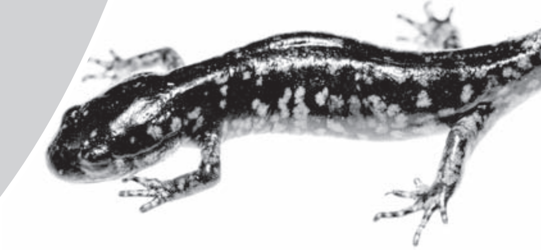
INQUIRY & INVESTIGATION

Salamander Antipredator Defenses: A Field Exercise to Engage Students in Ecosystem Dynamics



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Abstract

The addition of field experimentation to the theory of behavioral biology and ecosystem science creates a significant critical thinking link for students at all levels. First-hand observations of the responses of salamanders in the field permit students to see how organisms behave in their natural habitat. This exercise introduces students to the study of animal behavior, specifically the antipredator responses of salamanders. The students form hypotheses regarding the behavior of salamanders in their natural environment and then record responses of individual salamanders, compile data, interpret data, and draw conclusions about the behavior of salamanders in response to natural stressors.

Key Words: *animal behavior; interspecies interactions; predator-prey relationships; territoriality; antipredator postures; aposematic signals; "fight or flight" responses; taxis; kinesis; field experiment; field data collection; abiotic stressors; biotic stressors; natural selection.*

○ Introduction

The engagement of any learner and retention of information is increased through the process of doing, rather than hearing or observing. Although the application of "doing" is more commonplace in baccalaureate biology programs, the use of field studies in K-12 districts and general biology courses in higher education is often absent. This is likely not due to lack of interest, but rather lack of resources. Herein we provide the tools to conduct a basic-level field experiment assessing salamander behavior. The data collected from this study could be used in a myriad of ways in the classroom, some of which are presented as examples.

Why are salamanders well suited for observations by students? Salamander behaviors are easily observable and quantifiable. If a proper field site is identified, classes of various sizes can conduct a study under time and budget constraints. And because of the known needs for students to collect, process, interpret, and report scientific data, the data collected on salamander behavior is robust enough to address research questions and hypotheses, and be interpreted readily by students. Salamander studies can be used to address a variety of biological concepts associated with animal behavior (e.g., predator prey response, intraspecific competition, aposematic signals, habitat selection, ecology, and evolution). Although we focus primarily on the antipredator defenses of the salamanders, the role of food availability and competition for that resource (intraspecific competition) would be a significant extension and would lead directly in to the topics of habitat selection ("why do you live where you live?"), how prey can hide from or trick predators (aposematic signals), and how species evolve (natural selection acts on the individual who is able to survive and reproduce). Instructors should consider opportunities within their own curricula to use animal behavior as an applied investigation into topics that are otherwise term-heavy or less engaging. Additional salamander behavior and curricular development information is available in the Appendix.

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Antipredator responses are easy to observe in the field. The response of the salamander upon discovery is often immobility, mimicking a stick or twig (Figure 1), whereas antipredator responses are used as the threat of predation increases. Antipredator responses can also be observed in captured salamanders that are stimulated to respond using light taps. These taps on the anterior portion of the body or head will cause the salamander to arch its back and lean toward the point of stimulus, whereas taps on the posterior portion of the body or tail will cause the salamander to elevate its tail (Figure 2). These differential responses allow for the

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development of hypotheses regarding the attack "strategies" of the predator and the reasons for such responses by the prey.

○ Objective

Students collect, analyze, and interpret field-sampling data and apply those types of data to broad-reaching questions in evolution, genetics, and climate.

○ Use of Salamanders

Care should be taken to work only with salamanders that have a status of "not of concern" in the investigator's local area and not use those listed as "special concern." As the salamanders used in this investigation are living specimens, the students must be instructed on their proper handling to minimize the potential for stress and/or injury. Rough handling may cause them injury (skin abrasions, puncture, ulcers, etc.) and should be avoided at all costs. The National Wildlife Health Center (2001) recommends the following salamander handling procedures:

> Medium and large size salamanders . . . should be grasped in the middle of the body between the forelimbs and hindlimbs . . . Under no circumstances should salamanders be grasped by the tail or picked up by the tail.

Student health concerns are low for salamanders in the United States. It is possible that some may produce skin secretions that would be eye irritants, however, with proper technique (use of disposable gloves by any handlers) and avoidance of touching the face during any field activity, the risks are minimal. Amphibians may also carry bacteria in their intestines and feces that are human pathogens (*Salmonella* or *Leptospira*), but infection risk is minimal if students wear gloves and wash their hands thoroughly with soap and water. Although we did not require our students to complete any animal handling documentation, instructors may chose to do so depending on the students' grade level and any institutional regulations.

Figure 1. The blue-spotted salamander, *Ambystoma laterale*, in an immobility pose. All individuals herein were observed at Rochester Institute of Technology, Rochester, New York, April 2015.

Institutional Care and Use Committees (IACUCs) are increasingly concerned with field studies using live organisms. Some IACUCs are concerned only with research that may be disseminated beyond the university, but others are also concerned with animal use in the classroom. We were unable to identify any scientific studies, beyond circumstantial evidence, that suggest significant stress on the salamanders used in one-time observational studies. We were able to identify one publication that considered stress hormone levels in salamanders. Kinkead et al. (2006) compared marking techniques in salamanders, and found that even extreme marking techniques (toe-clippings), which are *not* utilized in this study, did not increase adrenaline levels in their study salamanders, even within increased handling time. Despite this, instructors should consult with their IACUC and determine if permissions should be obtained to handle the salamanders in the field or classroom. As we were not collecting any specimens, and were making only non-invasive behavioral observations as a class (not as research), we were not required to complete documentation, but this will be institute specific.

○ Class Design

In our implementation, we worked with a class size of 40 students with one instructor, one teaching assistant, and a designated photographer. If the activity will be implemented with only one instructor, we would recommend a field class size no larger than 24 for high school and college, 15 for middle school, and no more than six students for elementary level to allow close observation of or instructor-only animal handling. Instructors may choose to modify this activity for the classroom for younger students using captive-bred salamanders in the classroom.

It is recommended that this activity take place over several classes, if the instructor wishes to include an introduction to the subject matter and methodology and to conduct data analyses. In this design, three 90–120 minute blocks would afford time (Day 1) to introduce the topic and engage the students in the concept of field-sampling and behavioral biology, (Day 2) to collect the field data, and (Day 3) to analyze and report on the data collected. Although this timeframe could be condensed for higher-level classes, one would still need at least two class periods for thorough discovery and investigation.

Figure 2. Two *Ambystoma laterale* in two different antipredator defense poses commonly observed in the field.

○ Details of Activity

In the classroom, the instructor should introduce animal behavior and ecosystem factors that can influence behavior. Dependent upon class level, this can be as simple as “what do you do when the temperature outside gets warmer or colder?,” and can be expanded to higher levels if one also incorporates poikilotherms and homeotherms, predator-prey relationships, and the role of local climate on animal responses in the discussion. The study animal should then be introduced, and the students should be asked to consider how the salamander might respond to factors in its environment (climate, predators, finding food, etc.). The students should be asked to write questions and hypotheses that can be tested in the field or classroom using the salamanders. If this activity will be completed in two class periods, the design of the experiments conducted to answer the questions and hypotheses may have to be guided and modified by the instructor. Common hypotheses posed by students in our implementation included:

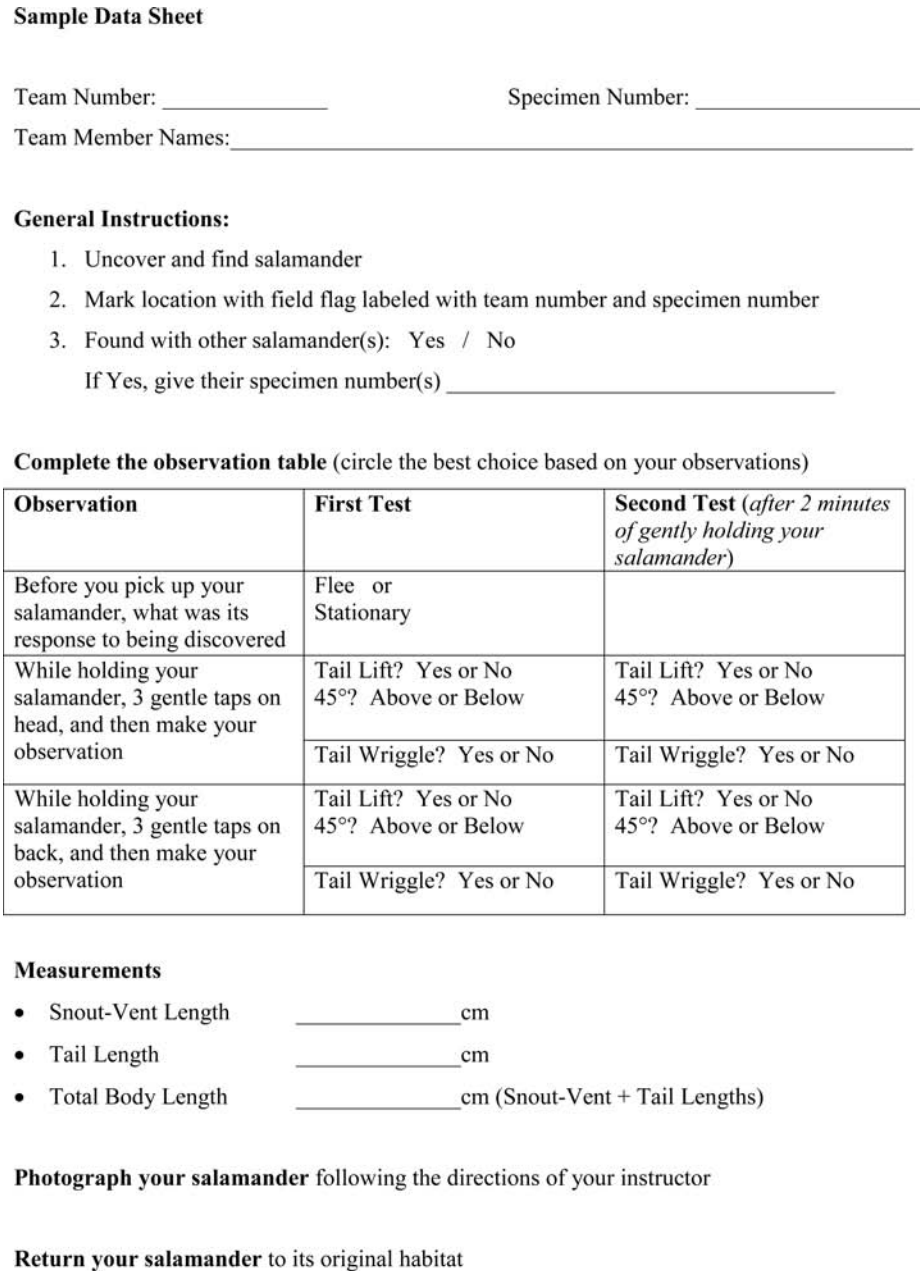
- Salamanders found together tend to be of similar size.
- Smaller salamanders will not display antipredator defenses.
- Salamanders in colder areas will display fewer antipredator defenses than those in warmer areas.
- Brighter colored salamanders will display fewer antipredator defenses than those with less coloring.

After the questions and hypotheses are posed and the experiments are outlined (as needed), the students should be given a worksheet that will be used for data collection, and a mock data collection exercise should be set up, depending on the age group of the students, so that there are fewer questions and concerns in the field. The students are now ready to find salamanders!

A suitable habitat for finding salamanders of the type described herein (*Ambystoma laterale*) or similar (e.g., *Desmognathus ochrophaeus*) would be moist but not inundated soils, with high debris (fallen logs and leaf litter), near streams or small ponds, which are required for reproduction. However, more terrestrial-based species, such as the Northern Redback Salamander (*Plethodon c. cinereus*), would also work well in this activity and can be found in the late spring and fall in deciduous forests under rocks and logs. In most areas throughout the United States, local wildlife management would be able to recommend sites.

If an instructor chooses to use captive salamanders for this activity, a reliable source for captive-bred salamanders should be used. We suggest Amphibian Aquatics (http://amphibianaquatics.com/); however, because of U.S. Fish and Wildlife Service bans on transport and shipment, only *Ambystoma talpoideum* may be available in some states. The Newt & Salamander Portal (http://www.caudata.org/) is also a terrific resource for instructors to identify local sources of salamanders.

For the highest efficiency of data collection, we split the students in to teams of three: one recorder of observations and data, one salamander handler, and one data collector. The number of students can be modified, and more than one student doing data collection is useful. If there is an additional student, they can be assigned to the role of photographer. The recorder will need the following items: clipboard, data sheet (Figure 3), pencil, and a field flag (or GPS to mark the exact location of the salamander when discovered). The handler must wear disposable gloves (and change gloves after *each*



Sample Data Sheet

Team Number: ______________ Specimen Number: ___________________
Team Member Names:____

General Instructions:

1. Uncover and find salamander
2. Mark location with field flag labeled with team number and specimen number
3. Found with other salamander(s): Yes / No
   If Yes, give their specimen number(s) _________________________________

Complete the observation table (circle the best choice based on your observations)

Observation	First Test	Second Test (*after 2 minutes of gently holding your salamander*)
Before you pick up your salamander, what was its response to being discovered	Flee or Stationary	
While holding your salamander, 3 gentle taps on head, and then make your observation	Tail Lift? Yes or No 45°? Above or Below	Tail Lift? Yes or No 45°? Above or Below
	Tail Wriggle? Yes or No	Tail Wriggle? Yes or No
While holding your salamander, 3 gentle taps on back, and then make your observation	Tail Lift? Yes or No 45°? Above or Below	Tail Lift? Yes or No 45°? Above or Below
	Tail Wriggle? Yes or No	Tail Wriggle? Yes or No

Measurements

- Snout-Vent Length ______________cm
- Tail Length ______________cm
- Total Body Length ______________cm (Snout-Vent + Tail Lengths)

Photograph your salamander following the directions of your instructor

Return your salamander to its original habitat

Figure 3. Sample data sheet.

salamander is observed) and must understand the need to carefully hold and manipulate the salamander without permitting the salamander to jump or fall from any height. The data collector(s) will need: sterile swabs or Q-tips™, a ruler, and a soil thermometer. Additional resources for the instructor(s) should include: a trash bag (for gloves, swabs, etc.), additional supplies for each team position, and a camera to photograph the salamanders. If a camera is not available for the class, students should use mobile devices to record images.

Figure 4. *Ambystoma laterale* in an immobility pose immediately after being discovered.

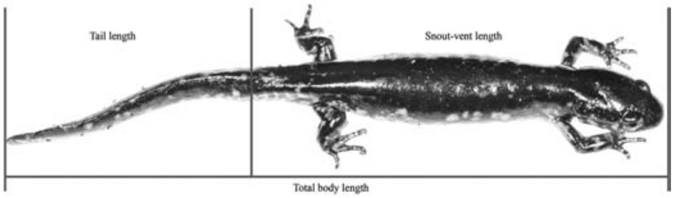


Figure 5. Measurement guide for snout vent length and tail length of a salamander. Note that the tail should be gently manipulated to be as straight as possible (unlike here) without harming the individual.

○ Field Materials

- Data sheets (see Figure 3)
- Pencils
- Clipboard
- Field flags or GPS
- Lab gloves
- Sterile swabs or Q-tips™
- Ruler (cm)
- Thermometer
- Trash collection bag
- Camera

○ Field Methods

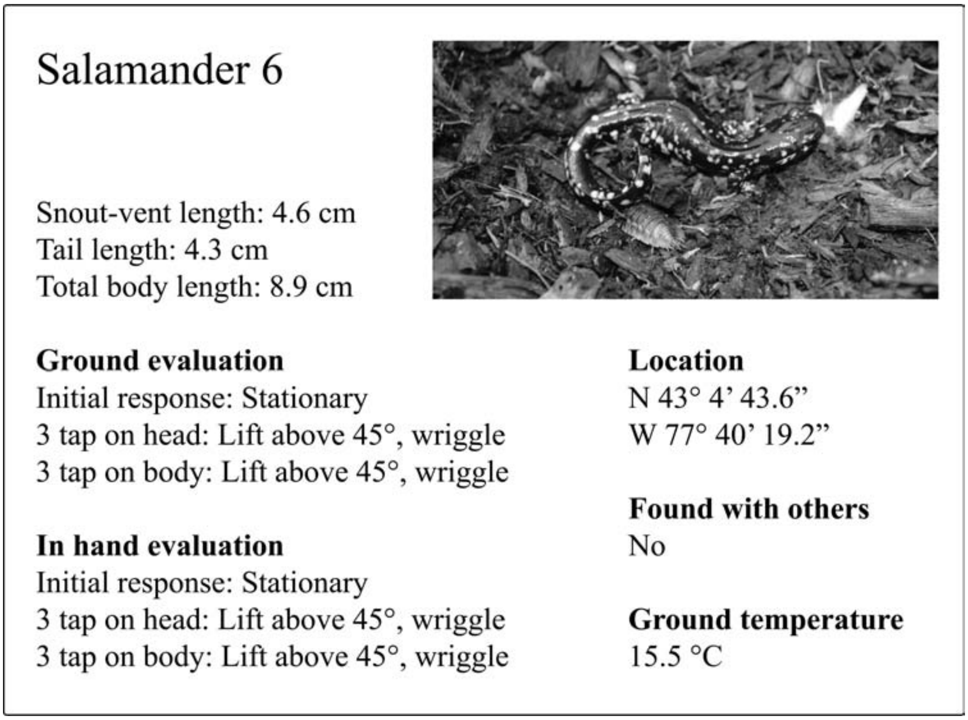
Each team is assigned a team number and should mark the field flags with their team number and the specimen number for each salamander found. Teams locate salamanders by overturning logs, boards, rocks, and such, in a manner that will not disturb the surrounding habitat. The preferred method is by rolling logs toward or away from oneself to observe the uncovered ground and underside of log. Be aware of any salamanders situated inside log crevices. Once a salamander is found, the location is marked with the field flag (already labeled with the team number and specimen number) or by using GPS. This will prevent other teams from collecting data on the same individual.

The team will note whether multiple salamanders are found in that particular location, and whether the initial response of the specimen was “Flee” (salamander attempts to return to cover as soon as being uncovered) or “Stationary” (salamander remains motionless, a primary antipredator defense; Figure 4). Students should not touch the specimen(s) at this time, but only watch and record their observations. If the salamander flees, the handler may pick up the salamander for captive data measurements, if it is possible to do so without harming it. The group will not have a data set for the first evaluation on the ground if the salamander flees.

To begin the first antipredator evaluation, the salamander handler will use a sterile swab to gently tap the salamander, simulating a predator. Complete three taps on the back of the head and record observations. Then complete three taps on the middle of the back and record observations. The team will determine if the salamander demonstrates antipredator postures following taps on the head and back.

The handler may now pick up the salamander for the second antipredator evaluation. Again, students will observe (1) the salamander’s initial response when held (flee or stationary), (2) its posture after three head taps, and (3) its posture after three back taps. The salamander handler will then measure the snout vent length (SVL) and tail length. Although these measurements can be taken from the dorsal side to decrease handling time, the most accurate measurements would be taken on the ventral side. On the ventral side of the salamander, one should be able to identify the cloaca, an enlarged glandular-like protrusion used primarily for excretion and reproduction, that is a clear divide for the SVL and tail measurements. Depending on the species, the cloaca may or may not have an obvious opening, and may or may not differ in color from the underbelly. Instructors may chose how to direct the collection of the snout vent and tail lengths based on the age and number of students, and whether the data are being collected from field salamanders or in a classroom. The two body measurements are then summed for total body length (Figure 5). The data collector should insert the thermometer in to the top 1″ of soil where the salamander was found for one minute and record the ground temperature.

When body measurements are complete, the salamander handler should take the specimen to the photographer station to be photographed, if a student or helper has been assigned this role. Best practice dictates that students photograph their data sheet with all information completed and then photograph the salamander. Alternatively, the team members may use their camera phones to take a photo of their data sheet and then place the salamander on a piece of white paper (card stock or foam board is preferable) to photograph the salamander. Following all data collection, students must turn the logs back to their original position and then allow salamanders to crawl back to their original habitat. If students place the salamanders and then move the log, there is a risk of crushing salamanders when the log is repositioned.



Salamander 6

Snout-vent length: 4.6 cm
Tail length: 4.3 cm
Total body length: 8.9 cm

Ground evaluation
Initial response: Stationary
3 tap on head: Lift above 45°, wriggle
3 tap on body: Lift above 45°, wriggle

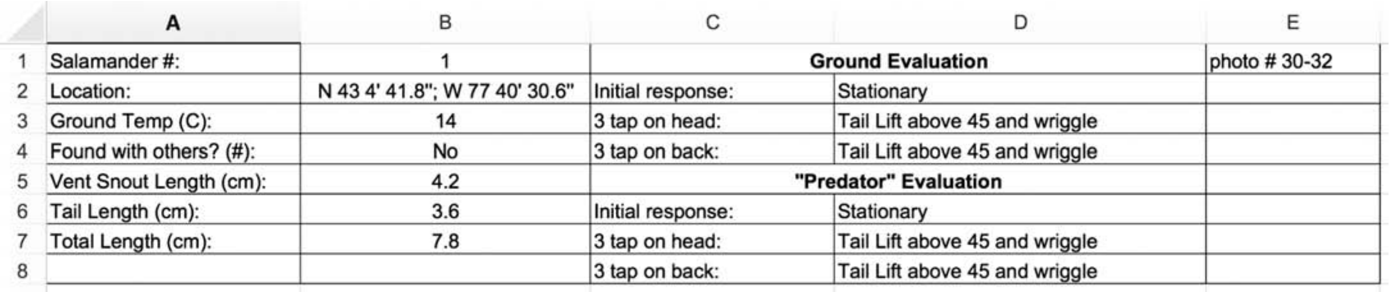
In hand evaluation
Initial response: Stationary
3 tap on head: Lift above 45°, wriggle
3 tap on body: Lift above 45°, wriggle

Location
N 43° 4' 43.6"
W 77° 40' 19.2"

Found with others
No

Ground temperature
15.5 °C

Figure 6. Data compilation for "Salamander #6" on a given collection day. This layout is easier to visualize in a presentation in the classroom, but the data are harder to analyze.



	A	B	C	D	E
1	Salamander #:	1	**Ground Evaluation**		photo # 30-32
2	Location:	N 43 4' 41.8"; W 77 40' 30.6"	Initial response:	Stationary	
3	Ground Temp (C):	14	3 tap on head:	Tail Lift above 45 and wriggle	
4	Found with others? (#):	No	3 tap on back:	Tail Lift above 45 and wriggle	
5	Vent Snout Length (cm):	4.2	**"Predator" Evaluation**		
6	Tail Length (cm):	3.6	Initial response:	Stationary	
7	Total Length (cm):	7.8	3 tap on head:	Tail Lift above 45 and wriggle	
8			3 tap on back:	Tail Lift above 45 and wriggle	

Figure 7. Data compilation for "Salamander #1" on a given collection day. This data layout is easier to compare and analyze statistically than Figure 6.

The students will have a significant amount of data to compile, analyze, and interpret—depending on the success of finding salamanders. To assist this process, it is recommended that the instructor(s) guide the students to compile each salamander's data in an orderly and consistent fashion so that all data are accounted for, and analysis and interpretation is more readily conducted (e.g., Figure 6 and Figure 7).

○ Conclusions

The data collected in this type of field experiment have potentially endless interpretation and application possibilities. The students were asked to write hypotheses regarding the behavioral responses (as noted above), and by using the class data collected, to determine if their hypothesis would be supported or refuted. We focused on basic data analysis techniques (mean and variation in body sizes), behavioral analysis (percentage of individuals exhibiting a given response), and the effects of body size and ground temperature on behavioral responses. Data analyses that could be conducted using the salamanders include:

- Total body length vs. Antipredator Defense
  - *Is there a correlation between salamander size and an antipredator defense?*
- Tail length vs. Antipredator Defense
  - *Is there a correlation between the salamander's tail length and the chosen defense (tail lift vs. tail wriggle)? Is it harder to lift or wriggle the tail if it is longer?*
- Coloration vs. Antipredator Defense
  - *Is there a correlation between the coloration of the salamander and the chosen defense (stationary at discovery vs. antipredator posture)? Do brightly colored salamanders have different responses than those with less or no coloration?*

This application of data may not be advanced enough for some classes, but that should not limit the instructor's imagination! Future implementations in our classroom may include the collection of this behavioral data and its analysis in an evolutionary biology course where the students will also collect toe-clip or tail-clip samples and conduct genomic and proteomic analyses. In addition, a workshop-based interdisciplinary biology and chemistry course will use the same field techniques, with the addition of soil quality analysis and regional climate analysis to further investigate the role of climate change on animal behavior.

The improved engagement of students in science and data analysis is critical, and we believe that field applications such as those described herein are perfect to spark the curiosity and wonder that many of our students have lost!

○ Acknowledgements

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APPENDIX

Supplemental Background: Curricular Applications and Design Using Salamander Behavior Studies

Let us consider the need for real-world science in K-12 curricula. The College Board identifies taxis and kinesis in animals as an illustrative example to foster student understanding in the "AP Biology: Curriculum Framework" Essential Knowledge 2.C.2 (organisms respond to changes in their external environments). The behaviors of salamanders are easily recognizable by students in the field. Further, the students are able to obtain body measurements as an independent variable. The collection of behavior and body size data will allow students to draw conclusions regarding salamander health.

In the "AP Biology: Curriculum Framework" Essential Knowledge 2.E.3, the College Board also recognizes that learning occurs through interactions with the environment and other organisms. Specifically they go on to identify the triggering of animal behaviors by environmental cues as being vital to the organisms' reproduction, natural selection, and survival. The direct observation of salamander behavior in the field is easily applicable to enhancing the understanding of natural selection by the students in the classroom, and could be used as a lead-in to a discussion of the effects of climate on natural selection in populations for older students.

Beyond K-12, the role of general education courses in curricular mapping and design at institutes of higher education has come front and center in recent years (e.g., Rochester Institute of Technology, General Education Curriculum). Even students in technical-based programs are typically required to complete general education science, technology, engineering, and math (STEM) courses. Institutes often go so far as to say each student must complete one natural science, or lab science, course for their degree. With this comes the added responsibility of the general education science instructor to engage highly variable majors and interests. The addition of active learning and engagement to any general education classroom is not a new strategy, however, the use of a field study component is rare. Many suburban and rural college campuses are well poised for the collection of natural data sets on the campus itself, making the logistics and cost of field trips moot. We have found that students are more engaged in a general science biology course that involves learning activities that tie in to something with which they are familiar—the campus around them! Because each general science course is different, how instructors integrate this approach will be highly variable. The data collection outlined in the associated manuscript lends itself very well to discussions of habitat restoration, environmental pollution, evolution of traits and behaviors, and organismal response to climate change.

Let us consider the significant breadth of knowledge regarding salamander behavior and the ease by which data can be compiled from such studies, even by groups of K-12 students. Brodie (1977) first described in detail the antipredator postures in salamanders. In most field observations, salamanders will exhibit either immobility or antipredator postures when exposed. Studies prior to Brodie (1977) often suggest the immediate collection of the salamanders upon discovery, rather than first making observations. This change in protocol provides much greater detail and applicable information regarding the salamanders' natural behaviors. It has been suggested that the choice of the salamander to exhibit immobility or antipredator postures may be closely tied to body position at the time of discovery. As a prey, the salamander may have a higher probability of survival by choosing immobility if the predator is at a great enough distance to mistake the prey for a piece of debris (e.g., twig, stick, etc.; see Figure 1). However, if the salamander is a species with bright coloration, noxious skin secretions, or if the predator is proximate, the antipredator posture may increase survival rates.

Brodie and Howard (1973) reported the aposematic function of bright coloration to visual predators in salamanders. Aposematic coloration is always associated with noxious skin secretions and serves as a visual warning to predators. Although pseudoaposematic coloration has been observed in some species of salamanders, the predator must have knowledge of the aposematic coloration (Batesian mimicry: an understanding that the bright coloration is a warning of an animal's toxicity despite the displaying animal being nontoxic). Depending on the location of the bright coloration, the antipredator posture will change, permitting the salamander to expose those bright color patterns as a warning. While the extent of the response is dependent on the intensity of the stimulus (location of predator, etc.), the antipredator postures most often include some degree of the following: raised head, raised tail (often coiled or wagging), and lateral flexure of the body. The antipredator posture will then be maintained, in a rigor or immobile state, until the stimulus is removed, or the response will be heightened to compensate for a potential attack. The wagging of the tail attempts to misguide the attack, with the predator potentially either biting off part of the tail (the most dispensable part of the salamander), or being exposed to glands containing noxious secretions along the dorsal margin of the tail. Either result will likely increase survival.

Thus far we have discussed the impact of biotic stressors on the response behaviors of salamanders. The question remains, however, whether abiotic stressors can alter those responses, and, if so, to what extent that alteration will affect the survival rates of the salamanders. Lang and Jaeger (2000) address the defense of territories by the red-backed salamander, specifically considering the role of abiotic and biotic factors in the defense tactics. Historically, territories for organisms were defined as space that is defended (e.g., Noble, 1939). Studies have also considered that defenses can be both beneficial (an established niche) and costly (potential harm while defending) (e.g., Lang & Jaeger, 2000; Quinn & Graves, 1999; Mathis, 1990; Toft, 1985). Most often, availability of resources (e.g., food, water, and habitat) and mates are cited as the driving forces behind the establishment of territories—a direct linkage to Darwinian fitness. Recent studies have also considered the "reward" of such defense strategies as having a positive effect on the genetic fitness of the organism or population, wherein the strongest individuals survive and reproduce (e.g., Agrawal, 2001; Lang & Jaeger, 2000). More advanced students may find it interesting to extrapolate their animal behavior data that will be collected by this study and consider the role of climate change in natural selection, specifically how changing resource availability (habitat and nutrients) may drive evolution (Ockendon et al., 2014; Sorte et al., 2013).