**DAY 2 Lady Beetle Lab Protocol:**

**Procedures for measuring Chill Coma Recovery Time (CCRT)**

*Protocol: Recording Chill Coma Recovery Time (CCRT)*

1. Set up an “arena” to assess CCRT. The arena can be a round piece of filter paper or any other clean, dry, flat and smooth (i.e. non-folded or wrinkled) piece of paper. Secure the edges to the lab bench with tape to prevent movement or vibration.
2. Have the following ready for each student pair:

* Arena (see above)
* Timing device (ideally a smartphone with “lap” capability in stopwatch)
* Blank data table
* Pen or pencil
* Paper towel (to dry the tube)
* Forceps (hard style to remove cotton; soft to capture bugs)

3. Beetle manipulation/data collection tips and suggested practice:

* When emptying tube, do it close to the arena, but don’t touch arena.
* Beetle Catcher: As soon as beetles have been emptied onto arena, use closed forceps tip to flip beetles onto their backs and space them at least 2 cm apart. Only manipulate beetles if necessary; those that are already on their backs and reasonably spaced should not be handled. (**The goal is to stimulate the beetles as little as possible, as stimulation can alter their CCRT).**
* Other tips to avoid excess stimulation of beetles: don’t lean closely to beetles and breathe on them; avoid tapping the vial or the arena; avoid vibrating the lab bench.
* When picking up beetles, pick them up from the top, not the side, to avoid pushing them into other beetles or vibrating the arena. Place removed beetles into original vial unless directed otherwise.
* Watcher/Timer: As soon as all beetles are on their backs and spaced, start the timer. If using the “lap” feature on your smartphone stopwatch, hit “lap” every time a beetle completely rights itself.

4. **CCRT PROCEDURE: After 24 hours:**

* One student is the watcher/timer; the other student is the Beetle catcher.
* Have stopwatch ready to start.
* Beetle catcher pulls one 24-hour tube, QUICK wipe with paper towel to dry the exterior; unscrew the cap; remove cotton plug; tip beetles out onto arena.
* Beetle catcher makes sure all beetles are flipped on back and spaced (see above). AS SOON AS all beetles are on back and spaced, Watcher/Timer starts timing.
* Record the time at which each beetle completely rights itself. If using the “lap” feature on a smartphone stopwatch, just tap “lap” without stopping the stopwatch.
* Beetle catcher needs to remove each recovered beetle from the arena without disturbing other beetles. *Beetles can be put back in the tube; keep loosely capped to prevent escapes!*
* Watcher/Timer stops time after all beetles are righted, or after 15 minutes have gone by.
* Students transfer time data to blank data tables. Clearly indicate any beetles that were unable to right themselves after 15 minutes.
* Beetles may be released to a natural environment outdoors.

Data can now be graphed and analyzed as directed by the teacher.