FINAL SCRIPT: APPROVED FOR FILMING

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Title: Field-based Thermal Physiology Assay: Cold Shock Recovery Under Ambient Conditions

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, done**

3. Interview statements: Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 17
Number of Shots: 38

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Emily Khazan:** This protocol is an extremely low-cost method of collecting data on organismal physiology in an accessible and standardized way. This tool can be used to explore physiology at several levels, it can be employed by intrepid field biologists as well as young school children.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Emily Khazan:** The main advantage of this technique is that it relies on readily available materials and is extremely low cost. It can be performed nearly anywhere and by researchers with a range of expertise and goals.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Emily Khazan:** This method can provide insight into physiological, behavioral, and ecological processes. As the key metrics for eliciting a physiological response are standardized, this method can increase our ability to understand and compare key traits across groups of individuals, species, and ecological communities.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.4. **Emily Khazan:** Handling insects without damaging them takes practice, but practice makes perfect. Defining parameters of the experiment, like how long to keep insects in ice water, can take time, as it requires experimentation and understanding of your focal taxa.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.5. **Emily Khazan:** Demonstrating the procedure will be Heather Rohrer and Matt Standridge, a Ph.D. student and lab manager from my laboratory.

1.5.1. INTERVIEW: Author saying the above.

1.5.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Identification of species of interest and conducting a pretrial

2.1. Begin with identifying the species of interest ~~to determine cold shock recovery time,~~ keeping in mind that each group will differ in the time it takes to induce a chill coma **[1]**. Based on the organism and use of data, choose different cutoff points for the experiment if the focal individual does not fly **[2].**

2.1.1. WIDE: Establishing shot of talent carrying insects in appropriate container.

2.1.2. Talent entering details in spreadsheet.

2.2. Conduct a pretrial on a small sample to determine the key parameters like time required on ice to induce a chill coma **[1]**. Choose a cutoff time based on the ecology of the species, keeping in mind that after many minutes of being incapable of flight, some insects are predated **[2].**

2.2.1. Talent making data entries for few parameters, with ice-water containers labelled as 30, 60, 90 minutes visible in the frame.

2.2.2. Talent recording the cut off time.

3. Collection of insects

3.1. Collect insects using appropriate methods such as baited traps and entomological nets **[1].** Place each individual in a separate glassine envelope with a unique ID **[2]** and store the animals in a shaded, cool place protected from direct sun, wind, and predators **[3].**

3.1.1. Talent collecting insects. NOTE: There are several times in the clip where they swing the net, but there is one particularly good catch in Take 2 at 01:18

3.1.2. Talent placing insect in a glassine envelope.

3.1.3. Talent placing envelopes in a shaded place.

3.2. Always expose the animal to the experimental treatment within 24 hours of being captured to standardize this time as much as possible across trials **[1].**

3.2.1. Insects in envelopes.

4. Set up the cold shock experiment

4.1. Fill a cooler with water and sufficient ice to maintain the environment in the water at 0 degree Celsius **[1].** Choose between 1 and 4 focal individuals for a round of experimentation, making sure that each individual is identifiable **[2].** *Videographer: This step is important!*

4.1.1. Talent adding ice and water to cooler.

4.1.2. Talent noting down distinct identification marks of insects.

4.2. When using multiple species, use only one of each to avoid confusion **[1].** If the experiment is not related with wing coloration, mark the wings with unique IDs with a fine felt-tipped marker to distinguish individuals **[2].**

4.2.1. Talent working with insects of different species.

4.2.2. Talent marking wings of insects.

4.3. If the experiments meet neither of the above criteria, conduct the experiment on one individual at a time **[1].** Populate the rows of the data sheet with the information about each insect assayed, including their unique ID and a useful identifier in the notes **[2].** NOTE: Shots 4.3.1 and 4.3.2 are combines as one shot

4.3.1. Talent working with an insect.

4.3.2. Talent making entries in a data sheet.

4.4. Place all focal individuals in a sealed plastic bag with a weight **[1]** and place the bag in ice water for 60 minutes to induce chill coma **[2].** *Videographer: This step is important!*

4.4.1. Talent placing insect in a plastic bag.

4.4.2. Talent placing the bag in ice water.

4.5. Use a simple thermometer to record ambient temperature at short intervals by hand **[1].** To record ambient data with datalogger software, connect the data logger **[4.5.1.1],** go to **Device**, **Launch**, and select the attached device by clicking '**OK**' **[2].** Enter the trial **Name**, select **Temperature and Light Intensity** and enter the units **[3].**

4.5.1. Talent recording temperature with thermometer.

4.5.1.1. Added shot: Talent preparing to program data logger with computer in the field

4.5.2. SCREEN: 62218_screenshot_1.mp4. 0:00 – 0:27.

4.5.3. SCREEN: 62218_screenshot_1.mp4. 0:28 – 0:42.

4.6. Set the **Logging Interval** to 10 seconds, enter **Date** and **Time** for the experiment and select **Delayed Start** to start data logger at the selected time**.** Ensure that the data logger information, including time of day, is synchronized with experiments so that data on ambient conditions can later be matched with each individual focal insect **[1].**

4.6.1. SCREEN: 62218_screenshot_1.mp4. 0:43 – 1:09.

~~4.6.2. Talent observing the stopwatch and entries in datasheet.~~

4.7. Decide on the experimental parameters like shade or sun and twilight or mid-day to associate with recovery time that can be measured without a data logger **[1].**

4.7.1. Talent recording different parameters on spreadsheet.

4.8. Place a mesh cage for the insects in an appropriate location so that the temperature and light environments are homogenous within the cage **[1].** Keep the base of the cage elevated so that it can be tapped by the observer **[2]** and place the data logger just outside or inside the cage where it can be kept undisturbed **[3].**

4.8.1. Talent placing the mesh cage in the appropriate location.

4.8.2. Talent elevating the base of the cage.

4.8.3. Talent placing data logger.

NOTE: Videographer mentioned that there is a shot 4.10.3 take 2, extra wide shot to show the gauge above the net. I think she's talking about shot 4.8.3

5. Start cold shock experiment

5.1. Remove the animals from the ice water bath after 60 minutes **[1]** and immediately remove the insects from the plastic bag **[2].** Then remove each individual from its envelope quickly with minimal handling **[3]** and start the stopwatch as soon as the animals are in the mesh cage **[4].** *Videographer: This step is difficult and important!*

NOTE: Shots 5.1.1 – 5.1.4 are combines as one shot

5.1.1. Talent removing insects from water bath.

5.1.2. Talent removing insects from plastic bag.

5.1.3. Talent removing each insect from an envelope.

5.1.4. Talent starting stopwatch.

5.2. Tap the base of the cage with a pencil frequently and strongly enough just to agitate the recovering insects without causing a response **[1].** Mark the trial as complete once an individual has flown **[2].** End the trial and consider the insect to have achieved a full recovery if it does not move after 30 minutes **[3].** *Videographer: This step is difficult and important!*

5.2.1. Talent tapping the cage.

5.2.2. Shot of the insect flying.

5.2.3. Talent stopping a stopwatch.

5.3. Remove the insects from the mesh cage **[1]** and place the individuals back into their labeled glassine envelopes **[2].** Liberate the animals or keep them for further data collection **[3]** and process the data as described in the text manuscript **[4]**. NOTE: Shots 5.3.1 and 5.3.2 are combines as one shot

5.3.1. Talent removing insects from mesh cage.

5.3.2. Talent placing insects in glassine envelope.

5.3.3. Talent liberating the animals.

5.3.4. Talent making entries into a spreadsheet.

5.4. To access the recorded environmental data, plug the HOBO logger back into the computer [1]. Go to **Device**, **Readout**, select **Stop Logging** and save the files in the desired folder as hobo files. After a pop up is displayed, confirm the **Units** of the parameters and select **Plot** to obtain a graph for experimental conditions **[2].**

5.4.1. Added shot: Talent plugging data logger into computer NOTE: Use shot 4.5.2, same shot as 5.4.1

5.4.2. SCREEN: 62218_screenshot_2.mp4. 0:00 – 0.56.

5.5. To export the data table, select **File, Export Table Data** and **Export** it as a CSV file to save in the appropriate folder **[1].**

5.5.1. SCREEN: 62218_screenshot_2.mp4. 0:56 – 1:30.

Results

6. Results: Examination of interaction between ambient environmental conditions and cold shock recover

6.1. In this protocol, the interaction between ambient conditions important to organismal physiology and cold shock recovery was explored for variable species of butterflies **[1].** It was observed that as the average temperature of trial increased, cold shock recovery time decreased, showing variability across taxa **[2].**

6.1.1. LAB MEDIA: Figure 1.

6.1.2. LAB MEDIA: Figure 1 A.

6.2. Similarly, the inverse relation between the mean light intensity of the trial and cold shock recovery time was observed, indicating that both temperature and light conditions contribute to the recovery of the butterflies **[1].**

6.2.1. LAB MEDIA: Figure 1 B.

6.3. 181 wild species of butterflies, collected by three observers over five months, showed distinct recovery from chill coma induced by cold shock, highlighting the taxonomic breadth across which this experiment can be successfully applied **[1].**

6.3.1. LAB MEDIA: Figure 2.

6.4. The ambient field conditions and experimental conditions were plotted, demonstrating the ecological relevancy of conducting physiology assays under ambient conditions **[1].**

6.4.1. LAB MEDIA: Figure 3.

Conclusion

7. Conclusion Interview Statements

7.1. **Emily Khazan:** When performing this protocol, be sure to pay close attention to all individuals in the mesh cage, as changes in behavior can be subtle and happen quickly.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1*

7.2. **Emily Khazan:** The data generated by this protocol shed light on the eco-physiological traits of the organisms tested. Many other traits contribute to organismal physiology like size, color, and phylogeny. Data on additional traits will further explain and contextualize measures of cold shock recovery, adding to the ecological and life history context of this information.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

7.3. **Emily Khazan:** This method was used to characterize the eco-physiology of entire communities of butterflies in the Colombian Andes. Because the protocol is accessible, cheap, and simple enough to implement in rural settings, data was collected on hundreds of individuals in a short period of time.

7.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.