**Azolla collection protocol.**

**Field**

Supplies for field collections:

* Rectangular storage containers (6”x9”x3”?) ([Amazon link to 12pk](https://www.amazon.com/Rubbermaid-TakeAlongs-Rectangle-Container-1824171/dp/B01FEBT6LS/ref=sr_1_14?crid=3HXA9T6VNAAPT&dchild=1&keywords=large%2Brectangular%2Bfood%2Bstorage%2Bcontainers%2Bwith%2Blids&qid=1599837499&sprefix=large%2Brectangular%2B%2Caps%2C208&sr=8-14&th=1))
* GPS
* Camera, Color Scale, Scale Bar and Tripod
* Label tape and sharpie
* Write in rain notebook, mechanical pencil
* Paper towels
* Large coolers for samples
* Physical printout of Azolla key & hand lens
* Physical printout of permits, etc
* Nalgene bottles with FPA
  + Vials for FPA-ed samples? Extra pencils/paper for labels
* Plant press, silica
* Waders? Dip net?
* Spraybottle (for cleaning the FPA ones, etc.)
* [Surface sanitizer (?)]
* [LN2 transport (?)]
* [15ml plastic tubes for flash freezing samples (?)]

**Field collections - on site**

* Upon arrival at the site
  + Search for fertile ones, as priority,
  + Survey for morphological variation, make multiple collections as warranted. [[I think morphotypes should be given different numbers, rather than “If two morphotypes are present (such as in terrestrial forms), both kinds should be preserved in separate containers but with the same collection number”. Because you never know when different morphologies might end up being different taxa with Azolla]]
* Photograph the landscape and habitat.
* Photograph the plants in situ with the color palette and scale bar, preferably using the macro.
  + Include root photos (trial run, see if it’s easier in the field or if this should be done in lab)
* Record the collection number, photo numbers and take a GPS waypoint.
* Collect ca. 5” x 6” of Azolla (for herb. voucher, etc)
  + For terrestrial forms, use a spade or hori-hori knife to gently lift patches from the soil and transfer to the container. Place wet paper towel into the container to keep Azolla moist.
    - Rinse as best as possible
  + For aquatic forms, use the container lid to gently skim the surface of the water and push the Azolla into the larger container. Keep a small amount of water in the container.
* For three individual plants:
  + Place single plant into a separate container (small ziplock with paper towel?), for freezing and subsequent DNA extraction. Aim for as large a plant as possible (while still keeping morphotypes separate, etc.), ideally have it be fertile.
* Place tupperwares into cooler, keep on ice, recharge ice as necessary, check on and potentially ventilate containers ~daily.
  + For this, might want to bring a cold-bag into the field, if the site is a ways from the car (have a freezer pack in one of those insulated bags?). If it’s hot, at least, and there’s a risk of the plants cooking before they get back to the car.

Field collections - at camp

* Make a preliminary ID of the samples using the key.
* Take several pieces of Azolla and preserve in FPA. In each bottle, include a small label, written in pencil, that includes the date and collection number.
  + Include whole-plant (roots, sporangia)
* [[\*\* Clean a small subsample of the plants with the surface sanitizer and flash freeze in LN2. (May not be possible/advisable)]]

**Back at Lab**

Supplies for lab work

* Stereoscope
* Scale and color palette
* Digital camera and tripod
* Silica archive packets and silica bin
* Surface sterilizer
* 15ml plastic tubes for -80 storage.

Dry lab

* For trial run:
  + Surface sterilize two of the for-extraction samples. And then compare the DNA yield (qubit) of the two surface-sterilized and two unsterilized samples to see whether surface-sterilization is ok, and how much material needs to be included in a sample.
* For main runs (contingent on results of the trial run):
* Remove roots and clean each for-extraction individual, and one of the for-silica-archive individuals with surface sterilizer. Henriette’s paper -- soap and bleach (New Phyt paper).
  + [[Deluxe solution, put wash water on LB and see if anything grows. If nothing grows you’ve done a great job. ]]
  + Place into a 5ml tube and freeze at -80°C.
* Place one surface-sterilized, one non-sterilized, and one population sample (a few individuals) into their own coin envelope and dry on silica for archival purposes. (labeled was collection number.1, collection number.2, and collection number.pop).
* Take a ca. 1cm piece of the sample used for macromorphology and photograph it under the stereoscope at “low” and “high” magnification with a scale and color palette. Photograph both the adaxial and abaxial faces of the central axis.
* Press remaining materials as the herbarium voucher.
* Note preservation date

Wet lab - Azolla DNA extraction

* Extract DNA of specimens in a 96 well plate using standard CTAB extractions.
* Extant from the surface-sterilized individual plants (one individual per extraction), contingent on the trial runs (does one individual give enough DNA)

Trial run – test the tupperwares for a couple days, check impact of surface sterilization on the DNA yield. See if one thumbnail is sufficient (need to check with UCLA for how much DNA they want). FW – 100ng to 1ug should suffice (100ng is low). By qubit.