eliminates both questions and character states within questions that do not help resolve the identity of the species remaining on the list. Clicking the SIMPLIFY button also adds those appropriate questions that were not included in the initial list of questions present when the guide was first opened. Additionally, hitting the SIMPLIFY button will also reorder the questions alphabetically.

Both the SEARCH and SIMPLIFY buttons can be clicked as often as you wish. We usually click on the SEARCH button after answering a question, just to get a sense of the questions that best

help eliminate species the quickest and to make sure that we haven't made some fatal error. We suggest waiting to click on the SIMPLIFY button until you have a reasonably small list of species left or have answered most of the questions you are comfortable with on the first page. If you hit the SIMPLIFY button earlier in the process it will bring up a potentially very large list of additional questions that may not be as useful or as easy to answer as the initial ones.

Strategy

Especially when you are unfamiliar with the species within a genus, it is very useful to take some extra time to double check your initial identification. In many cases, there will be pictures and extra information stored as a link to the species name. Those can be compared to your specimen (be aware that males and females of the same species can often look quite different from one another, that is, they exhibit strong sexual dimorphism).

The next step to verifying your species identification is to compare your specimen to the complete list of the scored characteristics of that species. To get a list of those characteristics, click on the MENU link (in the second row of links in the header at the very top of the Web page). At the top of the left hand column, select the CHARACTERS option. Next, click on the name of the species you wish to review. Finally, hit the SUBMIT button (

) to get a list of scored characteristics.

The Discover Life guides offer many paths to the final answer or correct species identification. This feature can be exploited when checking your identifications. By clicking on the SIMPLIFY button at the very beginning, you will display ALL the questions for the guides. By answering a different set of initial questions, a different species will remain on the list. These new questions and species may expose some flaw in your initial identification which will become obvious if you don't return to the same species identification at the end.

Hint 6: These guides are easier to use than dichotomous keys. However, answering questions incorrectly will still yield incorrect identifications. So be careful and conservative in your answering.

The RESTART link, located in the second row of links in the header at the very top of the Discover Life Web page, restarts the guide at the beginning.

Advanced uses of these guides

By clicking on the MENU link (in the second row of links in the header at the top of the Web page), the simple species list found normally in the left hand column is replaced with a set of new options used by individuals building or editing guides. Some of these options are also useful when exploring the identity of a species. But don't worry about exploring any of the features found in the MENU page, as only the guide developers have permission to use them to make permanent changes.

The CHARACTERS option will give you the scored characters for any of the species you have checked.

The DIFFERENCES option will give you the differences in scoring among any two or more species you click.

Clicking on the HAS link restarts the guide but brings up ALL the characters for that guide in alphabetical order. Additionally, a new set of 2-3 checkboxes has been added at the top of each characters section in the guide: The ONLY, HAS, and NOT, checkboxes. If you don't select any of these 3 checkboxes the guide acts as it normally does. If, however, you select the HAS checkbox along with one of the character states, clicking on the SEARCH button will generate a list of species on the left that will include only those species that have been scored as having that character. What will be missing are those species that were never scored for that character at all. Similarly the ONLY checkbox provides a list of species that have been scored for that character alone. This means that if a species was scored as possibly having all or more than one of the possible states, it will not be displayed if the ONLY checkbox was selected. The NOT checkbox provides a list of species that have not been scored for the selected character state(s).

The Discover Life Web site also has a HELP link (in the second row of links in the header at the top of the Web page), which provides even more details on some of the more advanced features.

If you have questions about any of the bee guides please contact Sam Droege (sdroege@usgs.gov, Phone 301.497.5840). His lab in Beltsville, MD is open to anyone who would like to learn to process and identify their collection of bees. Most of the time they have space, computers, and microscopes available, as well as access to their synoptic collection.

Using previously identified specimens as an aid in learning your bees

When first starting out, you will learn how to identify bees far more quickly if you use pre-identified specimens for comparison than if you try to immediately key out the bees you have collected. Because you already know the identity of the specimen, you can track your progress and reflect on your errors while using the guide and the mind/eye/guide learning loop will take place more quickly. If you use unidentified specimens, you may find it difficult to initially feel 100% confident that your identification was correct.

There are two ways to approach bee identification using previously identified specimens. One is to use the guides directly. After selecting each state of each character you believe your specimen expresses from the options available in the guide, click the SEARCH button. You can then watch the list of matching specimens on the left side of the screen to see if your species or genus remains on the list. If it does not, you know which state of which character you entered that lead to the incorrect match.

Alternatively, you can go to the menu section of the guide and call up the entire list of scored states/characters of the species or genus of your previously identified specimen. Once you are in the menu section, select the option next to "score," then select the box next to the species you want to investigate, and finally click the SUBMIT button. All the information for that species will be displayed and you can compare every scored character in the guide to the characters you see on your specimen, thus familiarizing yourself with all the characters in the guide. You will also find that you can "see" certain characters easily and others may remain difficult for you to interpret or find, thus helping you decide which characters you will preferentially use when keying out that group.

Feel free to contact Sam Droege (sdroege@usgs.gov, Phone 301.497.5840) for a set of identified specimens to use.

Stylopized bees

As you identify bees you will, at times, come across bees that have an infestation of mites and more rarely bees that have been parasitized by a Strepsipteran (i.e., stylopized). Strepsiptera is a mysterious order of unclear position within the holometabolous insects. They are endoparasites of various other insect orders including a diverse array of Hymenoptera. Families Andrenidae, Halictidae, and Colletidae are the most frequently parasitized bees.

One can find male puparia (MP), empty male puparia (EMP) and adult females (F) in bees. MP are usually very large spherical extrusions, however findings of these are quite rare. More frequently you can find EMP, which are sometimes hidden and difficult to recognize. In some cases, an EMP appears as an obvious deformation. Female cephalothoraces are most commonly encountered in bees and appear as small orange/brown plate-like extrusions that emerge from beneath the rim of the tergites of the abdomen. Upon seeing one you will have the impression of a small head peeking out from beneath the rim. Sometimes the apical rim of the tergite covers most of the parasite's body (as in most Halictidae) and will appear almost invisible from the dorsal view. However, the rim of the tergite is usually lifted upwards and the Strepsipteran can be viewed when looking under the rim.

Strepsiptera can modify not just the morphological features of the site where they are attached, but the morphological characteristics of the entire bee, including the sexual characters of bees. At times the characteristics of the bee are changed enough to partially disguise the species identity of the specimen. Deformations occur among all bee hosts, but they are quite rare. Sexual character changes are manipulated by the parasites and occur only in some groups - most bees of the family Andrenidae and some *Hylaeus* (Colletidae).

Jakub Straka, PhD, Aculeata Research Group, Charles University in Prague, (www.aculeataresearch.com/index.php/people), is working on the taxonomic and ecological facets of *Strepsiptera*. He is very interested in collecting host records for this group, parasitism rates, and specimens for DNA analysis. If you come across any stylopized specimens in your collecting activities, please contact Sam Droege (sdroege@usgs.gov) who will mail your specimens to Jakub (jakub.straka@aculeataresearch.com or straka-jakub@vol.cz). This group occurs uncommonly, so even single records are of great interest.

Standard Operating Procedure 7: Netting and Non-lethal Techniques

If the objective is to do a comprehensive inventory of bees on a NWRS station or there is a target of sampling bees visiting a specific group of plants, then netting is one of the techniques that should be employed. Netting can be part of a program to collect specimens for lab identification, or as part of non-lethal surveys, along with the other techniques described at the end of this SOP.

Capturing bees with a net is the most traditional way to sample bees. However, because it requires a skill that evolves and improves with practice (unlike the deployment of traps) there is a great deal of variation among individuals in their ability to collect and find uncommon and rare bees using a net. Even if time and location are well documented, there are still many factors affecting capture efficiency such as eye sight, strength, speed of handling a net, speed of taking bees out of a net and/or transferring to a specimen container (e.g. a 'kill jar' containing cyanide, ether, alcohol etc.), preferences for detecting and capturing or locating large bees versus small bees, where to watch or listen for bees, speed and timing of a person in traversing a site, the amount of time spent netting at an individual plant or clump of plants or in locating plants in flower, accessibility of flowers (e.g. netting canopy foraging bees), and preferences for capturing bees from certain types of plants.

Such preferences and skills lead to significant differences between and among individuals in total number of individuals and species of bees captured. These captures or sampling events are consequently not often representative or repeatable, making their use in detecting changes in the number and taxonomic composition of bees over time or among sites difficult. Instead the variation reflects differences in the collector's skill. Thus the data are often not comparable and effectively negate the use of netting as a standardized approach to surveying bees. Furthermore, netting can only occur when weather conditions are appropriate. Additionally, throughout the day the probability of detecting and capturing bees changes, further complicating the use of nets in standardized surveys.

However, when the objective is to inventory bees at a station or from a particular plant or habitat within the station, netting is the appropriate complement to using pan traps because some larger common bees rarely appear in pan traps, e.g., bumble bees (*Bombus*), large carpenter bees (*Xylocopa*). Netting, when applied to an inventory by even an unskilled person tends to bring in an additional set of species that pan traps rarely capture.

There are clear and dramatic differences between the number of bees captured by a novice first time netter and those of someone who has been netting bees for many years. Even professionals have large and known differences in capture rates and the capture of uncommon and rare species among themselves. So be aware, that simply providing a volunteer or someone inexperienced with a net in no way is comparable to bringing a professional to hunt down uncommon bees. Finding and netting bees is a skill that takes years to perfect. This is yet another reason to consider netting as ancillary information that augments the more standardized pan trapping survey technique.

Conditions for capturing bees with hand nets

Bees can be captured with a net under most weather conditions with the exception of rain or moderate to high winds, and temperatures below which bees are not active. Netting is most productive in the morning in North America usually from 9:00 AM until 2:00 PM. Depending on the location and the temperature, bees can be out prior to and after these times. In very cool areas, bees may not become active until midday or after temperatures reach 60^{o}F or higher, winds die down, and/or fog or overcast conditions dissipate.

Netting technique

Always hold the net in a "swing-ready" position. One hand should be below the head and the other towards the back or middle of the pole (not unlike the starting grip on a tennis racket). Hold the tip of the net lightly against the pole with the hand nearest the head so that it does not drag on the ground or in vegetation. When or as you start your swing, drop the tip of the net. For example a right handed netter usually holds the pole towards the middle or just above the bottom with their dominant sweeping hand (or right hand); while the left hand lightly holds the tip of the net against the pole just below the head (or net end) – ready to release it quickly.

Bees can be detected by their motion, and sometimes by their shadows or sounds (e.g. buzzing inside [sonication] or between flowers), rather than their size and shape. The mind detects motion much faster than it can process colors and shapes into bee/not bee categories. Train yourself to key in on movement or sound; over time you will become more adept at separating bee motion from plant motion and the sonication of some different genera (e.g. leafcutter, carpenter, and bumble bees). Bees can easily escape your net when you hesitate or check your swing. If you see something that looks like a bee, capture it in your net. Once in your net you can decide whether or not to keep it. If you spend any significant time thinking about whether you should or should not swing, the capture opportunity may be missed.

Always keep a mental check for the presence of thorny plants in the area where you might swing -for obvious consequences to your net. Additionally, in some areas some plants have seeds that can implant themselves directly into the netting; if that is the case then you might try moving from the usual coarse weave net bag to the fine weave type.

When swinging a net, speed is important as well as follow-through. Bees are very visual and very fast. If you are timid in your swing or cut your swing short bees will evade the net. Center your net on the bee if at all possible even if it means having to plow through some vegetation. When a bee is flying low to the ground, it is better to slap the net over the bee and onto the ground than it is to try to catch it with the edge of your net by swinging just above the ground.

All else being equal, it is better to swing at a bee that is just flying into or away from a flower than a bee that is actually on a flower. Particularly if you are trying not to damage the plant, a less than vigorous swing of the net will simply push a bee on a flower under the net and it will fly away afterwards. After some practice you can bring your net up to a bee on a flower, wait for the bee to leave the flower, push the flower out of the way with your net and still easily capture the bee.

Note: Station staff will appreciate your being mindful of not completely destroying flowering plants by scything all of the flowers off of them during netting.

When looking at a clump of flowers that could contain bees, stand 4 to 8 feet away and try not to let your shadow fall across the flowers. Most people stand too close to the flowers, which can scare away some of the bees you are interested in, limit both the number of flowers (and therefore bees) in your field of view, and limit your depth of field. In this way you can view a large area of flowers, spot a bee, and either lean or take one step forward to capture that bee in your net. If you have to take two steps or more, you are too far away.

On any flower patch, concentrate on the difficult to obtain bees first. In particular, look for bees that are moving very quickly, from flower to flower, and try to predict where they will move next. Usually there is some pattern to their flight and often they will come back to the area after making their circuit. Some of these individuals never really come to rest and you have to swing ahead of where you think you are going to catch them. It also pays to look below flower clumps for low-flying bees. Some of these are nest parasites, while others simply prefer to move between clumps of flowers just above the ground or grass.

Open soil of any kind and, in particular, south facing slopes, overturned root masses, clay banks, and piles of construction dirt or sand should be scanned both for bee nests and for low cruising nest parasites. Nest parasites (in particular those in the genus *Nomada*) usually fly just above the soil in erratic flight paths. The best way to capture them is to slap the entire head of the net over the bee and onto the ground, and quickly lift the tip or end of the net bag up while keeping the rim of the net on the ground. The bee will instinctively fly upwards rather than trying to sneak under the rim. Often this can take several seconds, so patience should be applied. This ground-slapping technique is often also very effective for capturing bees foraging on ground-hugging or low growing plants such as bees in the genus *Perdita* that forage on native species of ground-hugging plants in the genus Euphorbia.

For those more experienced in the use of hand nets, there are two ways to catch multiple individuals in a net. One way is to turn your net head sideways (90°) after capturing each bee, allowing the net bag to close over the head or rim of the net and hoping that the bee will not find a way out when you swing for the next bee. The other is to physically hold the bag closed above the tip of the net containing the bees (note: in between swinging at bees, you will be holding the closed net against the pole as you carry it from place to place). In both cases you will have to periodically snap the contents of the net (bees you have already captured) back to the bottom or tip of the net. Do this vigorously or some wasps (in particular) may not go to the bottom, and you could end up grabbing them through the net with obvious painful consequences to your hand.

In general it is easier to see and inspect bees through the mesh of your net if you go into the shade or shade the net with your body. Some people favor green nets over the traditional white ones to reduce the glare from the white net making it difficult to focus on the bee inside. However some collectors also prefer white nets because they contrast strongly with usually dark-colored bees and other insects.

An instructional video that demonstrates how to use a net to collect bees can be viewed online at: www.youtube.com/watch?v=n6ZFlz3uA7E

Removing bees from a net

Time spent removing bees from the net is time spent not capturing bees; therefore, as you become more experienced at catching bees, think about how you are removing bees from your net to see if you can speed the process up.

In the beginning, there is usually a great fear of being stung by your subjects. In reality, in North America, only bees and wasps in the *Polistes*, *Vespinae*, *Bombus*, *Apis*, and *Pompilid* groups, and perhaps a few others have significant stings. These are large insects and can be readily distinguished from your target species. However, even these species usually do not sting while caught in a net, unless they are physically grabbed or trapped against the net. Thus, over time you should concentrate on diminishing your fears, and spend more time sticking your hand and 'kill jar' directly into the net. If you are putting your net on the ground to remove bees, you are taking too much time. Kill jars should be fully charged (with cyanide, ethyl acetate, ether, alcohol, etc.) in order to quickly kill your specimens, and it helps to have multiple jars available (see section on kill jars, Pg. 39).

Note: We recommend that those who are truly allergic to bee stings and bee venom should not participate in any aspects of these surveys that involve actually handling bee specimens directly.

The most efficient means of collecting large numbers of bees from your net is to use vials or containers of soapy water (water plus dish liquid). In that way you can fill your net with bees and only have to empty the net into a kill jar periodically rather than after catching each individual bee or small numbers of bees. However, cleaning and processing bees killed in liquids requires some care to do properly (see section on washing and drying bees).

Once you have captured a bee or bees in your net, there are several ways to remove and transfer them to a kill/collection jar. In all cases, it is best to vigorously snap the net to drive the insects to the bottom or tip of the net. You can then safely grab the net just above where the bees are resting. Even the larger and more aggressive bees can't get at the hand that is closing off the net, due to the bunching of the netting. If you are worried about the specimen(s) escaping, or have numerous insects in the net, you can kill, or at least pacify your catch, by stuffing the specimens and the netting into your kill jar and closing the lid loosely for a few seconds. Keeping your kill jar(s) well charged with cyanide or ethyl acetate will ensure that the specimens quiet down quickly, and you will not waste a lot of time waiting. Once your specimens are immobilized (you will see them gradually become still), you can open up the net and drop the bees directly into the kill jar without worry of them escaping.

Most collectors take a more direct approach and bring the open kill jar and its lid into the net, trapping the bee against the netting. Bring the opening of the kill jar up to the inside of the net, trapping the bee(s) between the net and the solution in the jar. Then gently tapping the net or slapping your hand on top of the net over the opening of the kill jar is enough to release the bee from the net and drop it into the jar. Replacing the cap onto the jar inside the net can also help prevent bees from escaping. More than one bee at a time can be put into a kill jar this way, but at some point, more escape than are captured.

Because seeing the bees through the netting can be difficult, (use your body to shade the netting to better see the bees), some collectors have taken to hanging the net on the top of their head

while they transfer bees into their insect collecting jar or "kill jar(s)". With your head (and sometimes shoulder) inside the net, use one hand to hold the net out and up, and then use the other hand to reach in and collect the specimen with the kill jar. It is important in this situation to keep holding the net out so the bees move away from your head. Try to use small collecting jars or large test tubes that can be handled easily with one hand and practice removing and replacing the cap with one hand. Despite having your hand (and sometimes your head) in the net with the bees, most collectors are rarely stung.

In general, bare hands are recommended when removing bees from nets. Bees and wasps will almost never sting in a net, if you don't trap them in your hands or against the netting. Use of a centrifuge tube filled with soapy water (water plus dish liquid) makes removal easy, as you can keep well away from the bees. Some people will use gloves, such as handball gloves, welder gloves, latex dishwashing gloves (though stinging can occur through latex), and goatskin beekeeper gloves.

An instructional video that demonstrates how to remove bees from a net can be viewed online at: www.youtube.com/watch?v=n6ZFlz3uA7E

Note: Although the instructional video referenced here recommends not worrying about clipping off flower heads, when on refuges and other protected lands, station staff will appreciate your being mindful of not completely destroying flowering plants by scything all of the flowers off of them during netting.

General How-to Videos on how to work with insect collections are available at: www.bugs.nau.edu/learning_modules.html

Sampling units (hand net)

Because netting insects and bees is considered ancillary rather than primary data, this frees up the biologist from constraints of strict sampling location and procedures. Consequently, a net should be kept handy when in the field and if interesting bees, interesting blooming plants, nesting aggregations, or spare time become available, netting should occur.

Part of the skill in using nets is becoming familiar with where bees nest, detecting bees that are looking for nests or are nest parasites, working on your net handling skills, and becoming familiar with what the common and unusual bee plants are. For example, Willow (*Salix* spp.), while not a species of plant that most people associate with bees, is visited by a group of bees that only use its pollen for creating provisions for their young. Thus, if you do not collect bees off of blooming willow trees in the spring then you most certainly will not be obtaining the species associated with willow trees on the station unless you have traps that are immediately adjacent to willow trees. Even with pan traps deployed, some species of bees may not be detected or collected because they forage in trees and large shrubs. These canopy-foraging bees often occur at only very low rates in pan traps that are placed on or near the ground.

That said, if a person is going out collecting bees with a net for an extended period of time then they should note how long and where they are netting along with what plants are blooming and, more specifically, what plants they are collecting these bees from. One method for tracking what bees were collected off what plant species (especially when multiple kill jars might be used to

collect bees from the same plant species and/or the collector may not be familiar with the plant or able to identify it) is to place a flower from the plant in the kill jar with the bees (although the flower should not be transferred with the bee specimens into a wet sample bag). Bead containers that screw together, work well and can be purchased from craft stores; as do plastic vials from BioQuip. These also fit well in fly fishing vests, which may be worn as handy 'equipment carriers' while netting in the field. Plant species information will be a valuable addition to the collection information associated with the individual specimens. Patterns may emerge as to where and when uncommon or rare species occur that may provide direction and insight into future research, management, or conservation. Most groups who undertake an inventory of bees inevitably find rare or uncommon species or unusual distribution and locality information. Proper note-taking and documentation of all collecting activities and conditions helps link information about where a bee was caught and what factors such as the presence or absence of certain plants, nesting materials or other habitat attributes may be influencing the bee's site use.

Other non-lethal methods

These non-lethal techniques are more effectively applied to very specific or narrowly focused research projects rather than large-scale monitoring efforts for generating long term baseline datasets.

Using photographs to identify live bees

Taking photographs of live bees has low systematic repeatability, but can be used to document presence of species. As with any photography-based monitoring, there will be varying degrees of success and accuracy depending on the quality of the photograph(s), photographer(s), photography equipment, and the entomological and melittological identification skills of those providing the identification service. However, there are communities actively conducting and researching to improve bee identification through photography as a non-lethal sampling/monitoring method. Some quite sophisticated though expensive techniques have already been developed for taking higher quality photographs of bees in the field (see Thomson and Zung, 2015).

Important bee identification characteristics can include colors and patterns and the presence or absence of hair on the bee's face; upper or under side of the thorax and/or abdomen; and legs; the positioning of physical features and appendages; and the wing venation pattern (the patterns of the wing veins and cells). It's important to try to include as many of these characters in focus, in a photograph of a bee. It is often necessary to take multiple photographs of the same individual in order to capture good quality, sufficiently focused photos showing enough of these characters.

Some existing programs and projects focusing on using photography to identify live bees are:

1. The Bumble Bee Watch project (http://www.bumblebeewatch.org/photo-tips/) a citizen science project supported through a partnership of The Xerces Society, the University of Ottawa, Wildlife Preservation Canada, BeeSpotter, The Natural History Museum, London, and the Montreal Insectarium, offers tips on taking good quality photographs for successful identification of bumble bees.

2. The BeeSpotter project (https://beespotter.org/topics/photos/) supported through a partnership among the Office for Mathematics, Science, and Technology Education,

College of Education, University of Illinois; Department of Entomology, University of Illinois; and the University of Illinois at Urbana-Champaign, also provides instructions for what characters to capture in a good bee photograph.

3. BugGuide.net, hosted by the Department of Entomology, Iowa State University, is "an online community of naturalists who enjoy learning about and sharing our observations of insects, spiders, and other related creatures" and offers tips on taking good quality insect photographs (http://bugguide.net/node/view/137046). BugGuide.net is also supported by a highly qualified and active community of volunteer entomologists and mellitologists (scientists who study bees). See also the section below on chilling bees.

The USFWS has also setup a project page on iNaturalist, where bee photos taken on refuges can be uploaded and bee experts will periodically visit and attempt to identify the bees in the photos. Use the "Bee & Wasp" link on the NWRS page: (http://www.inaturalist.org/projects/usfws-national-wildlife-refuge-system). In order to identify observations that were made as part of a formal survey (as opposed to casual observations of refuge visitors), please add the keyword "IMBeeSurvey" in the Tags field on the Observation Submission page. These observations can then be selected with a query using that tag. If you would like to send a notice to Sam Droege that photos have been uploaded, so that he might attempt to identify when he has the opportunity, add the comment "@sdroege" to the observation (below the "Activity" panel). Add the iNaturalist observation URLs to the appropriate fields in the Observations Table of the NWRS Bee Access Database (See *SOP 8: Data Collection and Management*). Once the bees in the photos are satisfactorily identified, the identifications can be added to the Access database.

Refuge visitors who photograph bees may also post their pictures on iNaturalist where they can be viewed and identified.

Mark-release-recapture and genetic sampling

Although not a commonly used or easily implemented technique for broad-scale species monitoring, mark-release-recapture of insects has been used for more focused individual-species monitoring for many decades, indeed centuries where managed honey bees (e.g. *Apis mellifera*) are concerned (consider the practice of bee lining in the 1700-1800s) (Visscher and Seeley, 1898); and often accompanies non-lethal genetic sampling. Modern genetic sampling techniques usually involve removal of an appendange (e.g. an antennae) or piece of an appendage (e.g. a leg or wing tip) or a small volume of an excretion (e.g. faeces) or fluid (e.g. hemolymph) from the bee e.g. Holehouse et al. 2003; Châline et al. 2004; Akemi Oi et al. 2013; Scriven et al. 2013. And in some cases where the 'sampling' is not necessarily self-evident, methods are employed to 'mark' those individuals that have been sampled.

Individual insect marking methods vary with the size of the species and how long the researcher needs the 'mark' to last. The material used to mark the bees is carefully selected in order to avoid any significant negative impact on the bee's health and survival after sampling. Various examples of field and hive-based bee and other insect mark-recapture methods are described and evaluated in this sampling of available literature on the subject: Gary 1971a and 1971b; Walker and Wineriter 1981; Matteson and Langellotto 2009; Hagler and Jackson 2001; Hagler et al. 2011; Butler et al. 2012; and Yamamoto et al. 2014. Those interested in using these methods

should receive training in order to hone the chosen technique(s) and work flow (order of procedures) before venturing out into the field.

Chilling bees for sampling/marking/photography

Bees and other beneficial insects such as lady beetles (Coccinellidae) are regularly transported and stored in a chilled condition prior to release. When attempting to photograph or collect DNA samples from bees during the day or when they are more active, the chilling method can be used to temporarily slow them down for sample collection, marking, and/or photography. Captured bees need only be placed inside a small, clear, dry ventilated container and cooled in a refrigerator or a portable cooler with ice in it for 1-5 minutes (depending on your chilling apparatus, size and activity level of your bees, and ambient temperatures). Do not put bees directly on the ice and monitor them (by checking on them regularly) while they are chilling. You may need to experiment to find just the right amount of time needed to chill your bees in order to slow them down sufficiently for you to sample, mark or photograph them before they warm up enough to fly away. When finished photographing or sampling and/or marking the chilled bee, be sure to place it in an open container in a warm, preferably sunny, spot where it can warm up safely. Again, it should be noted that this technique does not lend itself well to generating large scale, repeatable or long term baseline monitoring datasets.

Collection of ad hoc/ancillary observations

The trapping and netting techniques described in detail in other SOPs in this protocol offer a standardized and repeatable survey method for establishing comprehensive baseline and long term datasets. With a little knowledge about a bee species' behavior, you may also be able to add a few more records to your baseline data and/or species list for some species of bees that can often be found just by knowing where to look through the collection of adhoc observations. Note that this should not be considered a standardized or repeatable technique for the purposes of generating scientifically comparable or comprehensive long term monitoring datasets. Larger bees – mainly bumble bees (*Bombus* sp.) will often rest overnight on the undersides of leaves, stems, or flowers. Various species of smaller bees are also well known for congregating overnight inside flowers (e.g. Bradley, 1908; Chemsak and Thorp, 1962). Bumble bees (*Bombus* sp.) often rest/sleep at the base of thistle flowers (e.g. *Carduus* sp., *Cirsicum* sp.) (Elizabeth Sellers, personal observation). Male squash bees (*Peponapis pruinosa*) spend the night in the closed flowers of cucurbits (e.g. pumpkin, squash, melon vines, etc.) (Hurd et al. 1974). Some smaller bees can also sometimes be found inside the closed, and even dried up flowers of Queen Anne's lace (*Daucus carota*) (Elizabeth Sellers, personal observation). With this information in mind, bee spotters can head out into the field early in the morning, particularly on cooler mornings when temperatures are below 60^{o}F and before the morning sunlight has reached the target plants, and find these bees before they have warmed up enough to fly away. But unless you are targeting monitoring towards specific bee species that exhibit these behaviors – or their preferred plant species, this technique should only be used to supplement other more comprehensive bee monitoring methods like those described in this protocol framework.

References

Akemi Oi, C., M. M. López-Uribe, M. Cervini, and M. Antonio Del Lama. 2013. Non-lethal method of DNA sampling in euglossine bees supported by mark-recapture experiments and microsatellite genotyping. Journal of Insect Conservation, 17(5):1071-1079. http://dx.doi.org/10.1007/s10841-013-9582-8

Bradley, J. C. 1908. A case of gregarious sleeping habits among aculeat hymenoptera. Annals of the Entomological Society of America, 1:127-130 http://biodiversitylibrary.org/page/9950518
Butler, S. R., Jurzenski, J., and W. W. Hoback. 2012. Evaluation of marking techniques, mark retention, and mortality due to marking of burying beetles (Coleoptera: Silphidae). The Coleopterists Bulletin, 6692):149-154. http://dx.doi.org/10.1649/072.066.0212
Châline, N., F. L. W. Ratnieks, N. E. Raine, N. S. Badcock, and T. Burke. 2004. Non-lethal sampling of honey bee, *Apis mellifera*, DNA using wing tips. Apidologie, 35:311-318. http://dx.doi.org/10.1051/apido:2004015

Chemsak, J. A., and R. W. Thorp. 1962. Note on the sleeping habits of males of Melissodes robustior Cockerell (Hymenoptera: Apoidea). Pan-Pacific Entomologist, 38:53–55

Gary, N. E. 1971a. A new tagging and recapture method for evaluating honey bee pollination activity. California Agriculture, June:4-6

Gary, N. E. 1971b. Magnetic retrieval of ferrous labels in a capture-recapture system for honey bees and other insects. Journal of Economic Entomology 64(4):961-965. http://dx.doi.org/10.1093/jee/64.4.961

Gezon, Z. J., E. S. Wyman, J. S. Ascher, D. W. Inouye, and R. E. Irwin. 2015. The effect of repeated, lethal sampling on wild bee abundance and diversity. Methods in Ecology and Evolution 6(9):1044-1054. http://dx.doi.org/10.1111/2041-210X.12375

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: Current techniques and future prospects. Annual Review of Entomology, 46:511-543. http://dx.doi.org/

Hagler, J., S. Mueller, L. R. Teuber, A. V. Deynze, and J. Martin. 2011. A method for distinctly marking honey bees, *Apis mellifera*, originating from multiple apiary locations. Journal of Insect Science, 11:143. http://dx.doi.org/10.1673/031.011.14301

Holehouse, K. A., R. L. Hammond, and A. F. G. Bourke. 2003. Non-lethal sampling of DNA from bumble bees for conservation genetics. Insectes Sociaux 50(3):277-285. http://dx.doi.org/10.1007/s00040-003-0672-6

Hurd, P. D., Jr., E. G. Linsley, and A. E. Michelbacher. 1974. Ecology of the squash and gourd bee, *Peponapis pruinosa*, on cultivated cucurbits in California (Hymenoptera:Apoidea). Smithson Contributions to Zoology, 168:1-17
http://www.sil.si.edu/smithsoniancontributions/zoology/pdf_hi/sctz-0168.pdf
Matteson, K. C., and G. A. Langellotto. 2009. Bumble bee abundance in New York City community gardens: Implications for urban agriculture. Cities and the Environment, 2(1), article 5:1-12

Peakall, R., and F. P. Schiestl. 2004. A mark-recapture study of male Colletes cunicularius bees: implications for pollination by sexual deception. Behavioral Ecology and Sociobiology, 56(6):579-584. http://dx.doi.org/10.1007/s00265-004-0816-3

Scriven, J. J., L. C. Woodall, and D. Goulson. 2013. Nondestructive DNA sampling from bumblebee faeces. Molecular Ecology Resources 13:225-229. http://dx.doi.org/10.1111/1755-0998.12036

Thomson, J. D., and J. L. Zung. 2015. A restraining device to aid identification of bees by digital photography. Journal of Pollination Ecology, 17(21):145-147. http://dx.doi.org/
Thorp, R. W., and J. M. Leong. 1998. Specialist bee pollinators of showy vernal pool flowers. Pages 169-179 in: C. W. Witham, E.T. Bauder, D. Belk, W.R. Ferren Jr., and R. Ornduff (Editors). Ecology, Conservation, and Management of Vernal Pool Ecosystems – Proceedings from a 1996 Conference. California Native Plant Society, Sacramento, CA. 1998

Visscher, P. K., and T. D. Seeley. 1989. Bee-Lining as a research technique in ecological studies of honey bees. American Bee Journal, 129(8):536-539

Walker, T. J., and S. A. Wineriter. 1981. Marking techniques for recognizing individual insects. The Florida Entomologist, 64(1):18-29

Yamamoto, M., C. N. Junqueira, A. A. A. Barbosa, S. C. Augusto, and P. E. Oliveira. 2014. Estimating crop pollinator population using mark-recapture method. Apidologie, 45(2):205-214. http://dx.doi.org/10.1007/s13592-013-0238-1

Standard Operating Procedure 8: Data Collection and Management

Most NWRS stations will not have in-house bee identification experts, so this SOP is written with the assumption that stations will send bees collected in surveys to a lab that has bee identification expertise. If a station has a local expert, they can carry out all of the steps outlined here and send their data to a national database to facilitate regional and national level assessments of bee populations. It is recommended that all bee data collected be shared with the USDA-ARS Pollinating Insects Research Unit (PIRU) in Logan, Utah regardless of where the bees are identified so that the NWRS can contribute to large scale assessments of the status of native bees. With arrangements PIRU can also identify bees and will update their national database and station databases with bees they identify. Also, the USGS Native Bee Inventory and Monitoring Lab (BIML) at the USGS Patuxent Wildlife Research Center in Beltsville, Maryland may be able to identify bees for stations if they make arrangements ahead of time. Some museums and universities may have experts that would be willing to identify bees and enter the identifications into station databases.

Data repositories such as DataOne (www.DataOne.org) are also important resources for information about data management, the data life cycle and as a place to store data. The set of best practices developed by DataOne are found at: www.dataone.org/best-practices.

The U.S. Geological Survey also provides a comprehensive Data Management Web site that includes guidelines and templates for development data management plans and best practices (www.usgs.gov/datamanagement/index.php).

Data collection

A standalone Microsoft Access-based NWRS Bee Database has been developed. An empty database can be obtained from the same USFWS ServCat site where this protocol framework is stored. This database can be used for bee surveys on stations to store all of the data about surveys being conducted by the station (or region). The database can then be sent to a lab with bee specimens for identification and the lab can enter the bee identifications into the database. Once the bees have been identified and entered into the standalone database, it should be shared with the USDA National Bee Lab so the data can be pooled for regional and national scale assessments.

A data sheet is attached at the end of this protocol framework that can be used to collect data in the field and then entered into the database, or data can be entered directly into the database. The use of (hard copy paper) field data collection sheets or field notebooks does however provide some level of safety and redundancy for data backup and review, and comparisons of level of effort (e.g. matching up sampling dates and locations with samples). The database is designed so that information about the station and each survey site only needs to be entered once (e.g., station name, survey site) and then only the particulars for individual survey events need to be entered thereafter (Figure SOP 8.1).

The types of information to be recorded for each sample site and for individual specimens have been described in previous SOPs. If ‘wet labels’ (see Figure SOP 2.2) are used, they should be

linked to matching records in the database and data sheets, as well as to the samples or individual specimens. A researcher encountering any one of these information records, whether in hard-copy or electronic format, should be able to follow the information trail in either direction – from the field to the sample or individual specimen to the database or digital record to any related materials (e.g. photos) and publications and vice versa.

We strongly encourage incorporation of the data formatting, management, and metadata standards (see recommended standards described below) into all aspects of data recording, digitization, storage, and publishing. Adherence to international standards can increase the usefulness and quality of your data and in general makes it easier to integrate with other datasets for modeling and analysis. The application of and adherence to data standards during data collection and digitization can greatly reduce the opportunity for error that are presented when badly formatted or missing data must be interpreted or located after the fact – often when the original collector or dataset curator is no longer available for consultation. It is important to describe (e.g. in the Site-specific Protocol) what standards you used for data collection and curation – especially if the standard you followed is not readily apparent in the data itself. The standards that follow are incorporated into the NWRS Bee Database.

Data Management

Standards for Species Occurrence Data:

The Darwin Core Archive (DwC-A) is a biodiversity informatics data standard that makes use of the Darwin Core terms to produce a single, self-contained dataset for species occurrence or taxonomic (species) data – the kind of data that is likely to result from the bee surveys described in this protocol. It is a standard for data sharing that facilitates easy comparison of and integration of the same data fields across multiple datasets. Here is a very simplified explanation of how mapping your species occurrence dataset to Darwin Core Standard terms can be useful for data integration: A species occurrence dataset from a refuge may have a data field labeled with the term ‘Name’. Their ‘Name’ field contains the scientific names of all of the species represented in their survey dataset. Another species occurrence dataset from another refuge might label their similar data field with the term ‘Identified’. Normally this might pose a problem for someone seeking to identify and compare the scientific name fields of these datasets. But if the owners of the datasets map or associate their scientific name fields with the Darwin Core’s Standard term “Scientific Name” then a complete stranger will know that the ‘Name’ field in the first dataset contains the same type of data and can therefore be compared with the ‘Identified’ field in the other dataset.

To take this concept one step further, a dataset owner can choose to use actual Darwin Core Standard terms to label the fields in their dataset – instead of using field labels that others might find difficult to interpret or understand (Darwin Core Terms Quick Reference Guide - http://rs.tdwg.org/dwc/terms/). This approach greatly facilitates dataset interpretation and integration with other similarly standardized datasets into the future (especially after the original data creators or curators are no longer around to consult). The Darwin Core Standard is also the preferred standard for labeling dataset fields published via the Global Biodiversity Information Facility (GBIF, www.gbif.org).

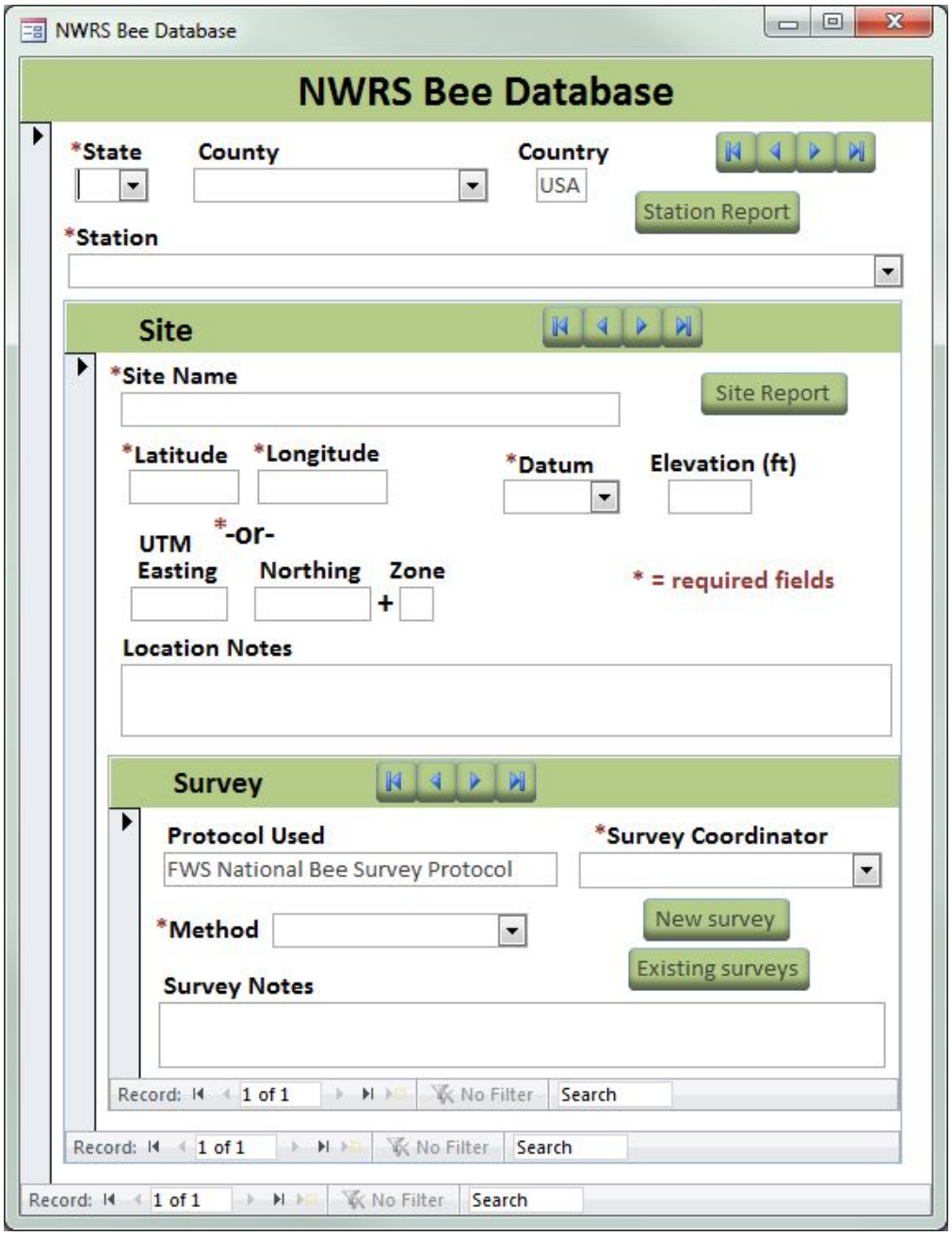


Figure SOP 8.1. NWRS Bee Database using Microsoft Access to collect and store station bee data.

Recording scientific names

Care should be taken in recording valid or accepted Latin scientific names for both animals and plants recorded as part of any species survey data. Binomial, trinomial, and quadrinomial names should be written out in full without abbreviations wherever possible. Care should be taken to use syntax accepted by the general taxonomic community for qualifiers such as subspecies, variety, hybrid etc. in scientific names – especially/mainly for plant names. Do not include taxonomic authors and dates, or other artifacts such as the taxonomic qualifiers 'nr.', aff., cf., in the same database field where the scientific name is recorded. Taxonomic authors and dates, and other artifacts should be recorded in separate database fields. Some dataset curators choose to

store the genus and specific epithet names in separate database fields but this can make it difficult to maintain the association between these two names that is necessary in scientific name syntax. The NWRS Bee Database has drop-down boxes with standardized scientific names for bees.

Standards for Scientific Names:
The Integrated Taxonomic Information System (ITIS, www.itis.gov) is the taxonomic authority of the U.S. Department of the Interior and as such should be used to ensure use of valid and accepted scientific names for bee species and plants wherever possible. These are the scientific names used in the NWRS Bee Database.

The World Bee Checklist (Ascher and Pickering 2015) (available online at (www.discoverlife.org/mp/20q?act=x_checklist&guide=Apoidea_species&flags=HAS) should also be used as an additional taxonomic reference, especially for valid scientific names for bee species that are not yet represented in ITIS.

Recording and referencing (species) common names
Plant and animal common names are highly ambiguous but can be useful for gaining a general familiarity with a taxonomic group, individual, and a dataset in which they are recorded. Common names should be recorded in a separate database field and spelled out in full (no abbreviations). An additional field may also be necessary to indicate the language of the common names, especially if they are from a language other than English e.g. tribal names.

Recording dates
Dates recorded in field notebooks and on field datasheets by hand, should be recorded with a 1 or 2 digit numeric day and the name of the month either written out in full or as a short 3 or 4 letter alphabetic abbreviation written in all capital letters; and all 4 digits of the year included e.g. 27 September, 2013 or 27 SEP 2013. This format avoids confusion that can occur when field technicians or volunteers record either the day or the month first in numeric syntax e.g. dd/mm/yy or mm/dd/yy; and for example future users of the data cannot be sure if the date 06/07/YYYY indicates an event on June 7, or on July 6.

Standards for Calendar Dates:
The International Standards Organization (ISO) 8601 international standard date format specifies a numeric format of yyyy-mm-dd with a four digit year being the minimum acceptable. Consider following this standard when digitizing the contents of date, time, or year fields in your datasets. Note that this standard format relates to how date information is best entered and stored in an *electronic* format (e.g. database or spreadsheet). However, as mentioned in the *Recording dates* section above, date information can be written on a paper field data sheet or typed into a software interface (e.g. mobile phone application) in formats other than the ISO format/syntax. These can be easily and accurately interpreted (transformed) into the ISO 8601 digital standard date format either by a data digitizer (person) or in some cases, by software applications.

Recording time:
Remember to note the time zone in which collection or sampling times are recorded, including noting when/if daylight saving may be in effect.

Standards for Time:
The ISO 8601 international standard also offers a format/syntax for recording times either separately or as part of a date record e.g. hh:mm:ss where 'hh' is the number of hours since midnight. If the date and a time are displayed on the same line / in the same database field, always write the date in front of the time e.g. yyyy-mm-ddThh:mm:ss where 'T' indicates the beginning of the time record. The NWRS Bee Database formats all date and times.

Recording geographic information
The recommendation is to record latitude and longitude in the field using a global positioning system (GPS). Differences in coordinates or 'datum shifts' that exist between different geodetic systems can be hundreds of meters or even several kilometers or miles, so it is very important to note which geodetic datum you or your GPS used when your latitude and longitudinal data is recorded.

All longitudes recorded in the United States and its Territories in the Western hemisphere, west of the Prime Meridian or that would be recorded with the West suffix should be recorded as negative. The negative sign should be included in the longitude field when digitizing these data.

Note: The negative symbol in longitudes recorded in the United States must be recorded and digitized as negative data. This is especially important for data sharing and integration at national and international scales. If the negative symbol is not included with a U.S. longitude coordinate, the corresponding point may appear in the ocean when displayed on a map.

If you record UTMs instead of latitude and longitude, you must also record the Zone you are in.

State and County names: When not accompanied by a Federal Information Processing Standard (FIPS) code (see Standards below), always record state and county names spelled out in full. In both handwritten and especially in digital datasets, avoid abbreviating state and county names.

Country names: When not using the ISO Standard 3166 Country Codes (www.iso.org/iso/country_codes.htm and en.wikipedia.org/wiki/ISO_3166-2) for country name abbreviations (see Standards below), country names should be spelled out in full. This should not be an issue for data collected solely in the U.S. and its Territories (the focus of this protocol). Note that U.S. Territories are referenced with ISO codes that are different from those used to refer to the United States. ISO 3166-2 Country Codes for the United States, U.S. Territories and Minor Outlying Islands (these are available as selections in the NWRS Bee Database):

AS : American Samoa
GU : Guam
FM : Micronesia, Federated States of
PW : Palau, Republic of
UM : United States Minor Outlying Islands (includes Baker Island, Howland Island, Jarvis Island, Johnston Atoll, Kingman Reef, Midway Islands, Palmyra Atoll, Wake Island, Navassa Island)
MH : Marshall Islands, Republic of
MP : Northern Mariana Islands

PR : Puerto Rico
US : United States
VI : Virgin Islands, U.S.

Standards for Geography:
FIPS codes are numeric and alphabetic codes for identifying U.S. states (e.g. FIPS 5-2, en.wikipedia.org/wiki/Federal_Information_Processing_Standard_state_code), counties (e.g. FIPS 6-4, en.wikipedia.org/wiki/FIPS_county_code), and territories. These codes can be used or incorporated into a dataset to compliment human observations about locations, latitude and longitude coordinates; and to standardize geographic references such as for indexing electronic data for search and for mapping species occurrence data. These codes are automatically saved when using the NWRS Bee Database.

The Geographic Names Information System (GNIS) may also be used to include standardized names for physical and cultural features throughout the U.S. and its territories. It is a gazetteer developed by the USGS in cooperation with the United States Board of Geographic Names.

ISO 3166 (www.iso.org/iso/country_codes) is the International Standard for 2 and 3 character alphabetic and 3 character numeric country codes. The ISO 3166 two and three character standard alphabetic codes for the United States are US and USA respectively. Note that U.S. Territories are referenced with ISO codes that are different from those used to refer to the United States.

Recording and Registering Metadata
Comprehensive standard metadata records should be created and published for all datasets developed as a result of or through application of this protocol and others. Metadata describe information about a dataset, such that a dataset can be understood, re-used, and integrated with other datasets. Information described in a metadata record includes where the data were collected, who is responsible for the dataset, why the dataset was created, and how the data are organized. Federal agencies are mandated by Executive Order 12906 to use the Federal Geographic Data Committee (FGDC) Content Standard for Digital Geospatial Metadata. A transition to the ISO Standard is occurring, the adoption of which is endorsed by the FGDC.

Metadata records are important tools for documenting and preserving the provenance, context, methodologies and standards, and other information associated with and used to characterize a dataset. Metadata records are usually developed following a standard (see Standards below) and numerous software tools are available for creating, formatting, and maintaining metadata records. These records are then published, usually through a metadata clearinghouse or catalog, where online search engines can index them thereby advertising the existence of digital and non-digital datasets and increasing their discoverability.

Examples of metadata records and more detailed information about what metadata is and how it is used can be found on the USGS Data Management Web site online at: www.usgs.gov/datamanagement/describe/metadata.php

As an example, the metadata record for the National Parks Lichens dataset can be viewed online at: mercury-ops2.ornl.gov/clearinghouse/send/xsltText2?fileURL=/data/Mercury_

instances/clearinghouse/csas/harvested/www1.usgs.gov_metadata_mdata_MISC_nps_d_NPSLichen.xml&full_datasource=Metadata%20Clearinghouse%20Principal%20Node&full_queryString=%20National%20Park%20Lichens&ds_id

Standards for Metadata:

In 1994, Executive Order 12906 called for the establishment of the National Spatial Data Infrastructure (NSDI) defined as the technologies, policies, and people necessary to promote sharing of geospatial data throughout all levels of government, the private and non-profit sectors, and the academic community. The FGDC develops geospatial data standards for implementing the NSDI. Many agencies have incorporated the FGDC Content Standard for Digital Geospatial Metadata (CSDGM) and Biological Data Profile as part of their regular data management and markup practices. However, the FGDC has also endorsed the international ISO 19115 Geographic Information Metadata standard and encourages federal agencies to follow or transition to this standard wherever and whenever possible. More information about FGDC and ISO metadata standards can be found online at: www.fgdc.gov/metadata/geospatial-metadata-standards. Using the NWRS Bee Database will ensure that all required metadata is recorded.

Standard Operating Procedure 9: Associating Standardized Vegetation Classes with Survey Locations

Authors: Lee E. O'Brien, Melinda G. Knutson

Introduction

Linking natural resources surveys to a standardized set of vegetation classes increases the long-term value of the survey data and supports data analysis at broad spatial scales (landscapes or ecoregions). The vegetation class associated with each survey location is an important attribute (covariate) that may be needed for future, currently unanticipated, applications of the data set. *At a minimum, most natural resource surveys should document the standardized vegetation class associated with each survey location.* For some surveys, this will suffice for documenting vegetation conditions. For other surveys, additional environmental attributes (plant species cover estimates, stem counts, water temperature, etc.) will be needed and separate SOP's for collecting this information will be needed.

This SOP provides guidance for associating standardized and mapped vegetation classes (hereafter referred to as 'vegetation classes') with natural resources data collected at points or polygons. The SOP can be used in any terrestrial or wetland survey when a minimum documentation of vegetation is needed. (Marine systems are not included at this time.) Survey coordinators can link sample locations with vegetation classes in advance of the field season and print them on the field data sheets and project maps. One advantage of this approach is that field staff with minimal botanical training can verify that the associated vegetation class is found at the survey location or, if the assignment is incorrect, can assign another vegetation class from a short list of those found in the study area.

How Are Vegetation Classes Standardized and Mapped?

Ecological systems are recurring groups of biological communities that are found in similar physical environments and are influenced by similar dynamic ecological processes, such as fire or flooding. These ecological systems are represented by standardized and mapped vegetation classes that are readily identifiable by conservation and resource managers in the field (Comer et al. 2003). Several federal and NGO agencies employ these standards and have developed useful tools; we employ the USGS National Gap Analysis Program (GAP) Land Cover Map. The GAP map uses vegetation classes from NatureServe's Ecological System Classification (Comer et al. 2003) and the National Vegetation Classification (NVC) System; these are the same vegetation classes used by the LANDFIRE program to model fire behavior and predict disturbance potential. The GAP map covers the entire U.S. including Alaska, Hawaii and Puerto Rico.

The standardized vegetation classifications (defined as Class, Formation, Macrogroup, and Ecological System) for a state, county, or Landscape Conservation Cooperative (LCC) can be perused with the GAP Land Cover Data Viewer. If you click on a location on the map, a description of the class and a range map pop up. This tool can be used to generate a master list of the land cover classes in the vicinity of the study area. Full descriptions of the classes are available from NatureServe Explorer for states, provinces, Forest Service Ecoregions, and MRLC 2000 Map Zones. For example, a search for 'oak', with Wisconsin selected as a state,

turns up a list of classifications, one of which is 'North-Central Interior Dry Oak Forest and Woodland'. A detailed description is provided.

Linking Vegetation Classes to Sample Locations

The survey coordinator will oversee the assignment of vegetation classes to sample locations. GIS technical skills are required to conduct the overlay analysis. With the sample location coordinates (and datum) in hand, the GIS technician will overlay the survey location coordinates on the GAP land cover map (available for download by regions, LCCs, states or for the whole country: here) and create a site-specific map showing the vegetation classes that the sample locations fall within and the list of sites with their expected vegetation class. Additionally, a master list of all the vegetation classes found in the study area is needed for reference, in the event that the assigned vegetation class is in error. Descriptions of the vegetation classes can be downloaded from NatureServe for states, ecoregions, or map zones: here.

The survey coordinator will prepare data sheets for each survey location and print the associated vegetation class on the data sheet. The fields shown in Table SOP 9.1 should be added to the data sheets and databases that are used for the survey. The database should provide a pick-list of all potential vegetation classes likely to be documented during the survey (these fields are included in the NWRS Bee Database).

Table SOP 9.1. Fields to be added to wildlife survey data sheets or databases.

- Sample Site ID # _____
  (Geographic coordinates should have been recorded with survey data)
- Survey Date _____
- Vegetation Class NVC Subclass: [Full name from the GAP database – to be filled in by the survey coordinator]
- Vegetation Class Ecological System: [Full name from the GAP database – to be filled in by the survey coordinator]
- Is site within the designated Ecological System? Y or N
- If not, what Ecological System is it in? (refer to local list)
- _____
- Is the site within 100m of an edge or ecotone? Y or N
- If yes, what is the secondary Ecological System? (refer to local list)
  _____
- Disturbances (from list, multiple disturbances can be recorded):
  _____
- Notes about the site:
  _____

  _____

Recording Disturbances

Disturbances, both natural and human-induced, can affect the condition of the vegetation and be observed at the survey location. In addition to verifying the associated vegetation class, the field observer should document disturbances (Table SOP 9.2). This includes any recent management or natural disturbances that have changed the structure or composition of the vegetation. The disturbance should be detectable by the field observer at the time of the survey; most observable disturbances will have occurred within the last two years. Some disturbances, such as tree blow-downs, may be visible much longer than two years and should be documented. If a recent

disturbance occurred (e.g. mowed), but there is no observable change to the expected structure or composition of the vegetation (vegetation has regrown), then do not record as a disturbance.

Categories of disturbance can be presented as a pull-down menu in the database and multiple sources of disturbance ($\leq$ 3) can be selected (Table SOP 9.2). 'No disturbance' is the default value.

Table SOP 9.2. Disturbances that may affect the structure and composition of the vegetation.

Disturbances	
Animal damage	Invaded by exotic species
Chained	Mowed
Construction: building	Plowed/Disked
Construction: road	Prescribed burn
Construction: trail	Treated with fertilizer
Destructive use (non-harvest)	Treated with herbicide
Drought damage	Treated with insecticide
Flooded	Wetland: drained
Forest: clear-cut	Wetland: fall drawdown
Forest: selective harvest	Wetland: spring drawdown
Grazed	Wildfire
Ice damage	Wind event/blow down
Insect damage	No disturbance

Workflow and Detailed Instructions for Documenting Vegetation Classes and Disturbances

- Download a GAP map for your region.
- Overlay your survey locations on the vegetation classification map and derive the NVC Subclass and the Ecological System associated with each location.
- Print the Subclass and Ecological System name on each datasheet along with the Site ID (Location name/number). Print a list of all Ecological Systems likely to be encountered at survey locations on the back of the data sheet as a reference.
- Enter the NVC Subclass and Ecological System name into the database when the locations are set up. Ensure that pick lists for the vegetation classes and disturbances are correctly set up in the database for data entry.
- Print the pick-list of potential disturbances (Table SOP 9.2) on the data sheet.
- Train observers to recognize, on the ground, the Ecological Systems associated with survey locations in the study area and any other potential Ecological Systems they may need to record.

- Field observers will verify, in the field, that the primary Ecological System assignment to each survey location is accurate or note on the data sheet what the correct classification should be (referring to the list on the back of the data sheet).
- Secondary Ecological System designations will be made on location (in the field) by the observer or recorder. *The secondary Ecological System is identified only if a different Ecological System is located within 100 m of the sample site.* Stated another way, locations that have secondary Ecological Systems have an edge or ecotone within 100 m. The error associated with many digital maps requires that this designation be made in the field. The secondary Ecological System name field in the database will be 'NA' as a default and will be updated as needed by the survey coordinator after field verification.
- Field observers will document up to 5 types of disturbances that they observe at the survey location on the data sheet; record 'none' if no disturbances are observed.
- Enter the vegetation classification information into the database, along with other field observations.
- Archive the GIS maps used to select the sample locations and the GAP maps used to assign the classes, along with other survey materials, in ServCat. This will allow for post-hoc analysis of attributes such as point count distances to edges, level of fragmentation, size of patches, etc., that may prove useful in the future.
- If the survey coordinator needs assistance with GIS maps and overlays, contact the AKN Node administrator, or the Refuge System Inventory and Monitoring Program for assistance.

References

Comer, P., D. Faber-Langendoen, R. Evans, S. Gawler, C. Josse, G. Kittel, S. Menard, M. Pyne, M. Reid, K. Schulz, K. Snow, and J. Teague. 2003. Ecological systems of the United States: a working classification of U.S. terrestrial systems. NatureServe, Arlington, Virginia. www.natureserve.org/library/usEcologicalsystems.pdf

NWRS Bee Survey Data Sheet

Station

*Refuge or Other Location		
***State**	***County**	***Country**
		USA

Site

Site Name	Elevation
Back 40	

*Decimal Latitude	*Decimal Longitude	*Datum
	-	

(e.g., WGS-84, NAD-83, NAD-27)

~ or ~

UTM Easting	UTM Northing	UTM Zone

Location Notes:

Ecosystem Type

NVC Subclass: [from the GAP map – to be filled in by the survey coordinator]		
NVC Ecological System: [from the GAP map – to be filled in by the survey coordinator]		
Is site within the designated Ecological System? Y or N (circle one)		
If not, what Ecological System is it in? (refer to local list)		
Is the site within 100m of an edge or ecotone? Y or N (circle one)		
If yes, what is the secondary Ecological System? (refer to local list)		
Disturbances (from list), multiple disturbances can be recorded:		
1)	**2)**	**3)**
Ecosystem Notes:		

*** Required**

Site:_____ Date:_____

Page:_____ of _____

Survey

Protocol Used	*Survey Coordinator
***Survey Method:**	
Survey Notes:	

Date/Time/Weather

Trapping Collection Start (time bowls, arrays, or other traps set out)

*Time	*Day	*Month	*Year	Temp	Wind	Weather Conditions	Phenology

Trapping Collection End (time bowls, arrays or other traps picked up)

*Time	*Day	*Month	*Year	Temp	Wind	Weather Conditions	Phenology
Nearest Weather Station:							

Survey Method – Traps (Bowls, Malaise)

*Batch ID	*Disposition	*Traps		Yellow		Blue		White	
		#	Failed	#	Failed	#	Failed	#	Failed
Associated Plants		**Scientific Name**						**Distance (m)**	

*** Required**

Site:_____ Date:_____

Page:_____ of _____

Survey Method – Netting or Observation

 # People Netting/Observing: _____ Batch ID:_____

Caught/Observed

*Label ID Species#	*Collector	*Specimen Disposition	Behavior	Associated Plant	#

*** Required**

Site:_____ Date:_____

Page:_____ of _____

Specimen ID

*Label ID	*Disposition	*Genus	Species	Subspecies	Sex	#

*** Required**

Look-up Values

Wind	MPH	Condition
Calm	< 1	Smoke rises vertically
Light Air	1-4	Smoke drifts and leaves rustle
Light Breeze	5-7	Wind felt on face
Gentle Breeze	8-11	Flags extended, leaves move
Moderate Breeze	12-18	Dust and small branches move
Fresh Breeze	19-24	Small trees begin to sway
Strong Breeze	25-31	Large branches move, wires whistle, umbrellas are difficult to control
Near Gale	32-38	Whole trees in motion, inconvenience in walking
Gale	39-46	Difficult to walk against wind, twigs and small branches blown off trees
Strong Gale	47-54	Minor structural damage may occur (shingles blown off roofs)
Storm	55-63	Trees uprooted, structural damage likely
Violent Storm	64-73	Widespread damage to structures

*e.g. Beaufort Wind Scale

Weather
Full Sun
Partly Cloudy (> 50% sun)
Partly Sunny (< 50% sun)
Cloudy (some shadow cast)
Completely Cloudy (no shadow)
Rainy

Phenology
Initial vegetation growth
Young leaves or needles
Leaves or needles
Colored leaves or needles
Falling leaves or needles
Flowers or pollen cones
Open flowers or pollen cones
Fruits or seed cones
Ripe fruits or seed cones
Recent fruit or seed drop

Behavior
Flying
Resting
Nectaring

Disturbance	
Animal Damage	Ice damage
Chained	Insect damage
Construction: building	Invaded by exotic species
Construction: road	Mowed
Construction: trail	Plowed/Disked
Destructive use (non-harvest)	Prescribed burn
Drought damage	Treated with fertilizer
Flooded	Treated with herbicide
Forest: clear cut	Treated with insecticide
Forest: selective harvest	Wildfire
Grazed	Wind event/blow down

Disposition
Observed
Collected
Held
Held - ID’d
Pinned
Sent to lab for ID
Held at lab
Held at lab - ID'd
Destroyed
Destroyed - ID'd


U.S.
FISH & WILDLIFE
SERVICE
NATIONAL
WILDLIFE
REFUGE
SYSTEM