An Inquiry-Based Exercise for Demonstrating Prey Preference in SNAKES

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The use of live animals at all educational levels has declined in recent years (Abramson et al., 1999c). Despite this trend, the curiosity many students have toward animals can be utilized to teach biological concepts in the classroom. Live animals continue to be used in the high school and undergraduate science classroom to teach physiology, ecology, and behavior (Abramson, 1990; Abramson et al., 1996; Abramson et al., 1999a,b; Darling, 2001; French, 2001; Rop, 2001).

The recent promotion of inquiry-based learning techniques (Uno, 1990) is well suited to the use of animals in the classroom. Working with living organisms directly engages students and stimulates them to actively participate in the learning process. Students develop a greater appreciation for living things, the natural world, and their impact on local environments by interacting with live animals. Through the process of experimenting with animals, students also meet many of the *National Science Education Standards* set forth by the National Research Council (NRC, 1996) and the Benchmarks for Science Literacy set forth by the AAAS (AAAS, 1993).

The goal of this paper is to describe an inquiry-based project in which snakes are used to illustrate the relationships between predator and prey and the importance of sign stimuli in attraction. Our exercise is also useful for sharpening the observational skills of students and for reinforcing the importance of gathering and analyzing quantitative data. This exercise is also beneficial for instructors wishing to meet the *National Science Education Standards* (NRC, 1996). Our experience suggests that this exercise is appropriate for students in middle school, high school, and college.

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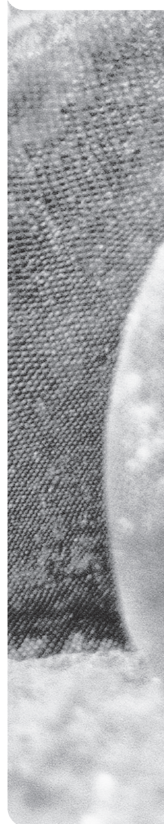
Snakes have much to recommend them for classroom study. They are readily captured in the field and available from pet stores and biological supply houses such as Wards Scientific and Connecticut Valley Biological Supply House. Snakes can also be purchased from reptile dealers such as Glades Herp, Inc. (http://www.gherp.com). In addition to wide availability, snakes are easy to handle and maintain. Generally, most snakes can be housed in appropriately-sized plastic storage containers with a paper substrate and water available *ad libitum*. Appropriate food should be offered every 7-10 days for large species and every 2-3 days for small species. Much information is also available about their natural history, behavior, and maintenance (e.g., Conant & Collins, 1998).

A word of caution is in order when considering using this exercise. Some snakes bite. For example, rat snakes (*Elaphe* spp.) and kingsnakes (*Lampropeltis* spp.) often bite when initially captured, but lose their aggressive tendencies in captivity. A thorough washing with soap and water and a bandage are all that is necessary in most cases. As in any laboratory or classroom situation in which animals are used, a first aid kit should be available.

We begin by presenting some background information for teachers unfamiliar with snakes and their habits. We then outline a general protocol for testing prey preferences in snakes. Guidelines for discussion are provided with general questions helpful in leading students through an inquiry using snakes. We conclude by relating a few aspects of this project to the *National Science Education Standards* for Life Science (NRC, 1996).



Background Information

Although all snakes are carnivorous, there is a wide array of items in the diets of snakes. Some snakes are generalists and consume various prey items, whereas others are specialists and limit themselves to one or two specific prey types. Nearly all snakes swallow their prey whole [the crab-eating snakes *Gerarda prevostiana* and *Fordonia leucobalia* may be exceptions (Jayne et al., 2002)]. The maximum prey size a snake can consume is limited by how far a snake can open its mouth. Thus, snakes are sometimes referred to as "gape-limited predators." Being "gape-limited" is a definite constraint on what prey a snake can consume (Pough & Groves, 1983). For example, the diminutive smooth green snake (*Liochlorophis vernalis*) cannot swallow an adult mouse.

The distribution and availability of prey are important ecological factors affecting prey choice in snakes. The habitat in which a snake resides influences the species upon which it can prey. A snake's diet is limited by what it encounters in its habitat. For example, a yellow-bellied sea snake (*Pelamis platurus*) is unlikely to encounter a deer mouse in the Pacific Ocean.

Evolutionary history can also affect prey preference in snakes. Research by Burghardt (1966, 1967, 1969, 1975a) suggests some species of snakes have innate prey preferences. Field studies have shown that the Northwestern Gartersnake (*Thamnophis ordinoides*) is a slug-specialist (Gregory, 1978), and experiments with newborn *T. ordinoides* that have never eaten (known in the scientific literature as "ingestively naïve") have shown that they have a preference for slug extract. Likewise, naïve newborns of the Pacific Coast Aquatic Gartersnake (*Thamnophis atratus*) responded only to extracts of amphibians and fish (Burghardt, 1969).

Snakes have a strong reliance on chemical signals (Zug, 1993). Most chemical information perceived by a snake is processed through the vomeronasal (a.k.a. Jacobson's) organ (Zug, 1993). Chemical information is picked up in the environment by the tongue. These molecules are then scraped off the tongue by the sublingual plica (Gillingham & Clark, 1981) and are transferred to the vomeronasal organ located in the roof of the mouth.

Because snakes rely so heavily on chemical cues, and the tongue is protruded to gather these cues, prey preferences may be easily observed and measured in the laboratory by simply measuring changes in tongue flicking rates as an indication of increases in the central excitatory state (i.e., preferences). If excited enough, snakes may even attack the source of the chemical signal, regardless of its shape or size.

Materials & Methods

Animals

This experiment can be done if only one species is available, but is best done with several different species. Wild snakes are available commercially, but we advocate using locally-captured animals because they can be returned to the capture site following the experiment, thus reducing the impact on wild populations. A less obvious advantage to using locally-captured animals is that both the teacher and student gain a better appreciation of environmental issues such as the importance of habitat. Students also learn to appreciate regional fauna when locally-captured animals are utilized. Be sure laws regarding animal collection are followed. Permits may be required.

Numerous species make excellent test subjects. Garter snakes (*Thamnophis* spp.), water snakes (*Nerodia* spp.), rat snakes (*Elaphe* spp.), kingsnakes (*Lampropeltis* spp.), ringneck snakes (*Diadophis* spp.), hognose snakes (*Heterodon* spp.), and redbelly snakes (*Storeria* spp.) are among the most common species encountered in the United States. The species listed vary from prey specialists such as the hognose snakes, which specialize on toads (*Bufo*), to garter snakes, which are generalists that consume insects, fish, amphibians, and small mammals.

The *Guidelines for Responsible Use of Animals in the Classroom* (National Science Teacher's Association, 1991) should be followed. These guidelines include safety protocols for both animals and students. Some students may exhibit apprehension toward snakes. However, it has been our experience that the fear most students exhibit is due to a lack of knowledge regarding snake behavior. For example, one author uses a snake in an undergraduate Psychology of Learning class to demonstrate habituation. Students exhibiting initial apprehension toward the snake are holding the snake by the end of the class (Freudian connotations aside). It may be helpful to spend some time before beginning the experiment so students can get accustomed to handling the snakes they will use in the laboratory.

Appropriate references for the capture, care, and handling of snakes as per the National Science Teacher's Association guidelines can be found in Conant and Collins (1998). Species-specific care guidelines are given in Perlowin (1997) for kingsnakes, Bartlett (1996) for rat snakes, and Rossman et al. (1996) for garter snakes. We do not recommend the use of venomous snakes (i.e., rattlesnakes, coral snakes) in the classroom, as these species are potentially deadly and should only be handled by trained professionals.

Prey Species

Snakes as a group eat a wide variety of vertebrate and invertebrate prey. Common invertebrates available through biological supply houses and commonly-consumed by snakes include: night crawlers (*Lumbricus*), crickets (*Acheta*), cicadas (*Magicada*), and crayfish (*Orconectes*). If local snakes are used, invertebrate prey species could be captured simultaneously from the same habitats as the snakes. Vertebrates commonly consumed by snakes include fish, frogs, salamanders, lizards, snakes, birds, and rodents. Most of these are available from biological supply houses. Frozen rodents and birds can be purchased from most pet stores.

Preparation of Prey Extracts

Since snakes rely so heavily on chemical signals, it is possible to present the test subjects with cotton swabs impregnated with various prey-derived chemicals instead of using the whole prey animal (Burghardt, 1967). Hence, fewer prey animals are required to conduct the experiment than if individual prey were presented to each test subject.

Extracts can be prepared by placing one or more intact prey animals in distilled water (1.5 g body mass of prey per 10 ml of water) at 50˚C and gently stirring for 2-3 minutes (Burghardt, 1967). For example, a 3 g night crawler should be prepared with 20 ml of distilled water at 50˚C. Extracts should be prepared on the day of use and stored in the refrigerator until needed. Because of the low volume of extract on the swab, the extract reaches room temperature rapidly.

In preparing the extracts, we recommend purchasing frozen birds and mice from a pet store or biological supply house. The animals should be thawed prior to preparing the extract. Alternatively, cage bedding from birds and mice can be used in lieu of the whole animal. Students may also experiment with food items brought from home. They may find it interesting to test snake preferences toward chicken, hamburger, or pet food. While snakes may not exhibit an innate preference for these items, repeated exposure may help them associate the items with food.

Table 1.
A partial list of some common snake species that can be used in an experiment demonstrating prey preference in snakes and potential prey species that can be used to create prey extracts used in the demonstration. In many of these species, fish extract can be used as a stimulus that does not elicit a response.

SNAKE SPECIES	PREY SPECIES EXTRACT
Garter snakes (*Thamnophis*)	Night crawler, crayfish, frog, toad, minnow, mouse
Water snakes (*Nerodia*)	Crayfish, frog, toad, minnow
Rat snakes (*Elaphe*)	Frog, bird, lizard, rodent
Kingsnakes (*Lampropeltis*)	Frog, bird, lizard, snake, rodent
Hognose snakes (*Heterodon*)	Frog, toad
Ring-neck snakes (*Diadophis*)	Night crawler, cricket, cicada, salamander
Redbelly snakes (*Storeria*)	Night crawler, cricket, cicada, salamander

Rationale for Experimental Procedure

This experimental procedure is based on the work of Burghardt (1966, 1967, 1969, 1975a,b). The specific protocol outlined here is a modification of Burghardt's (1975b) teaching demonstration. There are several differences between Burghardt's 1975b exercise and ours. First, we advocate using multiple species of snakes rather than focusing on a single species. The use of multiple species encourages students to address a broader range of hypotheses. Second, we suggest testing snakes in novel environments rather than the home environment. Such an arrangement makes performing the experiment for large groups easier. It should be noted that by using a novel environment for the testing situation, students can investigate the effect of environment on predatory behavior as an independent variable. Third, our experimental protocols are designed to fit the *National Science Education Standards* for Life Science (NRC, 1996). Anecdotal evidence suggests that such standards are becoming commonplace in the science classroom. Fourth, we encourage the student to use more quantitative measures such as latency, and the tongue flick attack score. There is also something to be said for making the exercise more accessible. The original exercise is available in an out-of-print laboratory manual that is difficult to obtain.

Experimental Procedure

The materials needed for this experiment include:

- 1 plastic storage container or glass aquarium to house the snake during testing
- 2 stopwatches (one to record latency and the other, session time)
- cotton swabs
- data sheet
- snakes
- extracts.

For the convenience of the instructor, Table 1 provides a list of species and some suitable extracts. The extracts we selected have been found to work in previous experiments. During the experiment, students are encouraged to provide their own ideas as to possible extracts. The data sheet contains such standard laboratory information as name, date, species, extract. There should also be a column to write down the number of tongue flicks, attack latency, and number of attacks. We suggest that the instructor ask the students what should be included in the data sheet.

The experiment begins by placing an individual snake into the testing chamber (e.g., 19-38 liter glass aquarium or similarly sized Rubbermaid® storage container work well, though any vessel large enough to contain a snake will suffice) and allowing it to acclimate undisturbed for 15 minutes. The snake should be motivated to search for prey. One way to ensure that the snake is motivated is to conduct the exercise before its regularly-scheduled feeding. An interesting variation would be to use time since last feeding as an independent variable.

Following the adaptation period, saturate the tip of a cotton swab with the desired prey extract and place the swab within 2 cm of the snake's snout, being careful not to touch the swab to the bottom of the container. Six-inch cotton tipped applicators work best, as they allow the student to be as far away from the snake as possible. A student hovering over the snake tends to be perceived as a predator and may cause the snake to act defensively.

Each snake is tested one time with each extract. For example, if the class uses four extracts, each snake will be tested four times. Each extract is presented for one minute, and the number of tongue flicks counted during this one-minute period. If the snake attempts to bite the cotton swab (considered as an attack), record latency for the first attack and count the total number of attacks. Latency is defined as the time from the introduction of the cotton swab to the attack. Additional attacks should be noted but their latencies need not be recorded. The student should start both timers at the beginning of the one-minute period. One timer is stopped if an attack occurs and the second continues until the one-minute session is over. The timers are reset after the one-minute period is up.

Following the one-minute period, the snake is left to rest for 15 minutes. During this 15-minute period, the student prepares to present the next extract. When the 15-minute period is over, the student repeats the procedure used for the first extract. The experiment is complete when each snake is tested with each of the extracts. Following the last extract, the snake is returned to its home environment. It should be noted that when different individual snakes are used, the testing chamber must be thoroughly cleaned with soap and water and dried to eliminate the possibility of pheromones of the just-tested snake influencing the next snake. For example, when the first snake has been exposed to all extracts, it is returned to its home environment and the test chamber is cleaned before Snake #2 is tested.

Data Analysis

Data can be analyzed qualitatively and/or statistically. Middle school students can analyze the data qualitatively by creating bar graphs of the average or cumulative number of tongue flicks, number of attacks, and attack latencies for each species by prey extract. High school and college students might construct box plots for each species by prey type or can perform statistical analysis on the number of tongue flicks or attack latencies by employing *t*-tests or analysis of variance (MS Excel has these options).

Additionally, Burghardt (1967) devised a composite score known as the tongue-flick attack score (TFAS) that combines the number of tongue flicks and the attack latency into one score. Under this behavioral scoring system, all trials not resulting in an attack are scored as the total number of tongue flicks exhibited during the trial. If the trial did result in an attack, the TFAS for that individual is scored according to the formula:

$$\text{TFAS} = \text{base unit} + (60 - \text{attack latency})$$

where the base unit is the maximum number of tongue flicks exhibited by the same species during all of the test trials, and the attack latency is recorded in seconds. For example, if ten individual snakes of the same species were tested and the maximum number of tongue flicks exhibited by any individual was 40, the base unit would be 40. If one of those individuals attacked the swab 20 seconds after initiation of the test trial, the calculated TFAS would be:

$$40 + (60 - 20) = 80$$

This composite score gives more weight to an attack than to tongue flicks based on the assumption that an attack is a stronger response than tongue flicking and that preferable stimuli elicit stronger responses. The TFAS can be analyzed qualitatively and statistically as described for tongue flicks, attacks, and attack latencies.

Discussion

Inquiry-based activities directly involve students in the learning process and help facilitate retention of biological knowledge (Uno, 1990). These inquiry-based teaching techniques are being advocated at all levels of science education (AAAS, 1993). The experiment outlined in this paper is very flexible, and the procedure is so simple that it is appropriate for students from grade five through college. Students can work individually, in small groups, or as an entire class. Furthermore, this exercise can be done as an in-class activity or as an independent study outside of class. The instructor can also modify the exercise to include a host of independent variables including species, extract type and concentration, motivational state, environmental condition, age, sex, and prior experience.

We recommend that the instructor engage the students in one or more discussions prior to performing the experiment. A few directed questions may be necessary to stimulate discussion. Some questions include: What habitats do different snake species occupy? Upon what organisms do snakes prey? Do all snakes eat the same prey? How do snakes find their prey? How are prey captured and con-

Table 2.
A list of some general questions for an inquiry exercise testing prey preferences in snakes.

1. What factors affect prey preferences in snakes?
2. How does the habitat a snake species resides in affect its prey preferences?
3. Do all snakes prefer the same prey?
4. Does size affect prey preferences in snakes?
5. How would prey preferences in snakes be determined experimentally?
6. Does previous experience affect prey preferences in snakes?

sumed? At this point, the teacher can assess the level of knowledge the students have regarding snakes and determine if a fact-finding trip to the library and/or Internet search is necessary.

For an inquiry exercise such as this one, it is often helpful to pose a general question to the students and have them develop and test their own hypotheses. Table 2 lists a few of the possible general questions suitable for this exercise. Table 3 gives a number of suitable hypotheses for one of these questions. Hypotheses for the other questions posed in Table 2 will have a similar structure. Application of the scientific method (making observations, developing hypotheses, considering control groups, etc.) gets students directly involved in the process of scientific thinking.

Table 3.
Some hypotheses addressing the general question "What factors affect prey preferences in snakes?"

1. If habitat affects the prey preferences of snakes, then a terrestrial snake species such as kingsnakes will prefer rodent prey to fish prey.
2. If the concentration of the prey extract affects tongue flicking rates (and hence our perception of prey preference), then more-concentrated extracts will elicit higher tongue flick rates than less-concentrated prey extracts.
3. If prey preferences in garter snakes are innate, then ingestively-naïve snakes will tongue flick at a higher rate in the presence of fish extract.
4. If hunger level affects prey preference, then snakes that have fasted will exhibit an overall higher tongue flick rate than snakes that have not fasted.

Following the experiments, numerous ecological and evolutionary topics can be discussed. After the students have determined the preferred prey of the species they examined, they should be asking questions about why particular snake species prefer a specific prey. Is it habitat related? Is certain prey too large for some species to consume? Encourage students to take the experimental procedure in other directions. Are there other groups of reptiles that flick their tongues to collect chemical information? Besides information about food, what other information could snakes gather using their tongues? Snakes do many other things besides eat. Males must find and court females during the breeding season. All snakes must avoid predators. Chemical signals may also be important for these activities. Would this experimental protocol work to test hypotheses related to these topics?

Several of the *National Science Education Standards* for Life Science can be addressed using snakes and the experimental protocol outlined above. The content standards for grades 5-12 suggest that students should develop an understanding of:

1. structure and function in living systems
2. reproduction and heredity
3. the behavior of organisms
4. populations and ecosystems
5. biological evolution and adaptations of organisms
6. the process of scientific inquiry
7. the skills necessary to perform scientific inquiry (NRC, 1996).

The project outlined in this article addresses nearly all of these. Through the inquiry process students will be observing snake behavior and should be able to use critical thinking skills to make the connection that snakes are living beings composed of cells, tissues, and organs that require energy to sustain life. The act of predation transfers energy from the prey (often primary consumers) to the snake (a secondary consumer). This is a key concept in ecology and is one of the processes by which energy is transferred through an ecosystem. The students will directly observe the snake's behavior toward different stimuli.

Students should also understand how those stimuli are relevant to the snake in its ecosystem. For example, black rat snakes (*Elaphe obsoleta*) do not respond to fish odors because they are an upland species that rarely encounter fish in its natural environment. Snakes also exhibit adaptations that allow them to consume prey whole. The bones in the snake skull are connected with ligaments that allow the skull to stretch out of shape while the snake is swallowing a meal. Snake skeletons can be purchased through a biological supply house to illustrate this point. This is a result of millions of years of evolution, an excellent segue into a discussion regarding natural selection.

Life Science Content Standards 1, 3, 4, and 5 are directly addressed as the students proceed through the inquiry. The actual process of scientific inquiry itself aids the students in developing an understanding of the scientific method and the process by which science proceeds (Standards 6 and 7). Included in Standards 6 and 7 are developing writing skills, library research, and skills in experimental design. The experimental protocol outlined in this article is relatively simple. Regardless of the questions students address, the experience of working with live animals will ensure they remember the experiment for their entire lives.

Acknowledgments

An earlier draft of this manuscript was greatly improved by comments from Don French (Oklahoma State University). Jim Gillingham introduced the first author to this technique in his Animal Behavior class at Central Michigan University.

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