Sky Islands Collection 2018

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Abstract
This protocol details the Ponisio Lab's collecting protocol for the 2017 Sky Islands season. Insects were collected into cyanide kill jars and pinned later the same day.

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Protocol

Site Setup

Step 1.
Supplies:

- transect tape
- field flags
- flagging tape
- compass
- silver sharpie
- GPS

Site Setup

Step 2.
Each meadow (site) will be separated into three plots (subsites). Each plot will be 50m x 50m, and collection will only occur within the designated plots.

Plot centers for most sites have already been established from 2017 season and are saved on the lab GPS (and on the lab Dropbox). For any site with three established plots, find the center on the GPS and put a red flag in the center. This flag should be labeled with the site initials, plot number, and the letter C (for center) using a silver sharpie (silver will not fade in the sun like black will).

Using a compass, find the four cardinal directions. Using a transect tape (and a buddy), walk 25m in each direction and put a flag down with the site initials, plot number, and cardinal direction (i.e. JC1 W, CH3 N, etc.). If possible, use a different colored flag for each cardinal direction, and be consistent across all sites. In 2017, the flag colors were as follows:
Center: Red
West: White
North: Pink
South: Yellow
East: Orange

To distinguish between the three plots, use a different color of flagging tape for each plot (try to stay

consistent among sites) and tie that color of flagging tape under each flag for that plot (so for example, all the flags for SC1 might have yellow flagging tape tied underneath the flag, all the flags for SC2 might have red flagging tape tied underneath the flag, and all the flags for SC3 might have pink flagging tape tied underneath the flags). This helps to identify flags that are close together during sampling.

For any new sites, be sure to add a GPS point in the center of the plot for data analysis/future collection. To do this, you will turn on the GPS and wait until the device finds your coordinates (this might take a while in remote sites). Use the 'mark' button to save your location. At the top (next to the flag icon) you should put the site initials and plot number (i.e. JC1). Do not edit any of the location or elevation information. Click 'Done' when you have finished and this will save the point.

Pan Trap Setup

Step 3.

Supplies:

- pan traps (5 blue, 5 yellow, 5 white)
- 1L bottle of water
- 1mL dish soap
- kestral
- weather data sheet
- pen/pencil/sharpie

Pan Trap Setup

Step 4.

At the beginning of each sampling day, pan traps must be setup at every flag for each plot. Generally the most efficient way to do this is to assign each team member one plot to place pan traps at.

Add 1mL of dish soap (should be prepared in eppendorf vials already) into 1L of water. Shake vigorously to mix the soap and water.

At each flag, place one pan trap of each color (white, yellow, blue) and fill about 2/3 full with soapy water. Try to place the pans in a flat, sunny location. Do not nestle them into tall grass or a bushy plant. If necessary, the pans do not need to be right next to the flag, if you need to move them further to find an acceptable location. But, try to keep them close enough to the flag that you can find them again when you pick them up.

You should nearly use all of your 1L bottle of water for the 15 pans in a single plot.

Insect Collection

Step 5.

Supplies:

- net
- stopwatch
- collecting jars
- collection belt

- fanny pack/tote
- ice pack
- butterfly box
- butterfly envelopes
- kestral
- sharpie(s) and minuten(s)
- weather data sheets
- dry ice (in the car)

Insect Collection

Step 6.

A note about sample round numbering:

Collection will occur in 30-minute sampling rounds. The numbering protocol for these rounds is X.Y, where X is the number of times you have visited the site during the season (1, 2, or 3), and Y is the 'net number,' or the number of 30-minute rounds that you have done within that larger sampling round (usually 1-7). So if it is the first time I visit Hannagan Meadow, and I am starting my third 30-minute round for that visit, it is round 1.3. The second number does **not** reset with subsequent sampling days within the same round (so if it takes me two days to complete sampling at Hannagan Meadow, and the first day I completed rounds 1.1, 1.2, 1.3, and 1.4, the second day I start with round 1.5.

Assign each team member a separate sub-plot (or in the event that there are more collectors than plots, try to evenly distribute the collectors). With each subsequent 30-minute sampling round, rotate your sub-plot chronologically (so if I start round 1.1 in plot HM2, then in round 1.2 I go to plot HM3, and in round 1.3 I go to plot HM1, etc.).

Insect Collection

Step 7.

Collection will occur in rounds of 30-minutes of active sampling. When you are ready to begin sampling, use your Kestral to collect weather data. **Note: you can only sample when the temperature is between 17-24C and the windspeed is below 2.5 m/s.**

Check that your stopwatch is set to Countdown and shows 30 minutes. Then, start it and begin looking for bees. You will stop the stopwatch every time you catch a bee, and start it again after you have put the bee in a vial and are ready to begin searching for a new specimen (so you are not counting any time to get the bee out of the net, into a jar, or labeled in the sampling time).

You should collect any pollinating insect that touches the reproductive parts of a flower within your assigned plot. This can include bees, flies, butterflies, and wasps. You should ignore any beetls that you know do not pollinate, grasshoppers, ants, ladybugs, spiders, etc. Do not collect an insect if it is just sitting on the petals or leaves - only if it seems to be foraging and actively engaging in pollination.

Once you have a specimen in your net, stop your stopwatch and transfer it into a clean jar. You should label this jar using your sharpie with the plot number (JC1, HM3, etc.), date, your initials, sample round (2.4, 3.1, etc.), and the flower that you collected the bee from. If you are not sure about the flower, you can write a description, but make sure to collect a pressed sample after you finish your sampling round for

later identification.

Store the collected insect in your fanny pack/tote on an ice pack, to keep the insect cool and calm. Once you have completed your sampling round, put the insects into a ziploc bag labeled with the site, plot number, sampling round, date, and your collector initials. Put that bag into the dry ice cooler to kill the insects, and leave it there for the rest of the day.

Insect Collection

Step 8.

A note about flower labeling:
For this project, we use a labeling system for flowers in which we only write the first three letters of the genus and species (so Hymenoxys hoopesii becomes "HYMHOO"). Capitalization and spacing does not matter, as long as you can tell what the 6-letter code is when you copy head labels back in the lab.

If you are uncertain about the flower ID, take pictures and write as distinct a description as possible (so something like "white flower" is not great, but "small, white, 5-petals, opposite leaves" would be more helpful in finding this flower later on for identification). After you are finished with your sampling round, you should try to field ID the plant with the plant guides, and take a pressed specimen that include a full flower head, leaves, stem, and roots. This will allow the team to confirm your ID later.

Insect Collection

Step 9.

Collection at a site is complete once 10 total hours of active sampling have occurred. This generally takes 2-3 days with a team of 3 people.

Try to distribute this sampling as evenly as possible, with one person completing 6 sampling rounds (3 total hours of sampling) and two people completing 7 sampling rounds (3.5 total hours of sampling). This generally means that people will finish their sampling at different times. If someone has completed their assigned sampling, they can move on to vegetation surveys, quadrats, or flower sampling (below).

Pan Trap Collection

Step 10.

Supplies:

- 18-20 eppendorf vials filled with ethanol
- pan trap label(s)
- micron pen
- scissors
- flat forceps
- kestral
- weather data sheet

Pan Trap Collection

Step 11.

Pan traps should be out for as close to 4.5 hours as possible. Do not pick the pans up earlier than 4.5 hours, but try to also not have them out for much beyond 4.5-5 hours.

Note - if weather becomes inappropriate for sampling (i.e. temperature drops below 17C, windspeed goes over 2.5m/s, it becomes too overcast to cast a shadow, or begins to rain), make a note of the times that it is inadequate samplng weather on the Pan Weather sheet. Any time that is not appropriate for sampling does not count toward the 4.5 hours of pan trap time (so for instance, if you put out the pan traps at 10:00am, and at 12:30, it begins raining and rains until 1:00pm, you need to add an extra half hour to the pan trap time, so instead of picking them up at 2:30, you will pick them up at 3:00).

Once you have determined that it is time to collect pan traps, take the weather and note it on the pan weather sheet. Then, fill out the pan trap labels **using a micron pen** and take a bag of eppendorf vials filled with ethanol. Collect the insects in each pan trap and put the appropriate label into the vial (so if you are collecting the white pan at the south flag, put the label that says S W in the vial).

If there are too many insects to fit into a single vial, you can use a second vial, but make sure to copy all of the relevant information onto a second label (or blank piece of label paper). Do not just write the information on the vial, since this will get rubbed off.

Only insects that would act as flying pollinators need to be collected from the pan traps (so you do not need to collect ants, grasshoppers, ladybugs, spiders, etc.).

Vegetation Quadrats

Step 12.

Supplies:

- 1m PVC pipes
- quadrat data sheet
- pen/pencil
- plant guides

Vegetation Quadrats

Step 13.

For each sampling round (so three times throughout the season), you must fill out a quadrat data sheet for each plot. To do this, you will use the 1m PVC pipes to make a square and categorize every flower within that 1m^2 quadrat. Identify each floral species in the quadrat using the vegetation guides, assess whether it is beginning to bloom, fully blooming, or finishing bloom (this is a value judgement - basically does it look like most of the flowers are still buds, does it look like most are blooming, or does it look like most are wilty/falling off), and count how many individual plants (not individual flowers) are present in taht 1m^2 quadrat. If you are not sure of a flower ID, describe it as best as you can, take photos, and take a pressing for future identification; however, at this point, you should work together to have all of the flowers at the site identified to the best of your abilities.

You will do this at every flag (N, S, E, W, C), and at the halfway point between the center flag and each directional flag (MN, MS, ME, MW [M stands for Mid-]). So for plot 1, you would have N1, MS1, C1, etc. For

plot 2 you would have N2, MS2, C2, etc. ...

Site Blooms

Step 14.

Supplies:

- bloom data sheet
- pen/pencil
- plant guides

Site Blooms

Step 15.

For each sampling round (so three times throughout the season), you must fill out a bloom data sheet for the entire site. This is a coarser-scale assessment than the quadrats. For each plot, you will list every flowering plant that is present (regardless of whether or not you caught anything on it), assess **on average** whether they are beginning to bloom, fully blooming, or finishing bloom, and estimate the number of plants (this is a very rough estimate - the bins are broken down as <10, 10-100, 100-1000, 1000-10,000, and >10,000). This does not need to take too long - it is a coarse-scale assessment. However, be careful not to forget any plants. Generally this is best done as a group at the culmination of sampling (so that at that point, every team member has visited every plot 2-3 times).

Vegetation Sampling

Step 16.

Supplies:

- glass screw-top vials
- eppendorfs
- ethanol
- plant press (large and small)
- flat forceps
- pan trap label paper
- micron pen

Vegetation Sampling

Step 17.

For every plant species present on-site, we want to get two samples.

First, we want a pressed sample that includes petals, leaves, stem, and roots (if possible). For large plants, you might have to cut the sample in 2 to get all of these components). Use the large plant-press for these voucher specimens. Label the paper next to this pressing with the site you collected it at, the date, and your identification.

Second, we want to get at least one flower head in ethanol to get pollen samples. Generally, you will use the large screw-top glass vials for this, but you can also use eppendorfs for small flowers. Do **not** use snap-caps - they will leak all of their ethanol! Label each specimen **using pan trap paper and a micron**

pen with the site, date, and your identification. Fill the vials with ethanol.

For each site, we only need one of each type of sample for the season (so for instance, if you have already gotten both a pressed specimen and an ethanol specimen of Hymenoxys hoopesii from Hannagan Meadow at your first sampling round, you do not need to get a new sample on your second sampling round). However if you find the same species of plant at multiple sites, you do want to get a new sample at every site (so even if you already sampled Hymenoxys hoopesii at Hannagan Meadow in SR1, when you then go to Jack's Creek for SR1 and find more Hymenoxys hoopesii, you want to get new samples).

Lab Work

Step 18.

Laboratory processing of insects will be done in two different ways, based on the type of bee.

Bombus and Apis (bumblebees and honey bees): transfer specimen from collection vial into a 2mL microcentrifuge tube (small screw-tops). These specimens will undergo microbiome assessment, so you need to be very careful not to contaminate them with anything from your body or the environment (so don't breathe on them, touch them with your bare skin, let them touch the lab bench, etc.).
1. Separate all of the jars containing Bombus and Apis specimens.
2. Put on nitrile gloves and wash them with bleach and ethanol.
3. Dunk your forceps in bleach and ethanol, then flame sterilize them.
4. Using your forceps, transfer the bee from the collection jar to the 2mL microcentrifuge tube. Avoid touching the specimens with your bare skin or breathing on them.
5. After finishing a single specimen, re-sterilize your forceps (bleach, ethanol, flame).
6. If you feel like your gloves have gotten contaminated (they accidentally touched a specimen or your body), re-sterilize them with bleach and ethanol.
7. Once all of the Bombus and Apis specimens have been transferred to 2mL microcentrifuge tubes, put them into a ziplog bag labeled with the site and date and note that entry on the freezer checklist.
8. Store the specimens in the -80C freezer at Sevilleta (or if you cannot access the freezer, store on dry ice until you can put them in the freezer).
Note: it will be impossible to get the working conditions as sterile as we would like them to be while camping, so at camping sites, do not process the Bombus or Apis specimens - just leave them in the collection vials on dry ice, and process them as soon as you return to Sevilleta.

All other insets: pin the insect as you normally would. Be sure to copy their label carefully. Rather than individually labeling each specimen, you can group specimens that all have the same label information (site/subsite, date, collector, sampling round, plant) and pin all of them as a group with one head label - this generally speeds up the pinning process.

Lab Work

Step 19.

After you finish processing insects/before you start your next field day, you must sterilize your collection jars. To do this, open all jar and put them (top and bottom) in a tub with bleachy water (no particular ratio, just a splash of bleach and then enough water to fully submerge the jars). Stir the vials around so that they are all touching the bleachy water/filled with the bleachy water (remember, the most important part is to

make sure that the insides are getting sterilized). Let them sit for 5-10 minutes. Then, dump out the bleachy water and rinse with clean water until there are no more bleach suds. Leave the vials open until they are completely dry (generally overnight). When camping, leave them open in the car, so that they do not get covered in dirt/plant material from the campsite.

Lab Work

Step 20.

Prior to your next site, it is important to make sure that the following preparations are made:

- Enough eppendorfs are pre-filled with ethanol and separated into individual bags for at least the next day, but often it is more efficient to do a lot of these at once. Fill the eppendorf with ethanol, cap it, and put it in a ziploc bag. Fill the ziploc bag with 20 vials (enough for one plot, plus 5 extras in case of over-filled pans). Add one eppendorf filled with 1mL of dish soap to each bag for pan trap setup as well.
- Enough data sheets are ready for every site that you will do before returning to Sevilleta. That means at least one quadrat and bloom data sheet for each site that you plan to visit, and 23 weather data sheets for each site (20 for insect collection, and 2-3 for pan trap weather).
- Any batteries that need to be replaced (kestrals, GPS) have been taken care of.
- All camping supplies are ready (water, food, etc.)