Native bee biodiversity long-term monitoring/survey/inventory protocol

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dx.doi.org/10.17504/protocols.io.wfhfbj6

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ABSTRACT

This is the full sampling protocol for a long-term native bee biodiversity monitoring/survey/inventory set up in 2011 at Pinnacles National Park in California. The park was sampled using these methods in 2011, 2012, and may be sampled again using the same methods in 2019 and 2020 to add an important additional time-step to earlier sampling to aid determination of trends in native bee ecology/decline/status over time.

More details about the 2011-2012 work can be found in the following masters thesis:
Meiners JM. Biodiversity, Community Dynamics, and Novel Foraging Behaviors of a Rich Native Bee Fauna across Habitats at Pinnacles National Park, California. Masters of Science, Utah State University. 2016.

These methods are also develped from a similar protocol that can be found at:
LeBuhn G, Griswold T, Minckley R, Droege S, Roulston T, Cane J, et al. A standardized method for monitoring bee populations–the bee inventory (BI) plot. 2003; Available:
http://cybercemetery.unt.edu/archive/nbii/20120111121317/http://online.sfsu.edu/~beeplot/pdfs/Bee%20Plot%202003.pdf

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Meiners JM. Biodiversity, Community Dynamics, and Novel Foraging Behaviors of a Rich Native Bee Fauna across Habitats at Pinnacles National Park, California. Masters of Science, Utah State University. 2016.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

Plot Establishment (at least a two-person job, more is ideal)

- Park map
- 100’ tape measure
- Range finder to help estimate distances when positioning plot
- GPS unit to record corners of plot and to check plot dimension distance measurements
- Red flags to mark outside boundary of plot (good color for visibility and to minimize influence on bee activity)
- Pink flags to quarter inside of plot to guide sampling walks (also good color)
- Camera to record images of plot from all corner angles

Net Collecting (designed for two regular collectors)

- 4 complete nets (2 for regular use, 2 extra)
- 2 extra net bags
- Extra wire to repair nets (necessity depends on type of net ring)
- 3 collection shoulder bags (2 for regular use, one for guest collector)
- 40 cyanide/killing vials (12 per each of 3 collection bags, 4 extra)—only need this many if trying to keep collections on different flowers separate per area
- 3 pair small scissors
- String to tie scissors to bag
- Kestrel or other device for measuring temperature, wind speed, other desired metrics
- Ethanol resistant pens

- Pre-printed data books (one sheet for each expected day and collecting location)
- “Stackers” or other plastic container with lots of small divisions to keep bees separated out of vials

Pan Trap Collecting (designed for sampling two sites at once)

- 3 sets of 30 painted “pan traps” (one per site to be sampled simultaneously, plus an extra set to replace broken bowls). MAKE THESE BEFORE HEADING INTO THE FIELD by spray painting 2-oz clear ‘Solo’ brand plastic shallow cups, with fluorescent blue, fluorescent yellow, or white spray paint (10 of each color per set)
- 4 gallon jugs (2 for regular use, 2 extra)
- ‘Dawn’ Brand blue dish soap (this specific kind is used to control for influence of particular soap scent or appearance on bee catch)
- 400 ‘Whirl’ packs, (4 for each time a site is sampled (one for each pan trap color, one to put all three bags in all together), plus extras)
- 9 tea strainers (3 for each site to be sampled simultaneously (one for each pan trap color), 3 extra)
- Masking tape to mark pan trap color on strainers
- Sharpie to label masking tape and Whirl packs
- Ethanol-resistant pens
- Pre-printed labels for pan trap colors (incorporated into pre-printed data books above)
- 4 gal. 75% (Usually buy as 95% and dilute down) Ethanol (about 10 - 16 oz. per collection site between 3 Whirl packs)
- 2 16 oz. plastic soda bottles to carry Ethanol into field
- Funnels to use to pour pan trap liquid back into gallon jugs and to dilute Ethanol into empty plastic soda bottles

Vegetation Monitoring

- Printed loose data sheets and binder to keep them organized
- Meter stick or piece of PVC with decimeter increments marked
- Homemade handy field guide with pictures for remembering common plants
- Ziploc bags for collecting plant vouchers
- Camera to record more plant detail if taking only a small section or voucher not possible
- Notebook with extra sheets or notecards to write more information about full plant size, location, etc to put in ziplocs with plant vouchers
- Pruning shears and/or small trowel for collecting woody plants (optional)
- Cooler for storing plant vouchers in car while continuing field work (optional)
- Plant press for vouchers (small one for field, then transfer into larger one at night)
- Newspaper and cardboard for blotting and absorbing voucher moisture
- Newspaper or loose-leaf sheets on which to tape vouchers when dried
- Masking tape to lightly tape down vouchers to paper
- Sharpie to mark collection number and information on voucher sheet
- Jepson Manual for identifying plants (for California)
- Herbarium reference collection and botanist experts to help with identification if possible!

Personal Field Gear (recommended)

- Radio or other method of reliable communication in the event of an emergency
- First aid kit
- Durable, non-cotton, long-sleeve and long-pant field clothes
- Wide-brimmed hat
- Sunglasses
- Sunscreen
- Backpack
- Water bottles
- Snacks
- Camera (for fun or to help with plant vouchers)
- Multi tool knife
- Snake chaps, if relevant in area

Durable footwear and hiking socks

Duct tape, to keep grass seeds out of your socks, and because it’s really cool

Specimen Pinning

- Field pinning ('Schmidt') boxes
- Pins (#1, #2)
- 'Elmers' non-washable glue or wood glue for gluing bees too small to pin
- Large plastic bowls for rinsing pan trap bees in 90% Ethanol
- 90% (undiluted) Ethanol
- Paper towels for drying and fluffing pan trap bees out of Ethanol
- Pen for labeling pinning boxes with date, location, collectors, frozen status, etc
- Freezer to store pinned bees and Whirl packs for 48 hours after pinning, then repeatedly check for Dermestid invasion and re-freeze
- Very large Ziploc bags for storing Schmidt boxes after freezing to prevent contamination

Collection and Database Management (don't need this equipment in the field)

- Computer with Microsoft Office and Access Database software
- ODBC database and entry form in Access for inputting field notes and assigning a unique ID accession number, with searchable collection information, to each specimen
- Printer for producing specimen labels
- Card stock paper for labels
- Fine, sharp scissors for precisely cutting labels
- Pinning block to mount labels evenly
- Unit boxes to move pinned, labeled specimen into
- Sealed wooden drawers for storing specimen in a museum collection safe from desiccation, mold, and attack by Dermestid beetles
- High quality dissecting microscope
- Light source for microscope
- Identification guide for bee genera
- Identification guides specific to each genera for species level determinations
- Card stock species determination labels
- Ethanol-resistant labeling pens
- Experts in bee identification who are willing to train or help with species determinations!

SAFETY WARNINGS

BEFORE STARTING

See steps

Equipment checklist/Pre-field season prep

1 Make sure you have the following equipment before heading into the field to set up a bee monitoring project. You will want to make the pan-traps (by spray-painting 2-oz solo cups) before heading into the field.

NOTE

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Set up long-term monitoring sites

2 Select sites:
We began our study by scouting out locations within our study area that represent a variety of habitat types so that we could survey all the different major landscapes within our broader area of interest (in this case, Pinnacles National Park in California). We looked to establish plots that had less than 50% shade cover to ensure adequate bee activity, and that occurred along available gradients in elevation, grass cover, and microclimates across the range of the park. Sites needed to be in areas where there was at least a hectare in size of a single habitat type, with a surrounding buffer of several hundred meters before it transitioned to any other habitat type. Sites also had to be flat enough to place pan traps (passive monitoring bowls) of soapy water (more on this later) across the entire plot without them tipping over. Sites also needed to be separated by at least 500m from each other to ensure that we were sampling distinct resident bee communities. The number of sites will depend on the planned sampling schedule (how much time you will have available) and how close the sites are to each other. If you can walk between them in less than 20 minutes, then you can sample two in the same day (daily schedule included in a later step).
We ended up setting up 10 sites, 8 in pairs and 2 that were set apart from the others and had to be sampled as the only plot for that day. These 10 sites spanned the area of the park and the four main different habitat types: alluvial, grassland, blue oak woodland (higher elevation), and live oak woodland (lower elevation).

3 Measure & mark sites:
We used a 100m tape and a handheld monocular laser rangefinder distance meter to measure the dimentions of the plots. There was some variation in proportions based on the contours of the landscape, but all plots were a total area of one hectare. Most ended up in a rectangular shape of about 200m x 50m.
Once we established the exact layout of each long-term plot, we recorded the GPS coordinates of the four corners and the centerpoint. We then marked each corner with pin flags. Outside of a National Park where more permanent markers are allowed, we may have marked the corners more permanently with rebar.
We then put additional pinflags (of a different color) along the sides and down the longer centerline of the plot to help guide movements during sampling.

Daily plot sampling protocol/schedule

4 The field sampling protocol for the project required two full-time collectors in order to sample two plots in one day and cycle through ten plots in a two-week period. Two regular collectors is also ideal to ensure safety in remote areas, to distribute and control for potential collector biases, to get pan traps set out and picked up at the same time in both plots simultaneously, and to capture diurnal bee diversity by sampling in pairs at specific times of the morning and afternoon.

We began our field seasons in both 2011 and 2012 in mid-February before most bloom had commenced, and ended the season in late June after most bloom had faded. We sampled ten plots in six days over a period of two weeks using the daily schedule included below, then started the cycle over for a total of eight sampling cycles of ten plots in 2011 and seven in 2012. Days deemed appropriate for sampling were fairly sunny, without high winds, and over 15 degrees Celsius if possible. Weather permitting, we typically sampled two pairs of plots per day Monday through Wednesday, and included additional trail collecting and work on side projects where possible. Thursdays and Fridays were spent collecting bees in other areas of the park for the diversity inventory, processing bee and plant samples, and entering data.

Each sampling day began and ended on site with the placement of pan traps (aka “bowls”). Pan traps were made from 2-ounce plastic Solo cups, spray painted one of three colors known to be attractive to bees (white, fluorescent blue, and fluorescent yellow). We placed 30 pan traps, ten of each color, evenly spaced in an “X” shape connecting the four corners of the rectangular plots at 9 am each sampling morning. Pan trap bowls were placed directly on the ground and filled ¾ full of soapy water (using a small squirt of blue Dawn dish soap, standardized within the bee research community to control for influences of soap scent or color). In grassland habitats, we made an effort to flatten an area in the grass on which to place bowls so that they were easily visible to bees from the air. We placed bowls in approximately the same position each time we sampled a plot because the bowl locations served as sample points for a companion vegetation monitoring protocol (*see Meiners et al., 2015*). At 4pm, after all net sampling had been completed, each collector picked up bowls in the same order in which they were placed out, straining the bee contents into three labeled mesh tea strainers, one for each pan trap bowl color, then emptying the strainers into separate plastic Whirl packs, filled with 75% ethanol until all bees were fully submerged in liquid, and stored in the freezer until specimens could be removed, washed, and individually pinned.

On days with sufficiently good conditions to anticipate bee activity, we began plot net sampling in the two plots at 10am and 11:15am, respectively. Immediately before these times, we recorded the cloud cover category (full sun, partly cloudy, cloudy, or mostly cloudy), and used a Kestrel monitoring device to record the ambient temperature (in the sun), wind speed, humidity, and barometric pressure at the plot. We began net sampling with the two collectors starting at opposite corners of the rectangular plot, then systematically working their way through one long half of the plot towards the opposite end for fifteen minutes, before switching sides to spend another 15 minutes covering the area on the other side, such that each collector evenly paced themselves through the entire plot area over 30 minutes and were not sampling in the same location at the same time. Because the study goal was to capture and record the plot bee community and relate it to measured habitat characteristics, we focused on moving consistently throughout the plot, rather than spending inordinate amounts of time in richly floral patches promising higher bee captures.
Net sampling involved visual and auditory-focused collecting of bee individuals encountered, rather than sweep-netting or otherwise randomized sampling methods. We quickly learned to visually distinguish and avoid collecting wasps and flies, but attempted to avoid bias in collecting certain bees, such as larger more charismatic fliers, by actively keeping our search images broad. Specimens were placed into

cyanide killing vials immediately after net capture, and without stopping the clock, as it is possible to efficiently put bees into vials with practice, and thereby avoid the human error and schedule setbacks inherent with starting and stopping the sampling timer. Bee collections from different floral hosts or surface category (i.e. “ground” or “air) were kept in separate vials during sampling in order to associate each bee with the plant species or surface on which it was collected. We took and pressed vouchers of all unknown flowering species, regardless of whether or not bees were collected from them, for later identification using keys and herbarium collections. Once the plot sampling period was over, we labeled each vial with the unique number of the corresponding data card and plant voucher, and moved bees into plastic storage containers.

We finished each plot sampling day by mounting all net-caught specimens on size 1 or 2 pins into field boxes with associated vial field labels. Medium to large bees were pinned through the right mesosoma; bees too small to pin were glued directly to size 2 pins by carefully placing a tiny dab of Elmer’s washable glue directly on the pin and then cleanly attaching it to the right side of the bee mesosoma. We froze full field boxes for at least 48 hours and then sealed them into giant Ziplock bags to prevent contamination and beetle infestation. Bees from pan traps were stored in the freezer in whirl packs to pin on rainy days or at the Logan Bee Lab, before which they were briefly rinsed with 90% ethanol, and thoroughly dried by placing on paper towels and/or ‘fluffed’ with a hair drier.Plant vouchers were pressed with collection information in a large wooden plant press, separated by cardboard sheets and newspaper to blot moisture from plants.

5 On days where we were sampling two plots at once, we followed the schedule below, with two collectors:

NOTE

Time	Task	Equipment
8:45am	One collector arrives separately at each plot to be sampled that day Lay out pan traps in “X” shape across plot	One set of pan traps Gallon of soapy water
9:00am	Collectors meet at first plot to sample for day Vegetation monitoring protocol for the first sampling plot of the day (this will take the whole 45 min allotted)	Vegetation datasheets Ethanol-proof pen Meter stick with decimeter marks Plant voucher bags
9:45am	Record Kestrel weather measurements	Databook Ethanol-proof pen Kestrel monitoring device
10:00am	Each collector walks to opposite long corner of plot Start collecting timer, begin walking	Net, Collection bag & killing vials Watch
10:15am	Collectors reach long end of plot, switch sides, and walk back other side	
10:30am	Record plants on which bees were collected Make corresponding vial labels from databook Transfer bee groups with vial labels to stackers	Databook Ethanol-proof pen Stackers or other small storage
10:45am	Hike to other paired plot for sampling that day *OR if only sampling one plot, collect bees in a new area to increase diversity until Noon*	Bring all gear except active pan traps
11:00am	Record Kestrel weather measurements at second plot	Databook Ethanol-proof pen Kestrel monitoring device
11:15am	Repeat protocol 10-10:30am to take morning sample of second plot	Net, Collection bag & killing vials Watch
11:45am	Finish collecting time for second plot Record flowers on which bees collected Store bees from vials with labels	Databook Ethanol-proof pen Stacks or other small storage
12:00pm	Lunch break	Food!
12:30pm	Walk back to first sampling plot of the day	Bring all gear except active pan traps
12:45pm	Take afternoon Kestrel measurements at first plot	Kestrel monitoring device Databook & pen

1:00pm	Repeat protocol 10-10:30am to take afternoon sample of first plot	Net, Collection bag & killing vials Watch
1:30pm	Finish collecting Record plants and label bees from vials	Databook & pen Stackers or other small storage
1:45pm	Walk back to second sampling plot *OR if only sampling one* *plot, collect bees in a new area to increase diversity until 3:30pm*	Bring all gear except active pan traps
2:00pm	Take afternoon Kestrel measurements at second plot	Kestrel monitoring device Databook & pen
2:15pm	Repeat protocol 10-10:30am to take afternoon sample of second plot	Net, Collection bag & killing vials Watch
2:45pm	Finish collecting Record plants and label bees from vials	Databook & pen Stacks or other small storage
3:00pm	Vegetation monitoring protocol for the second sampling plot of the day	Vegetation datasheets Ethanol-proof pen Meter stick with decimeter marks Plant voucher bags
3:45pm	Two collectors split up and walk to separate plots to pick up bowls	Tea strainers Gallon jug Funnel Whirl Packs (labeled on inside with ethanol-proof tag + on outside with Sharpie) Ethanol in plastic soda bottle
4:00pm	Each collector picks up pan traps at one plot	(all of immediately above)
4:45pm	Finish sampling day Collectors meet and return to car	All gear
5:00pm	Lay out gear for next day File datasheest	New Whirl packs Refill Ethanol Refill soapy water gallon jugs Replace useless vials, etc.
Evening	Pin all bees collected that day into field boxes	Field (Schmidt) boxes Pins (#1 & #2) Pinning blocks

6 We recorded data while in the field on the following sheet template:

NOTE

A3. Sample Data Collection Card Template

Collection ID#	START Date (like ## /JUN) _____/_____/2012	END Date (like ## / MAY) ____/____/2012	☐ Set Plot Collection ☐ Random Collection site ☐ See Notes on Back
Other data card #'s	Collector(s):		
Plot/Location Name:			
DD.dddd°(start)	w/lon	N/lat Input into GPS as	Elev.
DD.dddd°(end)	w/lon	N/lat Input into GPS as	Elev.
DD.dddd°BOWL	w/lon	N/lat Input into GPS as	Elev.
# of bowls run_____ Bowls Spilled >>>		Yellow	Blue	White
startAM Time (24hrs ex10:00)	endAM Time (ex10:30)	AM Temp °F	AM Wind Speed	AM Humidity
startPM Time	endPM Time (ex17:00)	PM Temp °F	PM Wind Speed	PM Humidity
startBowl Time- (24hrs)	endBowl Time- (24hrs)	AVG Temp °F	Avg Wind
BAR:	Other weatherNotes:		

Sunny AM PM	Partly Cloudy (>50% sun) AM PM	Cloudy (some Shadow cast; <50% sun) AM PM	Completely Cloudy (NO shadow cast) AM PM	Inclement Weather *see notes on back AM PM

Radius of collection in(m): ☐10/ ☐25/ ☐50/ ☐100 / ☐200/ ☐>200 / ☐>1km

eCard_Numbe re-A Au: ☐ Collector Det: ☐ Initials	Plant details_____ ☐NOT-Flowering/ ☐Voucher /☐Bee Not Foraging: Plant Name/info Abundance	eCard_Numbers-A COL: Date- / /2012 au/pu LOC: plant-
eCard_Numbe re-B Au: ☐ Det: ☐	Plant details_____ ☐NOT-Flowering/ ☐Voucher /☐Bee Not Foraging:	eCard_Numbers-B COL: Date- / /2012 au/pu LOC: plant-
eCard_Numbe re-C Au: ☐ Det: ☐	Plant details_____ ☐NOT-Flowering/ ☐Voucher /☐Bee Not Foraging:	eCard_Numbers-C COL: Date- / /2012 au/pu LOC: plant-
eCard_Numbe re-D Au: ☐ Det: ☐	Plant details_____ ☐NOT-Flowering/ ☐Voucher /☐Bee Not Foraging:	eCard_Numbers-D COL: Date- / /2012 au/pu LOC: plant-
eCard_Numbe re-E Au: ☐ Det: ☐	Plant details_____ ☐NOT-Flowering/ ☐Voucher /☐Bee Not Foraging:	eCard_Numbers-E COL: Date- / /2012 au/pu LOC: plant-
eCard_Numbe re-F Au: ☐ Det: ☐	Plant details_____ ☐NOT-Flowering/ ☐Voucher /☐Bee Not Foraging:	eCard_Numbers-F COL: Date- / /2012 au/pu LOC: plant-
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eCard_Numbe re-Q Au: ☐ Det: ☐	Plant details_____ ☐NOT-Flowering/ ☐Voucher /☐Bee Not Foraging:	eCard_Numbers-Q COL: Date- / /2012 au/pu LOC: plant-

PAN TRAPS Y / N	Col.ID#-eCard_Number- Yellow Date - '2012 col- site/plot#	Col.ID#-eCard_Number- Blue Date - 2012 col- site/plot#	Col.ID#-eCard_Number- White Date - 2012 col- site/plot#

Specimens processing & data management (back at the lab)

7 All bee and plant specimens were transported to Logan, Utah and incorporated into the USDA-ARS Pollinating Insect Research Unit collection except for reference and display collections housed at Pinnacles National Park. Students and experts at the Logan Bee Lab collaborated to identify all bees to the species level wherever possible. Bee identifications were completed using high quality Leica dissecting microscopes, one of the best collections of reference specimens in the world (the Logan Bee Lab houses approximately 1.5 million curated bee specimen), and the appropriate taxonomic literature. Plants were identified on rainy field days using 'The Jepson Manual' (*Baldwin and Goldman, 2012*) and help from Pinnacles botanists, or were transported to the Logan Bee Lab and identified with help from the reference collection and experts at the Utah State University Intermountain Herbarium.

8 All collection information from data cards was entered into the Logan Bee Lab's existing relational database using SQL and Microsoft Access as a front end. Corresponding individual ID numbers and barcodes were assigned to each specimen, and labels with this information were printed, double-checked, and pinned below each bee. Once each of the tens of thousands of bees for each sampling year were entered into the database and labeled, bees were moved into permanent museum storage unit drawers and sorted to morphotype by the naked eye. After species identification, all specimens were re-scanned, updated with their species and sex in the database, then organized in glass drawers by taxonomic group. The various iterations of the work described in the above two paragraphs took approximately two years.

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