TIEE

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EXPERIMENTS

The very hungry (and bold) caterpillar – A classroom experiment testing relationships between animal behavior and herbivory

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ABSTRACT

Students identify, measure, categorize, and summarize different animal behaviors (i.e., bold vs. shy response to identical stimuli) using individual lepidopteran larvae and test correlative relationships between behaviors. Students then use these herbivores to examine leaf consumption in an environment with high predation risk. The experiment is suitable for the exercise of hypothesis generation and carry-over to subsequent experiments with the same cohort of herbivores (e.g., oviposition studies). The experiment generally takes two class periods separated by >48 hours to complete. Activities include group discussion and hypothesis generation, animal behavior assays, herbivory studies, and performing regression analyses.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** Animal behavior, Biotic factors, Boldness, Feeding strategies, Herbivores, Lepidopterans, Predation risk

- **Science Methodological Skills Keywords:** Collecting and presenting data, Data analysis, Experimental design, Graphing data, Scientific writing

- **Pedagogical Methods Keywords:** Bounded inquiry, Brainstorming, Iterative writing practice, Scoring rubrics

CLASS TIME

Behavioral trials and the setup for the herbivory experiment can be completed in a three-hour lab period. Measuring the completion of the herbivory trial requires ~1 hour of class time >48 hours after the herbivory trial has been completed. I typically spend some in-class time (~1 hour) immediately following conclusion of the herbivory trial helping students enter data and conducting data analysis. I also include 2-3 hours of lab time over the following couple weeks to workshop data analysis or report writing problems.

OUTSIDE OF CLASS TIME

I structure this assignment as a group research project requiring students to generate a short, well-written abstract of the study (~300-350 words) with at least one figure appended after the abstract. Student groups that perform well on this assignment typically spend 4 hours outside class time analyzing their data, drafting their abstracts, and preparing their figures. I also encourage student groups to meet with me outside class time at least once for ~1 hour to check progress, help one-on-one with analysis, and help structure writing.

STUDENT PRODUCTS

Students generate a short *abstract*-like report that summarizes 1) the background, rationale, and questions that build towards testing linkages between animal behavior and herbivory, 2) their methods, 3) and the results and a brief discussion of those results in a conclusions section. The assignment also requires them to submit the citation of one peer-reviewed manuscript that explores similar research questions and asks students to include a figure summarizing their results.

SETTING

This experiment is predominantly an in-class experiment that can be completed with materials readily available from biological teaching supply stores. However, add-ons to this experiment can include field components. For example, if instructors prefer to use wild-caught organisms, trips to the field can be made to capture wild adult *Pieris rapae* or *Manduca sexta* (or other lepidopterans) for rearing and egg laying at the laboratory.

COURSE CONTEXT

This experiment has been performed in an upper-division, writing-intensive ecology laboratory courses with 12-15 students. A simplified version of this lab examining only relationships between insect behavioral assessments was conducted in introductory biology labs for biology majors.

INSTITUTION

This activity was originally taught at a 4-year institution with a significant proportion of class instruction focused on undergraduate education. Thesis and non-thesis master's degrees in biology are conferred at this institution, but this lab has not been trialed at the graduate level yet.

TRANSFERABILITY

This activity is extremely flexible and can be run simultaneously across multiple lab sections. If multiple lab sections test similar hypotheses, data could be pooled to generate strong power for detecting trends. This lab in not limited by geographical considerations save that the shipping of the live specimens may be restricted to certain states or provinces. *Pieris rapae* and *Manduca sexta* are lepidopterans found widely in North America and are an important pest on cruciferous and solanaceous crops. These species can be readily found in many urban, agricultural, and rural locations and frequently found in and around community gardens.

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SYNOPSIS OF THE EXPERIMENT

Principal Ecological Questions Addressed

1) Do multiple measures of lepidopteran behavior correlate to assess animal boldness?
2) Do measures of lepidopteran boldness correspond to amounts of herbivory in habitats that simulate differences in predation risk?

What Happens

Students conduct animal behavior assessments on lepidopteran herbivores to quantify and assess multiple measures of animal boldness. Students then use these individual lepidopteran herbivores to determine how measures of boldness correspond to amounts of leaf herbivory in artificial habitats that simulate a gradient of perceived risk. Ultimately, students will test the relationship between animal boldness and how that influences other species interactions, such as herbivory.

Experiment Objectives

By the end of the unit, students will demonstrate competencies in:

1. generating synthetic hypotheses relating animal behavior to herbivory
2. developing and executing experimental protocols quantifying animal behavior and herbivory.
3. practicing data management using spreadsheet applications (e.g., Excel, Google Sheets, Numbers)
4. applying formulas to analyze collected data
5. conducting relational statistical analyses (e.g., linear regression) to compare relationships
6. sharing results with succinct writing and clear, simple figures and tables.

Equipment/ Logistics Required

Stopwatches, plastic or glass petri dishes (4 per group), plastic condiment cups (4 per group), plastic paint mix containers, pencils, rulers, cardboard boxes, sticks, paint brushes, tweezers, dissecting needles, lab tape. Organic cabbage or other brassicaceous or solanaceous leaf material. Lepidopteran herbivores in 3rd

or 4th instar (preferably *Pieris rapae* or *Manduca sexta*, 4 or 5 per student group) available from Carolina Biological Supply or other educational suppliers as eggs or young instars (e.g., https://www.carolina.com/moths/hornworms/FAM_143880.pr).

NOTE: students may request additional equipment for their behavioral assessments.

Summary of What is Due

Assessment of this work comprises a short-written abstract that can be turned in multiple times so that students get iterative writing practice through the repeated process of submission, review, and revision. Students are provided with the grading rubric at the onset of the experiment to make certain assessment expectations are transparent.

DETAILED DESCRIPTION OF THE EXPERIMENT

Introduction

Animals can display remarkable variation in behavior, with individuals within a population exhibiting more bold behaviors or more shy behaviors in response to similar stimuli. Differences in behavior between individual animals can directly influence their capacity to acquire resources, encounter and escape predators, encounter mates, acquire parasites, and may influence many other ecological and evolutionary dynamics (Sih et al. 2004). In predator-prey ecology, for example, there is a direct relationship (and tradeoff) between the boldness of an animal and its overall activity under conditions of high predation risk. Bolder prey individuals are more likely to acquire resources during these “risky” times, but they are also more likely to be detected or consumed by predators while they are actively foraging. Conversely, the more shy (less active) prey may be safer from predation but are also less likely to acquire adequate resources and may struggle to meet their metabolic demands.

While variation in individual animal behaviors likely influences many ecological and evolutionary phenomenon (e.g., speciation rates, disease transmission rates), many fundamental questions still exist. Among these questions scientists are still working to determine 1) how to effectively evaluate or classify individuals along a spectrum of behavioral syndromes (e.g., bold-shy) and 2) what are the ecological consequences of variation in individual animal behavior on other species interactions. Using the larvae (caterpillars) of a common lepidopteran herbivore (either *Pieris rapae*, Lepidotera, Pieridae or *Manduca sexta*, Sphingidae) as the focal caterpillar, you will first identify two independent tests that will allow us to estimate boldness for each individual. Next, with a feeding trial, you will determine how your estimates of individual animal boldness relate to consumption of plants in a feeding arena that differs in perceived predation risk.

Lepidopteran larvae are an ideal group of organisms for evaluating variation in individual behavior and the effect it can have on rates of herbivory. Lepidopteran larvae are subject to a vast number of predator and parasitoid threats and display a remarkable diversity of behavior to either dissuade or escape attack. Some escape/defense behaviors include fleeing, head rearing, thrashing, biting, regurgitating, audible clicking, curling, cringing, or remaining unresponsive (summarized by Cornell et al. 1987 for buckmoth caterpillars [*Hemileuca maia*, Hemileucinae]). By simulating non-lethal attack on individual larvae, you should be able to characterize and quantify some of the variation in individual defense response and generate an estimate an individual's behavioral syndrome (Table 1).

Lepidopterans are also important herbivores with far-reaching impacts in both agricultural and natural ecosystems, and these insects are the active focus of many pest management projects. The extent of herbivory (i.e., how much is eaten) in agricultural and natural plant systems varies considerably and ecologists are still trying to determine the ecological processes governing herbivory. The degree to which differences in herbivore behavioral syndrome contribute to variation in herbivory is largely unknown and forms the basis of our series of studies here. Collectively, you will be addressing the relationship between herbivore behavioral syndrome and consumption rates in environments with high perceived predation risk, but in this bounded inquiry lab you will be choosing two animal behavior metrics to evaluate and developing methods to perform those evaluations.

Table 1. Examples of measurable behaviors of lepidopteran larvae and estimates of how such differences in behavior may reflect a “bold” or “shy” categorization. “Movement time” and “number of head rears” are estimates that can be made in an open arena. Defensive curling or thrashing are behaviors that can be measured after a simulated attack with forceps or paintbrushes. Behaviors that require stimuli are indicated with an asterisk (*).

Measurable Behaviors	**“BOLD”**	**“SHY”**
Movement in open arena	More time moving per minute	Less time moving per minute
Head rears	More head rears per minute	Fewer head rears per minute
*Audible Clicking**	Requires more perturbation to induce clicking	Requires little perturbation to induce clicking
*Defensive Curling**	Shorter time in curled position per minute	Longer time in curled position per minute
*Thrashing**	Shorter time spent thrashing per minute	Longer time spent thrashing per minute

MATERIALS AND METHODS

Study Site(s): This study conducted over two consecutive lab sessions within the classroom. These experiments are conducted best if the laboratory temperatures range from (18℃ to 24℃) and out of direct light.

OVERVIEW OF DATA COLLECTION AND ANALYSIS METHODS

Lab Session 1:

A. Design tests of lepidopteran boldness. With a partner, review Table 1, the suggested literature, and the equipment that is available (list above). Then, please identify/hypothesize two ways you could estimate and measure boldness/shyness in your lepidopteran larvae. Additionally, feel free to propose measures not included in Table 1. Time permitting, explore primary literature for other techniques used to assess lepidopteran boldness, but make sure that the animal’s response is something that can be measured on a continuous scale (i.e., not discrete data). For definitions of discrete versus continuous variables, please see this supplementary appendix provided by Vassarstats (Lowry 2021, Chapter 3; http://vassarstats.net/textbook/). If you need equipment that is not listed it may still be available, ask your instructor to see if it is available.

Group measure one: ____
Group measure two: ____

a. Briefly, describe the protocol you would use to quantify each measure of boldness. Use additional scratch paper if necessary.

Ideally, independent measurements (i.e., measure one, measure two) reporting on the same behavioral dichotomy (i.e., bold vs. shy) will be consistent for each individual. Put another way, regardless of the type of measurement, bold individuals should typically respond as "bold" and shy individuals typically respond as "shy". For example, individual lepidopteran Beet Armyworm (*Spodoptera exigua*) larvae that spend less time moving actively around an open arena are also individuals who typically spend a longer time in a defensive curled position following a simulated predator attack (Figure 1).

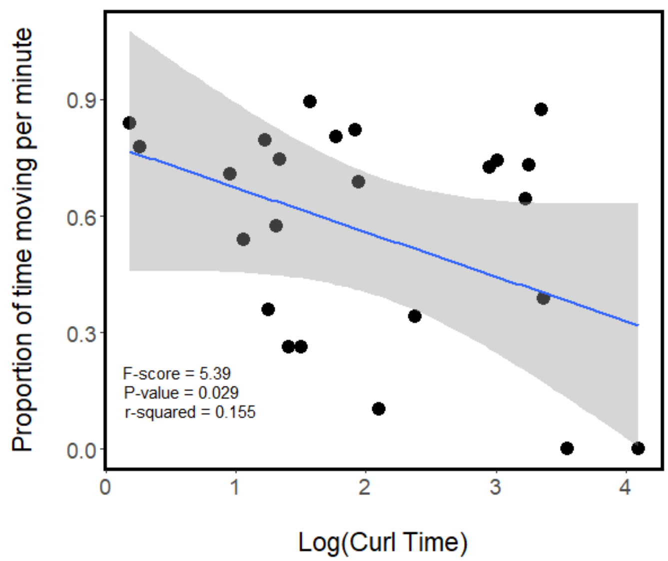

Figure 1. Correlation between the amount of time Beet Armyworm (*Spodoptera exigua*) larvae spent moving in an open arena and the amount of time these same larvae spent in a defensive curled position after a simulated predator attack. Movement time is reported as the proportion of a one-minute observation session. Curl duration is log-transformed. Data reported in the figure was collected in a preliminary study by BMC (n = 24 larvae); solid blue line indicates best fit linear trendline and the shaded region surrounding the best fit line indicates the 95% confidence interval band.

b. Assuming that both your measurements are able to capture whether an individual is bold or shy, please provide a predictive plot below estimating the relationship between measure one and measure two.

B. Class consensus, picking class behavioral metrics, and assessing behavior. Each group should go up to the board and write down their ideas for measure one and measure two. As a class, discuss the metrics and benefits/drawbacks of the measurement techniques. At the end of the discussion vote on the two measures that the class would like to evaluate.
Class measure one: ____
Class measure two: ____

In your two-person groups, you will be given four larvae. Generate your data sheet using the table provided below and the bolded guidelines to populate the table with the best categories and responses. Think critically about the factors being tested and the responses that you will record (i.e., how are measure one and measure two quantified?).

Important experimental notes:

- Each measure should be repeated three times (*technical replicates*) on each animal in order to generate an average response and each animal should have at least five minutes between trials to let the animal rest and to improve the likelihood of independence and reproducibility between trials.
- Scents and visual cues can influence animal behavior. If you conduct open arena movement trials or use tools to interact with the larvae (e.g., forceps or paintbrushes) make sure these tools are cleaned and dried before each use.

- An important part of this work is identifying and testing differences in individual animal behavior. As with the tool cleaning mentioned above, strive to have every trial conducted in an identical environment with identically applied stimuli. Such consistency will result in a greater likelihood that the response measured accurately reflects the animal's response to the stimuli or area you present and not conditions unique to that particular trial.

Factors tested and measured	Larva Identity	Replicate Number	Measure one	Measure two
Each unique observation	A	1		
	A	2		
	A	3		
	B	1		
	B	2		
	B	3		
	C	1		
	C	2		
	C	3		
	D	1		
	D	2		
	D	3		

Each group should now assess the behavior of the larvae. Individual identity is important so make sure the containers housing each individual larva (e.g., small solo cups or condiment containers with lids) are uniquely marked and individual identity can be tracked. If it helps, think up fun names for each larva (e.g., "Shemp", "Larry", "Moe", and "Curly") to help keep track of who is whom. Populate the table above with your measurements.

C. Herbivore behavioral syndromes and perceived risk. In this section of the lab, you will be testing how the behavioral characteristics measured above relate to leaf consumption under conditions of high perceived predation risk (i.e., exposed conditions). Each group will be provided with leaves of a Brassicaceous or Solanaceous plants for the feeding trial. To setup the herbivory trial, please do the following:

a. Get four plastic petri-dishes. You should have enough dishes so that each larva can be housed individually in its own petri dish. These petri dishes will serve as feeding arenas. Cover half of the top and half of the bottom of petri dish with duct tape; the resulting feeding arena should be completely clear on one half of the dish [the **LIGHT** side of the dish] and completely covered on the other half [the **DARK** side of the dish].

b. From the leaves provided by your instructor, cut four leaves into circles (4-5 cm diameter) using either scissors or a leaf disc cutter. Then cut these circles in half so you have two semi-circle shaped leaves. Record the pre-trial mass of each leaf semi-circle and assign them randomly to petri dishes. Make sure to record leaf identity on the petri dish. Place the leaf on the light side of the arena, centered ~3 cm from the light-dark border (Figure 2).

c. Randomly assign one of your larvae to each feeding "arena". Record the pretrial mass of each larva and then introduce one larva to each petri dish on the dark side of the arena, 3-cm from the light-dark border (Figure 2). Initial placement within the feeding arena is important because you do not want to influence the animal's consumption by placing it right on the leaf. As gently as you can, wrap the perimeter of the petri dish with a strip of parafilm wax. This wax seal will prevent the leaf tissue desiccation and larval escape.

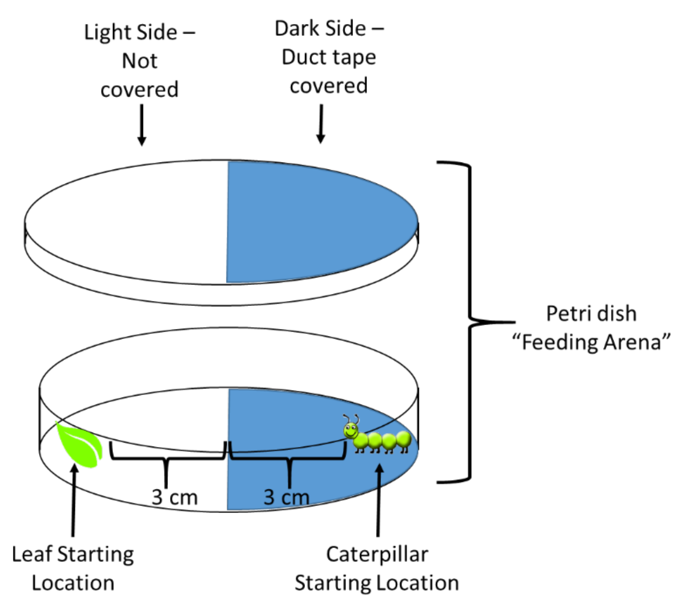

Figure 2. Schematic of the feeding arena setup

d. Place the feeding arenas in secluded location within the lab where they will not be disturbed by frequent activity and where temperature is likely to remain constant. Avoid locations that will receive direct sun. Bench tops in the corners of the lab or lab cupboards are good locations. Do not stack dishes, but lay them flat on the surface, side-by-side. Position a fluorescent light bank approximately 1 meter above the dishes and leave the lights on for the duration of the feeding trial; these lights should visibly illuminate the uncovered half of each dish, but generate shade in the covered half of the dish.

Lab Session 2:

A. Collecting herbivory data: After 48-72 hours measure the extent of herbivory that has occurred. Start by making a visual estimate (to the nearest 5%) of the overall leaf tissue lost. Next, remove the larva from the feeding arena back to their individual plastic solo cup. Clean insect frass (insect excrement from the feeding trial) from the leaf with a fine tip paint brush and then reweigh the leaf to get a post-trial leaf weight. Leave the larvae in their solo cups for 40-60 minutes and then reweigh the larvae to get a post-feeding trial mass.

Larva Number	Pre-trial Leaf Mass	Pre-trial Larva Mass	% damage Leaf	Post-trial Leaf Mass	Post-trial Larva Mass	PLTL
1						
2						
3						
4						

B. Data calculations and compiling a class data. You can use the data collected to calculate and quantify several important metrics that characterize plant-herbivore interactions. Here, you will focus on calculating proportion leaf tissue lost (PLTL), but keep in mind the other data you collect may also be useful for considering how herbivores and plants responded in this experiment. For example, you can also use your approximate estimates of percent damage to leaf or use the amount of mass gain (or lost) by the herbivore over the feeding trial to estimate the extent of herbivory. You can also generate more accurate visual estimates of leaf tissue loss using photographs of leaf tissue before and after the feeding trial and comparing leaf consumption with image analysis software (e.g., ImageJ, https://imagej.nih.gov/ij/).

For each leaf calculate the proportion of leaf tissue lost (PLTL) over the duration of the feeding trial according to the following equation and enter you values into the tables above:

$$PLTL = \frac{(Pre\ trial\ Leaf\ Mass - Post\ trial\ Leaf\ Mass)}{Pretrial\ Leaf\ Mass}$$

Once you have completed calculating PLTL for your trials, enter <u>all</u> your collected data on a shared class spreadsheet (e.g., Google Sheets). You can now make and test predictions regarding the relationships between insect behavior and herbivory in "high risk" locations.

C. Results and analysis. You will now use the compiled class data to test hypotheses regarding herbivory and animal behavioral syndromes

a. Using linear correlation and regression (available at www.vassarstats.net [Lowry 2021]), test the relationship between *measure one* and *measure two* of boldness. During lab time, generate hand drawn graphs of these relationships.

Test 1: Measure one versus Measure two
Linear Equation: ________________________________
R^2:___________ t-score:___________ df:________
p-value:____________

Below, please generate a hand-drawn graph the class data to demonstrate how measure one and measure two of larval boldness are related.

b. Test the relationship between *measure one* of larval boldness and leaf consumption.

Test 2: Measure one versus leaf consumption
Linear Equation: ___________________________________
R^2:___________ t-score:___________ df:________
p-value:____________

Please graph the class data to demonstrate how leaf consumption differs as a function of measure one of larval boldness.

C. Test the relationship between *measure two* of larval boldness and leaf consumption.

Test 3: Measure two versus leaf consumption
Linear Equation: ___________________________________
R^2:___________ t-score:___________ df:________
p-value:____________

Please graph the class data to demonstrate how leaf consumption differs as a function of measure two of larval boldness.

Questions for Further Thought and Discussion:

1. What other measures of animal boldness could you have used to quantify differences in animal behavior?

2. What are some issues with the sampling protocols you used for this experiment? How could you have modified your approach to make the experiment more rigorous?

3. Here you test the relationship between animal behavior and herbivory under conditions of high predation risk (i.e., high visibility). Can you think of alternative feeding arena setups that would also allow us to manipulate perceived predation risk?

4. Herbivory often also depends on leaf quality. How might your results change if the plant quality was altered say by growing the plants in more or less haline soils? What are some other ways you might modify leaf quality?

5. This experiment was conducted in highly controlled lab settings. How do these results relate to patterns you might encounter under natural field conditions? What are the benefits of conducting controlled lab studies like this? What are limitations to this approach?

6. Here, you have evaluated how individual animal behavior corresponds to plant-animal interactions. Using a primary ecological publication as a reference, what are some other interspecific interactions that animal behavior may modify? Describe how intraspecific variation to modify the outcomes of those interactions.

7. What would you expect to happen if, instead of having a leaf only on the light side of dish, you had leaf discs available in both the light and dark side of the petri dish?

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Tools for Assessment of Student Learning Outcomes:
The assignment for this lab entails a written report on the data collected from this experiment. While the worksheet provided above is not turned in for credit, taking the time to fill in the provided questions will greatly facilitate generating a clear written summary of the work. You may have the opportunity to submit this work a total of up to three times for comments and revisions prior to the final assessment. Please also feel free to have your peers review your work. See the assignment structure guidelines below and the corresponding rubric to gain familiarity with how you will be assessed.

ABSTRACT: Produce a 300-350 word abstract in which you describe relevant **background** information, the research question you are addressing, your **research hypotheses** (null vs. alternative hypotheses), **methods** (very briefly; typically, two short sentences), **results** and **conclusions/interpretation** of the data collected from today's lab. Each abstract will need a relevant and **informative title**. Using abstracts from other peer-reviewed articles as a model for outline construction will be helpful. Remember to be direct, concise, and efficient in your writing.

CITATION: In addition, please conduct a peer-reviewed literature search for one other ecological study that measured insect behavior, insect herbivory, or both (i.e., a **primary research reference**). Include the proper citation for this article at the bottom of the abstract; it will not count towards your word limit.

PLOT: Finally, generate a **plot** using your class data to visually represent the hypotheses your class tested. Remember it will be important to clearly label your axes and provide the correct units. The text legend for your plot should indicate the study variables and when and how the data were collected. Make sure to follow graphing requirements outlined by your instructor.

Example Grading Rubric:

Hungry Bold Caterpillar Abstract	Points Received	Points Possible
Abstract		
Experimental question and background clear and direct		1
Research hypotheses are clear and coherent		1
Accurate, comprehensive description of experimental tools and methods		1
Fully summarized relevant results		1
Insightful discussion of implication/application of results		1
Identified and correctly cited paper from primary literature		1
Figure w/ Descriptive Legend		
Figure is correct and clear (e.g., labeled axes)		1
Legend provides clear context for the data and figure		1
Writing/Grammar		
Smooth transitions between sentences / No run-on sentences / proper grammar, spelling, punctuation		2
Total Points		10

NOTES TO FACULTY

Challenges to Anticipate and Solve

1. Animal behavior data can be notoriously messy and highly variable. When conducting this lab, students may note that the larvae display different extremes in response to similar stimuli. This is why 1) multiple responses are measured and then averaged for each animal in response to the same stimulus and 2) it is important to try and keep each behavioral assessment as uniform as possible and minimize stochastic outside factors that may throw off data collection (e.g., cell phone usage, student talking, being too close to the larva during assessment).

2. For some students this is an introduction to regression analysis. Supplemental materials provided by the free online data analysis engine www.vassarstats.net can be great pre-lab reading for students to insure they are comfortable with how the data are being analyzed and what conclusions can (or cannot) be drawn from their study. Good starting spots would be the free online textbook provided by Vassarstats (http://vassarstats.net/textbook/), with an emphasis on Chapters 3 and 4 in this online textbook for this lab.

3. Students may be unsure about how best to test lepidopteran behavior when they are brainstorming measures in small groups. When I have encountered this situation, I encourage students to research during lab time methods used in published literature with lepidopteran behavior experiments. During this time, I also give the students an opportunity to interact with one larva (outside the context of the controlled behavior studies) and observe how it moves and how it responds to different kinds of stimuli. These two steps help the students make informed decisions about which hypotheses to test and underscores the importance of literature review and personal observation in conducting science experiments.

Comments on Introducing the Experiment to Your Students:

I introduce the topic of animal behavior and the effects it can have on structuring natural or disturbed ecosystems. I highlight that many questions pertaining to the drivers and consequences of differences in animal behavior are still unknown or little explored, including how intraspecific differences in behavior could influence herbivory. I then underscore the importance of hypothesis generation and novel, but bounded inquiries within the college classroom. Time permitting, it can be fun and informative for students to generate their own measures of animal behavior rather than those listed in Table 1. Student generated measures have worked well in some of my classes; for example, one group of students tested boldness by measuring caterpillar movement time in response to different types of bird calls (e.g., insectivorous versus granivorous). These measurement ideas, however, can take a long time to derive and I would often send students into the primary literature during lab time to validate the methods they proposed. If these additional steps take more time than permitted for the lab section, consider just focusing on the set of measures provided in Table 1.

Comments on the Data Collection and Analysis Methods:

I typically have all the behavioral assessment tools set up at a central station and allow students to review these tools after I discuss the lab timeline. I try to keep group and class brainstorming and decision-making to around 10 minutes each. If this kind of work is wholly new to an instructor, I would encourage the instructor to explore a little with the larvae and their responses to different stimuli well prior to lab time. Being familiar with what different types of behavioral responses look like will help you guide student inquiry and data collection. I would also encourage instructors to structure and post a shared data sheet (e.g., Google sheet) so each group can enter their collected data into a shared file and can access the data later while working on report generation. Instructor familiarity with the Vassarstats.net portal will greatly help guide student analysis; I use this online tool regularly throughout the semester and it serves as the analytical engine that I use for most of my labs.

Comments on Questions for Further Thought:

1. What other measures of animal boldness could you have used to quantify differences in animal behavior?

- This will depend on the measures the class decided to evaluate, but I do find it helpful to spend a little time with the students considering the differences between the stimulus-based responses (e.g., simulated predator/parasitoid attacks) and the more observation-based measures (e.g., movement in an open arena). It may also be helpful for students to reflect on their trials and discuss any observations that they made that could be quantified but were not the focus their study.

2. What are some issues with the sampling protocols you used for this experiment? How could you have modified your approach to make the experiment more rigorous?

- This requires reflecting on how well the students think they did conducting the experiment. I would use this opportunity to point out how unique factors (e.g., the amount of sunlight in the room or on the student's arena, the position of groups with relationship to the room's door) can all have effects on how an animal behaves. I would use this time to reflect with students about what the ideal behavior testing facility would look like and maybe explore online what some of those facilities look like.

3. Here you test the relationship between animal behavior and herbivory under conditions of perceived predation risk (i.e., high visibility). Can you think of alternative feeding arena setups that would also allow us to manipulate perceived predation risk?

- This encourages students to link experimental questions with experimental design. Other than visibility, what other types of cues might they manipulate to generate fear response? The presence or absence (or concentration) of particular predator or parasitic insect pheromones in spatial proximity to the available food may be useful. The presence of a predator (e.g., ground beetle) in a small makeshift cage within the feeding arena and at different distances from the leaf discs would also help determine the effect of perceived risk.

4. Herbivory often also depends on leaf quality. How might your results change if the plant quality was altered say by growing the plants in more or less haline soils? What are some other ways you might modify leaf quality?

- Leaf quality is easy to manipulate through the use of fertilizers or mineral salts that change leaf quality. Different gradations of leaf quality may make herbivores more or less likely to venture into "risky" areas to feed and these responses may scale with the boldness or shyness of the individual lepidopteran. Plants can also be induced with methyl jasmonate or physical wounding to reduce their leaf quality. Comparing herbivore response to induced or not induced plant leaf tissue may similarly vary as a function of herbivore boldness.

5. This experiment was conducted in highly controlled lab settings. How do these results transplant to patterns you might encounter under natural field conditions? What are the benefits of conducting controlled lab studies like this? What are limitations to this approach?

- This is an important discussion about the role of laboratory/greenhouse studies in the context of ecology and evolution. When I encounter these questions in lab, I laud the acknowledgement and underscore that the artificial environment in a lab or greenhouse may not be representative of what happens in natural settings. I conclude by noting, however, that controlled experiments can directly inform the hypotheses you choose to test under field conditions and often allow researchers the latitude to explore possible mechanistic drivers behind certain phenomena.

6. Here, you have evaluated how individual animal behavior corresponds to plant-animal interactions. Using a primary ecological publication as a reference, what are some other interspecific interactions that animal behavior may modify? Describe how intraspecific variation to modify the outcomes of those interactions.

- During this discussion, I elaborate on the different ways timing of animal activity influences ecological interactions. In particular, I expand on how predator-prey interactions often structure animal activity timing and how activity timing can influence an organism's contact with zoonotic disease carriers. Students seem to enjoy discussion and exploring the multiple different environmental factors that contribute to an animal's activity timing and then trying to anticipate how bolder or shier individuals within a species would respond to different types of conditions. These discussions are excellent foundation for further hypothesis testing activities and student research projects. Journals such as *Ethology*, *Animal Behavior*, and *Ecology* regularly publish research articles demonstrating how variation in animal behavior links to other interspecific interactions.

7. What would you expect to happen if, instead of having a leaf only on the light side of dish, you had leaf discs available in both the light and dark side of the petri dish?

- This is an important follow-up question and experiment that helps solidify the concept that light equates to perceived risk for the herbivore. Including some additional replicates or additional feeding trials will help demonstrate that, regardless of individual boldness and given identical quality food items, herbivores will preferentially feed in low-risk environments (i.e., the dark side of the Petri dish) before feeding in the higher risk environments.

Comments on the Assessment of Student Learning Outcomes:

I would encourage instructors to be open to receiving multiple (2-3) submissions of this assignment prior to the final graded assessment or adopting a sliding scale or grading where earlier submissions are worth fewer class points than later drafts of the same assignment. Commenting on multiple drafts of the same written assignment can generate significant changes in both student conceptual writing and their writing mechanics. Shorter written assignments also help the instructor maintain focus for the duration of the student's assignment and help allow for formative comments to be made throughout the abstract report. I do not typically collect the handout/worksheet provided above but leave it to the students to use this handout as a reference for their writing and data analysis work.

Comments on Formative Evaluation of this Experiment:

I use a DELTA/+ assessment structure in each of my labs/lecture sections. The "DELTA" section of the feedback asks the students to indicate what they would change about the lab and the "+" section asks students to reflect on what they liked about the lab. This feedback informs recommendations for what to change and what to retain regarding the structure and execution of the lab. Students provide anonymous handwritten feedback on scraps of paper placed in cardboard boxes by the door of the classroom or lab. This insect behavior lab was very popular among students; students often indicated that they liked the activity and the in-depth focus on the link between behavior and herbivory. Students and colleagues also continued to help shape the structure of this lab by commenting on things they think needed clarification, methods that would work better, or small valuable experimental add-ons.

Comments on Translating the Activity to Other Institutional Scales or Locations:

1. This experiment scales well with class/university size. As with any behavior study the greater the number of replicates the greater resolution can be attained. This lab does require productive conversation among individuals within the class, so it would probably be best suited for labs smaller than 25 individuals.
2. This experiment would translate well to other parts of the United States or world as long as instructors could acquire a sufficient number of lepidopteran larvae (wild or store bought) and their preferred plant food source.
3. As a laboratory activity, this experiment provides a unique opportunity for students with disabilities to explore rigorous questions in animal behavior without being concerned about the potential barriers repeated trips to the field may present.
4. This lab may transfer well to K-12 education. In these situations, I would suggest separating the two experiments and running them individually (an animal behavior study and/or an herbivory study). These labs would be great ways for K-12 students to generate data to practice scientific dialogue and data graphing. For much younger audiences, parts of this lab may need to be simplified to help with understanding core concepts.

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

A cleaned data set from Eastern Michigan Universities Bio 311W (Winter 2018) is included as a csv file. Please note some responses required transformation in order to adhere to model assumptions of normally distributed error. This provided an opportunity to discuss the role of data transformation with a student collected data set and was a great add-on.

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