**Determining CCRT Variability in Different Lady Beetle Populations**

*Scientific Question*: What is the variation in chill coma recovery time of lady beetles from different geographic populations?

*Summary of Background Research:*

Caroline and her team are interested in how species such as lady beetles that rely on show cover in the winter will respond to climate change. When soil temperatures drop below freezing (0℃), lady beetles go into a chill coma (a temporary, reversible paralysis). When temperatures rise back above freezing, they wake from their chill comas. Scientists can measure how long it takes lady beetles to recover from chill coma, called **chill coma recovery time (CCRT)**, and use this as a measure of their performance.

Caroline thought that beetles that spend the winter in different geographical locations would show variation in the mean chill coma recovery time (CCRT) for each population. She and her team measured the CCRT for beetles from the states of Washington, Arizona, Northern California, and Southern California.

**Record Your Prediction**:

1. In Lesson 1 (Handout # 1.2), you were asked to predict whether you think there will be variation in the mean CCRT for populations of beetles from different geographic locations. Re-evaluate your prediction in light of the learning you have done since then; revise your prediction if you think you need to. If you are still satisfied with your original prediction, you do not need to make any changes. *Record your latest prediction on the Lab Report handout, if you are using it.*
2. Justify your prediction.

**Record the geographic source of your beetle population:**

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**Placing beetles into chill coma - overview**: *Follow your teacher’s specific instructions.*

1. Label conical tubes as directed.
2. Pack styrofoam box with crushed ice.
3. Place 10 beetles in each tube; push a small wad of cotton about halfway into each tube to keep beetles in the bottom section of the tube. Screw on the lid.
4. Secure tubes in ice for 24 hours. *Be sure that beetles are submerged in ice and can’t crawl into the section of the tube that’s above the ice!*

**Protocol: Recording Chill Coma Recovery Time (CCRT)***: Read and understand these instructions BEFORE letting your beetles recover from chill coma!*

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 (rigid forceps to remove cotton; flexible entomology forceps, such as Bioquip item #4748 or 4750, to capture beetles)

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 out of ice, 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 and place it back in the original tube, without disturbing other beetles.
* 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.

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