Sky Islands Collection 2021 V.2

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Version 2

Jun 24, 2021

2 *Works for me* Share This protocol is published without a DOI.

Ponisiolab

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ABSTRACT

This protocol details the Ponisio Lab's collecting protocol for the 2021 Sky Islands season.

PROTOCOL CITATION

Lauren Ponisio 2021. Sky Islands Collection 2021. **protocols.io**
https://protocols.io/view/sky-islands-collection-2021-bvy2n7ye
Version created by Lauren Ponisio

CREATED

Jun 21, 2021

LAST MODIFIED

Jun 24, 2021

PROTOCOL INTEGER ID

50938

Field station prep

1 Prior to collection, it is important to make sure that the following preparations are made:

Shared sampling box:

- GPS
- spare batteries
- flagging tape
- pin flags
- spare sharpies, microns etc

Shared collection equipment/consumables:

- sterile, screwtop collection vials for insects
- non-sterile vials for pan traps
- 70% ethanol jug
- 10% bleach
- dry ice in Yeti (in the car) or dry shipper charged with liquid nitrogen
- freezer zip locks
- freezer boxes
- pre-printed labels/label printer

Personal sampling box + net:

- net
- stopwatch
- insulated fanny pack

- ice pack (frozen)
- butterfly box + butterfly envelopes
- kestral
- flat tweezer and pointy tweezer
- 2x thin sharpie(s) and 2x minuten(s)
- weather data sheets
- gloves
- 10% bleach squirt bottle
- 70% ethanol squirt bottle
- Plant guide

Pan trap box:
- 4 set of pan traps (blue, white, yellow *5 each set)
- 3, 1L Nalgenes
- blue soap bottles
- ethanol squirt bottle
- strainers
- scissors

Pollination experiment box:
- plant labels
- exclosure baggies
- metal plant tags
- gopro + lens
- tripod

Plant trait box:
- Camera
- Capillary tubes
- Calipers x2
- Tally counter
- Refractometer
- Printed 1cm grid
- Tubes with 1ul of distilled water

Communal camping supplies:
- solar panels + battery charger
- laptop + charger for data entry
- folding table
- pop up cover
- cooking supplies

Datasheets:
- Weather Net
- Weather Pan
- Specimen
- Quadrat
- Bloom Count
- Plant trait
- Pollination

To prep before leaving to go sampling:
1. Freeze ice packs
2. Go over all electronic equipment and check batteries
3. Charge laptop(s) battery
4. Charge backup battery(s)
5. Charge camera battery
6. Charge go-pro batteries
7. Charge AA rechargeable batteries
8. Check over pan traps for cracks

9. Buy dry ice from Socorro Lowes (delivery on TU weekly) or charge dry shipper at Albuquerque AirGas (11 L liquid nitrogen)
10. Assemble consumables (vials, ethanol, bleach) **NOTE: do not pre-fill spray or squirt bottles, they will leak with the elevation changes**
11. FIll up giant water container for pan taps + other water needs
12. Check over individual sampling supply boxes
13. Print datasheets
14. Print additional labels if needed

Optional:

- Enough pan trap tubes can be 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 tube with ethanol, cap it, and put it in a ziploc bag. Fill the ziploc bag with 20 vials (enough for one subsite, 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.

Site Setup

2 Supplies:

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

Each meadow (site) will be separated into **three** subites. Each plot will be 50m x 50m, and collection will only occur within the designated plots, labeled 1,2 or 3.

Subsite centers for most sites have already been established from 2017 season and are saved on the lab GPSs. 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, subsite number, and the **letter C (for center)** using a silver sharpie (silver will not fade in the sun like black will).

A	B	C	D	E
Site code	SubSites	State	Mt Range	Meadow
CH	1,2,3	AZ	Chiricahua	Barfoot Park
HM	1,2,3	AZ	Gila	Hannagan Meadow
JC	1,2,3	NM	Pecos	Jack's Creek Meadow
MM	1,2,3	NM	Magdalena	South Baldy Meadow
PL	1,2,3	AZ	Pina Leno	Hospital Flat
SC	1,2,3	NM	Sandias	Kiwanis Meadow
SM	1,2,3	NM	San Mateo	La Mosca Lookout Tower Meadow

Table of site codes and locations

These are the only acceptable site abbreviations. Please use them on all specimens and datasheets. To denote the subsite add a number after the site abbreviation, EX: CH1, CH2, CH3; HM1, HM2, HM3.

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, subsite number, and cardinal direction (i.e. JC1 W, CH3 N, etc.).

Use a different colored flag for each cardinal direction, and be consistent across all sites.

Center: Red
West: Green
North: Pink
South: Yellow
East: Orange

To distinguish between the three subsite, use a different color of flagging tape for each subsite (try to stay consistent among sites) and tie that color of flagging tape under each flag for that subsite (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.

Pan Trap Setup

3 Supplies:

- **Pan weather datasheet**
- 5 pan traps sets (1 blue, 1 yellow, 1 white) for each of 3 subsites = 45 pans at a site
- 1L bottle of water
- 1mL dish soap
- kestral
- pen/pencil/sharpie

A pan trap set is a white, a blue, and a yellow pan. At the beginning of each sampling round, a pan trap set must be setup at every single colored flag at each of the 3 subsite. Generally the most efficient way to do this is to assign each team member one subsite to place pan traps at.

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

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 subsite.

Insect Collection

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Assemble your supplies in from your supply box:

- **Net weather datasheet**
- net
- stopwatch
- sterile, screwtop collection vials
- insulated fanny pack
- ice pack
- butterfly box + butterfly envelopes
- kestral
- flat tweezer and pointy tweezer
- 2x thin sharpie(s) and 2x minuten(s)
- weather data sheets
- gloves
- 10% bleach squirt bottle
- 70% ethanol squirt bottle
- Plant guide

In the car

- dry ice in Yeti (in the car) or dryshipper charged with liquid nitrogen

Collection will occur in rounds of 30-minutes of active sampling for a total of **10 hours of active sampling per**

site, per visit. A 10 hour period is one sampling round. When you are ready to begin each 30-minutes of active sampling, use your Kestral to collect weather data. On the weather datasheet, each 30-minute interval is called the "net number" within the sampling round. It may take multiple days to achieve enough intervals to obtain 10 hours of active sampling. **At the end of a day, use the checklist to check off the number of 30 minute rounds each person did by writing their initials in a box.**

Assign each team member a separate subsite (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.).

Note: you can only sample when the temperature is between 17-24C and the windspeed is below 2.5 m/s.

1. Check that your stopwatch is set to Countdown and shows **30 minutes**. Wipe it with ethanol at the beginning of each sample round, but it will degrade the screen so be careful.
2. Put on your gloves, bleach, wait 30 seconds, then ethanol to remove residue
3. Start your stopwatch 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 vial, or labeled in the sampling time).
4. If you get pollen all of your hands/the net, re-sterilize everything again. **Only bleach degrades pollen**, but the ethanol removes the residue.
5. You should collect any pollinating insect that touches the reproductive parts of a flower within your assigned subsite. This can include **bees, flies, butterflies, and wasps (sometimes beetles).** Capture the insect into a sterile vial. For butterflies, use the envelopes located in the butterfly box.
6. You should **ignore any beetles that you know do not pollinate (just sitting in the flower), hemipterans, 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 vial. You should label this vial using your sharpie with the:

1. **subsite number (JC1, HM3, etc.),**
2. **your initials, SR and net number (2.4, 3.1, etc.)**
3. **the 6-letter code for the flower from which you collected the insect**

If you are not sure about the flower, create a temporary plant code based on the information you know such as color and family (small, white, 5-petals, opposite leaves), then you must write a description of the plant in as much detail as possible, and take multiple photos. Use the **Unknown plant datasheet** to write the description. **Upload the photos to the shared sky_islands_unknown_plants album using the label you created as the description.**

Store the collected insect in your fanny pack on an ice pack, to keep the insect cool and calm.

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.

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Once you have completed your sampling for the day:

Supplies

- **Specimen datasheet**
- **Sampling checklist datasheet**
- **Unknown flower datasheet**
- pre-printed numbered labels
- freezer box
- freezer ziplock
- sharpie

1. Add a pre-printed, numbered label to each vial. Ensure that each vial is labeled the above 3 pieces of essential data. If some flowers are still un-IDed, take some time to try to ID them and correct the plant code. If you cannot ID the plant, fill out the Unknown flower datasheet.
2. Record which label numbers were used on the specimen datasheets
3. Put all the vials from that collection day into a freezer box label the box with: **Site, date, numbers from that collection period. EX MM, 07/16/2021 #4567-4800**
4. place the freezer box into a freezer ziploc bag labeled with the site and date
5. Put that bag into the dry ice cooler or dry shipper to kill the insects, and leave it there until it can be put into the -80 at the field station or mailed to the lab.

Pan Trap Collection

6 Supplies:

- **Pan Weather datasheet**
- 18-20 eppendorf vials filled with 70% ethanol, or a squit bottle of 70% ethanol
- pan trap label(s)
- micron pen
- scissors
- flat forceps
- kestral

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, unless if rains and they need to be collected early.

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 all the insects in each pan trap and put in one vial with an appropriate label in the vial (so if you are collecting the blue pan at the south flag, put the label that says S B 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 (**you do not need to collect ants, grasshoppers, hemipterans, ladybugs, spiders, any insect/creature that is obviously not a pollinato).**

Vegetation Quadrats

7 Supplies:

- **Quadrat datasheet**
- 1 m^2 PVC pipes
- pen/pencil
- plant guides

For each sampling round (so three times throughout the season), you must fill out a quadrat data sheet for each plot. Place a quadrat 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. ...

The only possible quadrat labels are:

C1
C2
C3
E1
E2
E3
N1
N2
N3
S1
S2
S3
W1
W2
W3
ME1
ME2
ME3
MN1
MN2
MN3
MS1
MS2
MS3
MW1
MW2
MW3

Only quadrat code options

To survey the plants, you will use the 1m PVC pipes to make a square and categorize every flower within that 1 m^2 quadrat.

1. Identify each floral species in the quadrat using the vegetation guides, assess whether it is beginning to bloom, fully blooming, or finishing bloom (use your 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),
2. Count how many individual plants (not individual flowers) are present in that 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.

Site Blooms

8 Supplies:

- **Bloom datasheet**
- pen/pencil
- plant guides

For each site visit (each 10 hour sample round) , you must fill out a bloom datasheet 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. After you have spent 10 hours walking all around the site, you should have a pretty good idea of the plant and their abundances.

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).

Plant traits

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Supplies:

- **Trait datasheet for composite flowers**
- **Trait datasheet for tube/cup flowers**
- Plant Guide
- Digital calipers
- Tally counter
- Refractometer
- Pen
- Microcapillary tubes of various sizes
- Tubes with 1ul of distilled water
- Printed grid
- Camera

At each site, move systematically through each plot where we capture bees and collect the trait data for each **flowering** plant you encounter. We want to collect data 5 times for each plant species at each site, each time on a different plant. If a plant is not present 5 times, collect data for as many individuals that are present.

We already know the plants that are present at each site and have listed them on the trait datasheet, but there may be additional plants. We will collect plant data at the start of the field season and at end of the end of the field season, so you will repeat this protocol.

Notes: Sometimes it can be confusing to determine what is an inflorescence and what to count on each plant. Always take meticulous notes and include drawings of what you did. If you need more space to take notes or draw a figure, put a little check on the "notes" column and use the back of the datasheet.

Steps:

1. Identify the plant in front of you using the Plant Guide and find the corresponding plant name on the plant trait datasheet. There are separate datasheets for composite flowers and tube/cup flowers. If plant is not on the appropriate datasheet, add it to the bottom.
2. **In Site:** If you can't find a plant by the end of the sampling period, mark this as NO for the plant.
3. **ID Number:** Collect data for up to 5 plants per species per site.
4. **Inflorescence:** Y if yes, N if no
5. **Number Flowers/inflorescence:** If this plant has inflorescences (clusters of flowers), select one inflorescence. Count number flowers on entire inflorescence using the tally counter. If plant does not have inflorescence, write in NA.
6. **Number Flowers/plant:** Count number flowers on entire plant using tally counter. If the plant has inflorescences, count the number of inflorescences.
7. **Number Flowers/inflorescence:** If this plant has inflorescences (clusters of flowers), select one inflorescence. Count number flowers on entire inflorescence using the tally counter. If plant does not have inflorescence, write in NA.
8. **Shape**: cup (open, uplifted petals), tube (tubular corolla), small composite (diameter of disk is less than ray petal length), large composite (diameter of disk is greater than ray petal length). See examples below.
9. **Petal Length/Width:** use digital calipers of a single petal to nearest 0.01mm. If a composite, measure a whole composite petal that is comprised of multiple petals
10. **Corolla or Disk Length/Width:** use digital calipers to nearest 0.01mm.
11. **Color**: Select the primary color of the flower that appears most. Select color most similar.
12. **Sugar:**

- **Cap ul:** Select a microcapillary tube, write down the size selected (1ul, 5, 10, 20 etc). Place into nectar reserve of flower.
- **Col height:** Measure the height of nectar column you obtain using digital calipers (to 0.01mm)
- **Diluted**: if less than 1ul of nectar is obtained, check "diluted". Then dispense nectar into tube with 1ul distilled water using rubber top squeezer. Gently mix. Suck nectar/water mix back into microcapillary tube
- **Brix**: Dispense your nectar (or nectar/water solution) onto refractometer. Record Brix reading.

13. **Photo number in camera roll:**
- Take the first photo look directly down from birds eye view of the flower opening. Hold scalegrid directly underneath the flower.
- Take the second photo from a straight side view, with the opening of the flower facing straight up. This may require manipulating the angle of the flower for the photos. Hold scale grid directly behind the flower
- Record the numbers on the camera roll that correspond to the plant.

End of Day

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Supplies:
- **Laptop**
- **Scanner**

The team leader is responsible for ensuring that all data is entered on the laptop in the following folder:
Dropbox -> SI_2021_Fieldwork -> raw_data

The team leader does not need to enter all the data by themselves, but check that it's been done and do random spot checks in each datasheet to ensure quality of data entry.

After data entry, each datasheet should be scanned with the image scanner and uploaded onto Dropbox:
Dropbox -> SI_2021_Fieldwork -> raw_data -> scanned datasheets