


Version 4 ▼

Jul 27, 2022

# Sky Islands Collection 2022 V.4

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## ABSTRACT

This protocol details the Ponisio Lab's collecting protocol for the 2022 Sky Islands season.

## DOI

[dx.doi.org/10.17504/protocols.io.rm7vz39krx1/v4](https://dx.doi.org/10.17504/protocols.io.rm7vz39krx1/v4)

## PROTOCOL CITATION

Lauren Ponisio 2022. Sky Islands Collection 2022. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.rm7vz39krx1/v4>  
Version created by [Lauren Ponisio](#)



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## CREATED

Jun 01, 2022

## LAST MODIFIED

Jul 27, 2022

## PROTOCOL INTEGER ID

63683

### 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, snap cap collection vials for bees
- non-sterile vials for pan traps
- 70% ethanol jug
- 95% ethanol jug
- 10% bleach jug
- Yeti (in the car)
- Freezer packs in Yeti (in the car)
- quart freezer zip locks for pan trap tubes + misc
- freezer boxes

### **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 microns(s)
- weather data sheets
- 10% bleach spray bottle
- 70% ethanol spray bottle
- Plant guide

### **Pan trap box:**

- 3 sets of pan traps for 3 subsites (blue, white, yellow \*5 each set)
- 3, 1L Nalgenes
- blue soap bottles
- ethanol squirt bottle \* 2 (one for 70% one 95% ethanol)
- strainers
- scissors

### **Communal camping supplies:**

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

### Datasheets:

- Weather Net
- Weather Pan
- Specimen
- Quadrat
- Bloom Count
- pan labels

### To prep before leaving to go sampling (also see check lists):

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 AA rechargeable batteries
6. Check over pan traps for cracks
7. Assemble consumables (vials, ethanol, bleach) **NOTE: do not pre-fill spray or squirt bottles, they will leak with the elevation changes**
8. Fill up giant water container for pan taps + other water needs
9. Check over individual sampling supply boxes
10. Assemble datasheets

### Optional:

- Enough pan trap tubes can be 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
RP	1,2,3	AZ	Chiricahua	Ruster Park

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

### 4

Assemble your supplies in from your supply box:

- **Net weather datasheet**
- net
- stopwatch
- sterile, snapcap collection vials
- insulated fanny pack
- ice pack
- butterfly box + butterfly envelopes
- kestral
- flat 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

- freezer pack filled in Yeti (in the car)

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**.
2. 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).
3. If you get pollen all of your hands/the net, re-sterilize everything again. **Only bleach degrades pollen**, but the ethanol removes the residue.
4. 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). For butterflies, use the envelopes located in the butterfly box.
5. 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.**
6. 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:
  - **subsite number (JC1, HM3, etc.),**
  - **your initials, SR and net number (2.4, 3.1, etc.)**
  - **the 6-letter code for the flower from which you collected the insect**

Make sure to leave enough space for the unique ID number. Please write very legibly on the vials and ensure that it does not rub off when handling. When we add the unique number label, use this time to double-check the information is still clearly written.

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

### ***Unsure of a plant ID?***

*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 iNaturalist and to Dropbox labeled with the code you used on vials and the site (i.e., thistle\_white\_MM. Update the unknown plant datasheet you have an ID from iNaturalist.*

**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.

## 5

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. Put the vials in the vial box. For data entry try to keep different collector's specimens grouped together. Use this time to check over the data on the vials. 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. Count the vials. Place a cap sticker on the first vial and last vial. Referring to the unique IDs used in the last round, write the first and last number on the cap. You can alternative colors between days/sites. 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, number range from that collection period. Ex. MM 07/16/2022 #24567-24800
4. Place the freezer box into a freezer ziplock bag labeled with the site and date
5. Put that bag into the cooler or dry shipper to kill the insects, put it in the freezer when you can

### Pan Trap Collection

## 6

Supplies:

- **Pan Weather datasheet**
- 18-20 non-sterile vials filled with 70% ethanol, or a squirt bottle of 70% ethanol
- 95% ethanol squirt bottle
- 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 sampling 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).

1. Once you have determined that it is time to collect pan traps, take the weather and note it on the pan weather sheet.
2. Fill out the pan trap labels using a micron pen and take a bag of non-sterile vials filled with ethanol.
3. Collect all the flower-visiting **non-bees** in each pan trap and put in one **70% ethanol** 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). You can use the flat tweezers or the strainer, or a combination of both.
4. Collect all the **bees** in each pan trap and put in one **90% ethanol** vial with an appropriate label in the vial. This is because we would like to preserve them for DNA extraction. When in doubt as to bee vs. non-bee, collect it into 95% ethanol.
  - \*if all insects do not fit, make an additional vial. 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.
  - If there are no specimens in a trap, simply dump out the water, no notation is necessary.
5. Once all insects and traps have been picked up, finish filling out the weather datasheet that was started when the traps were set out.
6. Put all the vials in a labeled ziplock (site, date) and put the non-dry ice cooler (so keep the specimens cold not they do not need to be frozen at low temperatures). You do not need to put ID number stickers on these vials because they will be pinned individually.

Note: 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 pollinator**). **PLEASE** disregard **any non-pollinators because it makes a lot of work down the line**).

## Vegetation Quadrats

- 7 Supplies:
- **Quadrat datasheet**



- 1 m<sup>2</sup> 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<sup>2</sup> 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 wilted/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).

## End of Day

### 9

#### Supplies:

- **Laptop**
- **Scanner**

The team leader is responsible for ensuring that all data is entered on the laptop in the following folder:

Dropbox -> SI\_2022\_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\_2022\_Fieldwork -> raw\_data -> scanned datasheets