**SITE SELECTION AND NAVIGATION**

1. To aid your search, you have will have 2 sets of resources: 1) a laptop with GIS, topo software, and spreadsheet files, and 2) paper maps (e.g., gazetteers, USFS maps) and hard copies of spreadsheets. Use any and all of these in whatever combination makes sense. The directions below are the system that we’re envisioning right now, but it is open to revision…
2. First, a bit more about each material:
   1. GIS has everything you could want in one place, but the program it can be slow and cumbersome. GIS layers include the following:
      1. climate bins that were part of the stratified sampling design (“ClimateBins”)
      2. base maps with major roads and cities (“USA Base Map”)
      3. topographic layers (“USA\_Topo\_Maps”)
      4. your random sampling points (“RandomPoints”)
      5. previously recorded *M. cardinalis* populations (“Recorded\_pops”)
   2. To view maps in GIS, view the ArcReader file called “M\_card\_occ3\_lite” within the cardinalis occupancy folder in My Documents. Use this for areas where random points are close the edge of their climate bins. If, for some unanticipated reason, you need to add additional layers, you can use the ArcMap file called “M\_card\_occ3;” otherwise ignore this file and all others within the folder.
   3. TOPO! software is less cumbersome and buggy than the topographic layers within GIS. The only disadvantage is that you won’t be able to see when you cross out of the target climate bin and into a different one, so you should visually assess the position of your proposed access point on the GIS map but work primarily within the topo software.
   4. You can hook the handheld GPS receiver up to the laptop so that you have real-time tracking of your location on within the TOPO! software on the computer screen. This should be especially helpful for navigating unmarked FS and BLM roads.
   5. Spreadsheet files include
      1. All random sampling points and associated data like lat, long, climate bin, and gazetteer page (“Random\_pts\_clim\_FIELD.xlsx”)
      2. Table of target sampling points in each area (“Random\_pts\_summary.xlsx”)
      3. All previously recorded, georeferenced *M. cardinalis* populations (“Card\_records\_all\_jul09.xlsx”)
3. Random sampling points are tracked according to a Point ID number. These points are also waypoints viewable in the TOPO! and GIS software, labeled with the same ID number. These points were picked according to a stratified random sampling protocol in GIS to ensure that we sample evenly across latitude, temperature, and precipitation gradients.
4. We have a target number of sample points for each latitude x climate stratum (upper table in summary spreadsheet). There are sometimes more random point options than we actually need, so you can be somewhat choosy based on access and ownership. As you visit each site, you should tally each visit in the summary table so you can keep track of which cells are complete and which need further work (lower table in summary spreadsheet). *Keep in mind that it’s better to have some samples from everywhere than to get stuck for too long in one region.*
5. For each random point that you choose to sample, you need to identify an access point within a 5 km radius of the random point that will allow you to sample along a creek or river on any sort of public land (national forest, state forest, national park, state park, blm, county park regional open space, city park, etc). If nothing suitable is available within a 5 km radius, move up in 5 km increments to a maximum radius of 20 km around the random point.
6. To navigate to an access point, the passenger should connect the GPS to the laptop to view the file Random\_pts.tpo with real-time GPS tracking. Also use gazetteers or other maps if helpful.
7. Make every effort not to burn daylight on point selection. Use downtime while traveling to plan out the next series of site visits and reasonable places to camp.

**TRANSECT MEASUREMENTS**

At each site, you’ll sample 2 replicate 30m transects along the creek or river. Typically, one transect will be upstream and the other will be downstream of your access point. However, if you can’t move along the river in one of those directions (e.g., because of rapids, waterfalls, cliffs, etc.) then run the second transect wherever you can put it.

**Things to have with you every time:**

* GPS with extra batteries
* clipboard with random number table and blank datasheets
* 2 meter tapes
* 4 tally counters
* clinometer
* compass
* camera
* silica in baggies
* large empty ziplock bags
* coin envelopes
* rubber bands
* scratch paper
* field notebook

1. **Find your starting point**
   1. Use the random number table to determine how many paces to go upstream before starting the transect (between 15 and 75).
   2. Cross off the numbers as you use them.
2. **Set up the meter tape and record the endpoints**
   1. At the starting point, record the latitude, longitude, and altitude on the datasheet and mark it as a waypoint on the GPS. Name waypoints using this formula: [Random point ID]\_[A=upstream transect, B=downstream transect]\_[1= start, 2=end]. So, for example, waypoint “325A2” would be the end of the upstream transect for the access point that you chose for random point #325.
   2. Give the GPS, clipboard, 2 tally counters, and meter tape handle to person 2 so they can run the meter tape from the random starting point to the end point 30m upstream.
   3. The transect need not be a straight line; think of it as a 30m linear stretch of the creek or river. If the creek bends, try to wrap the tape around a tree or rock at the bend and keep going. Otherwise, make a temporary marker at the starting point and run the tape in segments until you’ve gone a cumulative 30 m.
   4. Record the end point’s lat, long, and alt on the datasheet and mark the waypoint.
   5. If the tape is running as one segment, secure the tape start and stop on whatever you can find – tree trunks, shrubs, rocks, etc. If you had to stretch multiple segments to add up to 30m, place another temporary marker at the stop point.
3. **Count *M. cardinalis***
   1. Walk the transect with 2 tally counters each.
   2. Work towards each other, each person taking a different bank.
   3. Person walking upstream should also search any midstream rocks or islands, if present.
   4. Search the entire riparian zone where cardinalis could reasonably occur.
   5. If the suitable habitat zone along the bank is fairly thin, wade in the creek alongside the bank while you search.
   6. If there are larger sand or gravel bars, walk them in parallel strips so that you search ~1m strips at a time.
   7. If certain spots are too deep or swift to wade, then walk up on the bank and try your best to look for plants from above. If you just can’t see certain areas, then make a note of how much of the transect was excluded.
   8. Click one counter for every reproductive *M. cardinalis* you see and click the other counter for every non-reproductive *M. cardinalis* ≥ 3cm tall from ground to the base of the last pair of leaves. You won’t count small seedlings, but see note below about suitable demography sites.
   9. Add your tallies within each category and record them on the datasheet.
4. **Make microhabitat measurements** (regardless of whether or not you find any *M. cardinalis*)
   1. **Searchable area**

*(To standardize plant counts by bank and bar area instead of transect length)*

* + 1. We will begin by assuming 30m long x 1m wide per bank = 60m2 searchable habitat. We then need to add additional area for any bars or islands.
    2. For any bars or islands, measure length (parallel to water) and width (perpendicular to the water). Measure width not at the widest point, but midway between widest part and the end. For bars, subtract 1m to account for the amount already included in the default 60m2. Multiplying [L x W] (islands) or [L x (W-1)] (bars) should give a rough area that adjusts for their triangular or elliptical shape.
    3. Record your calculations on back of datasheet and transfer the total area to the front of the sheet.
  1. **Stream gradient**

*(How steep is the pitch of the creek?)*

* + 1. One person should stand with the clinometer at the upstream end of the transect.
    2. The other person should walk to the downstream end of the transect (or as far as the person with the clinometer can see in one straight shot).
    3. With both eyes open, line up the black line inside the clinometer with something at a corresponding height on the other person.
    4. Record the slope as the number on the left side of the internal scale.
    5. If the creek makes a major bend, record the gradient for each segment.
  1. **Valley slope** at the transect midpoint

*(How steep are the canyon walls?)*

* + 1. One person stands at the 15m mark on the creek transect and holds one end of the second meter tape.
    2. The other person walks 30m up the steepest bank perpendicular to the creek transect. If it’s not feasible to go 30m, then try the other bank or shorten the distance as necessary and make a note.
    3. Standing on the bank (at the edge of where peak flow would be), line up the black line inside the clinometer with something at a corresponding height on the other person
    4. Record the slope (number on the *left* side of the internal scale).
    5. If the valley slope varies appreciably along the transect, make additional measurements at 5m and 25m.
  1. **Stream width** at transect midpoint

*(Is it a small creek or a large river?)*

* + 1. At the 15m mark on the creek transect, use the second meter tape to measure the width across the stream from bank to bank.
    2. If the stream width varies appreciably along the transect, make additional measurements at 5m and 25m.
    3. If it’s a big river and this is unfeasible, then guesstimate and record that it is not an exact measurement.
  1. **Aspect** at the transect midpoint

*(Which way is the creek flowing?)*

* + 1. Stand at the midpoint of the transect facing downstream.
    2. Hold the compass flat in front of your chest so that the compass needle floats freely and points to magnetic north.
    3. Rotate the entire compass until the red needle sits within the arrow stenciled onto the base of the compass housing. The zero degree mark on the compass housing and the north end of the needle should be aligned, both pointing to magnetic north.
    4. Keeping the orienting arrow and the compass needle both pointing north, twist the base plate until the direction-of-travel line points exactly downstream. Double-check to make sure everything is still properly aligned and record the declination where the direction-of-travel line intersects the compass dial.
  1. **Substrate**

*(Quick, qualitative assessment of substrate origin and texture)*

* + 1. **Origin**:
       1. igneous, granitic
       2. igneous, non-granitic (e.g., basalt)
       3. sedimentary, sandstone
       4. sedimentary, marine (e.g., limestone)
       5. metamorphic
    2. **Predominant texture** (can be a mix of more than 1):
       1. rock slab
       2. boulder (average size greater than 30cm)
       3. cobble (average size between 7.5cm and 30cm)
       4. gravel (average size between 0.4cm and 7.5cm)
       5. sand (average size between 1mm and 0.4cm)
       6. clay/silt (finer than 1mm)
  1. **Willow/alder development**

*(Index meant to capture the successional state of the riparian corridor)*

* + - 1. 1 = open, recently scoured bars with few willows or alders
      2. 1.5 = mix of, or intermediate between, 1 and 2
      3. 2 = filled in bars with shrubby willows and alders
      4. 2.5 = mix of, or intermediate between, 2 and 3
      5. 3 = mature tree canopy with shady but open areas underneath

1. **Take pictures**
   1. Be sure the camera is always set to include a date and time stamp.
   2. Take two photos from the transect midpoint, one facing upstream and the other facing downstream. If this position does not capture the characteristics of the site well, then take additional photos from other vantages.
   3. Record the time range that you took the photos on the datasheet.
   4. If the site is potentially suitable for demography (scores 3 or higher below), take multiple photos in the area and/or a low-res video and email them to Amy next time you can access WiFi.
2. **Repeat 1-5 for the downstream transect** (or x paces away from the endpoints of transect 1, where x is the number from the random number table that you would paced downstream).
3. **Collect leaf tissue and/or seeds** 
   1. Target = 30 individuals (maximum = 50, minimum = 5)
   2. Collect beyond transect if need be, even if no plants were detected on the transect
      1. Moving in the stream is slow, so hop out and hike up or down for up to ~0.5km to see if more promising areas are nearby before beginning, unless plants are abundant where you did the transect.
      2. Plants are often patchy, with stretches of unsuitable bank in between patches. In this case, again, don’t labor in the stream but hop out and move up to the next promising bar.
      3. If you’ve collected at nearby sites and are not readily finding plants, then skip collections and move to next site.
      4. If you haven’t collected recently (e.g., last collections 30-50 miles away), then consider spending up to 45 minutes searching this site or stopping at non-transect spots on your way to the next site (e.g., bridges with easy access to creeks). You can also use the spreadsheet and/or topo layer of known *M. cardinalis* localities from herbarium records to help guide you to places where you are more likely to find plants.
   3. Detailed methods for collecting:
      1. Probably best to work as a team: one person picking from the plant, the other person labeling and storing the samples.
      2. Aim for ~1m between individuals, although this distance can be shortened for younger plants that are obviously separate
      3. If plants are in large patches and it is difficult to distinguish individuals, focus on a single stem with nice, green leaves. When plants are dense it’s easy to lose track of what stem you are taking leaves from, so it helps to break off one stem and collect leaves only from that stem.
      4. Collect young leaves when possible. Axillary clusters of baby leaves are best, when possible. If young leaves are not available, any green leaf material is fine, and immature fruits and flowers can also work if leaf tissue is not available. A total amount about the size of a quarter should be good. Take note when poor-quality (yellowed, tough or diseased) leaf tissue is collected.
      5. Place leaves from a single individual into baggie with silica gel. Each collection should have a unique identifier that follows this formula: (Firstinitial LastInitial\_unique 4-digit number – e.g., AA0349, PB0001). Write this number on the sticky label on the outside of baggie, and place the completed label inside the bag so that it doesn’t fall off.
      6. Have two quart-sized ziplock bags—one with new silica-filled baggies, and another for baggies that contain leaf-tissue collections for a given site. It is useful to always store the silica-filled baggies in a larger ziplock since they tend to leak and silica gel can damage other equipment (e.g., camera, gps screen, etc.).
      7. If the individual from which leaf tissue was collected has mature fruits, collect 2 fruits (or just 1 if it is the only available at the right stage of development). Place both fruits in a single taped coin envelope, and label the envelope with the same unique identifier as the leaf tissue sample for that individual. If only young fruits are available, collect these and make note that they may be too immature. If seeds look light brown but still wet when a fruit is cut open, they will probably be good; if they are green, they probably are too young. Fruits that are beginning to dry and are yellowish in color are great. Old, dried, brown fruits provide good seeds, but be careful that the seeds do not spill out when you break the peduncle. Typically, you can lightly squeeze fruits and feel if there is a good amount of seed.
      8. Write the last collection number in your field notebook so you know where to begin next time.
      9. Fold the flaps of the coin envelopes down and bundle them into one secure package. Flip the first envelope in the stack around backwards before placing a rubber band around the bundle horizontally and vertically so that envelopes can’t escape. Keep the bundle upright at all times (breast pocket or vest pocket is best)!!! Back at the car, wrap scotch tape around the bundle and place in ventilated box. Place the Ziploc bag of leaf tissue in the tissue tub.
4. **Assess suitability as a demography study site**

*(Index that will help me identify sites to return to in September)*

* + 1. 0 = *no cardinalis*
    2. 1 = cardinalis present, but *very sparse* (fewer than 10 plants observed and/or some stages not found)
    3. 2 = like 5 in every respect except that plants are only *moderately abundant*
    4. 3 = cardinalis *abundant* and *all stages* observed (over ~1km, could probably find 200 seedlings\*, 200 juveniles, and 200 adults) but habitat is *compromised* (grazed, weedy, &/or heavy recreational use)
    5. 4 = cardinalis *abundant* and *all stages* observed (over ~1km, could probably find 200 seedlings, 200 juveniles, and 200 adults) but habitat is *inconvenient* (mostly rock slabs or other configuration that would make plot and plant marking difficult)
    6. 5 = cardinalis *abundant* and *all stages* observed (over ~1km, could probably find 200 seedlings, 200 juveniles, and 100 adults), plus habitat is of *high quality* (ungrazed, few non-native species, or heavily used) and *convenient* for plot setup (bars for rebar and tag placement)

\*seedlings = non-flowering plants < 3cm tall; juveniles = non-flowering plants >= 3cm tall; adults = reproductive plants of any size

**It’s difficult to make one system work for such a broad spatial and ecological gradient, so use your best judgment and add detailed notes, explanations, caveats, etc. to the datasheets and your field notebooks.**