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

VERSION 3.0 **MARCH 2025**

ON THE COVER
Four photo collage of native bees of the United States.
Photographs by: Joel Gardner

NWRS Survey Protocol Signature Page

Protocol Title: National Protocol Framework for the Inventory and Monitoring of Bees	**Version[1]:** 3.0
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Version[1]	Date	Author	Change Made	Reason for Change
2	2017	Droege, *et al.*		
3	2025	Maffei, *et al.*	Updated text of entire protocol framework and existing SOPs, and addition of new SOPs	To reflect latest science, align with cross-organizational standardization recommendations made by the National Bee Monitoring Research Coordination Network, and improve clarity and field-readiness of guidance

[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). Only two signatures are required: one from the submitter (lead author)[2] one from the approving official, which is dictated by the scope of the protocol[3,4,5].
[2] Signature of station or I&M representative designated lead in development of a site-specific survey protocol.
[3] Signature signifies approval of a site-specific survey protocol.
[4] Signature by Regional I&M Coordinator signifies approval of a protocol framework to be used at multiple stations within a Region.
[5] Signature by National I&M Coordinator signifies approval of a protocol used at multiple 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 found in the United States. The objective of this protocol framework is to provide guidance on how to select survey methods best suited to achieving bee inventory and monitoring goals on National Wildlife Refuges. The associated Standard Operating Procedures (SOPs) detail how to implement the selected field methods, process specimens, and manage data. The time and resources required for field collection, specimen processing, identification, and data management can be substantial. To that end, proper planning is essential to executing informative and impactful surveys. When executed well, surveys can provide crucial insight into the bee communities of a National Wildlife Refuge and help guide future management decisions.

This protocol framework updates the 2017 version, first developed in cooperation with the United States Geological Survey (USGS), the USFWS, and a worldwide network of bee researchers who have investigated study designs and methods for capturing bees. The updates made in 2024 incorporate the recommendations for bee protocol standardization made by the National Native Bee Monitoring Research Coordination Network (RCN). The RCN's multi-year collaboration of leading academic experts, government agencies, non-profit organizations, and the public to standardize bee monitoring in the U.S. provided the foundation for many of the updates herein and is referenced when more specialized protocols are required. Although this protocol framework was designed with the U.S. Fish and Wildlife Service (USFWS): National Wildlife Refuge System (NWRS) in mind, we welcome its use by our partners, as appropriate, for their bee inventory and monitoring objectives.

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

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Contents

List of Figures

List of Tables

Narrative

Element 1: Introduction

Background

Bees are an integral part of most ecosystems and are essential for the long-term persistence of flowering plant species via the pollination of trees, shrubs, and forbs. Bees provide a critical, yet often unrecognized, function in almost every aspect of wildlife and habitat management. This includes providing pollination services for the reproduction and survival of plants (Rodger et al., 2021) and the nectivores, herbivores, and frugivores that depend on those plants for forage. Pollinators can also be a forage source themselves for insectivorous birds and mammals. Because of the specialized nature of bees for their preferred (or sole) pollen sources, there is a direct link between biodiverse plant communities and a biodiverse bee community (Mathiasson & Rehan, 2020).

However, many bee species and communities are in decline due to the impacts of habitat loss, pesticides and herbicide use, pathogen spread, and climate change (Zattara & Aizen, 2021; Mull et al., 2022). Therefore, it is vital that the National Wildlife Refuge System (NWRS) maintain diverse habitats that support native bee communities on refuge lands. Standardized inventory and monitoring of bees is crucial for understanding the health of habitats and ecosystems within (and across) National Wildlife Refuges and thus guiding critical management decisions. Data collected on Refuges also contribute to the understanding of trends in bee fauna diversity, relative abundance, species richness, and distribution at a landscape scale.

Objectives

- The objective of this protocol framework is to provide general guidance on bee study design and standardized field methods that should be used to develop a site-specific protocol for a specific project. The site-specific protocol should incorporate station details such as survey locations and objectives.

- Methods found in this framework can be used to collect data on species occurrence, and basic phenology (period of adult activity) (Lebuhn et al., 2013; Gezon et al., 2015). When coordinated with an academic partner, they may also be applied to studies of relative abundance and species richness.

- The most appropriate field method is determined by project objectives. If your objective is a species inventory, combining passive (bowl traps) and active (netting) methods will best capture the bee community.

- This framework is NOT intended to provide guidance on study design for more complex projects such as estimating abundance, genetics studies, nesting studies, or statistically rigorous monitoring or efficacy studies. Although many of the methods described in this protocol would still be relevant, a more complex study design than is described here would be needed. We suggest referencing the Bee Monitoring RCN's 2025 protocols (*In* *review*, 2025; www.nativebeemonitoring.org) and consultation with the Refuge I&M Pollinator Team or another subject matter expert for guidance on study design and techniques for this type of study.

- Typical attributes to be measured during bee surveys, regardless of specific objective include:
  - Count of bees by morphospecies (groupings by morphological traits), genus, or species
  - Floral species being visited by different bee species (netting only)
  - Vegetation composition of sample plot
  - Count and relative composition of flowering plant species

- Application of this protocol framework should result in comparable data when applied across habitats and stations and provide the baseline information needed for developing management objectives.

Challenges

- *Designing your study and selecting field methods appropriately to enable inference regarding your question of interest.*
  It is often tempting to start with a method (e.g., bowl traps) and then design your study around that method. As is discussed later in this protocol, bee collection field methods all have different types of bias associated with them and are differentially suited for making inference, depending on your study question. There is not one design or method that is best for every project, and these topics are still debated in the scientific literature. The Bee Monitoring RCN is a useful resource for peer-reviewed literature on the state of the science (https://www.nativebeemonitoring.org/resources).

- *Properly processing insect specimens requires specialized equipment and training.*
  Field-based collection of bees is only one step in the bee identification process, and the one that requires the least training and expertise. All collected bees must be processed prior to identification, which requires specialized equipment and training. Project managers should plan and budget time and resources to either train their own staff to process specimens or to pay for a lab to provide that service. The Refuge I&M Pollinator Portal provides links to taxonomic experts, identification labs, training, and other relevant resources. Although there are labs that can both process and identify specimens, there will be additional costs incurred for processing.

- *Bee identification is costly, time consuming, and identification services can be difficult to attain.*
  It takes many years of training to be able to accurately identify most species of bees, and field staff cannot and should not be used for identification of lethally captured bees. In nearly all instances, bee specimens must be sent to a lab for expert identification. However, taxonomic identification services are extremely limited, leading to long turnaround times for identification. This is further exacerbated when identification experts are also relied upon for specimen processing. A realistic

expectation at this time is that it will take one to two years from the time specimens are collected to the time identifications are received back from a lab, depending on the scale of the project. In general, approximately 30 percent of the time required by a project that uses lethal collection is field work, and 70 percent of the time is lab and identification work. The timeline may be even longer in Western regions where there are considerable gaps in taxonomic guides and expertise. The notable exception to this timeline is the non-lethal capture of bumble bees, which are identified in the field and released.

- *Specimen management.*
  Specimens need to be properly organized and remitted to an appropriate insect research collection. This requires advance planning to identify a collections facility able to accept the specimens, time for prepping the specimens to the requirements of the facility, and the purchase of cabinets and unit trays for storage, prepping, and transport. The boxes that specimens are typically returned in from the identification lab are not sufficient or appropriate for long term storage. Although some specimens may be kept on the refuge for educational purposes, most must be sent to a collections facility as soon as identification is complete. Improperly stored and unprotected specimens will be eaten by dermestids and are vulnerable to mice, dust, and mold.

Element 2: Sampling

SOP 1 provides an in-depth discussion of sampling design.

Developing study questions and objectives

- Once you have decided to survey bees, it is important to define your survey objectives and identify desired data outputs. If you know how you will analyze the data and produce your summary information, the sampling design will be easier to determine. The project planning template below (Table 1) can be used to help clarify project objectives.

- Once you have answered the questions below, you can define your target population and select the appropriate data collection methods.

- Our recommended next step when initiating a new pollinator study is to reach out to the Natural Resource Program Center (NRPC) I&M Branch Pollinator Team. This team offers a complimentary advisory process whereby they can assist you with study design, methods selection, cost estimates, data management planning, and provide training materials, as needed. Submit this brief form and a pollinator team member will reach out you shortly.

> *The I&M Program Pollinator Site provides resources to assist with project planning & design, including project-specific guidance from the pollinator team.*

Although not required, the team will be able to provide more specific and comprehensive guidance if you have already completed Table 1.

Table 1. Project planning template to define project-specific objectives and desired outcomes. *It is recommended that a project identify a single objective and design the study to best meet that objective, as each objective may have different study designs.*

Do your project objectives include……	
A bee species list for the refuge?	
Determining whether a specific species or group of species are present?	
Determining whether any T&E bee species are present?	
Evaluating bee habitat quality and availability on the refuge?	
Understanding whether restorations or other management actions are yielding the expected results?	
Understanding how the bee species community has changed since an inventory was last conducted, or how it compares to neighboring land?	
Education or community outreach and engagement?	
Other (see Table 2 for other common project objectives)?	
Describe the data products you will generate…	
What type of data tables will you produce?	
How will you graph your data?	
What maps will you produce?	

Workflow

Field-based data collection is only one aspect of a complete bee study workflow. Additional important steps to anticipate and plan for include:

1. *Question development:* What information is your data intended to provide?

2. *Field methods selection:* Determined based on the question you want to answer.

3. *Field-based study design:* Dependent on your question, methods, budget, and staff resources. This should include the frequency of data collection and length of the study.

4. *Specimen processing and identification planning:* Subject matter experts for specimen processing and identification should be enlisted at the project planning stage before any data are collected. If specimens will be sent to an expert, determine who will process specimens and plan for appropriate resources and training.

5. *Data management planning:* A data management plan should be developed prior to data collection, including a plan for using standardized (BOMS/Darwin-core) data formatting, developing a database and data collection tools if needed, and uploading data to ServCat and GBIF. Reach out to your regional data manager for assistance on this aspect of project planning.

6. *Data analysis planning:* Analysis may be as simple as production of a table of species for a basic inventory, or it may require power analyses and advanced statistics for more rigorous research studies. Determining the length, complexity, and objectives of the study will inform analytical needs. Anticipated data products, such as maps, tables, graphs, and specific response variables, should be identified in advance of data collection. For complex analyses that may require outside assistance, reach out to your zone biologist or other partner before data collection begins. It is vital that study design is appropriate for the anticipated analysis. If in doubt, reach out to the I&M Pollinator Team, your zone biologist, or another expert data analyst during the project design stage.

7. *Collections management planning*: Develop a long-term storage plan for specimens.

8. *Field Training:* The hand netting process in particular requires practice to do successfully and efficiently. However, new field technicians benefit from practice locating plots, performing all field tasks, and learning the breadth of bee and vegetation species they might find during a survey. Approximately one to two weeks of training and practice prior to the beginning of formal data collection are recommended, including a few hours per day of hand netting practice. Ideally, initial hand netting training will be provided by an expert.

9. *Lab Training:* If technicians or other staff will be pinning and labelling captured specimens, some should be collected and sent to the identification lab for feedback and validation prior to the start of the field season.

10. *Data collection:* Conduct surveys and start sending specimens to your partner lab for processing and identification.

11. *Collections management:* Send specimens to your pre-determined partner institution, and document disposition (location) in reporting.

12. *Report writing:* A report may be written once at the conclusion of single year project or may consist of progress reports and a cumulative report for a multi-year project. If the project includes lab-based specimen identification it may be several years from the time data collection concludes until identification data is received. At a minimum, a progress report should be written to ensure preservation of project details. The frequency and extent of data analysis and reporting should be established in advance of data collection in coordination with the zone biologist, data manager, and/or inventory and monitoring coordinator.

13. *Data and report archiving:* Upload all data, reports, site-specific protocols, and other project materials to ServCat.

Capacity planning

As evidenced in the workflow outlined above, capacity planning may include field technicians, and staff or partners to process and identify specimens, manage and analyze data, and manage the collected specimens. When partnerships with labs or other experts are needed, those individuals should be contacted in advance to understand expectations, requirements, and cost.

Study design

Inventory & Monitoring

The most appropriate bee survey sampling design is contingent upon the question the project endeavors to answer. On Refuge lands, this question is often a simple inventory intended to understand species composition, or a series of inventories implemented before and after management actions, such as restoration, to evaluate efficacy. Typically, a statistically defensible pollinator monitoring study requires a sample size and degree of standardization that is beyond the resource capacity of a refuge. In most cases, the question of interest can be sufficiently answered through a series of inventories conducted at relevant time intervals. Understanding the composition and habitat 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. Question definition will guide sampling design and indicate when subject-matter experts should be engaged to assist with the design of more complex research studies (Table 2).

Research
Some refuges may be interested in conducting more in-depth research on genetics, nesting, specific plant-pollinator interactions, pesticide impacts, or a more rigorous evaluation of management impacts. For example, a refuge might want to understand the phenology of bee activity by vegetation community type to guide the timing or type of management actions implemented. The more complex study design required for this type of project, as well as some of the specific techniques, are not described in this protocol. We recommend the 2025 protocols produced by the Bee Monitoring RCN for this purpose, and consultation with a subject matter expert (e.g., university, USGS, or other partners).

Rare & Imperiled Species Considerations
At the time of writing, there are two bumble bees and seven Hawaiian yellow-faced bees on the endangered species list, and one bumble bee proposed for listing. Several bumble bees are considered on regional species of greatest conservation need (RSGCN) lists and solitary bees are receiving more attention as well. The bumble bee protocol included in this Framework reflects current requirements. If you think you might have T&E pollinators on your refuge or are designing a study to answer T&E species-specific questions, please connect with your local Ecological Services office for the most up-to-date guidance.

Table 2. Common Questions and Project Frameworks for Bee Studies on National Wildlife Refuges.

a. Common questions that may be answered with an inventory, or series of inventories

Common Study Questions/ Objectives	**General Design**
What bee species are found on my refuge?	Inventory—*include a representative sample of locations and habitat types*
What bumble bees (or other specific group) are on my refuge?	Inventory—*include a representative sample of locations and habitat types*
How has my refuge bee community changed from what was documented 10 years ago?	Inventory—*replicate the study design and locations of the original study*
How does bee species richness/composition on the refuge compare to that in the surrounding area, or other refuges in the region?	Inventory—*compare refuge inventory results between refuges, with GBIF, or similar data source for neighboring lands*
Am I achieving the desired results through restoration, native seeding, or other refuge management actions?	Before/ After Inventories—*the after-action inventories may take place at several time intervals to determine whether species richness or composition changes following the management action. This question could also be answered with just floral resource surveys (i.e., no bee surveys).*
Do I have any rare/ T&E species on my refuge?	Inventory—*target habitat types used by T&E species that might be expected to occur on the refuge.*
Do I have T&E species XX on my refuge?	Inventory—*target species of interest.*

Does the refuge provide high quality floral habitat for the bee community?	Inventory—*with surveys in spring through fall, and a vegetation assessment component. This question could also be answered with only vegetation assessments (i.e., no bee surveys).*
Does the refuge provide the specific habitat characteristics required for rare and imperiled pollinator species?	Inventory—*targeting specific habitat type of interest, with a vegetation assessment component. This question could also be answered with only vegetation assessments (i.e., no bee surveys).*
Which bee species are visiting T&E plants on the refuge?	Inventory—*targeting specific rare and imperiled plants of interest.*
The refuge would like to use bee surveys as a tool for education or community outreach and engagement	Inventory—*this objective may contribute to a larger refuge bee inventory objective with proper planning.*

b. ***Common project types that require a more complex study design and likely partnering with a subject-matter expert (e.g., University, USGS, etc.)***

Common Research Project Types	**Partners that have supported this project type on refuges in the past**
Genetic data collection	USGS, USDA Logan Bee Lab, Oregon State University
Investigation of eDNA and other novel non-lethal collection methods	USGS
Pesticides impacts analysis	USGS
Statistically rigorous investigation of management action efficacy	University partners (e.g., University of Minnesota)
Use of drones to evaluate habitat	Monarch Joint Venture
Statistically rigorous monitoring of changes in species richness, composition, or a specific species' population status over time	University partners, USGS, State partners
Species-specific or habitat surveys to support Species Status Assessments	Ecological Services, State partners, University partners

Capture Methods: Bowl traps versus hand netting

Bowl trapping techniques and hand netting are the primary bee sampling methods described in this document. Both bowl trap and hand netting methods have bias associated with the species they capture and the area they effectively sample. In short, bowl traps tend to disproportionately sample smaller bees, whereas netting disproportionately samples larger bees. Passive traps have the potential to collect bees from a large geographic area and may represent bees in the broader landscape, while a netting event tends to collect specimens visiting the habitat being surveyed at that point in time. In terms of staff time requirements, bowls can be put out at any time of day, but netting can old be done during certain times. Additional details about sources of bias associated with each method are described below.

Bowl traps and hand netting should be implemented simultaneously to conduct a thorough inventory—neither method accurately represents bee species composition when used alone. If your study objective is not a species inventory, the appropriate method(s) should be selected to fit your study objectives. For example, if you are primarily interested in documenting bumble bee species then hand netting would be the most appropriate capture technique. These same limitations and biases should be considered when designing a more complex study: unless your objectives are specifically monitoring sweat bees, or other small bees, bowl traps alone are likely insufficient.

An additional consideration for Refuges is the time and cost associated with these two sampling methods. Bowl traps have considerably higher capture rates than hand netting, resulting in a greater number of specimens to be processed and identified and, consequently, a higher cost and longer timeline until those identifications are completed. If the cost and timeline for identification of lethally collected specimens is outside of the tolerance or capacity of the station, the study question may need to be adjusted accordingly. For example, rather than an inventory of all bee species the refuge may elect to inventory bumble bee species specifically, which can be captured using non-lethal techniques, identified in the field (with training), and then released. We recommend reaching out to the Refuge I&M Pollinator Team or another expert for assistance in determining the necessary method(s) to answer your question of interest.

<u>Bowl Traps: Sources of Bias</u>

- Bowl traps tend to oversample small bees (e.g., 5 to 14 mm long, especially sweat bees, also mason bees) and under sample large bees (e.g., bumble bees).
- Bowl traps may capture more bees when floral abundance is low (Kuhlman et al., 2021; Lezzeri et al., 2024).
- Bowl traps do not collect all bee species in direct relationship to the population size and are consequently poor indicators of bee abundance.
- Bowl traps can capture rare and endangered species and should not be employed when listed species are thought to be present.
- Bowl traps tend to sample a wider area and are better suited for landscape-level questions than habitat-specific questions.
- Bowl traps can be knocked over by wildlife or extreme weather, be inundated by rain, or can dry out in arid conditions.

<u>Hand Netting: Sources of Bias</u>

- Netting disproportionately samples larger, more conspicuous species (e.g., bumble bees, carpenter bees, 15 to 25 mm long), therefore excluding smaller species from species composition and richness estimates.
- Netting may capture more species of bees when floral diversity is high (Kuhlman et al., 2021; Lezzeri et al., 2024).

- Netting does not collect all bee species in direct relationship to the population size and are consequently poor indicators of bee abundance.
- Catch rates may be influenced by the weather at the time of netting if care is not taken to standardize conditions under which netting is permitted.
- Netting capture rates and species representation can exhibit observer bias, such that more experienced observers detect more species and individuals than inexperienced observers and species detected varies among observers regardless of experience (Krahner et al., 2021). For example, collecting rare, parasitic, and highly specialized bees often requires a keen eye and sufficient practice.

Non-lethal sampling

The extent to which large-scale and long-term lethal sampling may negatively impact bee populations is currently a subject of debate in the scientific literature (Portman et al., 2020; Miller et al., 2022; Montero-Castaño et al., 2022). Currently, however, most bees can only be accurately identified to species under a microscope, requiring lethal collection of specimens. As databases of DNA barcodes and high-quality reference photographs are expanded, alternatives for species identification may become more viable (Levenson et al., 2024). Nevertheless, this dilemma underscores the importance of intentional, question-driven study design and method selection. If a lethal collection method is most appropriate to answer your question of interest, careful study design should be used to collect sufficient data while minimizing the potential to cause harm to the population.

Non-lethal methods are particularly attractive because it avoids issues with harm to populations and avoids the logistical issues of collections management. The primary method used is non-lethal hand netting and is implemented when the species or community being investigated can be readily identified in the field, or when morpho-group is an acceptable level of taxonomic detail. Bumble bee communities (*Bombus*) are the group most often investigated using this method, as most species can be identified in the field and many species are listed as threatened or endangered (T&E). 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 hand netting may also be used when there is a concern for impacting populations of rare species or vulnerable life stages, or when lethal capture might be perceived poorly by stakeholders. For example, non-lethal hand netting may be preferred for spring sampling, when emerging bumble bee queens are most vulnerable, or for volunteer-conducted surveys.

Environmental DNA (eDNA) is a type of non-lethal collection technique whereby organismal material (e.g., hair, excrement, etc.) is collected from flowers, and genetic barcoding is used to identify the bee species to which that material belongs. A key prerequisite for bee species identification from an eDNA sample is the existence of a sufficient bee genetic reference database to connect the sample with. This technique appears promising in that sampling requires much less time and can be more widely applied than methods that require capture of bees, but is limited by, among other things, lack of a genetic reference database for many species. At the time of this protocol update, the science of applying eDNA to bee studies is still in its infancy and further guidance for application in field studies is therefore not provided herein.

Methods Selection Decision Tree

To begin, refer to Table 2 above to determine whether your question of interest is considered an Inventory or Research study. Then follow the decision tree below to determine survey methods (Figure 1). Please note that even though the same field techniques can be applied to a variety of study questions, study design should be contingent upon your question of interest and will inherently differ by project goal.

1. Will you have access to taxonomic experts and collections management resources?
    a. Yes—go to (2)
    b. No→ You may be limited in your ability to answer your question of interest. The following decision tree is designed to help you select the most appropriate methods to answer different types of study questions. If you find that the recommended technique for your study question includes lethal collection, we recommend reaching out to the Refuge I&M Pollinator Team or another expert for further guidance. Lethal collection should not be undertaken in the absence of resources to support expert identification and collections management. In this situation, consider narrowing your study question or partnering with an institution that can provide identification and collections support. Go to (2)

2. Is your species/ group of interest rare, threatened, or endangered, or are you surveying in a location where T&E bees or proposed species are known to exist? If unsure, reach out to a subject matter expert or your regional Ecological Services office for guidance. Tools such as IPaC or The Bee Tool can help this question as well.
    a. Yes→*You will need to reach out to Ecological Services and a species expert for the appropriate permits and protocols for this project. Additionally, if you find that the recommended technique for your study question includes lethal collection, you will need additional study design guidance.* Go to (3)
    b. No—go to (3)

3. What type of project are you planning? (*See Table 2 above)*
    a. Inventory—go to (3A)
    b. Research—go to (3B)

(A) Is the goal of your inventory to….
- i. Understand something about specific species or groups?—go to (4)
- ii. Understand something about the entire bee community?—go to (4)
- iii. Understand something about plant-pollinator relationships?—go to (7)
- iv. Understand something about pollinator habitat—go to (7)

(B) Your project requires a more complex study design and, in some cases, more specialized techniques than are described in this protocol. We recommend consulting with the Refuge I&M Pollinator Team or another subject matter expert for guidance on design, sample size, and techniques.

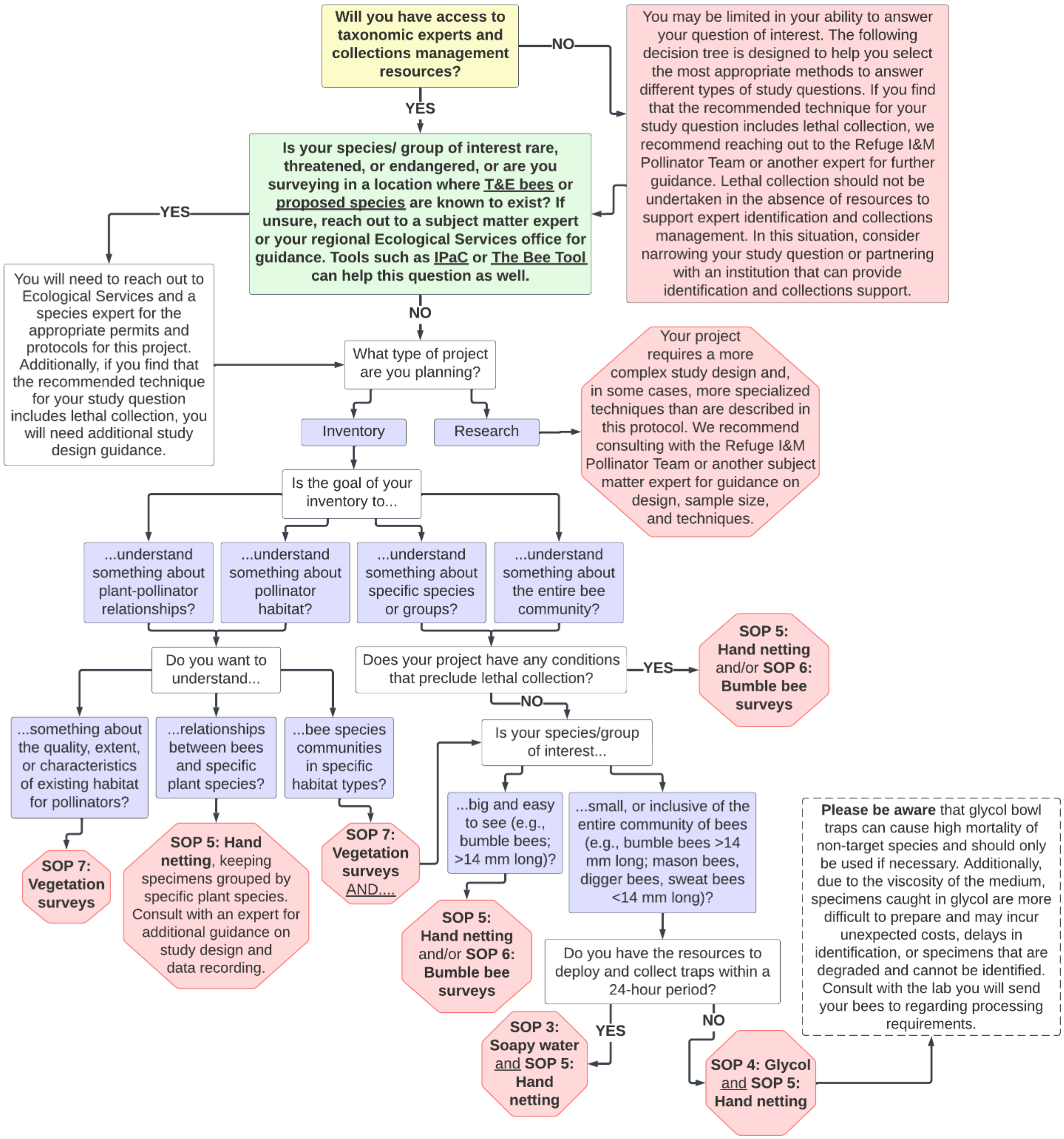
4. Does your project have any conditions that preclude lethal collection?
   a. Yes→ *Hand netting (SOP 5)* and/or *Bumble bee surveys (SOP 6)*
   b. No—go to (5)

5. Is your species/ group of interest….
   a. Big and easy to see (e.g., bumble bees; >14 mm long)→ *Hand netting (SOP 5)* and/or *Bumble bee surveys (SOP 6)*
   b. Small, or inclusive of the entire community of bees (e.g., bumble bees >14 mm long; mason bees, digger bees, sweat bees <14 mm long)—go to (6)

6. Do you have the resources to deploy and collect traps within a 24-hour period?
   a. Yes→ Bowl Traps: *Soapy water (SOP 3)* **and** *Hand netting (SOP 5)*
   b. No→ Bowl Traps: *Glycol (SOP 4)* **and** *Hand netting (SOP 5).* Please be aware that glycol bowl traps can cause high mortality of non-target species and should only be used if necessary. Additionally, due to the viscosity of the medium, specimens caught in glycol are more difficult to prepare and may incur unexpected costs, delays in identification, or specimens that are degraded and cannot be identified. Consult with the lab you will send your bees to regarding processing requirements.

7. Do you want to understand….
   a. Relationships between bees and specific plant species? →*Hand netting (SOP 5)* keeping specimens grouped by specific plant species. Consult with an expert for additional guidance on study design and data recording.
   b. Something about the quality, extent, or characteristics of existing habitat for pollinators? → *Vegetation Surveys (SOP 7)*
   c. Bee species communities in specific habitat types?—go to (4) **and** *Vegetation Surveys (SOP 7)*

Figure 1. Field methods selection decision tree.



Sampling units, sample frame, and target universe

Identifying the question you want to answer through data collection is critical to designing a study with the appropriate target universe. Once you have defined your necessary scope of inference, understanding what constitutes a sampling unit (i.e., unit of analysis) and the geographic and temporal limitations of different techniques will help you choose field methods and design your study appropriately. The following discusses some of the key considerations of study design in terms of the scope and limitations of different techniques, trap placement, and sampling frequency.

- *Target Universe.* The target universe refers to the population, community, or geographic area to which conclusions or inferences will apply. Also commonly referred to as *scope of inference.* For example, your goal may be to inventory bees on the entire refuge or just in a specific habitat type. Identifying your target universe will help you select the appropriate field methods and sampling frequency.

- *Sampling Unit.* The spatial area, such as a transect, in which data are collected and analyzed. The sampling unit can be a collection of traps (e.g., transect or an array) or a discrete netting event. The sampling unit is NOT the specimens associated with a single bowl or flower species. For this protocol we define site, plot, and transect as follows:
  - **Transect—**A sampling path, either linear or meandering, located entirely within one habitat type, that falls within a plot or site.
  - **Plot**—an area that could be sampled via a transect, also located entirely within one habitat type. There may be one or more transects in a plot.
  - **Site**—an area containing one or more plots, which may contain multiple habitat types, and is generally defined by what you are attempting to characterize (often a management unit or defined by ecology). A site could be the entire refuge, or a refuge could contain multiple sites, depending on your question of interest.

- *Zone of Influence.* A bowl trap's, or sampling unit's, zone of influence is the area within which it is visible, detectable, and able to entice or trap bees. Bowl trap transects should be at least 100 m apart to avoid influencing each other, and at least 1.5 km apart to be considered independent samples. Bowl trap transects placed within 100 meters of each other have potential to compete because they both fall within the foraging range of the same individuals. Consider the zone of influence of bowl traps when choosing where to place them, and whether the placement will sample the community you are interested in.

- *Sample Frame.* The sample frame is the entire spatial area with which the sample may be associated. This can be a specific habitat patch, management unit, or the entire NWRS station. It is important to recognize that sampling methods impact the extent of your sample frame and the inferences that can be drawn. For example, hand netting is more correlated with local habitat factors, while passive methods (bowl traps) are more associated with landscape factors (Rhoades et al., 2017). Given this, if your objective is, for example, to compare bee habitat use between two habitat types or management units on a Refuge then hand netting is more likely to reflect bee compositional differences, whereas bowl traps are likely to capture bees from non-target habitat types.

- *Sampling Timeframe: Trap Duration.* Trap duration refers to how long a bowl trap can be left out to collect specimens. 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 wildlife or other events. In general, bowl traps with soapy water can be deployed at dawn and retrieved at dusk or left out for a full 24 hours and are therefore considered short term traps. Bowls with propylene glycol can be collected weekly, due to slower evaporation rates, and are therefore considered long term traps. However, there is risk of trap disturbance during that time. Transects using soapy water bowls can be re-deployed once every 1-2 weeks throughout the sampling period.

- *Sampling Timeframe: Seasonal Duration.* Seasonal duration refers to how often, and throughout how much of the survey season, you should sample. Bee species composition changes throughout the survey season, often following important shifts in habitat floral phenology. A comprehensive species inventory can only occur through repeated sampling that captures the entire floral resource availability period for the vegetative communities of interest. Some objectives seek to understand the entire seasonal community, while others may focus on critical periods in the habitat's phenology (for example, spring flowering in Eastern deciduous forests). Whole-season sampling—that is, sampling that occurs throughout the survey season rather just once or a few times—will capture more uncommon and rare species, provide better relative abundance data, and provide more useful phenology information compared to short term or discrete sampling periods.

Sample selection and size

If bowl traps are being used, the sampling unit should be placed where it can be reasonably deployed and monitored. An accessible location that is representative of the habitat for which management objectives are being investigated can work as a sampling site, assuming the site is relatively open and has a variety of vegetation, structure, and substrate. Depending on objectives, this basic sampling scheme can be modified to sample specific habitats, compare types or intensity of management regime, or target specific plant-pollinator relationships.

A standard sample unit for a baseline inventory study typically consists of some combination of:

- <u>Soapy Water Bowl Traps:</u> 30 bowls, 5 m apart, in a linear transect (10 each of blue, yellow, & white bowls) for 8-24 hours
- <u>Hand netting:</u> Walking a 150 m transect for a 30-minute sampling period.
- <u>Propylene Glycol Bowl Traps:</u> 9 traps, 5 m apart, in a linear transect (3 each of blue, yellow, & white traps) for up to 2 weeks.

Because objectives vary, there is no set standard for the number of sampling units per study site, or the specific combination of the above techniques that is most appropriate. The number of sampling units, spatial configurations, and locations are determined during development of the station-specific protocol, and the combination of field methods should be selected based on the question of interest. It is recommended that bowl trapping and hand netting are jointly implemented if the goal is a comprehensive bee inventory for the study area (Cane et al., 2000; Roulston et al., 2007; Wilson et al., 2008). Multiple transects within the same area or habitat should be placed at least 100 meters apart to avoid influencing each other's capture rates.

Survey timing and schedule

A survey, consisting of one or more sampling periods, can be as short as a specific plant species' flowering period, or as long as the native floral blooming season. The survey season for bees will coincide with bees' spring emergence and end when bees are no longer flying, foraging, or become uncommon (often this occurs several weeks prior to the first hard frost in temperate locations). 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 two to six week window in which they are active and available for collecting as adults (Linsley, 1958).

Since both flowering plant and bee communities turn over throughout the season, it is necessary to conduct multiple surveys over the length of the active growing season to if the goal is a comprehensive inventory. As such, it is important to consider the phenological start of flowering for your community of interest and to time re-survey intervals to coincide with changes in species flowering over the season. A reasonable default in any region is to begin sampling when flowering plants begin to bloom and stop at that first frost. If a station's objective is to monitor shifts in phenology, scheduling sampling events one week or so early would be advantageous.

The suggested sampling frequency to attempt an inventory of the full bee community throughout the season is every two to three weeks for soapy water bowls and hand netting. If locations are inaccessible and alternative sampling techniques are not feasible, continuous operation of glycol bowl trap arrays is an option. A reasonable guideline is that the ideal number of sampling events is the number of weeks in the active bee flight season (i.e., the floral resource availability season) divided by four (Levenson et al., 2024). This frequency should be adjusted to fit project-specific objectives, such as inventories of a specific habitat type or monitoring studies.

Weather conditions can dramatically impact the types of bees observed as well as influence floral phenology. We provide general guidelines of appropriate weather conditions in which to conduct a survey in the following SOPs. However, prevailing conditions often vary by region (windier in the Great Plains, cloudier in the Northwest) and it may be necessary to modify based on specific location. Under these circumstances it is important to try to be as consistent as possible in the conditions under which you are surveying.

Sources of error

As station objectives and expectations become more specific and complex, increasing sources of error need to be considered. Regardless of methods, results can be affected by weather, observer bias/expertise, sampling effort, inter- and intra-annual variation in population life cycles, and regional trends. Method-specific sources of error are discussed in Element 2 (above).

Element 3: Field Methods and Processing of Collected Materials

Pre-survey logistics and preparation

SOP 2: Equipment and Preparation includes detailed information on survey preparation and equipment.

Establishment of sampling units

The same sampling locations are typically maintained from year to year when a monitoring program is established. In most cases, inventories should be conducted in new locations to maximize discovery of different species.

- Record the coordinates at the center of each sampling site.
- For transects, start and end points may be recorded as coordinates.
- Transects may meander if habitat conditions (excessive shading, terrain) dictate. If so, include a written description 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 the sample unit to be replicated upon subsequent visits. Consider whether the survey location will be mowed, burned, grazed, or frequented by elk or other ungulates that might eat flagging or rub on posts when determining the feasibility and type of survey marker to use.
- Thick grass, especially non-native species, can form near-impenetrable barriers to movements of small bees and conceal bowl traps. Consider elevating traps where fast-growing vegetation may impact bee movements and describe any sampling adjustments in the data sheets.

Data collection procedures (field)

SOPs 3, 4 and 5 describe how to setup and collect samples using soapy water bowl traps, glycol bowl traps, and hand nets. These three survey techniques differ in design and data collected and are often used in combination when the goal is a species inventory (Table 3).

Regardless of the survey method selected, it is imperative that all site and survey-specific information is documented during each survey event. *SOP 8: Data Collection and Management* details the data fields that should be collected by all projects and the Biotic Observation Minimum Specifications (BOMS) format in which data should be collected.

Table 3. Sampling Considerations by Field Method

	Bowl Traps: Soapy Water	**Bowl Traps: Glycol**	**Hand netting: Lethal**	**Hand netting: Non-lethal**
Survey Type	Lethal	Lethal	Lethal	Non-lethal
Standard Sample Unit	30 bowls	9 bowls	150 m transect for 30 minutes	30-minute meander of target area
Sample Frame	Landscape-level	Landscape-level	Habitat-specific	Habitat-Specific
Sample Location	Representative but convenient locations	Representative but convenient locations	Within habitat of interest	Within habitat of interest
Species Targeted	Small bees (5-14 mm)	Small bees (5-14 mm)	Large bees (15-25 mm)	Bumble Bees
Trap Duration	1 day (Dawn to dusk)	1-2 weeks	N/A	N/A
Trapping frequency	Once every 2-3 weeks	Continuously, with weekly trap checks	Once every 2-3 weeks	3x for counts, 6x for presence/absence
Field time required for set up and sampling upon arrival at plot	1 hr/ transect	1 hr/ transect	1 hr/ transect	1 hr/ transect
Relative Target-species Capture Volume per Time Invested	Medium	High	Low	Low
Relative Mortality Rate of Non-target Species	Medium	High	Low	None

Processing of collected materials

Bee specimen processing and identification

Decisions regarding bee specimen processing, identification, and collections management should be made prior to beginning any field work. The Survey Coordinator should develop an agreement with an authorized facility such as a lab, museum, university, or other research organization to process, identify, and archive any field specimens as necessary (per policy: 701 FW 5). This agreement must identify whether the station or the third party will be processing specimens prior to identification, and who will archive the collected specimens after identification. These entities typically have guidelines for how samples should be handled prior to receiving them, as this may influence the identification procedures, time-investment, and cost. Additionally, SOP 9 contains detailed information on how to prepare bees for shipping, identification, and archiving. ***If the project does not develop an agreement with an authorized facility prior to collecting data, their specimens might not be accepted.***

Bee identifications can be time-consuming and costly. It should be expected that it will take approximately 2-5 years from the time of collection for a field station to receive species-level identification data back from a lab, depending on the lab and number of specimens collected. Therefore, it is imperative to understand the level of identification needed to achieve the desired objectives. 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. Conversely, projects that aim to construct species lists or attempt to determine the presence of rare or uncommonly detected species may attempt to identify all specimens to species. Identification needs should be a key consideration during objective setting and study design. *All lethal collections must coordinate accordingly for the specimens to be identified to species level.*

Stations may also attempt to identify some easily identifiable specimens themselves after proper curation. Native bees can be difficult to identify to the genus and/or species level and require adequate training by working with other people or groups that are experienced in bee identification. It requires many years of specialized training to identify many bees to species. For those stations with capacity to begin preliminary identifications themselves, see *SOP 10: Identifying Collected Bees*. If NWRS stations plan to identify specimens on their own, their identifications will be considered provisional until reviewed by a taxonomic expert who can confirm the identification of specimens.

If threatened or endangered species (including other invertebrates) might be collected, contact the Regional Ecological Services Program prior to the beginning field work 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. Regional or local species lists may be available from state wildlife agencies or GBIF (Global Biodiversity Information Facility).

Collections management and disposition

All specimens collected lethally need to be properly curated, identified, and eventually archived within a proper institutional insect collection. Specimens held on-site should be stored in appropriate temporary pinning boxes, such as Schmidt boxes or equivalent. These boxes should then be stored in large sealable plastic bags or freezers to prevent infestation with collection pests, such as dermestid beetles. Due to the lack of air flow in sealable bags, allowing specimens ample time to dry, and storage in a humidity-controlled space, is recommended to avoid issues with rot and mold. If specimens are not kept in an appropriate storage facility, they should be frozen at -20° C for three days every six months to minimize pests. After the specimens are identified, they should be transferred to an institutional insect collection as soon as possible. Regional Universities and Natural History Museums often maintain large insect collections of the surrounding insect fauna and are an appropriate place for archiving insect specimens. These institutions are historically underfunded, so planning to share the cost of specimen archiving should be part of the project budgeting process. Official collections require cabinets, wooden drawers, unit trays, and curators time to accession specimens. The location ("disposition") of archived specimens should also be documented and included in the annual ServCat record.

End-of-season procedures

Upon season completion, temporary markers and equipment should be removed from the site and permanent transect or array markers should be secured in place for the off-season. All traps should be removed, cleaned, repainted if needed, and stored dry. Glycol bowl trap stands should also be removed and stored to increase their longevity. Data should be digitized, archived, and secured following guidance in *Element 4* and *SOP 8.*

Element 4: Data Management and Analysis

Data Management Plan: Data archiving & dissemination

All major data collecting endeavors require the creation of a Data Management Plan (DMP) per 274 FW 1. A data management plan "describes the data that will be acquired or produced; how the data will be managed, described, and stored; what standards will be used; and how data will be handled and protected during and after the completion of the activity where the data are involved." Your regional data managers can assist you in developing and filing a DMP.

Data should be digitized regularly and archived per your Data Management Plan. This data not only includes tabular data, but also the Site-Specific Protocol, maps, data forms, and project reports. Original and backup copies of raw and electronic data should be stored in a secure location. The Survey Coordinator will archive raw and digitized survey data, field notes, and photographs in compliance with relevant USFWS data standards and pursuant to the USFWS Policy on Service Information and Technology Architecture (270 FW 1) and the USFWS Policy on Electronic Records (282 FW 4). Details of archiving are described in *SOP 8: Data Collection and Management.*

As part of the National Wildlife Refuge Program, ServCat is an important aspect of archiving and preserving data. Any Data Management Plan should include developing a ServCat file structure and a process to upload data products into ServCat. For more information regarding the Inventory and Monitoring Branch's official policy regarding ServCat, see here.

In an effort to make pollinator data more publicly available, we recommend working with partners to upload final data products into a public biodiversity database such as GBIF or Symbiota. If specimens are sent to an external partner for identification or collections archiving, that facility may provide this service. Discuss a plan for data integration into a "free and easy access" external database with the partner facility prior to initiating data collection. The only exception to this should be sensitive data for T&E species.

Lethal Collection Data Considerations

Collections involving lethal sampling must contact a reputable taxonomist before starting surveys to establish an agreement around specimen identification and data products. While independent contractors may be appropriate for identification, it should be noted that individuals have had difficulty navigating federal contracts without institutional support. Taxonomic specialists often have limited capacity and engaging them early in the project process is necessary to ensure identification needs will be met.

If identification (and/or other services) are being provided by Museums, universities, or research organizations, it is important to note that they often have their own databasing systems and will prefer to utilize these systems for all bee data and identifications products. All federally-funded data must adhere to Free and Easy Access, which mandates that data are stored in repositories that provide *broad, equitable, and maximally open access to datasets and their metadata free of charge in a timely manner after submission*, per the 2022 Guidance on Desirable Characteristics of Data Repositories for Federally Funded Research. Data agreements to ensure these policies are followed need to be established prior to collection; often these will be part of a broader contract or cooperative agreement. For additional information on data entry and management, and for example data collection sheets, see *SOP 8: Data Collection and Management.*

Metadata

Metadata provide the information needed for other biologists to interpret and analyze the data you have collected. Data should be formatted using BOMS (Biotic Observation Minimum Specifications), and a template is provided that includes field formatting and metadata for common data fields. Adopting the BOMS format allows data to be easily compared and visualized across projects and integrated into external databases such as the Global Biodiversity Information Facility (GBIF). Additionally, a survey record should be created in the Planning and Review of I&M Activities on Refuges (PRIMR) database to store survey-level metadata.

Analysis methods

The data analysis has four main functions: (1) Provide basic summaries of the data, intended for use in quality control and annual reporting; (2) Analyze occurrence, relative abundance, annual variations, or long-term trends for species (3) Investigating elements of bee community biodiversity, including: species richness, diversity, and composition and (4) Examining potential differences among experimental treatments of the aforementioned measures, such as differences in species diversity between multiple habitat 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 should be conducted as needed to meet project objectives.

Element 5: Reporting

Implications, application, & schedule

A project report should be written upon study conclusion. This may include a species list when the objective is a species inventory, or more complex analyses relating species observed to vegetation type or management action history. If the project is meant to inform refuge management practices, annual meetings with refuge staff should be held to determine whether the data being collected is appropriate to do so and to interpret results as they pertain to management decisions.

The USFWS encourages the Survey Coordinator pursue publication of significant findings in peer-reviewed journals (Policy 115 FW 1) and to share their reports with the NRPC I&M Pollinator Team directly or through their Regional Pollinator or I&M Coordinator.

Report archiving

Reports should be archived in the USFWS Service Catalog (ServCat) and/or per the Data Management Plan.

Element 6: Personnel Requirements and Training

Roles and Responsibilities

The Survey Coordinator oversees and coordinates the implementation of the survey protocol, leads surveys, and ensures that survey data are managed, analyzed, reported, and archived properly. They provide biological expertise, advice on station-specific implementation of the SOPs, and report findings to station staff and leadership. The field crew will be responsible for implementing field methods specific to the project, specimen collection and preparation, and data entry.

Training

Staff involved in bee I&M activities should receive broad training in all aspects of the project and more detailed training for tasks specific to their role. Examples of skill sets biologists conducting pollinator work often need training in:

1. Insect identification
2. Plant identification
3. Specimen curation
4. Sampling – especially as it pertains to hand netting.
5. Specimen management

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 important.

The Pollinator Field Sampling and Laboratory Techniques course (CSP 22225) is periodically offered at the National Conservation Training Center (NCTC) and provides a general overview of pollinator survey and laboratory techniques. In addition, this document's SOPs and the Refuge I&M pollinator page provide links to other training and technique resources. All project staff should receive adequate training in the specialized techniques and foundational field skills relevant to the project prior to the field season.

We recommend that those who know they are severely allergic to bee stings or bee venom not participate in aspects of these surveys that involve handling bee specimens. However, it is possible to have an allergic reaction to bee stings or venom even with no prior allergic response. First aid training and availability of an on-site EpiPen is advisable.

Element 7: Operational Requirements

Budget

Field & lab equipment: Field and lab equipment lists specific to the techniques described in this protocol are detailed in SOP 2 and the method-specific SOPs. Although this needs to be budgeted for, the costs of most field and lab equipment are low relative to the costs of staff time and specimen processing and identification services.

Staff time: A two-person team can be expected to set up three to six soapy water bowl trap transects, or 6 or more glycol bowl traps transects per day, depending on the distance between them. Collecting trap specimens, hand netting, and recording the associated data can be expected to take two hours per survey location, depending on whether multiple techniques are employed at a single location. Note that soapy water bowl traps need to be set up early in the morning and retrieved shortly before sunset or the following morning, constraining the hours available to complete field activities.

At a minimum, additional time should be budgeted for travelling to and between sampling locations, data entry, verification, management, and analysis, as well specimen processing, packaging, mailing, and identification.

Third party services: The costs associated with sending specimens to an external institution for processing, identification, and/or collection storage can be significant. The Survey Coordinator should communicate with these institutions well in advance of initiating data collection to budget appropriately and to determine the scale of the field effort that will be financially feasible. An external institution may not accept specimens if they are not contacted prior to data collection.

Coordination

The Survey Coordinator should engage with the NRPC I&M Pollinator team during project development for guidance and resources on study design, protocols, and training. They should also work with their regional data manager prior to the field season to establish a database and data collection tools. Data analysis needs should be coordinated with the zone biologist. Finally, the Survey Coordinator should communicate with field station staff prior to the field season to select survey locations that will not conflict with management activities that may bias data (e.g., mowing, burning, herbicide application).

Element 8: Glossary of Terms

BOMS— Biotic Observation Minimum Specifications - a standardized data format derived from Darwin Core definitions that allows data to be easily compared and visualized across projects and integrated into external databases such as the Global Biodiversity Information Facility (GBIF).

Flight period— The seasonal activity period for a bee or bee species. An average individual bee might have a flight period of 2 - 6 weeks. Some species have longer flight periods, and some might have both a spring and fall flight period.

Glycol traps— Propylene glycol in bowl traps, used for whole-season sampling.

Inventory— A survey that estimates the presence, abundance, or distribution of bee species, habitats, ecological communities, or abiotic features during a particular time period.

Monitoring— A survey repeated through time to document changes in select attributes of bee communities or habitats. Baseline, or surveillance, monitoring documents changes over time but is not tied to specific predictions of a how a natural resource will respond to management or environmental stressors. Monitoring to inform management involves defining threshold values or the expected response and surveying to measure that response (U.S. Fish and Wildlife Service, 2013).

Morphospecies—A group of bees that are distinct from other species based on physical characteristics but whose exact species identity is unknown. This simplified classification system can be used to answer some basic questions about diversity.

Plot— An area that could be sampled via a transect, located entirely within one habitat type. There may be one or more transects in a plot.

Sample frame— The entire spatial area with which the sample may be associated - a specific habitat patch, management unit, or the entire NWRS station.

Sample unit—The transect along which specimens are collected from bowl traps or hand netting.

Seasonal duration—How often, and throughout how much of the survey season, you should sample.

Short-term trap—Bowl traps that must be collected within 24 hours of being deployed. A soapy water bowl trap is a short-term trap.

Site— A sampling area containing one or more plots. A site could be the entire refuge, a management unit, or defined by ecology.

Soapy water bowl traps—Traps for lethal bee sampling characterized by blue, yellow, and white cups each containing a mixture of dishwashing liquid and water.

Target universe— Refers to the population, community, or geographic area to which conclusions or inferences will apply. For example, your goal may be to inventory bees on the entire refuge or just in a specific habitat type and determining that target universe will guide sampling methods and locations.

Transect— A sampling path, either linear or meandering, located entirely within one habitat type, that falls within a plot or site.

Trap duration—How long a trap can be left out to collect specimens.

Whole-season sampling- Sampling repeatedly or continuously throughout the survey season to document bee composition throughout the entire floral resource period.

Zone of influence— The area within which a bowl trap or sampling unit is visible, detectable, and able to entice or trap bees.

Element 9: References

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Standard Operating Procedure 1: Sampling Design

The most appropriate sampling design is contingent upon the study objective. If the study objective is a comprehensive bee species inventory for either the whole refuge or for a specific habitat type, it is recommended that hand netting and bowl trapping are jointly implemented to maximize the breadth of species captured by sampling.

If the resources exist to inventory the entire Refuge, the sampling area can be stratified by habitat type for sampling. Habitat classifications relevant for stratifying bee communities should focus on plant floral communities that are diverse, distinctive, and native in origin. The more distinct in floral composition and phenology two plant communities are the more distinct the bee communities will be. For example, deciduous forest native plant communities are primarily spring blooming with distinctive, ephemeral, tree and shrub components. This is highly distinctive from prairie grasslands, with strong herbaceous perennial communities that peak in floral abundance and diversity in mid-late summer. Alternatively, specific habitat types may be targeted for sampling due to the presence of specific plants or nesting substrates known to be associated with specific bee species or taxonomic groups.

Regardless of whether the project endeavors to inventory bees across the entire refuge or only within specific habitat types, the sampling location should be representative of the habitat. For example, landscaped area or gardens surrounding a visitor center should not be chosen for sampling as they are not representative of the natural habitat on the refuge.

If the objective is to inventory a particular habitat type or management unit, multiple transects can be situated throughout the area of interest to capture variation in the vegetation composition (habitat) within that area. Multiple transects or arrays placed in a single habitat or area of interest should be placed at least 100 meters apart to avoid overlapping zones of influence (Element 2) and trapping bias. These transects should then be pooled for analysis (though not for specimen processing), as they are not considered independent samples. If comparing among areas of interest, the number of transects should be standardized among sampling areas to ensure equal sampling effort.

Transects should be placed at least 1.5 km apart to be considered independent samples, even if located in different habitat types. Solitary bee foraging distances can be 250m to 1500m from nest sites, and up to 3 km for hive/honey bees (*Apis mellifera*) (Abou-Shaara, 2014; Zurbuchen et al., 2010). Additionally, when you need to make inference beyond the location in which data are collected (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. We recommend that you reach out to your regional I&M coordinator, zone biologist, or the Refuge I&M Pollinator Team for assistance with developing a statistically defensible study design.

Survey Schedule

Sampling frequency for a bee community inventory should follow the blooming phenology of habitats being investigated. Typically, a two to three week resample interval for soapy water bowls and hand netting results in effectively capturing turnover in the species of plants in bloom, and, thus, the bee community as well. A reasonable guideline is that the ideal number of sampling events is the number of weeks in the active bee flight season (i.e., the floral resource availability season, see Element 2) divided by four (Levenson et al., 2024). This frequency should be adjusted to fit project-specific objectives, such as inventories of a specific habitat type or monitoring studies.

Soapy water bowl traps should be set up and collected on the same day (e.g., at dawn and dusk) or within 24 hours of deployment (see SOP 3). Glycol bowl traps are typically deployed continuously throughout the active bee flight season, with samples being collected at a maximum of once every two weeks (see SOP 4). Hand netting should be conducted at the same frequency as soapy water bowl traps and is typically conducted at the same time as traps are collected (see SOP 5).

Specimen & Data Management

In addition to field work requirements, data entry, specimen processing, specimen management, and identification need to be considered when estimating project resource requirements. Although resources required for field-based collection of bee specimens can be relatively minimal, the time and cost required for specimen processing, identification, and management are considerably higher. It is imperative that these additional costs are considered when planning the scale and breadth of field study to be implemented.

Standard Operating Procedure 2: Equipment and Preparation

Note: *Many of the pieces of equipment described in this SOP are illustrated in photos and diagrams in The Very Handy Bee Manual* (Anonymous, 2024).

Field Equipment

Equipment for Setting up Traps <u>plus</u>…

- ☐ Silica flat binder
- ☐ Florescent blue and florescent yellow pigments
- ☐ Primer
- ☐ Paint brush
- ☐ Wired flags/flagging tape/wood stakes/PVC
- ☐ Trapping liquid container/dispenser

…Soapy Water Bowl Traps <u>and/or</u>

- ☐ 3.25 oz plastic cups—30/transect
- ☐ Dawn Original Scent (blue colored) dishwashing liquid or unscented natural/ biodegradable dish detergent
- ☐ Wire plant support stakes <u>or</u>
- ☐ Heavy-duty wire and garden stakes
- ☐ Clothespins

…Glycol Traps

- ☐ Propylene glycol
- ☐ 12 oz plastic cups: 9/array
- ☐ PVC pipe
- ☐ Bolt and lock nut/ self-tapping screw
- ☐ Rebar/stake
- ☐ Mallet

Hand Netting

- ☐ Fine mesh insect net
- ☐ Ether/alcohol/soapy water/cyanide
- ☐ Clear, lidded container/ vials

Emptying Traps/ Nets

- ☐ Fine specimen strainers/filters
- ☐ White plastic spoons
- ☐ Funnel
- ☐ Nalgene bottle/bowl
- ☐ 70% denatured alcohol
- ☐ Nasco Whirl-Paks/Ziploc bags
- ☐ Printed/written tracking labels
- ☐ Datasheets

Vegetation Surveys

- ☐ 1 meter quadrat

Lab Equipment

Specimen Washing/Drying

- Fine mesh tea strainer/brine shrimp net
- Mason/canning jar
- Fiberglass window insect screen mesh
- Cloth/paper towels
- Hairdryer/air compressor/ “bee-dryer”

Specimen Identification/Pinning

- Stereoscope/dissecting microscope
- Reverse clamping tweezers/ feather-weight forceps/ small paint brush/ dissecting needle
- Insect pins (sizes 1-3)
- Pinning block
- Points/point punch
- Specimen glue (e.g., Aleene’s Original Clear Gel Tacky Glue)
- Parchment paper
- Cross linked/expanded polyethylene foam
- Cardboard/plywood
- Wood/white glue

Specimen Storage

- Archival/acid-free specimen labels
- Carboard boxes/insect storage cabinets/dressers
- Plastic bags/tightly closed bins
- Desiccants
- Mothballs and/or pest strips

I. <u>Equipment for Setting up Traps</u>

a. Soapy water bowl traps

White or clear 3.25 oz plastic cups are used for soapy water bowl traps. Solo brand 3.25 oz white cups (model #p325w-0007) work well and can be purchased in bulk online (e.g., webstaurantstore.com). The interior of the cups should then be painted so that each bowl trap transect is equally divided into white, blue, and yellow cups (Figure 2). At a typical 30-bowl transect, the interior of 10 bowls should be white (primed and painted white if using clear cups), the interior of another 10 should be primed and painted fluorescent blue, and the interior of the remaining 10 should be primed and painted fluorescent yellow. *See the 'Paint' section below for more information about paints.*

Figure 2. Soapy water bee bowls (3.25-oz), painted in fluorescent blue, fluorescent yellow, and white.

b. Glycol bowl traps

White 12 oz plastic cups are used for glycol bowl traps. Follow painting instructions for *Soapy water bowl traps*, such that the total number of glycol bowl traps at each transect is equally divided into white, yellow, and blue cups. Poke 4-5 small pinholes 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 (Figure 3). The holes should be small enough (1-2 mm) so that tiny bee specimens will not be lost through them. The glycol bowl traps should be set into stands to elevate them just above the ground or underlying substrate to prevent the cup from absorbing the heat of the surface of the earth. If the traps are deployed in an area with tall vegetation, use taller stands and elevate the traps to the vegetation canopy level to ensure the traps are visible to insects. 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.

Figure 3. Propylene glycol cup trap (12-oz), painted in fluorescent blue, fluorescent yellow, and white.

c. Paint

The interior surfaces of all traps (soapy water or glycol) are either left white or painted fluorescent blue or fluorescent yellow. To standardize the color used, we recommend ordering Silica Flat binder and Yellow Fluorescent and Blue Fluorescent pigments from Guerra Paint and Pigment. A primer intended for plastic will be needed to get the paint to adhere. Check bowls throughout the sampling season and repaint if fading or chipping is observed. Spray paint should not be used as it deteriorates rapidly.

d. Bowl trap stands

Soapy water bowl trap stands can be fashioned from commercially available wire plant support stakes that often include a perfectly sized loop at the top for holding a bowl trap by its lip. Alternatively, you can use pieces of heavy-duty wire (8-12 inches long) and garden stakes. The wire can be manipulated into a loop that fits underneath the rim of the bowl trap. Wrap the ends of the wire loop around the stake and use clothespins for further stabilization if needed.

For a glycol bowl trap stand, you can use PVC pipe or plastic electrical conduit as the stake and create a hoop to hold the cup by cutting plastic culvert pipe (Figure 4). 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, attached to a stake of its own, or pounded directly into the ground, depending on soil type. White PVC pipe should be painted grey or black so that only the trap itself will attract bees. The top of the pipe should be plugged to prevent bees and other organisms from becoming trapped inside. Instructions on how to make glycol trap stands can be viewed here: https://www.youtube.com/watch?v=x87CXM7mq54.

Figure 4. Propylene glycol cup trap

e. **Dishwashing liquid (soapy water bowl traps)**
Dishwashing liquid acts as a surfactant, breaking the surface tension of the water used in soapy bowl traps. We recommend standardizing by using Dawn Original Scent (blue colored) dishwashing liquid or an unscented biodegradable dish detergent. Do not use floral or citrus scented products. Add enough dishwashing liquid to a gallon jug of water to give the water a very light blue tinge (typically one large squirt of soap). This can then be poured directly into bowl traps in the field. Used soapy water from bowl traps can be poured onto the ground at the time of specimen collection if you are not in proximity to riparian or wetland habitats. Soapy water can be toxic to aquatic species and amphibians.

f. **Propylene glycol (glycol bowl traps)**
When glycol bowl traps will be deployed, it is recommended that clear, undiluted, food-grade propylene glycol is used, as it is the most benign formulation and has less environmental impact if spilled or ingested. Avoid red-dyed propylene glycol, as this may attract non-target organisms such as hummingbirds. Unless sampling in a hot area (i.e., a desert), use a 1:1 ratio of 100% propylene glycol and water to reduce costs. Add a large squirt of dishwashing liquid to each gallon of the glycol solution and gently mix before use. Dishwashing liquid is important in this mix, or bees will simply land on the propylene glycol's surface and fly away.

Unused propylene glycol can be repurposed in vehicle or building cooling systems or diluted and poured down the drain if you are not on a sewer system. Consult your local hazardous waste disposal regulatory authority if you are on a sewer system.
CAUTION: **<u>Ethylene</u>** glycol (antifreeze) is toxic to mammals and should not be used.

g. **Trapping liquid container/dispenser**
Propylene glycol can often be dispensed directly from the container it was purchased in after diluting and adding a squirt of dishwashing liquid. Heavy plastic, large-handled gallon bottles can also be rinsed and reused for soapy water or propylene glycol preparation and dispensing into traps. Remember to label any repurposed containers. Determine how much liquid will be needed to fill all bowl or glycol bowl traps before going to the field. One 30-bowl transect will require approximately one-half gallon of

soapy water. One 9-glycol bowl trap array will require approximately one gallon of propylene glycol.

h. Transect markers
Mark the beginning and end of line transects, or center of circular arrays, so that the sites can be relocated for trap collection and if repeated sampling is planned. Wired flags or surveyor's tape can be used as short-term markers but do not place brightly colored markers close to traps, as they may bias sampling by attracting or deterring bees. Do not use flagging that is the same color as the traps (white, blue, or yellow). Wooden stakes or capped rebar can be used as long-term markers of survey site locations and should be set up on or before the first sampling date.

II. Equipment for Emptying Traps/ Nets

a. Specimen filters/strainers
Use a strainer with extremely fine mesh when removing specimens from traps to catch the smallest bees, some of which may only be 2-4mm. White, disposable paint and coffee filters work well. They can be directly labeled in lead pencil (before getting wet) and placed in a funnel for support while straining the bowl contents through. The specimens can remain in the folded filter after they are strained and then be frozen in Ziploc or Whirl-Pak® bags.

b. Nalgene bottle/bowl
A Nalgene bottle, bowl, or similar container should be held under the strainer to ensure that no specimens are lost while emptying traps.

c. Plastic spoons
White plastic spoons are useful when removing wet bee specimens from traps or filters.

d. Ziploc or Nasco Whirl-Pak bags
Ziploc or Nasco Whirl-Pak bags are used for short term storage of bee specimens immersed in alcohol or propylene glycol. Nasco Whirl-Pak bags are preferable because Ziploc bags tend to leak. Bag size will depend on your expected sample size. Four-to-seven-ounce Whirl-Pak bags are often suitable for soapy water bowl traps; larger sizes may be necessary for glycol bowl traps.

e. Tracking labels
Tracking labels are small pieces of paper that contain the minimum data required for tracking bee specimens and are placed in each Whirl-Pak/Ziploc bag of bee specimens. Tracking labels should be printed using a laser printer or written in **dark lead pencil** on paper, heavy cardstock, or, ideally, Rite-In-The-Rain paper. Do not use pen or marker (including Sharpie brand) on the label or outside of the bag because the ink can dissolve from the preserving solution. See SOP 8 for the recommended template to be filled in using a dark lead pencil, inserted into specimen bags, and stored with specimens for 6 months or less. Labels ensure that station staff and volunteers include all the required

information on the label at the time the sample is collected. To save time in the field, prepared labels can be placed beforehand in the appropriate Ziploc bags.

f. Data sheets

The same data recorded on the tracking label that is placed inside the specimen bag must be recorded on separate data sheets. The tracking labels will be sent to the identification lab with specimens, and the data sheets provide the collection record data to be entered into a database by the collector. Data sheets should be used to collect additional, project-relevant data beyond what is required on the tracking label (e.g., habitat details, management treatments, etc.). See *SOP 8: Data Collection and Management* for example data sheets to use with this survey protocol.

III. Equipment for Hand Netting

a. Insect nets

Although a wide variety of net types may be used to catch bees, it is recommended to use an aluminum-handled aerial net with a 12-18" hoop. The larger the hoop, the greater the area of capture, but larger hoops are more difficult to swing quickly due to air resistance and a higher likelihood of snagging. Sweep and beating nets are not recommended. A fine mesh or muslin net bag, rather than the traditional medium to large gauge aerial net bag, can keep the smallest bees from escaping.

b. Insect kill jars

Bees collected from hand nets are transferred into an insect "kill jar": a clear, lidded container with a small amount of alcohol, soapy water, ether, or prepared cyanide inside. Clear containers allow you to confirm that you've successfully transferred bee specimens from the net to the container. Any small, clear, container with an easy-to-replace lid, such as a plastic film canister or specimen jar, will work. Ethyl acetate was historically used in kill jars but is now recommended against as it denatures the DNA from specimens. **CAUTION:** Use extreme caution if using cyanide and wrap glass jars in tape to prevent breaking.

IV. Equipment for Vegetation Surveys

a. 1 meter quadrat

A one-meter quadrat for vegetation sampling can be easily constructed out of PVC pipe purchased from a hardware store or ordered online from any number of biological suppliers. The quadrat is a square PVC frame measuring one meter in length on each side and used to define the area in which the floral community is quantified.

V. Equipment for Specimen Storage, Preparation, and Identification

See *SOP 9: Storing, Shipping and Pinning Collected Bee Specimens* for additional information on methods.

a. Specimen (emersion) storage solutions

Denatured alcohol is recommended for storing insects as it is easiest to work with and does not harden specimens as readily as some other alcohols (particularly isopropyl). Often alcohol needs to be diluted to achieve the right percentage (70%). Always check bottle labels for the percentage of alcohol before use. To dilute from 100% alcohol to 70%, choose a convenient sized container, such as a pint bottle, then fill it ~70% full of alcohol and the rest with tap water. This measurement does not need to be exact. A minimum of 90% alcohol is recommended for DNA preservation, but it is not necessarily ideal for specimen processing, as specimens can become brittle. Specimen storage in EtOH of about 70% is better for specimens that are to be processed within the short term (present to about 1 year).

b. 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/mason jars 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 screen mesh with the same diameter as the lid and place it under the cap section that screws on to the jar. Loose fiberglass screen can be purchased 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.

c. Tools for drying specimens

A 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 more padding for glass 'bee washing' jars. A handheld hair dryer can be used to dry specimens prior to pinning. An air compressor can also be used for larger scale specimen processing operations. If you are involved in collecting and processing many specimens, you may want to invest in the creation of an "autobeedryer" using instructions from these slideshow and video demonstrations.

d. Stereo or dissecting microscope (stereoscope)

Unfortunately, a good microscope is not cheap. Our experience is that an adequate microscope costs over $1000 new, and good ones run over $2000. Inexpensive microscopes usually have poor optics, very low power, small fields of view, are difficult to set to fixed heights, and their stands are usually lightweight and often designed in such a way that makes specimens difficult to manipulate. That said, microscopes with even moderate care are a one-time investment. Usually, used prices are about half the cost of new. Brands to consider include Nikon, Leica, Zeiss, Olympus, Wild, Wild-Heerbrug, Nikon, and Meiji. The USGS Bee Lab at the Eastern Ecological Science Center (USGS Bee Lab) uses Nikon SMZ745T and SMZ800N stereoscopes (as of 2024).

When purchasing a microscope, new or used, look for:

- Heavyweight base: Will ensure balance even when the microscope is fully extended and not topple with the addition of lighting.
- Working distance (distance between specimens and the objective lens): This will ensure that while you manipulate your specimens you have plenty of space to bring them in and out of focus without fussing too much with the height of the microscope.
- Magnification: Minimum maximum of 30x, preferable max of 60x.
- Adjustable and removable oculars: Important for individualized settings to accommodate vision differences, to facilitate cleaning, the addition of reticles, or to increase maximum magnification.
- Zoom nob placement: recommend a side nob.
- Field of view: 20mm at 10x magnification or wider.
- Customer Service: Are returns, installation, repairs, or adjustments available? This is important to know before shipping.

e. Small hand tools

- Reverse clamping tweezers—for untangling and positioning bees for pinning. They apply less pressure than standard tweezers.
- Feather-weight forceps—provide a firm but light pressure grip that lessens the threat of crushing or breaking a specimen.
- Small paint brush—for sorting bees and picking up very small bees without crushing them or breaking legs.
- Dissecting needles—for untangling bees or repositioning the legs of specimens.

f. Insect pins

Only size 2 pins should be used for all pinning activities. Pins size 1 and below are prone to bending and do not hold up well to long term museum curation. Size 3 and above are too large for general use. Stainless steel pins should be used in almost all scenarios.

g. Pinning block

The use of an insect pinning block is helpful to determine the correct height a specimen or pinning point should be placed and can be purchased from most scientific or biological equipment suppliers such as Carolina Biological.

h. Point punch

The use of points is traditional, especially for pinning very small 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 Home Science Tools (https://www.homesciencetools.com/biology/life-science/insect-collecting-supplies/). Pointing insects can be difficult and time consuming to do correctly and is typically eschewed in favor of gluing specimens directly to the pin.

i. Specimen glue

Glue gels (e.g., Aleene's Original Clear Gel Tacky Glue) are recommended when gluing smaller bees to pins, as they have a longer work time, dry crystal clear, and are easily reversible. To ensure the glue has time to set, leave the pin laying down flat, resting on the specimen for at least 5-10 minutes prior to moving.

j. **Parchment paper**
Parchment paper provides a non-stick substrate on which to glue bees, and to sort and position dried specimens without them sticking, catching, or breaking. You can secure the parchment paper by pinning it to a large foam pinning board.

k. **Pinning board**
Pinning boards provide a soft, springy surface that can accommodate pin pricks without damaging pins (unlike table-tops). Cross-linked polyethylene foam is the preferred pinning substrate. Styrofoam is inadequate: both labels and specimens will bend too much when pinned. The foam can be cut to your preferred size and glued to a piece of cardboard or plywood to create a longer lasting pinning surface. 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 for a nice and tidy look. You can cut an oval-shaped hole as a handle in one end of the pinning board to make it easier to carry.

l. **Specimen labels**
Paper**:** Archival, acid-free, 35–65-pound paper should be used for specimen labels to prevent deterioration and is readily available in office supply stores.

Label Production**:** The label generating program developed by Discover Life is recommended but can be initially confusing. Contact the USGS Bee Lab for guidance getting started (beelab@usgs.gov). Dan Kjar has generalized this Discover Life labeling program so that it will generate and print out insect labels.

m. **Specimen boxes and storage**
Pinned insect specimens must be stored in a clean, dry, pest-free environment and checked periodically for damage/deterioration. Simple cardboard insect specimen boxes with a completely detachable lid and Ethafoam board glued to the bottom are suitable for **short term storage** of pinned bees (e.g., *Folding Insect Storage Box* at www.ecologysupplies.com). 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.

For more permanent, longer-term storage, working with your institutional insect collection partner is essential. They will know the types of drawers and unit trays compatible with their collection and direct you to the proper vendors.

n. Specimen care and pest control

An excellent means of keeping your collection pest free if using cardboard boxes is to keep each box in a large Ziploc bag (2-gallon bags fit most boxes). Specimens need a month to thoroughly dry out after pinning before they should be enclosed in a plastic bag. Mothballs and Hot-Shot No-Pest strips can be effective but carry health risks with long-term exposure. In humid conditions, specimens should be stored in an air-conditioned space or put in plastic bags or tightly closed bins that contain active desiccants to prevent molding. Specimens stored in this way should be routinely cycled through the freezer for 3–4-day periods despite precautions.

Standard Operating Procedure 3: Setting Up Soapy Water Bowl Traps for 24-Hour Sampling Periods

Introduction

Bowl traps are apt tools for the inventory of bees as they are inexpensive, easily obtained, require no entomological training, and may catch small bee species not easily captured by net (McCravy, 2018; Krahner et al., 2024). Additionally, bowl traps are not subject to observer bias that may be introduced through netting. However, bowl traps are not exempt from taxonomic bias. Recent studies have found that bowl traps tend to oversample small bees (<14 mm, e.g., sweat bees) and under sample large bees (e.g., bumble bees), resulting in inaccurate estimates of species composition and abundance. Combining bowl traps with netting is recommended for a more complete inventory *(see Element 2: "Sources of error" for additional considerations*).

Soapy water bowl traps are small white, blue, and yellow colored plastic cups that are filled with water and liquid dishwashing soap. Bees are attracted to the bowl colors and trapped in the water. The dish soap decreases the surface tension of the water, causing even small insects to sink beneath the surface. Most insects perish within 60 seconds of becoming immersed in the soapy water but can revive if removed too soon. Thus, specimens should be frozen or stored in alcohol for at least 24 hours prior to processing. If specimens will not be processed within a few days of capture, they should be stored in a -20° freezer until pinned or shipped to a lab for processing and identification.

The best places to put bowl traps are exposed settings, elevated to the height of the surrounding vegetation, where bees are likely to see them (e.g., fields, roadsides). Avoid putting bowl traps in heavily shaded areas, such as under trees or thick herbaceous vegetation, as few to no bees will come to those bowls. If you can easily see the bowl trap, then bees can too. Flowers need not be apparent in an area for bee capture rates to be quite high in bowl traps. However, the presence of superabundant nectar and pollen sources appears to lead to low bowl trap capture rates (Kuhlman et al., 2021; Lezzeri et al., 2024). This context underscores the need for vegetation characterization data for accurately interpreting bee community data.

Work with the refuge/land managers to determine if the transect markers can be left throughout the survey season, or if they need to be collected at the end of every survey.

Habitat Characterization

Bee species composition can only be fully understood in combination with habitat data. Given this, all bee surveys should include a vegetation characterization component (see SOP 7: Vegetation Surveys for additional details). The simplest suggested approach to plant sampling in association with bee bowls is a meandering walk that documents a list of all flowering plant species present. This is the vegetation survey method described in SOP 1 of the Prairie Reconstruction Initiative's (PRI) National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions (McColpin et al., 2019). It uses the DAFOR (Dominant, Abundant, Frequent, Occasional, Rare) scale to make a general estimate of abundance of each species. Bee bowls attract and sample bees over a large area, and this vegetation sampling method is useful in describing those broad habitat characteristics. If a more complex or quantitative measure of vegetation is needed, see SOP 7. Habitat characterization is included as a step in the following field protocol, but the surveyor is referred to the PRI framework for specific methods—that SOP should be provided as a supplement to the protocol below.

Soapy Water Bowl Traps Field Protocol

Surveying Conditions

Bowl traps must be active for a minimum of 6 hours and a maximum of 24 hours. At minimum, traps need to be active 9:00 am and 3:00 pm or while the sun is up, unless your specific project goals require sampling outside of this time frame. Typically, bowl traps are deployed in the morning and collected either that same afternoon or the next morning. Soapy water bowl traps are not to be deployed for longer than 24 hours, as specimens will rot. If no bees were collected during the survey, it is important to document that data on your tracking label and data sheet.

Bowl traps should be placed on the ground. If necessary, flatten the vegetation just around the bowl to increase the bowl's visibility. If you are unable to flatten the vegetation, then bowl stands may be used to elevate the bowls to average floral canopy height.

At least two of these conditions must be met throughout the sampling period to deploy bowl traps:

- Temperature is above 50°F and below 110°F.
- Cloud cover does not exceed "Mostly Cloudy" (>75% of the sky covered by clouds).
- Winds mild (less than 10 knots or 3 on the Beaufort scale).

Do NOT deploy bowl traps during, or immediately following, precipitation, or if precipitation will occur while the bowl traps are deployed.

Minimum Required Supplies for the Field if Soapy Water Bowl Trapping

Day 1: Deploying bowl traps:

- ☐ Data sheets, and extras
- ☐ Tracking labels, and extras
- ☐ Pencil for each collector, and extras
- ☐ 10+ white bowls per sample unit
- ☐ 10+ fluorescent yellow bowls per sample unit
- ☐ 10+ fluorescent blue bowls per sample unit
- ☐ Dishwashing liquid
- ☐ 1-gallon jug with soapy water per sample unit

Day 2: Collecting bowl traps:

- ☐ Data sheets, and extras
- ☐ Tracking labels, and extras
- ☐ Pencil for each collector, and extras
- ☐ Nalgene bottle/small bowl/other similarly sized container.
- ☐ Fine strainer, brine shrimp mesh aquarium net, or net bag
- ☐ Whirl Pak/quart-size Ziploc bags
- ☐ 1-gallon Ziploc bags
- ☐ White plastic spoon

Minimum Required Supplies for the Field if Setting up a Transect

- ☐ GPS unit
- ☐ Map
- ☐ Compass
- ☐ Measuring tape
- ☐ Poles
- ☐ Flagging tape
- ☐ Wire

Additional Suggested Supplies for the Field

- ☐ 30+ elevated bowl stands per sample unit
- ☐ 1-gallon jug 70% denatured alcohol
- ☐ Small cooler with ice
- ☐ Clipboard for each collector
- ☐ Notebook
- ☐ Collecting permits
- ☐ Plant ID material
- ☐ Backpack/hip pack
- ☐ Boots
- ☐ Sunglasses
- ☐ Hat
- ☐ Sunscreen
- ☐ Insect repellant
- ☐ Hydrocortisone cream
- ☐ Water bottle
- ☐ Lunch/snacks
- ☐ Toilet paper
- ☐ Smart phone
- ☐ Chargers
- ☐ Two-way radios
- ☐ Camera
- ☐ Hand lens
- ☐ First aid kit

Instructional Videos
It is highly recommended that surveyors watch the following instructional video and practice these skills at a local site before conducting surveys.

Video on "Surveying Bees with Soapy Water Bowl Traps" is available at:
https://youtu.be/5Y8fpwdSOGM

Field Instructions:
<u>Day 1: Setting up the soapy water bowl traps:</u>

1. **Before leaving for your survey site:**
    a. Print out the provided tracking labels (Figure 13) and your project-specific data sheets. Use a **dark lead pencil** to fill out the following information on both forms for each sample unit that will be surveyed: state, county, site name, transect/sample unit name, and collector name(s). **Do not use pen or Sharpie on any labels or specimen bags as the ink will dissolve when exposed to water or preserving solution.** Make sure you have extras of everything in case anything gets lost in the field.
    b. Use the supply checklist to confirm you have all the necessary supplies.
    c. Prepare at least one 1-gallon jug of soapy water per sample unit you will survey by filling the jug with water and mixing in one squeeze of dish soap.
    d. Sort your bowl traps into sets for each sample unit that will be surveyed, alternating between fluorescent blue, fluorescent yellow, and white bowls.
    e. Measure out five meters to calibrate how many of your step's are required between traps.
2. **Upon arrival at your survey site:**
    a. Before you begin, record all data on your tracking label except for Day 2/bowl collection fields. Use the notes field or project-specific additional sheets to record additional data as required by your project.
3. **IF NEEDED: Setting up the transect:**

   a. Use a GPS or map to locate the first transect point. Place a post at the first collection point and mark with flagging tape. Record the GPS coordinates.
   b. When you reach the end of the transect, place the second post at the end point and mark with flagging tape.
4. **Deploying bowls**
   a. Stand at the start of the established transect/sample unit.
   b. Place a bowl down at the start of the transect.
      i. If the vegetation is short and is not likely to obscure the bowls…
         1. Place the bowl on the ground in an exposed, open setting where bees are likely to see it. Avoid setting the bowl in heavy shade or where they can be hidden by tall vegetation.
      ii. If the vegetation is tall and may obscure some bowls…
         1. Flatten the vegetation just around the bowl to increase the bowl's visibility. If you are unable to flatten the vegetation, then elevate all bowls in the transect. Use elevated bowl stands to bring the bowls to the plant canopy height. Ensure the bowl is stable.
   c. Fill the bowl three-quarters full of the soapy water solution. If there are two or more surveyors, one person can set bowls down while the other fills the bowls with the solution.
   d. Continue setting and filling bowls five meters apart, alternating between fluorescent blue, fluorescent yellow, and white bowls so that no two bowls of the same color are next to each other. Continue until all 30 bowls are placed.
      i. The bowls do not need to be laid out in a strictly straight line. The transect can meander around tall vegetation or other obstacles if traps remain 5 m apart.
      ii. You can use a measuring tape to measure five meters, or you can measure out five meters and count how many of your steps measure 5 meters.
   e. Record the placement of the bowls (ground or elevated).
5. **After survey:**
   a. Ensure all available data has been recorded on all tracking labels and data sheets. Do not leave until all fields are filled out.
   b. Use the supply checklist to confirm you have all your supplies.

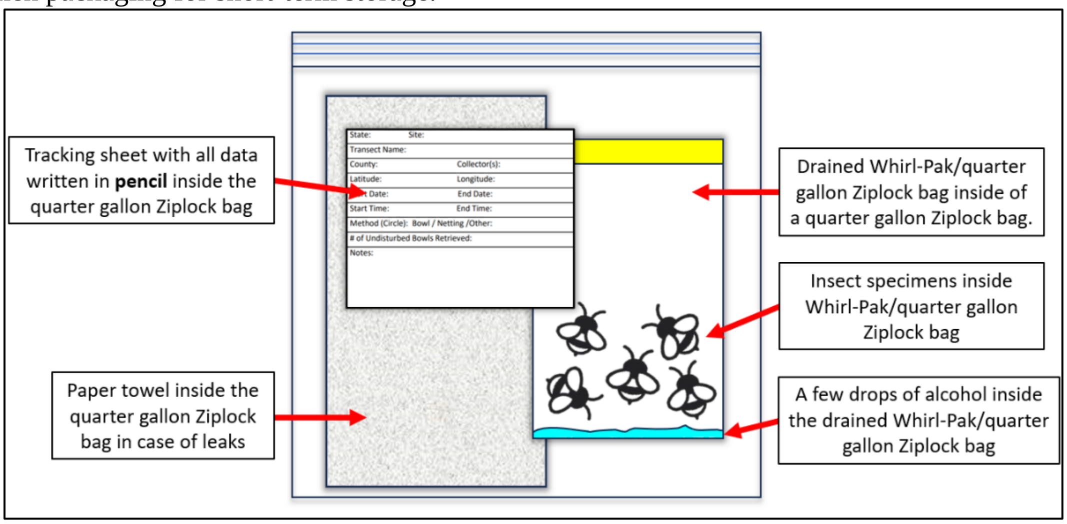
<u>Day 2: Soapy water bowl trap and specimen collection (~24 hours after bowl deployment):</u>

1. **Arrive at your survey site:**
   a. Record all Day 2/bowl collection data on your tracking label and data sheet. Use project-specific data sheets or the notes field on tracking label to record additional observations as necessary.
   b. 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.
2. **Collecting bowls and specimens:**
   a. Go to the first bowl in the transect.

        i. Using your fingers or a spoon, remove all moths, butterflies, slugs, snails, very large bodied non-Hymenoptera (e.g., grasshoppers and beetles), and large plant material from the bowl. **Be careful**: Check for any bees or other small insects that might be attached before removing the contaminant. Keep all small insects even if you do not think they are bees.
        ii. Pour the remaining insects and solution in the bowl through an extremely fine strainer, brine shrimp mesh aquarium net, or net bag. Strain the insects over a Nalgene bottle, bowl, or other container in case of spills. If there are still insects stuck to the bowl, rinse and repour the bowl until all insects in the bowl have been collected.
    b. Continue collecting bowls and straining insects out of the soapy water until all bowls (and bowl stands if used) are collected.
        i. Pool the insect specimens across bowl colors.
    c. Place all the specimens into a Whirl-Pak (or quart-size Ziploc bag). Use a spoon or your fingers to scoop out any specimens that do not easily go into the bag.
    d. Gently roll up the Whirl-Pak, trying to remove as much air as possible without crushing the specimens, and place it inside a gallon-size Ziploc bag, along with a fully filled-out tracking label.
        i. If you are surveying in a remote location and will be in the field for an extended time, pour enough alcohol in the bag to cover the specimens to preserve them while you continue surveying and/or store the bagged specimens in a small cooler with ice. Consult your project manager for instructions on their choice of preservation method.

3. **Habitat Characterization**
    a. Refer to SOP 1 of the Prairie Reconstruction Initiative's *National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions* for guidance on completing a meandering walk to characterize habitat in the survey plot.
4. **After survey:**
    a. Ensure all data has been recorded on all tracking labels and data sheets. Do not leave until all fields are filled out.
    b. Use the supply checklist to confirm you have all your supplies.
    c. If the refuge/land managers require the transect markers to be collected, remove the start and end posts before leaving.
5. **Short-term specimen storage:**
    a. If travelling, place the specimens in a cooler and store overnight in a fridge or freezer at your lodging.
    b. Upon returning to the lab, place a paper towel in the quart-size secondary Ziploc bag where the insect specimens are being stored. Ensure all fields in the tracking label are filled out (Figure 5).

Figure 5. Specimen packaging for short-term storage.



c. Store the specimens in a -20°C freezer until they can be pinned or shipped to a lab for processing, within 6 months of capture. For easier organization, all bags from a single event (same day or transect) can be grouped together in a gallon-size Ziplock bag.
d. Store the specimens in a -20°C freezer until they can be pinned or shipped to a lab for processing, within 6 months of capture.

Standard Operating Procedure 4: Setting Up Glycol Bowl Traps for 2–Week Sampling Periods

Readers of this SOP will also benefit from reading *SOP 3: Setting Up Soapy Water Bowl Traps for 24-Hour Sampling Periods* as some methods are the same.

Glycol bowl traps are a method that should be employed only when soapy water bowl traps or netting are not feasible, typically due to sampling in a remote location that is difficult to access regularly. Glycol trap can cause high mortality of non-target species and, due to the viscosity of the medium, specimens caught in glycol are more difficult to prepare and may incur unexpected costs, delays in identification, or specimens that are degraded and cannot be identified. Therefore, glycol trapping should only be used if necessary.

Glycol bowl traps catch bees continuously and are less susceptible to evaporation, rain, and disturbance than smaller and non-glycol traps, thus circumventing problems of shifts in phenology and weather-based scheduling. Glycol traps may require more effort and materials for initial installation but are less intensive in terms of field personnel time required for monitoring, tending, and replacing them.

Glycol traps should be arranged into transects consisting of 9 traps (3 each of 3 colors) that are each filled with a mix of propylene glycol and soapy water. Specimens must be collected from the traps every 1-2 weeks.

As with soapy water bowl traps, glycol traps should be placed in exposed, sunny areas during the trapping season. The traps should be set in stands so that the bottom of the cup is positioned just above the level of the ground or underlying substrate (e.g. matted down grass). If traps are set in tall vegetation, use taller stands so that the glycol trap is visible, but still within the floral canopy level. 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. Instructions on how to make glycol trap stands can be viewed here: https://www.youtube.com/watch?v=x87CXM7mq54.

Glycol traps should be spaced approximately 5 m apart in a linear configuration. 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.

Habitat Characterization

Bee species composition can only be fully understood in combination with habitat data. Given this, all bee surveys should include a vegetation characterization component (see SOP 7: Vegetation Surveys for additional details). The simplest suggested approach to plant sampling in association with bee bowls is a meandering walk that documents a list of all flowering plant species present. This is the vegetation survey method described in SOP 1 of the Prairie Reconstruction Initiative's (PRI) National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions (McColpin et al., 2019). It uses the DAFOR (Dominant, Abundant, Frequent, Occasional, Rare) scale to make a general estimate of abundance of each species. Bee bowls attract and sample bees over a large area, and this vegetation sampling method is useful in describing those broad habitat characteristics. If a more complex or quantitative measure of vegetation is needed, see SOP 7. Habitat characterization is included as a step in the following field protocol, but the surveyor is referred to the PRI framework for specific methods—that SOP should be provided as a supplement to the protocol below.

Glycol Bowl Traps Field Protocol

Surveying Conditions

Glycol bowl trap deployment and collection is not contingent upon ambient conditions, though you should be mindful of coming weather patterns. Storms and heatwaves both will require you to revisit the bowls sooner than the maximum 2-week interval. Storms may overflow or knock down traps, while collection medium can evaporate faster during heatwaves. Glycol bowl traps should not be deployed for over 2-weeks as specimens will rot. If you are collecting specimens continuously through the season, you may service bowls as convenient during the 2-week interval. If deploying glycol traps intermittently (e.g. 1 week a month), the collection period (effort) should remain consistent throughout the survey period.

If you are applying other sampling methods when servicing glycol bowl traps, then adhere to required conditions of that method. If no bees were collected during the survey, it is important to document that data on your tracking label and data sheet.

Do NOT deploy bowl traps during, or immediately following, precipitation.

Minimum Required Supplies for the Field if Glycol Trapping

Deploying glycol bowl traps:

- ☐ Data sheets, and extras
- ☐ Tracking labels, and extras
- ☐ Pencil for each collector, and extras
- ☐ 3+ 12-oz fluorescent blue stadium cups per sample unit
- ☐ 3+ 12-oz fluorescent yellow stadium cups per sample unit
- ☐ 3+ 12-oz white stadium cups per sample unit
- ☐ 9+ glycol trap stands per sample unit
- ☐ 1-gallon jug with propylene glycol per three sample units
- ☐ 3+ 12-oz fluorescent yellow stadium cups per sample unit
- ☐ Mallet

Collecting bees from glycol bowl traps:

- ☐ Data sheets, and extras
- ☐ Tracking labels, and extras
- ☐ Pencil for each collector, and extra
- ☐ White plastic spoon
- ☐ Nalgene bottle/small bowl/other similarly sized container
- ☐ Extremely fine strainer, brine shrimp mesh aquarium net, or net bag
- ☐ Whirl Pak/quart-size Ziploc bags
- ☐ 1-gallon Ziplock bags
- ☐ Large funnel

Minimum Required Supplies for the Field if Setting up a Transect

- ☐ GPS unit
- ☐ Map
- ☐ Compass
- ☐ Measuring tape
- ☐ Poles
- ☐ Flagging tape
- ☐ Wire

Additional Suggested Supplies for the Field

- ☐ Clipboard for each collector
- ☐ 1-gallon jug 70% denatured alcohol
- ☐ Notebook
- ☐ Collecting permits
- ☐ Plant ID material
- ☐ Backpack/hip pack

- ☐ Boots
- ☐ Sunglasses
- ☐ Hat
- ☐ Sunscreen
- ☐ Insect repellant
- ☐ Hydrocortisone cream
- ☐ Water bottle
- ☐ Lunch/snacks
- ☐ Toilet paper
- ☐ Smart phone
- ☐ Chargers
- ☐ Two-way radios
- ☐ Camera
- ☐ Hand lens
- ☐ First aid kit

Instructional Videos
It is highly recommended that surveyors watch the following instructional video and practice these skills at a local site before conducting surveys.

Video on "Surveying Bees with Glycol Bowl Traps" is available at:
https://youtu.be/d1SH81sU6Fw.

Field Instructions:
Setting up the transect and glycol bowl traps:

1. **Before leaving for your survey site:**
   a. Print out the provided tracking labels (Figure 13) and your project-specific data sheets. Use a **dark lead pencil** to fill out the following information for each sample unit that will be surveyed: state, county, site name, transect/sample unit name, and collector name(s). **Do not use pen or Sharpie on any labels or specimen bags as the ink will dissolve when exposed to water or preserving solution.** Make sure you have extras of everything in case anything gets lost in the field.
   b. Use the supply checklist to confirm you have all the necessary supplies.
   c. Prepare at least one 1-gallon jug of propylene glycol for every three sample units you will survey. Fill the jug with half propylene glycol and half water. Mix in one large squeeze of Dawn dish soap.
      i. If you are surveying somewhere hot where there will likely be high evaporation (e.g., a desert), increase the proportion of propylene glycol. Do not forget to add dish soap.
   d. Sort your stadium cup traps into sets of nine for each sample unit that will be surveyed, alternating between fluorescent blue, fluorescent yellow, and white cups.
   e. Measure out five meters to calibrate how many of your step's are required between traps.
2. **Upon arrival at your survey site:**
   a. Before you begin, record all data on your tracking label except for Day 2/bowl collection fields. Use the notes field or project-specific additional sheets to record additional data as required by your project.
3. **Setting up the transect:**

a. Use a GPS or map to locate the first transect point. Place a post at the first collection point and mark with flagging tape. Record the GPS coordinates.
b. When you reach the end of the transect, place the second post at the end point and mark with flagging tape.

4. Deploying bowls:

a. Stand at the start of the established sample unit.
b. Take the elevated bowl stand and place it in the ground. Use a mallet to sink the post into the ground. Elevate the bowl-holding loop high enough so the bowl sits at surrounding vegetation height.
    i. Place the stand in an exposed, open settings where bees are likely to see it. Avoid setting the stands in heavy shade or where they can be hidden by tall vegetation. If necessary, flatten vegetation just around the stand to increase the bowl's visibility.
c. Place a bowl into the stand.
d. Fill the bowl three-quarters full, do not fill past the holes under the rim with the diluted glycol solution. If there are two or more surveyors, one person can set bowls down while the other fills the bowls with the glycol solution.
e. Continue deploying traps five meters apart, alternating between fluorescent blue, fluorescent yellow, and white bowls so that no two bowls of the same color are next to each other. Continue until all nine bowls are placed.
    i. The bowls do not need to be laid out in a strictly straight line. The transect can meander around tall vegetation or other obstacles, if traps remain 5 m apart.

5. After survey:

a. Ensure all available data has been recorded on all tracking labels and data sheets. Do not leave until all fields are filled out.
b. Use the supply checklist to confirm you have all your supplies.

Glycol bowl specimen collection (every 1-2 weeks after bowl deployment):

1. **Before leaving for your survey site:**
    a. Print out the attached tracking labels. Use a **dark lead pencil** to fill out the following information for each sample unit that will be surveyed: state, county, site name, sample unit name, and collector name(s). Make sure you have extras of everything in case anything gets lost in the field.
    b. Use the supply checklist to confirm you have all the necessary supplies.
    c. Prepare at least one 1-gallon jug of diluted propylene glycol (50% water: 50% propylene glycol: one squirt of dishwashing liquid) for every three sample units you will be visiting.
2. **Arrive at your survey site:**
    a. Record all specimen collection data on your data sheet and finish filling out the tracking labels. Use project-specific data sheets or the notes field on tracking label to record additional observations as necessary.

    b. 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.

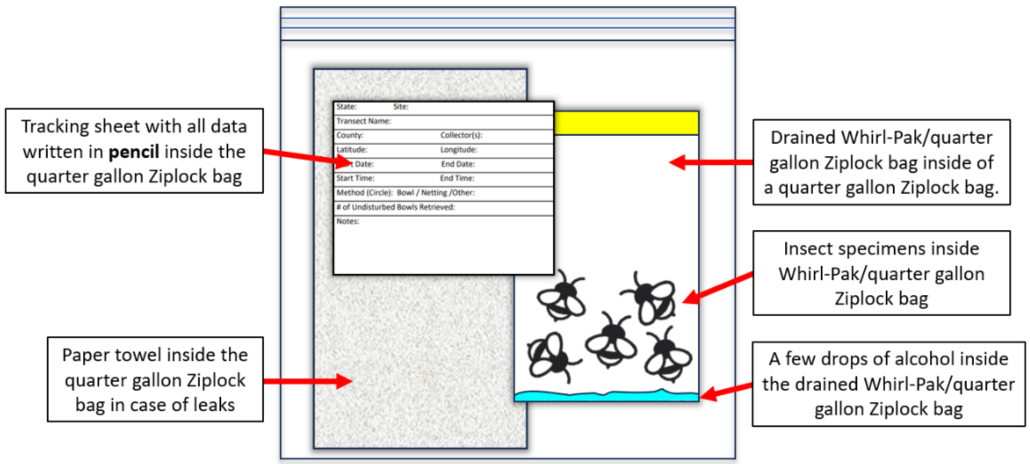
3. **Collecting specimens:**
    a. Go to the first bowl in the transect.
        i. Remove the bowl from the elevated bowl stand and pour the contents through an extremely fine strainer, brine shrimp mesh aquarium net, or net bag. Strain the insects over an empty glycol bowl trap or another container to recapture the strained glycol. Use a large funnel to prevent spills.
        ii. Pour back the strained glycol into the now empty bowl trap and return the bowl to its stand. Replenish the bowl with more diluted glycol solution if needed or replace the solution completely if the liquid becomes too cloudy or dirty.
        i. Using your fingers or a spoon, remove all moths, butterflies, slugs, snails, very large bodied non-Hymenoptera (e.g., grasshoppers and beetles), and large plant material from the net. **Be careful**: Check for any bees or other small insects that might be attached before removing the contaminant. Keep all small insects even if you do not think they are bees.
    b. Continue collecting bowls and straining insects out of the glycol solution until all the bowls have been strained and refilled with the glycol solution.
        i. Pool the insect specimens across bowl colors.
    c. Place all the specimens into a quart-size Ziploc bag or Whirl-Pak. Use a spoon or your fingers to scoop out any specimens that do not easily go into the bag.
    d. Gently roll up the quart-size Ziploc bag/Whirl-Pak, trying to remove as much air as possible without crushing the specimens, and place it inside a quart-size Ziploc bag, along with a fully filled-out tracking label.

4. **Habitat Characterization**
    a. Refer to SOP 1 of the Prairie Reconstruction Initiative's *National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions* for guidance on completing a meandering walk to characterize habitat in the survey plot.

5. **After survey:**
    a. Ensure all data has been recorded on all tracking labels and data sheets. Do not leave until all fields are filled out.
    b. Use the supply checklist to confirm you have all your supplies.
    c. If this is your final survey, remove the elevated bowl stands and transect markers before leaving. Collect the soapy glycol solution into a gallon jug and store for reuse until the liquid becomes very cloudy or dirty. Consult the propylene glycol label for proper disposal instructions.

6. **Short-term specimen storage:**
    a. If travelling, place the specimens in a cooler and store overnight in a fridge or freezer at your lodging.
    b. Upon returning to the lab, place a paper towel in the quart-size secondary Ziploc bag where the insect specimens are being stored. Ensure all fields in the tracking label are filled out (Figure 6).

Figure 6. Specimen packaging for short-term storage.



c. Store the specimens in a -20°C freezer until they can be pinned or shipped to a lab for processing, within 6 months of capture. For easier organization, all bags from a single event (same day or transect) can be grouped together in a gallon-sized Ziplock bag.

Standard Operating Procedure 5: Hand Netting

Introduction

Hand netting is an important tool for investigating the relationship between bees and their habitats. It places a special emphasis on recording the association of the bee with the flower it was collected from, allowing for inference regarding resource use by the species in the habitat of interest. It differs from bowl trapping in that it documents the direct relationship between forbs and bee species, and thus offers insight into strategies for managing plant communities (and habitats) for the benefit of bee diversity.

Hand netting tends to capture pollen specialists, kleptoparasites, and larger-bodied species (>14 mm) that are typically under-sampled by bowl traps (Rhoades et al., 2017; Pei et al., 2022). Hand netting and bowl traps also perform differently based on local floral resources, with hand netting tending to perform better in florally dense environments (Kuhlman et al., 2021; Pei et al., 2022). If the objective is to develop a species inventory, the combination of hand netting and bowl traps will provide the most comprehensive data on community composition. Hand netting is subject to bias due to the netter’s skill level and observational search experience. However, with proper experimental design and procedures that bias can be minimized significantly.

Survey Design and Addressing Bias

Hand netting, as with most active surveys, is standardized by both space and time. An individual netter will conduct a 30-minute timed survey that is bound by some form of spatial constraint (a plot or transect). While the time component is import for standardizing effort across surveys, the spatial constraint is important for controlling plant community inference and addressing surveyor bias. There are typically two kinds of transects, variable transect walks and strict transect walks. Regardless of which is used, the transect should be contained within one general habitat type (e.g., upland prairie, wet prairie, oak woodland, chaparral, riparian grassland, early succession old field, wet alpine meadow, aspen forest), which should be recorded.

Variable transect walks are free form walks that allow surveyors to meander within a bounded plot. Variable transects usually follow a sweeping path through the plot but allow for surveyors to focus on patches of flowering plants. This design is suited to maximizing catch in flowering communities that are patchy and heterogenous as it allows the surveyor to sample all available habitat rather than being limited to a defined transect. It constrains sampling effort to the plant community of interest but is but is less repeatable and does not address many surveyor bias issues. The lower repeatability of the actual transect means that this is often not the best design if vegetation surveys will be conducted in conjunction with hand netting.

The unit of area associated with a variable transect is the area of the plot within which it is contained. Plot sizes can vary in size, but the plot size should be measured and recorded on datasheets and marked in the field using flagging or another suitable plot marker.

Strict transect walks are predetermined lines along which a user must constrain netting to 1 meter on either side of the transect. The surveyor should intensely scan and net only from the flowers within 1 meter of the transect. This method is best suited to addressing surveyor bias by standardizing the search area to plants along the transect (meaning rare plants are more likely to be searched). It also helps avoid the propensity for surveyors to chase large-bodied bees (e.g. bumble bees) that are visible from greater distances, and thus improves detectability of small bees by the surveyor. It is less well suited to flowering plant communities that are more heterogenous, and sometimes results in large areas of a transect with no flowering plants to observe. It may also reduce catch rates by forcing surveyors to focus on plants along the transect, instead of the most fruitful patches.

The unit of area associated with a strict transect is defined by its length and the 1 m search area on either side of the transect. Strict transects can be any length, dependent upon your study area and question of interest, but should be a minimum of 25 m. Splitting a long transect into smaller units will facilitate easier comparison of data with studies that gather data along shorter transects. The transect length and width should be measured and recorded on datasheets and marked in the field using flagging or another suitable marker.

Surveying Conditions and Timing

The biology of both flowering plant and bee communities, as well as ambient weather conditions, can significantly impact the types of bees found and potentially bias samples and affect inference. If the goal of a survey is to compare treatments or locations, effort should be taken to standardize survey time and weather conditions. Optimal netting times will likely differ with the plant community of interest but can typically be accounted for with a mid-morning (~9am) survey and an early afternoon survey (~1pm).

Habitat Characterization

Bee species composition can only be fully understood in combination with habitat data. Given this, all bee surveys should include a vegetation characterization component (see SOP 7: Vegetation Surveys for additional details). If hand netting, the simplest approach to habitat characterization is measuring vegetation in quadrats along a transect. In this method, the surveyor should list each flowering plant species found in a one-meter square quadrat placed every 5 meters along the hand netting transect. Each species can then be quantified by the percentage of total quadrats sampled in which it was found. This method provides a quantitative measure of relative species composition.

Hand Netting Field Protocol

Surveying Conditions

Optimal netting times will likely differ with the plant community of interest and should be discussed with the Project Manager in advance. Be aware that ideal survey periods are mid-morning (~9-11am) survey and early afternoon (~1-3pm). Certain bee species, particularly specialists that forage on flowers with limited bloom periods, only fly at specific times of day. Mid-day heat can also restrict bee activity. Therefore, mid-morning and mid-afternoon are optimal for hand netting. If complementing the study with bowl traps, one netting period per day is sufficient, but if solely hand netting, aim for both morning and afternoon sessions. If no bees were collected during the survey, it is important to still document that data on your data sheet.

At least two of these conditions must be met to conduct a survey:

- Temperature is above 50°F and below 110°F.
- Cloud cover does not exceed "Mostly Cloudy" (>75% of the sky covered by clouds).
- **Mandatory:** Winds mild (less than 10 knots or 3 on the Beaufort scale, < 3m/s using a kestrel).

Do NOT conduct a survey during, or immediately following, precipitation.

Minimum Required Supplies for the Field if Netting

- ☐ Data sheets, and extras
- ☐ Tracking label sheets
- ☐ Printed Beaufort Scale
- ☐ Pencil for each collector and extras
- ☐ Net for each collector
- ☐ Replacement net bags
- ☐ Ice in a cooler for holding bees for observation *(non-lethal netting)*
- ☐ Camera or materials for collecting bee and plant vouchers
- ☐ 1-meter quadrat for vegetation surveys

If using lethal collection methods:

- ☐ Whirl Paks/quarter gallon Ziploc bag
- ☐ 1-gallon zip lock bags
- ☐ Collecting vials half-filled with alcohol or soapy water (ex: 50 ml centrifuge tubes)
- ☐ 1-gallon jug with soapy water or 1-gallon jug with alcohol

Additional Suggested Supplies for the Field

- ☐ Clipboard for each collector
- ☐ Notebook
- ☐ Collecting permits
- ☐ Open reel measuring tape
- ☐ Plant ID material
- ☐ Flags or flagging tape
- ☐ Backpack/hip pack
- ☐ Boots
- ☐ Sunglasses
- ☐ Hat
- ☐ Sunscreen
- ☐ Insect repellant
- ☐ Hydrocortisone cream
- ☐ Water bottle
- ☐ Lunch/snacks
- ☐ Toilet paper
- ☐ GPS unit
- ☐ Map
- ☐ Smart phone
- ☐ Chargers
- ☐ Two-way radios
- ☐ Camera
- ☐ Hand lens
- ☐ First aid kit

Instructional Videos
It is highly recommended that surveyors watch the following instructional video and practice these skills at a local site before conducting surveys.

Lethal hand netting techniques: https://youtu.be/nNfA4lXiveQ.

Field Instructions:

1. **Before leaving for your survey site:**
   a. Consult with your project manager to determine if the project will be keeping track of bee-plant interactions. If yes, then work with them to determine which plant species to collect bees from.
   b. Print out the provided tracking labels (Figure 13) and your project-specific data sheets. Use a **dark lead pencil** to prefill all the following information on both forms for each transect that will be surveyed: state, county, site name, transect/sample unit name, and collector name(s). **Do not use pen or Sharpie on any labels or specimen bags as the ink will dissolve when exposed to water or preserving solution.** Make sure you have extras of everything in case anything gets lost in the field.
   c. Use the supply checklist to confirm you have all the necessary supplies.
2. **Upon arrival at your survey site:**
   a. Before you begin, record all data on your tracking labels. Use the notes field or project-specific additional sheets to record additional data as required by your project.
3. **Netting bees:**
   a. When you are ready to begin netting, record the time on your data sheet and set a 30-minute timer. You will pause this timer every time you stop to process specimens from the net. **Expect 30 timer-minutes to require closer to 60 in-person minutes.**
   b. Hold your net in a "swing-ready" position by holding the net with your dominant hand below the head and your other hand towards the end or middle of the pole. Hold the tip of the net lightly against the pole with the hand near the head so that it does not drag in vegetation (Figure 7).

Figure 7. Photo of net held in the "swing-ready" position.

   c. Look for bees in exposed, open settings around clumps of flowers in a 1-meter radius on either side of the transect and in front of you. Stand a couple steps away from the flowers and try not to let your shadow fall across the flowers.

i. If studying bee-plant interactions…

1. Consult with your project manager on how to select what plants to collect from. Ensure that specimens from each focus plant species are kept separate and receive their own tracking labels.

ii. If netting at a site where bowl traps have also been deployed…

1. Collect bees along a transect through the plot boundaries.

d. When netting the bee…

i. If the bee is flying or resting on a flower…

1. Center the net on the side of the bee if possible, drop the tip of the net, and swing fast with an upward scooping motion (Figure 8).

Figure 8. Photo of net position for bee capture.

ii. If the bee is low to the ground…

1. Slap the net over the bee and onto the ground and placed your foot over the connection point between the handle and the net to stop the bee from escaping through the bottom. Hold the tip of the net up to encourage the bee to move up, further into the net. Once the bee has moved up, pinch the tip of the net closed and remove the net from the ground.

e. Flip the tip of the net over the net frame and snap the bee down into the tip of the net (see training video for technique). This will trap the bee deep in the net. Grab the net above where the bee is in the net to prevent it from escaping (Figure 9).

Figure 9. Photo of how to trap a bee in the net.

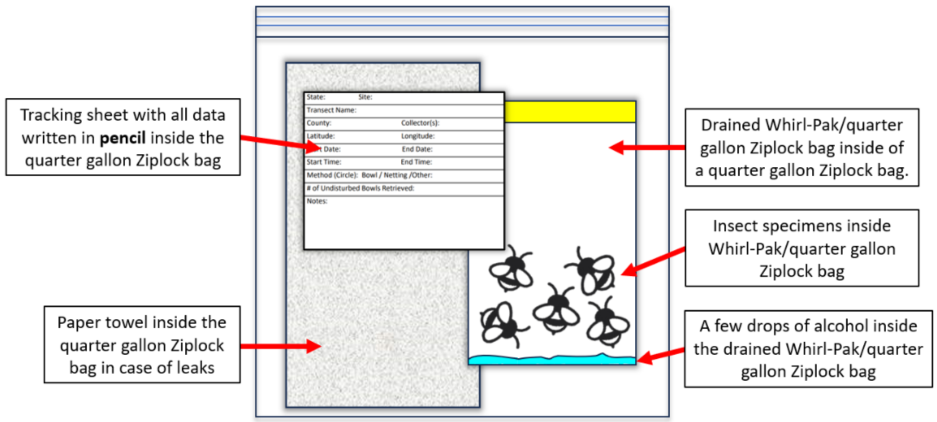
f. Upon successfully capturing the bee, immediately pause your timer, then transfer the bee into a collecting vial.
g. When you are ready to resume, start your timer again as you resume observing and netting. Stop and start the timer to process additional bees as they are collected.

4. **Removing bees from net** (see training video for technique)**:**
   a. Sharply snap the net down to force the bees down into the tip of the net. You can also sweep the net back and forth in a figure eight shape to push the bees into the tip of the net. If the bees are climbing up the sides, hold the tip of the net up to encourage them to move into the smaller end of the bag.
   b. Using your non-dominant hand, hold the net bag just below where the bees are resting to trap them into the tip of the bag and prevent them from escaping.
   c. Using your other hand, remove the cap from the container with alcohol or soapy water (for lethal collection), or an empty vial (for non-lethal capture-release), and insert the container into the net.
   d. As you slide the container into the net, slowly loosen your grip on the twisted net bag to allow the container into the net tip and toward the bees. Ensure the net is wrapped securely around the container to prevent bees from escaping out the sides.
   e. Scoop bees into the container as you move in, until everything in the net is collected. One method to encourage the bee into the container is to trap the bee against the side of the net, then tap or flick the bee through the net into the container.
      i. *For lethal collection:* While still in the net, swirl the container to ensure the bees are trapped in the collection medium. Put your thumb over the container or secure it along side of the net as you prepare to cap the container. Remove, immediately cap the container, then shake the container to ensure insects are euthanized quickly, minimizing prolonged distress.
      ii. *For non-lethal capture-release:* Put your thumb over the container or secure it along side of the net as you prepare to cap the container. Remove, and immediately cap the container.

5. **For non-lethal capture-release….**
    a. Take a photographic voucher of the captured bee immediately following capture. If the bee is too active to photograph, briefly chilling a specimen by putting the vial in a cooler with ice for a few minutes can make them calmer and easier to photograph.
    b. Photographic vouchers need to include (at minimum) close-up dorsal, lateral, and face views. Short video clips are often very effective.
    c. Release the captured bee once you have finished photographing it, restart the timer, and continue the survey.
6. **For lethal collection….**
    a. Depending on the project needs…
        i. If your project is **NOT** keeping track of specific bee-plant interactions…
            1. Collect all the bees in one container. Use a new container if the previous container is full. At the end of the survey, place all filled containers for the transect into a Ziploc bag with a tracking label detailing the containers' metadata.
        ii. If your project is investigating plant-pollinator interactions, every unique plant species collected from during the sampling event should have their own package and tracking label:
            1. Bees that are netted from the same plant species during each sampling event should be stored in unique whirlpaks
            2. Bees collected from multiple plants of the same species should all be placed in the same whirlpak
            3. A plant species voucher should be collected for each unique plant species that was sampled from. All unique plant species that are collected from should be photographed. If a physical voucher is needed to confirm species post sampling event, place the voucher specimen and the whirlpak containing the bees collected from that species into a Ziploc bag.
            4. Each whirlpak and Ziploc bag should contain a tracking label with the plant species name and image name(s) listed in the notes section.
        iii. For additional details on plant-pollinator interaction sampling design, refer to the Native Bee Monitoring Research Coordination Network protocols (*In review*, 2025) or consult with a subject-matter expert.
7. **Habitat Characterization**
    a. Place a one-meter quadrat every 5 meters along the hand netting transect.
    b. Record all flowering plant species observed in each quadrat, creating a separate list for every quadrat observed.
    c. Collect a physical or photo voucher for any unknown species.
8. **After survey:**
    a. Ensure all data has been recorded on all tracking labels and data sheets. Do not leave until all fields are filled out.
    b. Use the supply checklist to confirm you have all your supplies
9. **Short-term specimen storage (when lethally collecting specimens, or if collecting physical vouchers associated with non-lethal surveys):**

a. Upon returning to the lab, strain the bees out of the collection vessel and place the specimens into Whirl-Paks or quarter gallon Ziploc bags.
b. Roll up the Whirl-Pak with the specimens and place it into another quarter gallon Ziploc bag, along with a paper towel and the tracking label with all the metadata filled out (Figure 10).

Figure 10. Specimen packaging for short-term storage.



c. Unless your project is specifically investigating plant-pollinator interactions, all bees collected during a full 30-minute netting event should be combined into one quarter-gallon Ziploc with a single tracking label.
d. If your project is investigating plant-pollinator interactions, each unique plant collected from should have their own tracking label and package. Store the specimens in a -20°C freezer until they can be pinned or shipped to a lab for processing, within 6 months of capture. For easier organization, all bags from a single event (same day or transect) can be grouped together in a gallon sized Ziplock bag.

Standard Operating Procedure 6: Bumble Bee Surveys

This SOP was adapted from the draft National Bumble Bee Monitoring Protocol, in development by Ian Pearse (USGS), Clint Otto (USGS), and Tamara Smith (USFWS). Although many of the methods are the same as those found in the more general hand netting protocol, the study design is specific to bumble bees.

Bumble bees (*Bombus spp.*) are the most common species for which a Refuge might need to implement a standardized, non-lethal survey. Refuges may have many reasons for conducting non-lethal bumble bee surveys, such as determining presence/absence of an endangered species before a major management action. This SOP was adapted from the National Bumble Bee Monitoring Protocol currently in development at the time of publication of this protocol framework. Currently, most non-*Bombus* bee species cannot be identified to species using non-lethal collection methods, as their distinguishing characteristics can only be seen under a microscope.

Two sampling designs are described in this SOP, both of which employ a 30-minute timed survey along a variable (meandering) transect (see SOP 5) that enables the observer to focus on areas with high bumble bee activity or abundant floral resources. Bees do not need to be caught, captured, or marked as part of these surveys, but this may be done to obtain photographic or physical vouchers or for identification. The 30-minute timer will be paused while captured bumble bees are handled. The area of interest should be identified a priori, and variable transect boundaries set (and ideally captured via a polygon shape file). Therefore, although the survey time is standardized at 30 minutes, the survey area may vary between transects.

1) **Bumble bee counts**—*in which a tally of observations by species is recorded.*

Counts provide the rate of observations of a species over time. Although often referred to as “abundance sampling”, this design does not enable true estimates of abundance—neither of a particular species (absolute abundance) nor as a proportion of the bee community (relative abundance). In this design, at least six surveys should be conducted during the peak summer season to determine an annual count. Ideally, these will be executed by two observers surveying on the same day on three different days of the season, each two weeks apart. This scheduling may vary based on project needs but, at a minimum, surveys must be conducted on two days one week apart. For count surveys, the surveyor should be trained sufficiently to identify bees in the region, though ‘partial community’ surveys may be conducted for a subset of the bumble bee community if the focal bee species are clearly outlined.

The annual count per species may then be calculated as peak count (max of surveys), average count over surveys (mean of surveys), or an estimate of integration (area under the curve) of the count-date relationship over the period delimited as the peak season. Estimates of within-year phenology may be calculated based on the relationship between count and time of year. This design may be most appropriate if the project objective is to compare the count of a particular species between locations or time periods.

Note: this design is often referred to as “abundance” rather than “counts”, including in the National Bumble Bee Protocol from which this SOP was derived. The term “counts” is used here to clarify the metric that is being recorded and its limitations, namely that it cannot be used to estimate population abundance or relative community abundance.

2) **Bumble bee occupancy**—*in which a list of species (or castes) observed is recorded.*

Occupancy surveys document the species observed, but not the number of individuals of each species. If a focal species is of primary interest, the standard 30-minute survey can be truncated upon detection of the focal species and the actual survey duration noted, to expedite survey effort. In this design, a minimum of 2-4 independent 30-minute surveys should be conducted during peak summer season to assess annual occupancy. More surveys may be conducted if desired. Ideally, these surveys will be conducted by two observers on the same day on two different days during peak bumble bee season, approximately two weeks apart. A single survey may be conducted by two observers searching for 15-minutes, each recording a separate species list.

A photo voucher should be collected of any focal bee species observed and of any difficult-to-identify species observed at a site. Optionally, the exact GPS locations of focal bumble bee species may be recorded.

This design may be most appropriate if the project objective is to inventory which bumble bee species are present, or to determine whether a specific bumble bee species is present.

Bumble Bee Field Protocol

Surveying Conditions

Surveys should occur between 6:30 AM and 9:00 PM, and ideally will be conducted mid-day (9 AM – 5 PM). Weather requirements to conduct a bumble bee survey are specified below. Surveys may still be conducted outside of these conditions if a high level of bumble bee activity is observed prior to initiating the survey, if conditions are recorded.

The time spent conducting a survey should range from 30 minutes to 1 hour (depending how many bees are netted for identification) for count surveys, and from 5 minutes (if truncating survey to focal species observation that occurs quickly) to 45 minutes (if many bees are netted for identification) for occupancy surveys.

If no bees were observed during the survey, it is important to still document that data on your data sheet.

At least two of these conditions must be met to conduct a survey:

- Temperature is above 60 F.
- Cloud cover does not exceed "Mostly Cloudy" (>75% of the sky covered by clouds).
- **Mandatory:** Winds mild (less than 10 knots or 3 on the Beaufort scale, < 3m/s using a kestrel).

Do NOT conduct a survey during, or within 2 hours of, precipitation or when vegetation is extremely wet.

Minimum Required Supplies for bumble bee surveys

- ☐ Data sheets, and extras, or electronic data entry tool
- ☐ Pencil for each collector and extras
- ☐ Identification guide
- ☐ Net for each collector or collection cups for directly collecting bumble bees
- ☐ Replacement net bags
- ☐ Vials for captured bees
- ☐ Camera or materials for collecting bee and plant vouchers
- ☐ GPS unit
- ☐ Timer (a phone works for this)
- ☐ Printed Beaufort wind scale or handheld weather station that records temperature & wind

Additional Suggested Supplies for bumble bee surveys

- ☐ Ice for holding bees for observation
- ☐ Clipboard for each collector
- ☐ Notebook
- ☐ Survey permits
- ☐ Open reel measuring tape
- ☐ Plant ID material
- ☐ Backpack/hip pack
- ☐ Flags or flagging tape
- ☐ Boots
- ☐ Sunglasses
- ☐ Hat
- ☐ Sunscreen
- ☐ Insect repellant
- ☐ Hydrocortisone cream
- ☐ Water bottle
- ☐ Lunch/snacks
- ☐ Toilet paper
- ☐ Map
- ☐ Smart phone
- ☐ Chargers
- ☐ Two-way radios
- ☐ Camera
- ☐ Hand lens
- ☐ First aid kit

Instructional Videos

It is highly recommended that surveyors watch the following instructional video and practice these skills at a local site before conducting surveys. How to collect bees using a net: https://youtu.be/nNfA4lXiveQ

Field Instructions:

Foraging bumble bees are slow and can be collected directly into small containers for ID. This is similar to hand netting but requires many containers if many individuals are retained for batch identification. See SOP 5: Hand Netting for detailed instructions on hand netting if you will be capturing bumble bees during the survey.

Note: Maintaining protocols for a site over time is important, so if surveys deviate from protocol initially, note this and maintain those methods over time.

1. **Before leaving for your survey site:**
    a. Use the supply checklist to confirm you have all the necessary supplies.

2. **Upon arrival at your survey site:**
    a. Go to the center point of the site that has been defined in the site description.
    b. Before you begin, fill in site information (date, site name, weather info, observer names and experience level, site disturbance, etc.)
        i. Weather information that should be recorded includes temperature, cloud cover [estimated percent], precipitation [none, trace, light, full], wind (handheld or Beaufort category), humidity [estimated, optional]. Record whether conditions were documented on a handheld weather station (ideal), estimated, or determined retroactively using online weather data.\
    c. Estimate and record abundance of bumble bee-preferred flowers (true flowers, flower heads, capitula, etc.) in orders of magnitude (e.g. 1, 10, 100, 1000, 10000, etc.) and the percent floral cover of the sample unit. Identify flowers at least to genus, ideally to species. Take a voucher and/or use iNaturalist/ Seek for identity of flowers that you do not know.
    d. We recommend recording (check mark) which flowers were visited by any bumble bee.

3a. Bumble bee survey—Counts:
    a. When you are ready to begin netting, record the time on your data sheet, set a 30-minute timer, and begin recording your GPS track (optional).
    b. Meander through site recording tick marks for each bumble bee observed.
    c. Stop timer when catching bees and photographing bees. Restart timer when resuming active observation.
    d. For focal bee species, e.g. rusty patched bumble bee, record caste if known (caste for all species may be recorded but is not required).
    e. Imperfect identifications may be recorded under ‘other’ bee species, and vouchered for later ID.
    f. Provide a physical or photographic voucher of each bee species.

    i. If permitted to provide a physical voucher, see SOP 5: Hand Netting for detailed instructions on preserving specimens.
    ii. Photographic vouchers need to include (at minimum) dorsal, lateral, and face views. Short video clips are often very effective. Briefly chilling a specimen can make them calmer and easier to photograph.

g. Once a tally of 50 individuals of one bumble bee species or caste is found, note the time (from timer) elapsed to reach a count of 50, and stop counting that species.
h. When a rusty patched bumble bee is observed, note the floral resource on which it is found and take a voucher photo. Consider taking an exact GPS point of that observation (optional).
i. Once the 30-minute timer stops, record the time that the survey ended and record any survey notes. Note the abundance of any additional bumble bee-preferred plant species observed during the survey. Place an "X" next to the plant species where bumble bees were observed foraging.

3b. Bumble bee survey—Occupancy:

a. When you are ready to begin netting, record the time on your data sheet, set a 30-minute timer, and begin recording your GPS track (optional).
b. Meander through site recording the presence of bumble bee observed (e.g. with an "X" or checkmark).
c. For focal bee species, e.g. rusty patched bumble bee, note caste if known (caste for all species may be noted but is not required).
d. Imperfect identifications may be recorded under 'other' bee species, and ideally vouchered for later ID.
e. Ideally provide a physical or photo voucher of each bee species observed (optional).
    i. If permitted to provide a physical voucher, see SOP 5: Hand Netting for detailed instructions on preserving specimens.
    ii. Photographic vouchers need to include (at minimum) close-up dorsal, lateral, and face views. Short video clips are often very effective. Briefly chilling a specimen can make them calmer and easier to photograph.
f. Stop the timer for long periods catching, collecting, and photographing bees.
g. When a threatened or endangered bumble bee (e.g. rusty patched bumble bee) is observed, note the floral resource on which it is found and take a photo voucher. Consider taking an exact GPS point of that observation (optional).
h. Once the 30-minute timer stops, record the time that the survey ended and record any survey notes. Note the abundance of any additional bumble bee-preferred plant species observed during the survey. Place an "X" next to the plant species where bumble bees were observed foraging.

4. After survey:

a. Organize any photos or vouchers before leaving the site.
b. Ensure all data has been recorded on all tracking labels and data sheets. Do not leave until all fields are filled out.
c. Use the supply checklist to confirm you have all your supplies.

d. Confirm and update bee and plant IDs from vouchers upon returning to the office if there is any uncertainty.

Standard Operating Procedure 7: Vegetation Surveys

The bees collected through any of the methods described in this protocol framework are inextricably linked to the vegetation community in which they are found. Bee species composition can only be fully understood in combination with habitat data. Given this, all bee surveys should include a vegetation characterization component. This SOP provides guidance on two methods of documenting the surrounding vegetation community when collecting bees via bowl traps or hand netting.

The simplest suggested approach to plant sampling in association with bee surveys is a meandering walk that documents a list of all flowering plant species present. This is the vegetation survey method described in SOP 1 of the Prairie Reconstruction Initiative's National Protocol Framework for Monitoring Vegetation in Prairie Reconstructions (McColpin et al., 2019). It uses the DAFOR (Dominant, Abundant, Frequent, Occasional, Rare) scale to make a general estimate of abundance of each species. This method will provide species richness in the study plot and is best used if the goal is to determine which and how many species of flowers are present. It is the most basic habitat characterization that should be completed in conjunction with sampling via bee bowls. Bee bowls attract and sample bees over a large area, and this vegetation sampling method is useful in describing those broad habitat characteristics.

If hand netting, or if the study calls for a more quantitative measure of habitat characteristics associated with bee bowls, measuring vegetation in quadrats along a transect is more appropriate. In this method, the surveyor should list each flowering plant species found in a one-meter square quadrat placed every 5 meters along the hand netting transect. Each species can then be quantified by the percentage of total quadrats sampled in which it was found. This method provides a quantitative measure of relative species composition. Additionally, SOP 5: Hand Netting provides guidance on documenting the flower species on which a bee is captured.

Finally, if a study seeks to answer very specific questions about the floral resource characteristics of the survey site a more in-depth protocol is recommended. The most appropriate protocol depends on specific study objectives, and it is recommended that the project manager consult with the Refuge I&M Pollinator Team or another expert for guidance.

Standard Operating Procedure 8: Data Collection and Management

All major data collecting endeavors require the creation of a Data Management Plan (DMP) per 274 FW 1. A data management plan describes the data that will be acquired and how it will be managed, summarized, and stored. The Service provides comprehensive guidance on data management requirements, including templates for developing data management plans (https://doimspp.sharepoint.com/sites/fws-data/SitePages/DataManagementPlan.aspx). Your regional data managers can assist you in developing and filing a DMP.

International data formatting, management, and metadata standards should be used for all aspects of data recording, digitization, storage, and publishing. Adherence to international standards increases the usefulness and quality of your data, reduces the opportunity for error and information loss, and enables integration with other datasets for modeling and analysis. Recommended data standards are provided below and should be described in the project's Site-Specific Protocol and DMP.

Any Data Management Plan associated with a National Wildlife Refuge System project should include developing a ServCat file structure and a process to upload data products into ServCat. This includes tabular data, as well as the Site-Specific Protocol, geospatial files, data collection forms, and project reports.

To facilitate the public availability of pollinator data, final data products should be uploaded into a public biodiversity database such as the Global Biodiversity Information Facility (GBIF) or Symbiota. If specimens are sent to an external partner for identification or collections archiving, that facility may provide this service. Discuss a plan for data integration into a "free and easy access" external database with the partner facility prior to initiating data collection. The only exception to this should be sensitive data for Threatened & Endangered (T&E) species.

Data Standards

The Darwin Core Archive (DwC-A) is an internationally-recognized 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 and integration of the same data fields across multiple datasets.

The Biotic Observation Minimum Specifications (BOMS) were derived from Darwin Core standards specifically for the Fish & Wildlife Service by FWS data managers. BOMS identifies which Darwin Core terms are relevant for FWS surveys, without every user needing to read through and understand the entire Darwin Core library of terms. The Pollinator I&M team developed a comprehensive BOMS template of data fields specifically for bee surveys that can be easily adapted into a BOMS-compliant database. This template is intended to be shared with your data manager, or whoever is setting up the project database and data collection tools (e.g., Survey 123).

The use of data standards, such as BOMS/ Darwin Core, enables the integration and analysis of data from multiple independent project datasets for large scale analysis. For example, a bee survey dataset from one refuge may have a data field labeled with the term 'Name'. Their 'Name' field contains the scientific names of all species represented in their survey dataset. Another bee survey dataset from another refuge might label their similar data field with the term 'Identified'. Normally this discrepancy in field names would pose a problem for someone seeking to identify and compare the list of species collected on these two refuges. However, if the owners of the datasets map or associate their scientific name fields with the BOMS/ Darwin Core Standard term "Scientific Name" then any future user 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 second dataset. It also facilitates the integration of project data with global biological databases like GBIF, which use Darwin Core standards.

A dataset owner can choose to use actual BOMS/ Darwin Core Standard terms to label the fields in their dataset, or those terms can be "hidden" in the background so the user can see the more colloquial field labels. Regardless of how the database is set up, the use of BOMS/ Darwin Core terms 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.

Considerations for Bee identification Services

When contracting identification services with external entities, a data sharing agreement between FWS and the entity may be required (OCIO Directive 2022-001). A template data sharing agreement for specimen identification can be found in the ServCat reference for this protocol framework. For assistance creating the data sharing agreement, please contact your regional I&M data manager.

Data Collection

All data collected must adhere to the Biotic Observation Minimum Specifications (BOMS), derived from Darwin Core Standards. Template field data collection sheets for each survey method are available in the ServCat reference for this protocol framework. These can be printed and used as paper datasheets in the field. When 'tracking labels' (Figure 13) are used, the "eventID" field should be included to connect site and sampling event data in the datasheets, database, and specimen identification data. 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 individual specimen to the database or digital record to any related materials (e.g. photos) and publications and vice versa.

Data Management

At the conclusion of each sampling event, data sheets should be scanned and archived as a product under the survey's ServCat project reference. Data should also be entered into a digital database that is regularly archived as a product under the ServCat project reference. The database should include all project-level, site-level, event-level, and individual specimen-level BOMS fields (and associated metadata) relevant to the survey. A template for BOMS-formatted data fields relevant to the bee sampling methods outlined in this framework has been developed and should be utilized when designing this database.

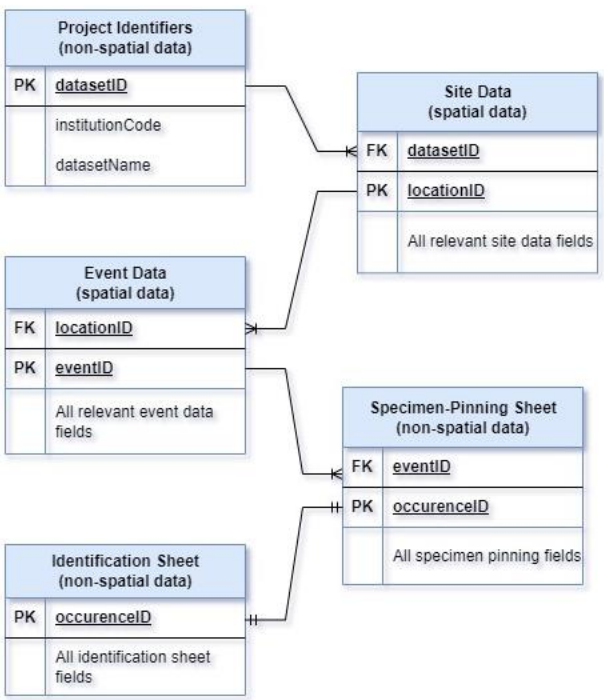
Archiving Survey Products in ServCat

ServCat is the approved data repository that should be used to archive all products associated with any Refuge inventory or monitoring effort. A unique project reference should be created for each site-specific protocol developed by the Refuge from this protocol framework and should be cross-linked to the National Protocol Framework. All data products, including tabular data, geospatial data, reports, photographs, and the Site-Specific Protocol, should be archived under the project reference. Consult with your regional I&M data manager or the Refuge I&M Pollinator Team for assistance constructing the ServCat project and product references.

Database Recommendations

It is recommended that all data be stored in a file geodatabase that is shared to ArcGIS Online (AGOL) and is archived in ServCat as a product under the survey's project reference. Hosting data on AGOL provides the benefit of utilizing the suite of field data collection and visualization applications provided by ESRI to facilitate field data collection, management, and summarization. The file geodatabase structure can relate non-spatial data tables (e.g., project identifying and specimen data) to spatial data (e.g., sampling sites, sampling event, FWS authoritative spatial data, etc.) while retaining all relevant metadata for each data source. A data schema for the file geodatabase is provided in Figure 11.

Figure 11. Recommended data schema for the file geodatabase.



Note that the primary key for each table in the figure above is identified in the left column by the letters "PK" (Figure 11). The primary key serves as a unique identifier for each row of information contained in the table. The arrows between the tables indicate the fields that will be used to relate the data from each table ("FK", or foreign keys). Refer to the template data field excel file to determine all other relevant fields to include in each table. For assistance creating or structuring the file geodatabase, sharing to AGOL, and developing field data collection applications or data summaries, consult with your regional I&M data manager or the I&M Pollinator Team.

The Project Identifiers table captures project-level metadata that is required as part of the BOMS/ Darwin Core standard. The institutionCode will always be "FWS". The datasetID is a unique identifier that distinguishes the data collected by this project from other datasets collected to support other FWS efforts. A one-to-many relationship connects the Project Identifiers table to the Site Data table; many different sites will likely be sampled during the life of the project, and all should share the same datasetID.

Each sampling site should be assigned a unique locationID in the Site Data table. If the survey method calls for the use of one or multiple transects, a unique transectID should be assigned to each transect in the sample site and captured in the Site Data table. A one-to-many relationship connects the Site Data table to the Event Data table; multiple sampling events will likely occur at a single sampling site during the project, and each sampling event that occurs within the sampling site should share the same locationID. If the specific survey design calls for tracking transect-level sampling events, then the transectID field should be used to relate these tables instead of the locationID field.

The Event Data table captures sampling event-level data, such as start and end times, weather conditions, and collection method. Each event should be assigned a unique eventID. A one-to-many relationship connects the Event Data table to the Specimen-Pinning Sheet; it is anticipated that multiple bee specimens will be captured during each sampling event and each individual specimen caught during the event should share the same eventID. This is extremely important for developing labels for each specimen (see SOP 9 for more information on developing specimen labels). Each specimen collected during the sampling event should be assigned a unique occurenceID.

The Specimen-Pinning Sheet and the Identification Sheet share a one-to-one relationship and are connected by the occurenceID field. Each pinned specimen will have a single entry in the Specimen-Pinning Sheet and the Identification Sheet. In cases when specimen identification is completed by an external party, both the Specimen-Pinning Sheet and the Identification Sheet should be shared with the individual or organization responsible for providing the identification service. Once the identification services are complete, the two sheets should be shared back to the Refuge and the Identification Sheet table in the geodatabase should be updated.

Open Data Sharing

Species occurrence data should also be shared to the Global Biodiversity Information Facility (GBIF). This can be accomplished in-house using the GBIF Integrated Publishing Toolkit. Alternatively, as part of the terms of the data sharing agreement, the institution providing the identification services can upload the occurrence records to GBIF on behalf of the Service. For assistance with publishing data to GBIF, consult with your regional I&M data manager or the I&M Pollinator Team.

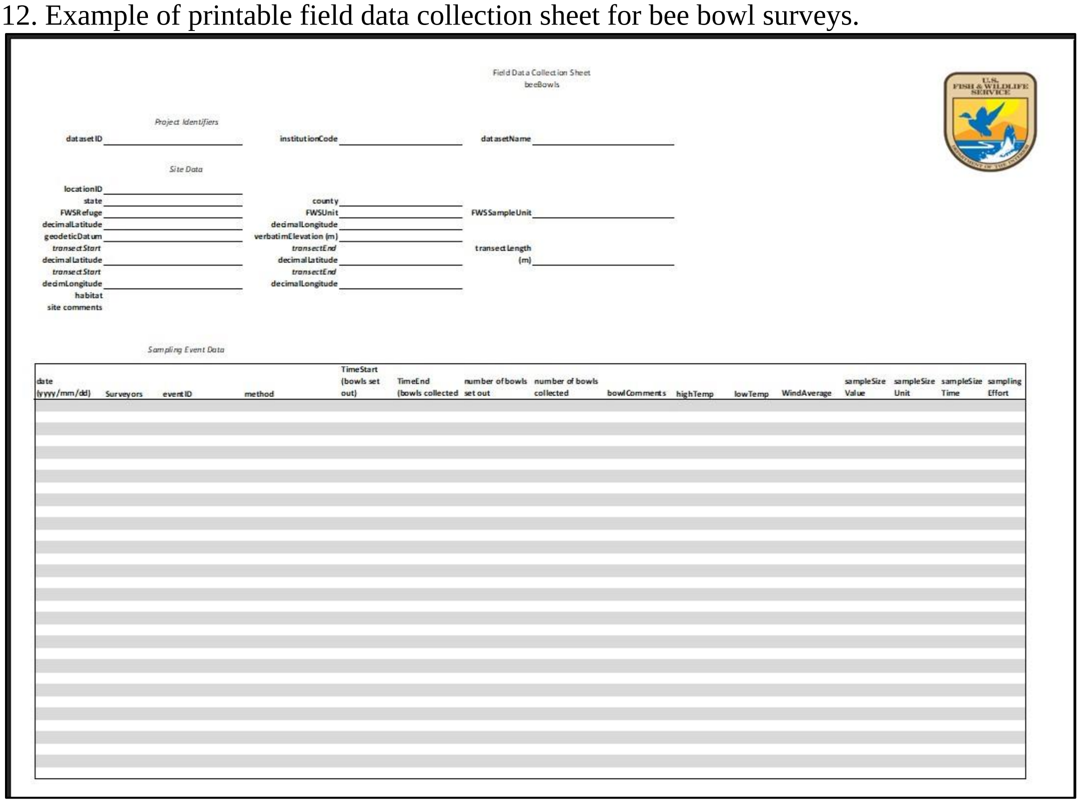
Recording Metadata

Comprehensive metadata records should be created and published for all datasets developed through application of this protocol. Metadata describe information about a dataset that enables it to 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. The FWS Data Management SharePoint site provides additional guidance on creating metadata records and metadata standards used by the Service: https://doimspp.sharepoint.com/sites/fws-data/SitePages/Metadata.aspx.

Template Data Collection Sheets

The ServCat reference for this protocol contains an excel file that can be used to generate data collection sheets for use in the field. Data collected in the field using printed data collection sheets should be digitized after each sampling event and archived in ServCat as a product under the project reference. An example of the available, printable field data sheets can be seen in Figure 12. The sections at the top of the sheet capture Project Identifier data and Site Data. The fields in the table capture event-level data for multiple sampling events at a single sampling site over the course of the study. Note that these templated datasheets should be printed using the landscape orientation.

Figure 12. Example of printable field data collection sheet for bee bowl surveys.



Field Data Collection Sheet
beeBowls

Project Identifiers

datasetID ____ institutionCode ____ datasetName ____

Site Data

locationID ____
state ____ county ____
FWSRefuge ____ FWSUnit ____ FWSSampleUnit ____
decimalLatitude ____ decimalLongitude ____
geodeticDatum ____ verbatimElevation (m) ____
transectStart *transectEnd* transectLength
decimalLatitude ____ decimalLatitude ____ (m) ____
transectStart *transectEnd*
decimLongitude ____ decimalLongitude ____
habitat
site comments

Sampling Event Data

date (yyyy/mm/dd)	Surveyors	eventID	method	TimeStart (bowls set out)	TimeEnd (bowls collected	number of bowls set out	number of bowls collected	bowlComments	highTemp	lowTemp	WindAverage	sampleSize Value	sampleSize Unit	sampleSize Time	sampling Effort

Template Tracking Labels

The tracking label below should be printed and included with bee specimens collected in the field and sent to a lab for identification (Figure 13). This tracking label can also be found in the ServCat reference for this protocol.

This tracking label is not that same as, or a substitute for, a field data sheet. The tracking label will travel with specimens through their identification process. A field data sheet is typically more comprehensive and will remain with the field biologist (see Fig 12.).

Figure 13. Template tracking label to be included with bee specimens.

State: County:	
Site:	
Transect Name:	
Latitude:	Longitude:
Start Date:	End Date:
Start Time:	End Time:
Method (Circle): Bowl / Netting /Other:	
# of Undisturbed Bowls Retrieved:	
Notes:	

Standard Operating Procedure 9: Storing, Shipping and Pinning Collected Bee Specimens

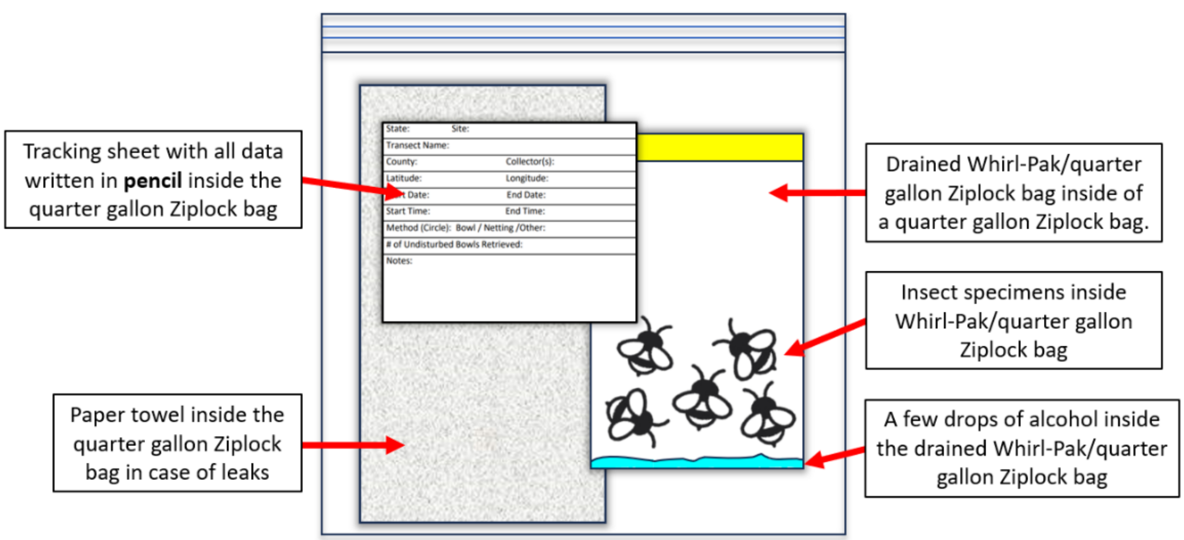
Refer to SOP 2: Equipment and Preparation for comprehensive information on all supplies referenced in this SOP.

Storing immersed specimens
For step-by-step instructions on storing specimens, please refer to “Short-term specimen storage” in SOPs 3, 4, or 5.

Bee specimens should be immersed in alcohol or propylene glycol and kept in Ziploc or Whirl-Pak bags inside another plastic bag. The specimens should be kept refrigerated in the short term (up to 2 months) or placed in a -20°C freezer if stored for longer periods (up to 6 months) prior to processing the specimens and/or mailing them to a processing center. Freezing reduces the evaporation of the liquid the specimens are immersed in and minimizes the risk of deterioration or rotting prior to processing and shipping.

If the bags will be shipped, the bags of specimens should be placed inside a second, larger Ziploc bag that also contains a paper towel to soak up any possible leaking liquids (Figure 14). That Ziploc bag should then be placed into a third Ziploc bag just to be sure that any spilled liquids are contained.

Figure 14. Specimen packaging for short-term storage.



Preparing immersed specimens for shipping/mailing
Immediately prior to shipping, drain excess liquid from the bags to prevent leaks while shipping. Leave a small amount of alcohol or propylene glycol in the bag to help preserve the specimens in transit. 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 specimens collected at 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.

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. A video instructing how to prepare specimens for mailing is available at: www.youtube.com/watch?v=POQmHUVwFjw (starting at 2:52).

Preparing specimens for pinning

Pinning and processing specimens should only be undertaken following proper training. Reach out to the Refuge I&M Pollinator Team, NCTC, or the lab that will be identifying your specimens for training opportunities.

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 were not kept in liquid and the hairs are not matted down, then can be pinned immediately, as it preserves the pollen load for future analysis (which is not the case for bees captured in bowl traps) and speeds up the entire process. However, if the bees are matted from too much water 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 collection in 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. The longer a specialist must look at a poorly prepared specimen, the less time and funding they have for identifying additional bees. Washing and processing bees using the process describe here will result in well-groomed specimens that exceed the quality of unwashed specimens pinned directly from the field.

Note that the best-looking bees are those that are cleaned within 24 hours of capture.

Strainer/'bee washing' jar method: Fill your specimen Whirl-Pak with water and then dump the contents into the strainer (tea strainers work well because of their fine mesh, brine shrimp nets also have sufficiently small mesh, but it is more difficult to remove specimens because of the flexibility of the netting). Dump the specimens into a plastic container with a lid. Add warm water and dishwashing liquid (more if the specimens were stored or collected in propylene glycol) and very vigorously shake the specimens for 60 seconds. Place specimens back into the strainer and rinse under warm to hot tap water until no suds are present. Use your hand to break the force of the water to protect the specimens. Shake off loose water and use a towel to blot out as much excess water on the bottom of the strainer or brine shrimp net as possible. Then use any of the drying techniques described below.

Whirl-Pak washing method: An alternative to using washing jars is using the original Whirl-Pak. Carefully drain the preservative from the Whirl-Pak, then add a drop of soap and warm tap water. 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. Rinse the sample carefully under the tap until clean.

Mosquito netting and net bag washing: Mosquito netting makes for a great vessel to wash bees. The first way to do so is to cut a square of mosquito netting that is at least 12 in by 12 in. Lay the mosquito netting into a large metal strainer (preferably one that has a flat base and is self-standing). Dump the bees into the very center of the square of fabric and fold the fabric around the bees until you have a small pouch, with the bees at the bottom and all the excess fabric at the top. Use a reusable zip tie to seal the bees in the fabric. We recommend using more than one zip tie in case one fails. Make sure that you are not securing the zip tie too close to the pocket of bees as you want them to be able to bounce around. In addition, ensure that there are no holes in the netting for the bees to escape through.

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 should 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 and wings can stick to the bees' bodies, obscuring identifying characters on the thorax and making important wing venation characters difficult to use for identification. Regardless of the drying method used, bees should be fully dried and appear lifelike before pinning and labeling. 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.

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. If you aren't in a hurry, you can leave them lying on the paper towel for up to 45 minutes before further fluffing. Then, 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 fluffy.

Mason jar drying method: For this method, you will need a small clear glass pint or half pint jar and a canning jar lid with a removable central metal disk. Replace the center of the canning jar lid with a similarly sized section of fiberglass or metal screening. The screen can either be left loose and the lid used to clamp it down, or it can be glued into the lid using waterproof glue.

Pour the specimens into the canning jar and put the lid back on, making sure the screen is snug around the entire lid.

Turn a 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 a folded hand towel and place the hair dryer pointing into the jar as close as possible. 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. 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 done once their wings are flexed away from their body and their hairs are not matted. Bumblebees and long-haired specimens take longer. Depending upon your hair dryer and your technique, this may take anywhere from 1.5 to 3 minutes. See the Very Handy Bee Manual for additional tips and tricks with this and other methods (Anonymous, 2024)

Compressed air-drying method: We have found that using compressed air results in the quickest drying of wet bee specimens. Compressed air can come from a small air compressor, a paint sprayer device (without the paint) or a compressed air duster for computers. 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. Bumblebees take about 2 minutes to have all the hair on their thorax fluff up.

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. Pour the 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 their 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 Fig. 19-D in Schauff, 2001; and Fig. 1 in Stephen et al., 1969). The midline of the scutum often contains useful identification characteristics which can be destroyed by a pin.

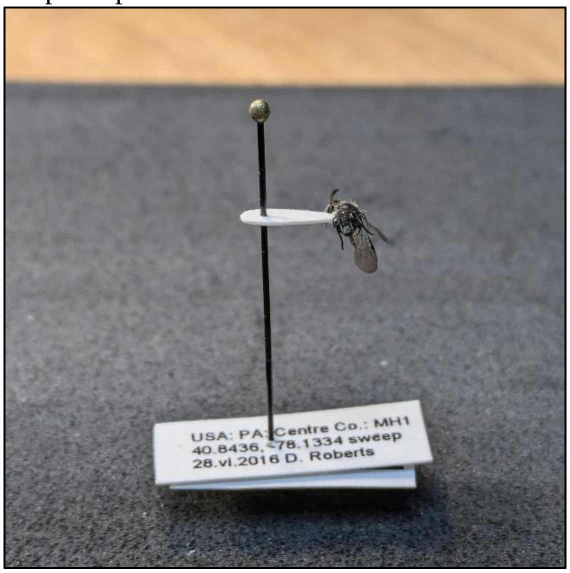
Leave enough room at the top of the pin so that the specimen can be safely picked up by the largest of fingers, and enough room at the bottom for two or more labels and insertion into the foam of a collection box.

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 or glued to the side of a pin (Figure 15). Consult with the lab that will be identifying your specimens to understand their preferred methods and requirements.

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.

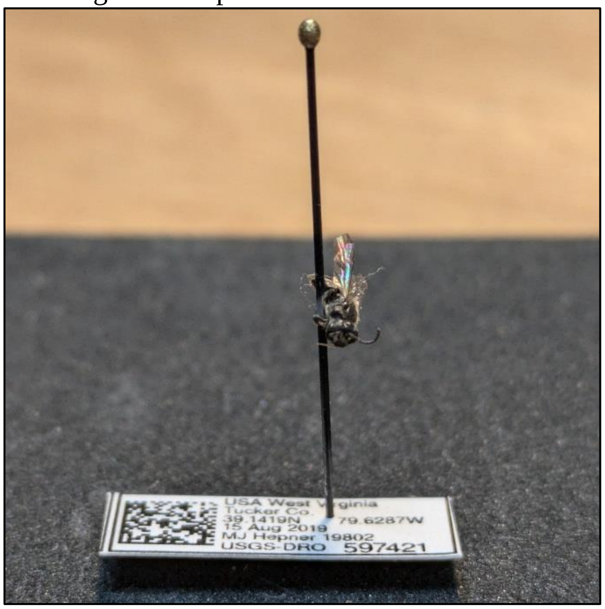
Figure 15. Photo of point-pinned bee.



Note: A paper point is 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 minutien pins or glue to mount very small insect specimens 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 (Figure 16). Again, most museums prefer that specimens are 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, matting down hair. 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. 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, 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

Figure 16. Photo of bee glued to a pin.



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

Storing pinned specimens

After a batch of specimens is washed, dried, and pinned, place them in a cardboard specimen box. 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 tracking label (now dried out) that was placed in the bag with a batch of specimens when first captured. 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 tracking label) belonging to each sample will begin a new row and so forth until the box is filled. In general, it helps if each box contains specimens from only one sample or site. 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.

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.

Unique specimen numbers are required for effective specimen and data management and for referencing and integrating digital data. Each 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 regarding 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 on printed and hand-written specimen labels, tracking labels, 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.

Speed up the process of cutting out printed labels by cutting out rows of labels, placing them in their corresponding specimen box, and then cutting the individual labels apart with scissors. See: www.slideshare.net/sdroege/preparing-insect-labels-a-faster-way and youtube/HqxrkC6xe40.

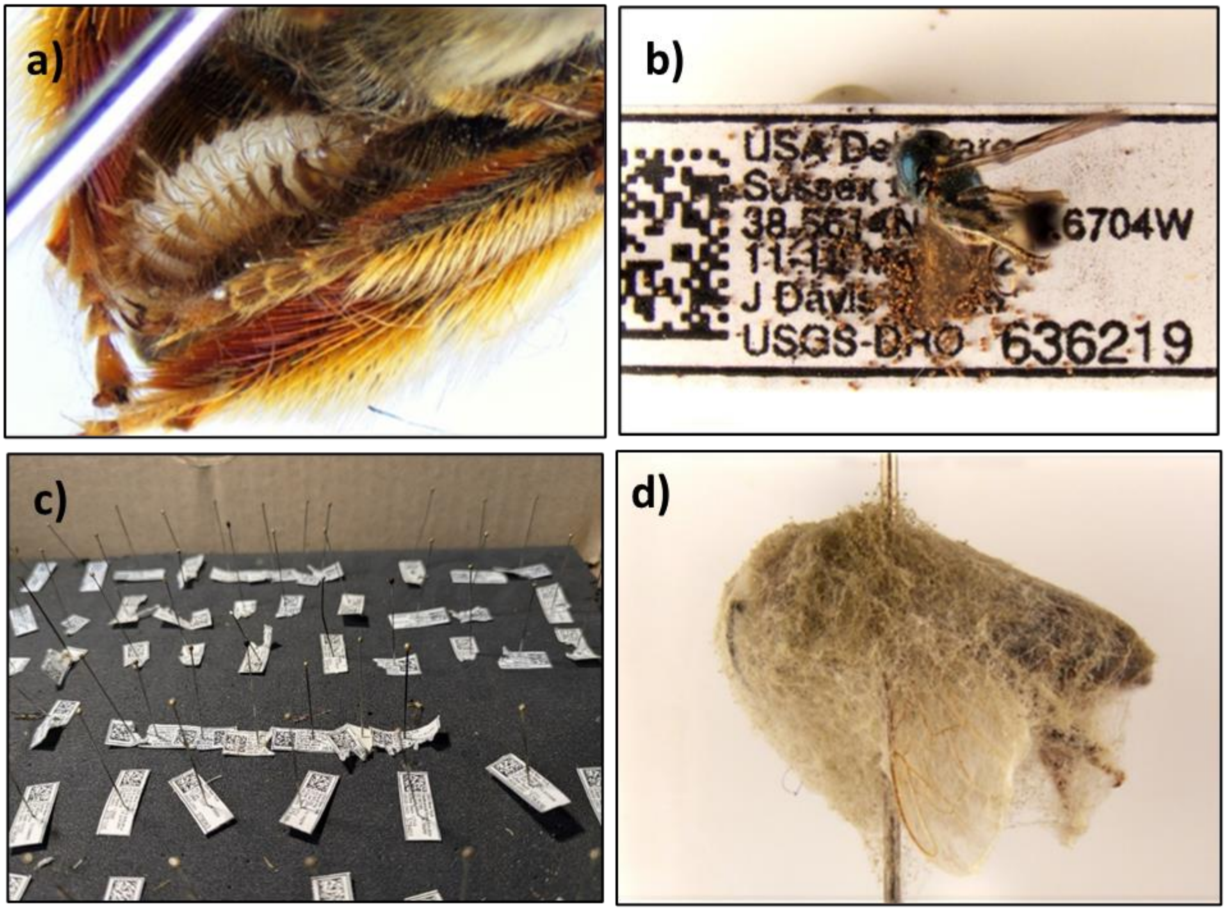
Specimen labels are quickly added to specimen pins by laying them across a piece of Ethafoam or a pinning board that is the same thickness and desired height of the label on the pin. Labels are oriented along the same axis as the specimen with the specimen's head over the left-hand end of the label – 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). Confirm label information matches the row tag prior to putting labels on specimens (if using the Discover Life labeling program).

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 (Figures 17 and 18a). 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 (Figure 18b). Mice will chew through boxes and tend to devour large bees, such as bumble and carpenter bees, but bees stored in Cornell boxes are protected (Figure 18c). Mold grows on bees that are prepared without thorough drying and/or when kept in humid conditions (Figure 18d).

Figure 17. Photos of a dermestid beetle.

Figure 18. Photo of a) live dermestid larva on a bee, b) a specimen with dermestid damage and frass, c) a collection with mouse damage, and c) a specimen with mold damage.



Control and prevent dermestids by freezing the box at -20° C (~0° F) for 3 days, thawing for a day, and then freezing for another 3 days. Freeze specimens for twice as long if using a household freezer. 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 BioQuip boxes, use 8-gallon bags to fit larger cardboard specimen boxes). Large plastic storage containers work well for protecting larger collections of boxes. Let the specimens dry out thoroughly after pinning (about one month) before enclosing them in a plastic bag. Before storing specimens long-term, put them through a thorough freeze cycle.

In humid conditions, 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.

Regardless of the storage method used, all collections should be regularly inspected to identify and address any potential threats to the quality of the specimens.

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 (Figure 19). 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.

Figure 19. Photo of how to use a cardboard top to stabilize pinned specimens for shipping.

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. Although it is good practice to fumigate boxes before shipping, do not leave loose fumigant, fumigant strips, or fumigant balls in the box with the specimens as they are 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. 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, add 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 leave at least 2 inches of free space around/on all sides of the boxes of specimens and surround the specimen boxes with packing peanuts or bubble wrap. 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 then be shipped via any major carrier, but those intended for genetic analyses should be shipped via FedEx as other carriers have been known to irradiate packages. Additional detailed information on shipping specimens is provided by Schauff (2001) and Hunter (2006). Consult with the lab that will be identifying your specimens for their standards and preferred shipping carrier.

Standard Operating Procedure 10: Identifying Collected Bees

Identification of bee fauna is difficult, time-consuming, and requires expertise that can only be acquired through many years of specialized training. Due to the large volume of bees that can be collected by even a single, moderately sized project, and the limited taxonomic expertise available to identify those specimens, identification can be expected to take several years. However, considerable information can be acquired from easily identifiable genera, species, and/or morphospecies, which may be sufficient for some project objectives. If NWRS station staff plan to identify specimens on their own, they should have subsamples reviewed by an expert to confirm the identification. Prior to starting any field-based data collection, you should identify and develop an agreement with authorized identification and collection facilities, such as a laboratory, university, or museum.

This SOP provides guidance for the use of the online Discover Life identification guides (www.discoverlife.org/mp/20q?search=Apoidea#Identification). A comprehensive guide on how to navigate Discover Life and its features can be found at https://docs.google.com/document/d/1ia3dcpmDCFSR2ERRsFMWLiaAyElY3TJi/edit?usp=sharing&ouid=100453343088071895016&rtpof=true&sd=true. Be sure to read the section at the end regarding the use of already identified specimens.

If you have questions about any of the Discover Life bee guides, please contact Sam Droege (sdroege@usgs.gov). His lab in Laurel, MD is open to anyone who would like to learn to process and identify their collection of bees. They typically have space, computers, and microscopes available, as well as access to their synoptic collection. Additionally, a set of identified specimens to be used while learning identification can provided at no charge.

Hint 1: If you are newly learning how to identify bees, we suggest that you look at the glossary of terms, vocabulary, identification tips, “Bee Body Part” figures, and pronunciation materials in *The Very Handy Manual 2.0: How to Catch and Identify Bees and Manage a Collection* (Anonymous, 2024). Contact the USGS Bee Lab for more resources (beelab@usgs.gov).

Training

The American Museum of Natural History (AMNH) offers a highly recommended annual two-week intensive training (“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, AMNH (www.amnh.org/our-research/staff-directory/jerome-g.-rozen).

Seminars for identifying bees to species level are held weekly and virtually by Clare Maffei (clare_maffei@fws.gov) and Sam Droege (sdroege@usgs.gov). The lessons on bee species identification are valuable for North American learners, as experts of niche groups often lead series on their published keys. Email Clare Maffei for more information on upcoming seminars. Archives can also be watched at: http://bio2.elmira.edu/fieldbio/beemovies/index.html

Viewing specimens under the microscope

When viewing specimens under the microscope, the USGS Bee Lab has found that it is most efficient for the observer to hold the specimen in their hand, being 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.

Organizing specimens for identification

Identification may begin after the specimens are labeled and the labels have been double-checked against the data records. When identifying specimens, first make a quick pass through the box, look at each specimen under the microscope, and identify those easily named without using a guide. As bees are identified they should be pinned in a separate box or rearranged in the original box if there is room. As new species are identified, write a determination label that states the genus and species and person completing the identifications (available as a modifiable Excel file with this protocol in ServCat). Pin the identification label to the board separately from the specimens (i.e., 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.

As you make your identifications, place the specimens in the box in rows starting at the upper left corner, working from left to right, top to bottom, pinning determination labels at the beginning of each new group of species. Position female specimens so that their labels (and the specimen itself) are vertical, and position male specimens so that their labels (and specimen) 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 ensure accuracy, identifications and digitized data should be independently error-checked.

Identification guides and materials

The taxonomy of North American bees, like that of many insects, is 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 *Bees in the Eastern United States*, are still very valuable sources for identification keys, illustrations, and species accounts, and are freely available online as a series of PDF files at https://catalog.hathitrust.org/Record/001515742 (Mitchell, 1960), and https://catalog.hathitrust.org/Record/011474482 (Mitchell, 1962).
However, 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 & Pickering, 2015) and within the 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.

Scientific names of species also change as our ability to identify and distinguish one species from another improves over time. 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'. Taxonomists maintain and map the relationships between old and new scientific names and publish them in lists such as those maintained at www.discoverlife.org or the Integrated Taxonomic Information System (ITIS, www.itis.gov). T

Additional Identification Resources:

- Laurence Packer's Lab has produced a guide to the bee genera of Canada: https://www.researchgate.net/publication/237841490_The_Bee_Genera_of_Eastern_Canada (Packer et al., 2007) and to the bee Families of the world: https://www.yorku.ca/bugsrus/resources/keys/BFoW/Images/Introduction/Introduction.html. A pictorial catalog of the genera of bees of the world is available online at: https://www.yorku.ca/bugsrus/resources/galleries/bgow

- All the Discover Life ID Nature Guides are located online at: www.discoverlife.org/mp/20q. The consolidated links to the bee guides and associated materials are online at: www.discoverlife.org/20/q?search=Apoidea. Many of these guides have been expanded to include Western species, with more additions anticipated in coming years.

- The Bee Identification with a Microscope weekly seminar has a Google Drive of resources for class members. Email bee.identification.resources@gmail.com or clare_maffei@fws.gov for more information.

- *The Very Handy Bee Manual* (2024) has detailed information on bee identification including a glossary and diagrams of bee characteristics, summaries of the traits of different bee genera, and how to use various forms of identification keys.

- University labs publish native bee identification material. Explore resources shared by the University of Minnesota, Ohio State University, and Oregon State University, among others.

Stylopized bees

As you identify bees you will come across bees that have an infestation of mites and more rarely, bees that have been parasitized by a *Strepsipteran* (i.e., stylopized). *Strepsiptera* are endoparasites of various 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 but are rarely found. 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 often encountered in bees and appear as small orange/brown plate-like extrusions that emerge from beneath the rim of the tergites of the abdomen (Figure 20). They look like 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.

Figure 20. *Strepsiptera* emerging from beneath the rim of the tergites of the abdomen.

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.