

FEEDING KINEMATICS OF THE GROTTO SALAMANDER,  
*EURYCEA SPELAEA*

by

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## ABSTRACT

Melanie M. H. Grigsby

### FEEDING KINEMATICS OF THE GROTTO

SALAMANDER, *Eurycea spelaea*

Thesis under the direction of  
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All larval salamanders that have been studied capture prey using inertial suction feeding, and the kinematics were at one time thought to be highly stereotypical. Many salamanders have been studied, but the life history of *Eurycea spelaea*, the grotto salamander, is unique among the troglobitic plethodontids. This cave salamander transforms at metamorphosis into a terrestrial adult, but the eyes recede and the adult is blind. The feeding behavior of larval *E. spelaea* was measured to determine stereotypy using high-speed videography and kinematic analysis, and then was compared across several taxa. The salamander feeds using the conserved aquatic feeding pattern with three observable stages: expansion, compression, and recovery. Like other inertial suction feeders, the prey item is brought into the buccal cavity using a strong hyoid depression that generates buccal expansion and therefore negative pressure within the buccal cavity. Four of thirteen kinematic variables were found to be stereotyped, and five more were slightly stereotypical based on a stereotyped to variable continuum. The length of the gape cycle of *E. spelaea* corroborates with earlier findings that the time of movements are directly proportional to linear body size. Future studies should include buccal pressure measurements and analysis of the feeding behavior of the transformed adult.

## INTRODUCTION

### ***Aquatic feeding***

Larval salamanders are carnivorous and all species that have been studied feed using inertial suction (Deban and Wake 2000), in which a jet of water is drawn into the mouth, carrying the prey along with it. Inertial suction feeding is common across many aquatic taxa, and consists of three to four stages as defined by movements of the skull (Lauder 1985). The first (preparatory) stage is not found in all taxa. During this stage, the predator reduces the volume in the buccal cavity by compressing the walls of the mouth cavity together, thereby reducing the volume of water in the buccal cavity before the next stage. The expansive phase occurs next, starting with the opening of the buccal cavity and continues until maximum gape is reached. During this phase the volume of the buccal cavity is rapidly expanded, resulting in a decrease in pressure that drives a high-velocity flow of water through the open mouth and into the buccal cavity. The prey item is generally brought into the mouth at this time. Labial lobes create a circular gape in larval salamanders, restricting the flow of water to be inward through the front of the mouth. The compressive phase is the time from maximum gape until the jaws close; the buccal cavity is compressed, and water exits either through the back of the head if possible (i.e. gill slits) or through the mouth opening. Finally, the recovery phase occurs from the time the jaws close until the skull bones return to their original resting position.

For salamanders, kinematic analysis alone will not distinguish a preparatory phase, as buccal pressure is an integral part of the preparatory phase and must be measured. Visibly, the salamander approaches the prey item, the mouth opens and the hyoid is retracted, drawing water and the prey into the buccal cavity by creating negative pressure. The mouth closes before maximum hyoid depression, and water exits through the gill slits in the back of the head. The movements of the head mostly mirror the movement of the jaws. The high density and viscosity of water has constrained the evolution of the feeding mechanisms of aquatic organisms, causing suction feeding to be present across many vertebrate taxa (Lauder 1985). The density, viscosity, and velocity of the water affect the inertia of the prey item, so the predator must overcome the inertia of the prey item in order to draw it into the buccal cavity, whereas this factor can be ignored in air feeding because air does not noticeably act upon the inertia of the prey item (Gans 1969, Lauder 1985).

For many years it was thought that all amphibians feed stereotypically, but it has been shown that the original models, *Ambystoma* and *Bufo*, are the most highly stereotyped of the salamanders and frogs, respectively (reviewed in Deban *et al* 2001). Confusion has occurred over the definitions of stereotypy and flexibility, and I have followed the guidelines set out by Wainwright *et al* (2008) here. Stereotypy refers to the amount of variability in a behavior of the organism within a set treatment condition. Thus, if a salamander has a low coefficient of variation during hyoid depression when feeding on a worm, then hyoid depression would be considered stereotypical. Flexibility is the ability of an

animal to change the hyoid depression when feeding under a different set of conditions, such as a different prey item. Accordingly, a salamander may be considered both stereotypical and flexible. Researchers have now discovered that living amphibians have a diverse approach to feeding mechanisms, and the kinematics are not as stereotyped as once thought (Deban *et al* 2001). Most salamanders are thought to be opportunists, choosing the prey based on availability and the constraints of the feeding mechanism (Roth 1987).

### ***Plethodontid feeding***

Several terrestrial plethodontid salamanders, such as those from the genus *Eurycea*, accurately feed using a “free tongue” system (Regal 1966, Roth *et al* 1983). The salamander is able to shoot the tongue out of the mouth and catch the prey, generally on the first strike, whereas salamanders with less specialized tongues have a less accurate strike (Regal 1966, Roth *et al* 1983, Reilly and Lauder 1990). Similar to frogs, the salamander tongue attaches at the anterior region of the salamander’s mouth, and flips forward out of the mouth during a feeding strike (Lombard and Wake 1977). Less specialized tongues, such as those of hynobiids, ambystomatids, and most salamandrids, are less differentiated and the lateral and posterior margins are loose, but still attached to the mouth’s floor. Salamanders with a slightly more specialized tongue (most plethodontid genera and the salamandrid genera *Chioglossa* and *Salamandrina*) have freed the lateral and posterior margins from the floor of the mouth, but the tongue is stabilized by small muscles and part of it remains in the mouth during a

strike. The most highly specialized salamander tongues (Central and South American plethodontines, *Hydromanthes*, and *Eurycea*) are carried entirely out of the mouth on the hyoid elements (Regal 1966).

With a highly specialized and highly accurate feeding mechanism, these salamanders must have also developed a highly accurate visual system to accommodate the need for depth and distance discrimination (Roth *et al* 1983). Additionally, plethodontid salamanders which exhibit tongue projection have more frontally directed eyes, as well as longer rod outer segments in the eyes, than non-tongue projecting salamanders and anurans (Roth *et al* 1983). Vision is generally the most significant sense in guidance of feeding for salamanders with sight (Duellman and Trueb 1986, Roth 1987). However, *E. spelaea* lives in caves, which have a very limited amount of light, and may not have any light where the salamanders are located within the cave. As such, the salamander does not have a need for a visual system. Most obligate cave-dwelling salamanders do not transform (8 of 10 in the United States), but rather remain aquatic, reaching sexual maturity while retaining larval morphology (a life history pattern termed paedomorphosis; Brandon 1971b). Interestingly, all obligate cave-dwelling (troglobitic) salamanders are members of the family Plethodontidae (Peck 1998).

Only two troglobitic salamanders transform, *Eurycea spelaea* and *Gyrinophilus subterraneus* (Culver 1982), and both seem to follow a pattern of ontogenetic eye degeneration (Besharse and Holsinger 1977). Unlike *G. subterraneus*, *E. spelaea* does not begin to lose its vision until transformation,

and this project will focus on this species. Because *E. spelaea* adults do not have the benefit of sight, one must question what senses are used to feed and whether or not their behavior is stereotyped like several other salamanders (Deban *et al* 2001). Some salamanders rely on chemical cues and have the benefit of smell to determine where to locate their food. Though thought to be capable of significant tongue projection (Roth 1987), there is no current evidence that *E. spelaea* does use its tongue. Stone (1964) fed adult *E. spelaea* in a vision test and found that they do not notice the food without tactile stimulation. Stone (1964) used beef liver to test his salamanders and until they became accustomed to the feeding methods, they were easily startled by the tactile stimulation, suggesting that they did not orient themselves to the prey before tactile stimulation occurred. However, beef liver is not in the natural diet of *E. spelaea*, which may have caused some confusion for the animal as the (lack of) movement and smell of the prey would not be typical. Overall, one may hypothesize that chemical and visual cues may not be used in adult *E. spelaea* feeding, while tactile stimulation plays a primary role in feeding.

### ***Life history and taxonomy***

First placed in the Family Desmognathidae based on its vertebral structure by Stejneger (Hilton 1909), *Eurycea spelaea* is now a member of the Family Plethodontidae, and Desmognathinae no longer exists (Frost *et al* 2006). The grotto salamander, *Eurycea spelaea*, begins life as an aquatic larva, inhabiting freshwater springs. Larvae are also found in caves and exhibit varying degrees

of pigmentation based on how far from the mouth of the cave they live (Fenolio 2003). A one to three year larval period has been suggested (Hendricks and Kezer 1958, Brandon 1971a). When metamorphosis occurs the salamander's eyelids cover the eye and fuse together (Durand *et al* 1993). This cave salamander does not exhibit paedomorphosis; instead, it fully transforms into a troglobitic salamander (Hendricks and Kezer 1958) which is generally found on walls or ledges in caves (Smith 1948) (Figure 1). Originally named *Typhlotriton spelaeus*, Bonett and Chippindale (2004) found this salamander to be closely related to *Eurycea multiplicata* and *Eurycea tynerensis* and suggested changing the name to *Eurycea spelaea* (Figure 2).

*Eurycea spelaea* inhabits caves of the Ozark Plateau (Fenolio 2003). They range from northeast Oklahoma, northwest Arkansas, and southern Missouri to extreme southeastern Kansas (Petranka 1998). Fenolio (2003) studied several populations in the Ozarks and determined that their populations are the highest when their environments have the most food available for consumption. Because caves are generally oligotrophic, it is thought that most troglobitic salamanders have evolved to exhibit paedomorphosis (Brandon 1971b) because they would be more likely to find food in the water than on land (Culver 1982, Fenolio 2003). However, this species is generally found in caves also occupied by bats (Fenolio 2003). Thus, the grotto salamander may have retained its biphasic lifecycle because it occupies caves with abundant food provided by the increase of guanobites correlated with an influx of guano (Fenolio 2003). Absence of bats does not indicate an absence of the

salamander, but it is thought the salamanders prefer caves with colonial bats (Fenolio 2003). The extreme temperature range for this species is 5.5-19.7°C, though if other species were present (with ranges mostly 11-20°C), the grotto salamander larvae were generally found in lower temperatures than the other species (Rudolph 1978). The larvae were most abundant in temperatures ranging from 16-19°C (Rudolph 1978).



Figure 1. Larva and adult *Eurycea spelaea*. The larva was collected from a spring outside of Locust Grove, Oklahoma. The adult was not collected, and the picture was taken in Tumbling Creek Cave, Protem, Missouri.

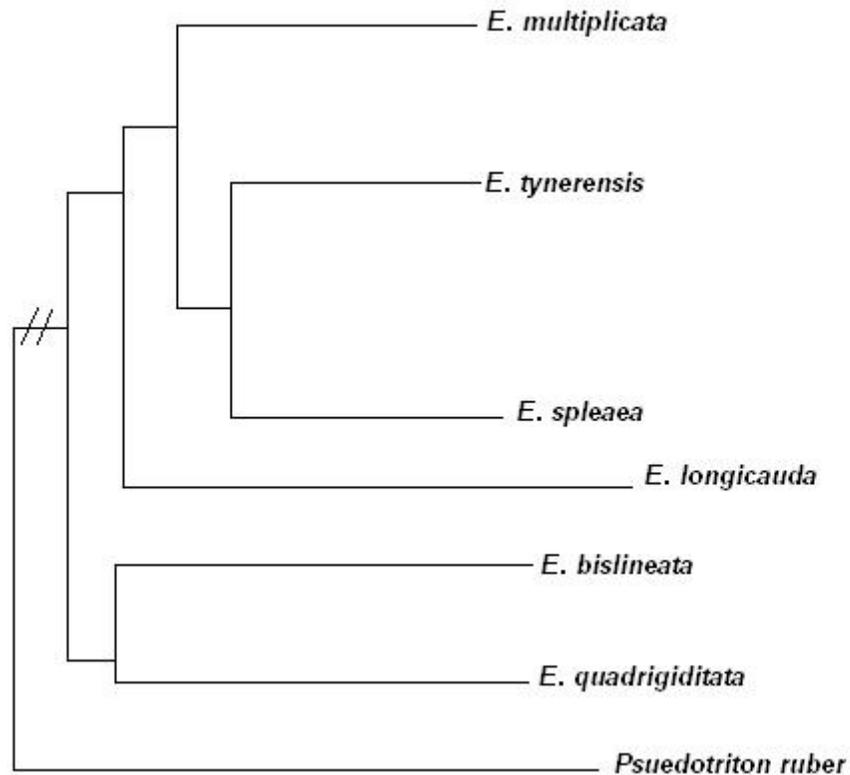


Figure 2. The phylogenetic tree of the *Eurcyea multiplicata* complex. *Eurycea spelaea* is most closely related to *E. tynerensis*, though specimens were not available for a comparative study. Adapted from Bonnet and Chippendale (2004).

### ***Vision and metamorphosis***

Most information about this species involves the degenerative eyes (e.g., Noble and Pope 1928, Stone 1964, Besharse 1972, and Besharse and Brandon 1976). During and after metamorphosis, the eyelids mostly fuse together and the eyes degenerate, leaving the metamorph functionally blind (Durand *et al* 1993, Stone 1964). The eyes stop growing during metamorphosis, and reduced eyes are a defining characteristic of the adult (Brandon 1971b, Durand *et al* 1993). The adults have a wide range of eye size because the larvae transform at many

different body sizes (Besharse and Brandon 1974). Noble and Pope (1928) found that degeneration of the eyes is due to the absence of light. Larvae kept in an environment with no light had either closed lids or degenerate retinas, whereas larvae kept in an environment with light showed fully functional, open eyes. Besharse and Brandon (1976) found that the animals transformed in the dark had slightly smaller eyes than animals transformed in the light, but the difference was not as clear as Noble and Pope (1928) found. It was also found that it takes over a year for the eyelids to close completely, though not all animals followed the same pattern. Some animals artificially transformed using L-thyroxin and placed in an environment without light had fused eyelids 179 days after initial exposure to L-thyroxin, while most were still open after day 366 (Besharse and Brandon 1976). Eigenmann (1909) found that the eyes in the larvae are unable to distinguish between objects, though they appear normal both histologically and anatomically (reviewed by Stone 1964). However, the conditions surrounding Eigenmann's tests are uncertain, thus it is impossible to determine the accuracy of his results (Stone 1964). Further research by Stone (1964) and Besharse (1972) have discovered that the eyes are normal when younger, but older larval salamanders lose their vision and rely more fully upon their other senses. Later, Durand *et al.* (1993) found that the cornea "sinks" into the eye and the retina folds to reduce in size during metamorphosis. The supraocular skin progressively replaces the supraocular eyelids which cover the eyes after metamorphosis.

### ***Feeding apparatus and methods***

The hyobranchial apparatus of adult *Eurycea spelaea* has three branchial arches (Hilton 1909). Like other plethodontids, its tongue attaches to the front and along the median line of the floor of the buccal cavity and the mouth has parasphenoid and vomerine teeth (Stejneger 1893). Unlike most plethodontids, the parasphenoid teeth are continuous with the teeth of the vomer instead of separate from each other (Regal 1966). The tongue of this salamander has been described as rather large (Stejneger 1893), and the salamander is thought to be capable of significant tongue projection (Roth 1987).

Generalized feeding behaviors for visually guided prey capture in salamanders, both larvae and adults, have been established. To begin with, the salamander uses orienting movements to locate the prey using the head, as opposed to the whole body. Then, the salamander approaches the prey, usually by walking to striking distance from the prey. Next, salamanders may perform an olfactory test. During this stage the salamander will lower its snout until the head is mostly vertical above the prey item. In some species, the olfactory test occurs by sniffing, or increasing the number of inhalations. Fixation on the prey occurs binocularly when light and eyes permit. This stage is species-dependent, as well as dependent on the level of movement of the prey item. Finally, snapping occurs in which the salamander brings the prey into the mouth. Aquatically, this capture is accomplished by sucking the prey into the mouth along with water. Terrestrially, the salamander will extend the tongue until it attaches to the prey and the tongue is retracted back into the mouth (Roth 1987). Aquatic

salamanders are also able to use olfactory, tactile, and lateral line receptors to detect prey, but vision predominates and even appears to inhibit olfaction unless there is no light available (Roth 1987). The lateral line system is able to detect prey within a few centimeters (Bartels *et al* 1990). When the lateral line receptors are combined with the visual, olfactory, and tactile receptors, most larval salamanders notice live, active prey and not dead prey (Deban and Wake 2000).

Suction feeding through hyobranchial depression occurs in all larval salamander feeding (Deban *et al* 2001), including *Eurycea spelaea*. All muscles involved in feeding are activated nearly simultaneously: adductor mandibulae externus, adductor mandibulae internus, depressor mandibulae, levatores branchiarum, epaxials, coracomandibularis, branchiohyoideus, rectus cervicis, pectoralis, and the hypaxials (Lauder and Shaffer 1985). The salamander opens the mouth and depresses the hyoid apparatus, which creates negative pressure in the buccal cavity, causing water and (hopefully) prey to enter the buccal cavity following the pressure gradient. Water exits through the gill slits in larval salamanders while the mouth closes and the hyoid apparatus is raised, thus pushing the prey into the pharynx (Roth 1987).

After metamorphosis, the grotto salamander is thought to feed terrestrially, perhaps through tongue projection because all terrestrial amphibians which have been studied thus far feed by utilizing the tongue (Duellman and Trueb 1986). The fastest vertebrate feeding mechanism, tongue projection (Roth *et al* 1983), occurs when all muscles associated with feeding are activated simultaneously: subarcualis rectus, rectus cervicis profundus, geniohyoideus medialis,

geniohyoideus lateralis, genioglossus, the tongue pad muscles, and depressor and levator mandibulae (Roth *et al* 1990). The subarcualis rectus is contracted, and the epibranchials are pulled and squeezed out, causing the tongue skeleton to be shot out of the mouth in plethodontids as the hyoglossus protracts the tongue pad (Lombard and Wake 1977). Folded to produce a compact projectile, the tongue skeleton is able to leave the mouth under its own momentum in many plethodontids (Lombard and Wake 1977). The tongue attaches to the prey (hopefully) and is retracted back into the buccal cavity. Once back in the mouth, many salamanders use the jaws and teeth to manipulate large prey. Plethodontid salamanders have a reduced genioglossus muscle which allows the tongue to be freed from the mouth. This tongue attachment, coupled with the lengthening of the epibranchials, allows the hyoid to be projected from the mouth, increasing the distance the tongue is able to travel (Roth 1987).

Examination of stomach contents in adults captured in Missouri reveals that adult *E. spelaea* ingests mostly aquatic isopods (Smith 1948), which suggests that the adult salamanders could return to the water to feed because they are rarely found more than five feet from the water during droughts (Hendricks and Kezer 1958). The adults found in Smith's (1948) study were not found in the water, suggesting that the adults take advantage of both terrestrial and aquatic feeding. This dual habitat use may provide the salamander with a more stable diet throughout the year, ensuring survival. The larvae also ingest aquatic isopods and other aquatic invertebrates, such as mosquito and fly larvae (Brandon 1971a). Larvae have also been observed eating bat guano after it

lands in the water (Fenolio *et al* 2006). The presence of fresh guano also correlates with an increase in invertebrates, and convergence of both larvae and adults in the cave (Fenolio *et al* 2006). Fenolio (2006) also suggests that both the larvae and adults are opportunistic and omnivorous feeders. Unsure if the larvae were ingesting the guano for nutritional purposes, Fenolio *et al* (2006) conducted a stable isotope analysis and determined that the guano is indeed a potential food source. Further investigation showed that the guano is comparable to stream amphipods in nutritional value to the salamander, cave sediment has no caloric value, and guano is more nutritious than a Big Mac. Guano has almost twice as much crude protein, and two thirds the amount of calories than the hamburger (Fenolio *et al* 2006).

### ***The Objective***

The objective of this project is to determine the kinematics of suction feeding in larval *E. spelaea*, and compare it to those of other salamanders and, more broadly, other vertebrates. It has been shown that aquatic feeding kinematics of *Ambystoma tigrinum* do not change during metamorphosis, but that the hyoid kinematics change significantly between aquatic and terrestrial feeding (Shaffer and Lauder 1988). However, Lauder and Shaffer (1986) also have found that transformed individuals are not as effective at catching prey once transformed. The amount of negative pressure generated by the salamander is significantly lower in terrestrial adults than in aquatic larvae (Lauder and Reilly 1988). If the same results are found in *Eurycea spelaea*, one would expect that

metamorphosing would not preclude the salamander from exploiting both feeding environments even though efficiency in the water is significantly decreased. In tiger salamanders, Shaffer and Lauder (1988) asserted that the changes in efficiency must be due to morphological changes because the kinematics do not change with efficiency. Though not as efficient, the salamander is still able to feed, thus supporting theories that *Eurycea spelaea* can feed both terrestrially and aquatically. This dual food basis allows the salamander more effectively to exploit its environment for prey and therefore nutrients. Closely related terrestrial animals are able to feed in the dark through chemical and mechanical mechanisms (reviewed in Deban *et al* 2001), so “blind” terrestrial feeding is possible. With only one other troglobitic salamander, *Gyrinophilus subterraneus*, exhibiting transformation (Culver 1982), different pressures and abundance of food for *E. spelaea* and its relatives may have caused the others to evolve into paedomorphic species while *E. spelaea* was able to maintain its biphasic life style (Brandon 1971a). Determining how larval *E. spelaea* feed will help to flesh out the current body of plethodontid kinematic knowledge, and provide further insight into how this species may differ from other vertebrates.

## MATERIALS AND METHODS

### ***Subjects***

Eight larval *Eurycea spelaea* were collected from a spring near Locust Grove, Oklahoma and were 2-3 years old based on the size as reported by Brandon (1980) (32mm+ = 2-3 years of age in larvae). Four were collected in July 2005, and four were collected in August 2006 (Figure 3). All eight salamanders were pooled because preliminary tests indicated the year of collection did not have an effect on the kinematic profile (Grigsby, unpublished data).

The specimens were housed in the Wake Forest University Animal Facility. They were kept in separate ten gallon aquaria with dechlorinated water and a Penn Plax® Sand Shark™ filter. Appropriate enrichment items were also in the aquaria. The animal holding room temperature was kept between 23 and 27 degrees Celsius with a twelve hour light, twelve hour dark cycle. The salamanders were fed black worms, *Lumbriculus variegates*, twice a week.

### ***Feeding Kinematics Procedure***

Preliminary tests showed the salamanders rely on vision to find the prey item (Grigsby, unpublished data), and the feeding trials were set up accordingly. The salamanders were placed in a 2 gallon glass aquarium with room temperature dechlorinated water. The salamanders were allowed to acclimate to the tank for approximately 30 minutes before trials began. Two high-speed digital video cameras were placed outside the tank: one below for a ventral view,

and one on the side for a lateral view of the feeding event. The specimens were encouraged to situate themselves inside a structure made of Apoxie<sup>®</sup> Clay so as to position them perpendicular to the axis of the camera. The prey item was then placed in front of the opening of the structure and the specimens were able to feed. The prey offered to the specimens was *Lumbriculus variegates*, which mimics the vermiform shape of natural prey items.



Figure 3. Spring at a local park outside of Locust Grove, Oklahoma. The salamanders were found by digging through the rocks at the base of the waterfall.

### ***High Speed Video***

The specimens were filmed using two digital video cameras (JVC GR-DVL9800U) (JVC Americas Corp, Wayne, NJ) at 240 frames per second. The laterally placed camera focused on the head of the animal, but most of the body was in view to the hind legs. The ventral camera captured the same portion of the salamander. The motion of the salamander was recorded from the time the prey item was positioned until after the feeding event. The cameras were synchronized by an event visible in both views, and both views contained a 1 cm grid for scale. Sequences where the salamander was approximately perpendicular to the axis of the lateral camera were selected for analysis. The videos were then transferred to a computer using Final Cut Express v. 2.0.3 (Apple Computer Inc, Cupertino, CA), and saved as a series of still-image JPEG files. Video frames were de-interlaced into their component fields using DeBabelizer Pro 5 v.5.0.2 (Equilibrium Technologies, Sausalito, CA). The pictures were then analyzed using Didge (Image Digitizing Software Version 2.3, Alistair Cullum, Creighton University, Omaha, NE, USA, available at <http://biology.creighton.edu/faculty/cullum/Didge/index.html>). This custom video analysis program provides (x,y) coordinates for eight points on the body of the animal as marked by the researcher (Figure 4). Thirteen video frames (each containing four pictures) were digitized for each sequence starting at time zero, the frame immediately preceding when the mouth begins to open. These fields covered the first 200 ms of the feeding event, approximately four times the length of the gape cycle. Laterally, the openings of the buccal cavity, two points on the

top of the head, the bottom of the hyoid, and two points along the trunk were marked; ventrally, the sides of the buccal cavity were marked. The distances were normalized using the grids in the viewing field of each camera during each trial. Additionally, another point was marked in each field to reduce the amount of noise in the data. These coordinates were then exported into Microsoft Excel (Microsoft Corp., Redmond, WA). A custom program, Gobbler, was written for Matlab R2008a (The MathWorks, Natick, MA) to calculate the distances and angles needed from the (x,y) coordinates. An algorithm based on a fourth central difference as described by Lanczos (1956) was also used to smooth the data, reducing digitizer error. The additional point in each field was used to normalize the data between the four pictures per frame that the camera produced. The time variables were calculated by analyzing the frames in Didge.

### ***Data Analysis***

*Kinematic Analysis.* Thirteen variables were compared for each feeding event. The time to maximum gape (TMG), time to maximum hyoid depression (TMHD), time to minimum head angle (TMinHA), time to maximum head angle (TMaxHA), time to maximum buccal expansion (TMBE), time of total feeding event (TTFE), the time length of the gape cycle (LGC), maximum gape distance (MGD), maximum hyoid depression distance (MHD), minimum head angle (MinHA), maximum head angle (MaxHA), maximum buccal expansion (MBE), and the number of hyoid depression cycles (HC) per feeding event were compared. TMG was defined as the time during the 200 ms measured that the maximum

gape distance was achieved, regardless of hyoid cycle, and MGD was defined as the largest distance between the upper and lower jaw tips. TMinHA, MinHA, TMaxHA, and MaxHA were also calculated during the first 200ms of the feeding cycle. Negative angles occurred when the snout was dipped below the line of the body, and positive angles occurred when the snout was raised above the body line. Time zero was defined as the frame immediately preceding the opening of the mouth, and TTFE was measured starting from time zero and ending when the salamander returned to rest with all four feet on the bottom surface of the tank. The LGC was measured from time zero and until the mouth closed. HC was a count of the number of times the hyoid depressed during a feeding cycle. TMHD was measured at the time of peak of hyoid depression (MHD), a distance which was calculated from a line perpendicular to line between points four and five and terminating at point six (Figure 3). To determine how much the hyoid depressed during the feeding cycle, the shortest line measured (while the salamander was at rest), was subtracted from the distances at all other time points. The MBE was measured from the line from point ten to eleven (Figure 3), and the TMBE was measured at the time point corresponding to the MBE. The shortest line measured (while the salamander was at rest) was then subtracted from the distance at all other time points to calculate the distance of change during the feeding cycle. All distance variables were then normalized by dividing by head length (distance from snout to gular fold).

*Statistical Analysis.* The coordinates were compared using repeated measures analysis of variance to determine if trial number had an effect on the kinematics of prey capture. Single-factor analysis of variance was used to determine if the individuals differed from each other. A post hoc Tukey test was conducted if the variable showed significance to determine where the variation was located. The accepted significance level was set using a sequential Bonferroni correction as described by Rice (1989). All tests were conducted in SPSS version 16.0 (SPSS Inc., Chicago, Illinois).

#### ***Permits and Animal Use Statement***

The collection of specimens of *E. spelaea* was covered under the Oklahoma Department of Wildlife Conservation special license, scientific collectors permit number 4034. The Wake Forest University Animal Care and Use Committee approved of the project (protocol #A04-150).

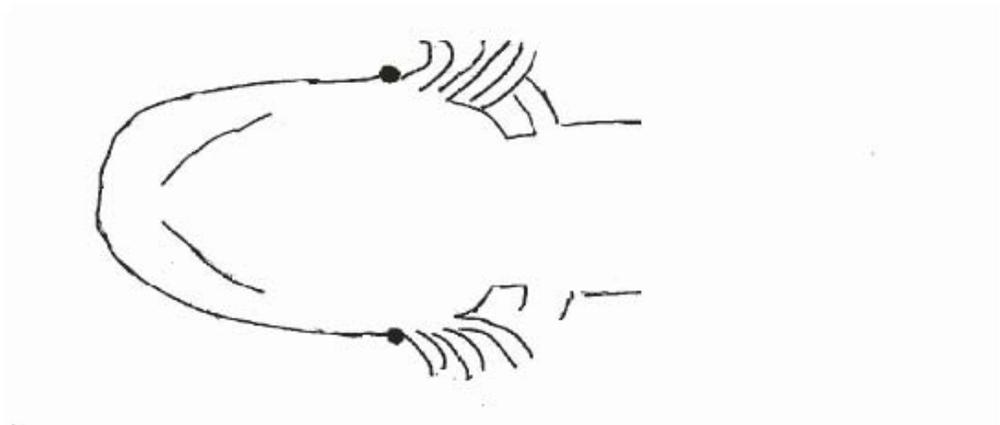
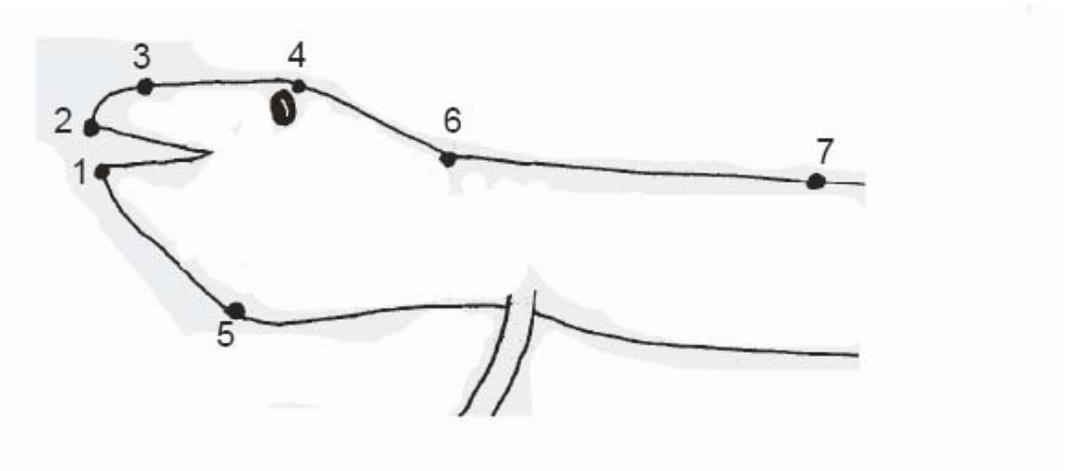


Figure 4. Anatomical landmarks digitized (black points) on the lateral (A) and ventral (B) high-speed video images of *Eurycea spelaea*. The landmarks are: 1, lower jaw tip; 2, upper jaw tip; 3, rostral tip of the skull; 4, skull above the eye; 5, tip of the hyoid; 6, base of the vertebral column; 7, mid-vertebral column; 8,9, lateral tips of the mandible.

## RESULTS

*Eurycea spelaea* larvae used inertial suction feeding in all recorded feedings (48 total feedings from eight individuals). The mouth opens, and reaches maximum gape distance just after the hyobranchial apparatus begins to move posteroventrally. The buccal cavity is expanded ventrally by the rapid depression of the hyobranchial apparatus, lowering the floor of the mouth and throat. Water and prey are drawn into the mouth, and the hyobranchial apparatus slowly returns to its resting state as water is expelled from the buccal cavity through the open gill slits. If the prey item is not fully engulfed during the first hyobranchial depression, additional buccal expansion-compression cycles (hyoid cycles) are used to draw the prey into the mouth. Figure 5 illustrates a representative feeding cycle as visible through the high-speed cameras.

Figure 6 shows the average kinematics of one salamander feeding through time. The preparatory phase could be present as the amount of buccal expansion at the end of the cycle (and therefore while the salamander is at rest) is higher than the amount of buccal expansion at the beginning of the feeding cycle. This lateral measurement is indicative of lateral compression of the buccal cavity, which would decrease the amount of water in the buccal cavity before the salamander opens its mouth. The expansive phase lasts until maximum gape distance (MGD) is reached, and the compressive phase lasts slightly longer than the expansive phase, though it still follows a typical bell-shaped curve. MGD always occurs before maximum buccal expansion (MBE), which occurs before maximum hyoid depression (MHD). Minimum head angle (MinHA) is reached

just before the end of the compressive phase, and maximum head angle (MaxHA) is reached last, during the recovery phase.

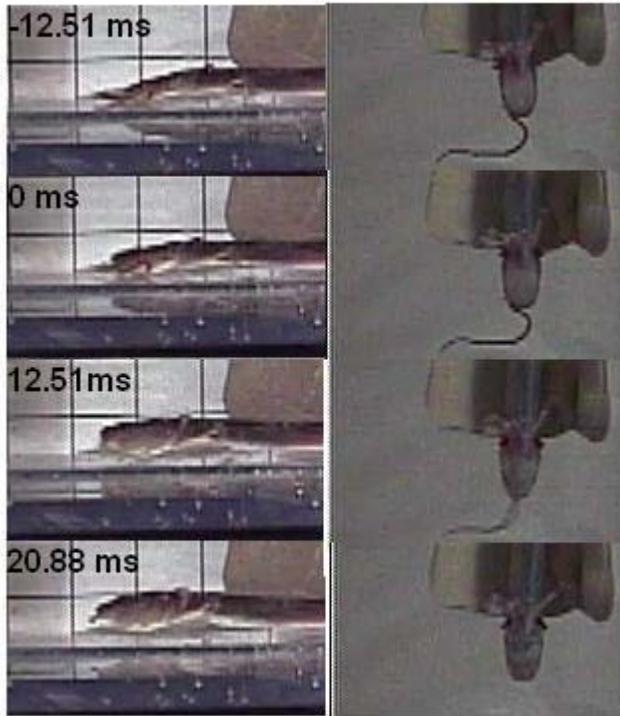


Figure 5. High-speed video of larval *Eurycea spelaea* feeding on a black worm using inertial suction feeding. The lateral view shows the hyoid depression, followed by the worm disappearing. The ventral view shows the buccal cavity expanding laterally, followed by the prey disappearing. The two views were synchronized by an event visible in both cameras. Time is in milliseconds, shown from the onset of opening at time 0 ms. Scale bar = 1 cm.

Repeated measures analysis of variance indicated that for all thirteen variables tested, trial number did not make a significant difference in the way the salamanders behaved (Table I). Single-factor analyses of variance were conducted on each variable (with a total of 48 feedings) to determine if each salamander behaved similarly to the other salamanders (Table II). If the single factor ANOVA was significant, a post hoc Tukey comparison was conducted to determine where the differences were present (Table III). For all tests, a

sequential Bonferroni correction was used to determine statistical significance because multiple univariate tests were conducted (Rice 1989).

Following Wainwright *et al* (2008), the coefficient of variation was used to determine the level of stereotypy for each variable. While stereotypy is a continuum and therefore not easily defined, I wanted to be able to conclude whether or not *E. spelaea* is stereotypical. For the purposes of this study, all variables with a coefficient of variation less than 0.50 were considered stereotypical, and all those over 0.50 were considered variable (Table II). Because each variable is tested individually, and may or may not have a high degree of stereotypy, I have not concluded that the salamander itself has a stereotypical feeding pattern, but rather that some aspects of the feeding pattern are stereotypical.

Figure 7 illustrates the individual variation in salamanders for the timing of events during the feeding cycle. Salamander A was the only specimen to reach MGD after the first hyoid cycle, whereas the others always reached MGD during the first hyoid cycle. This difference caused the standard deviation for TMGD to be much higher for A than for any other specimen tested. Overall, the time variables showed remarkable similarity, as none of them varied between individuals (Table II). Several of the salamanders individually exhibited stereotypical patterns for reaching TMinHA, but as a collective group, *E. spelaea* is more variable than stereotypical. The coefficient of variation shows that the timing variables are not stereotyped, as all values are greater than 0.50 (Table II).

Table I. Repeated Measure ANOVA

| Variable | df | F     | P     | P Crit |
|----------|----|-------|-------|--------|
| GC       | 47 | 1.167 | 0.345 | 0.0071 |
| GD       | 47 | 0.868 | 0.541 | 0.0100 |
| TMGD     | 47 | 0.326 | 0.593 | 0.0167 |
| MBE      | 39 | 1.562 | 0.179 | 0.0045 |
| TMBE     | 39 | 0.474 | 0.235 | 0.0050 |
| MHD      | 47 | 0.803 | 0.576 | 0.0125 |
| TMHD     | 47 | 0.935 | 0.484 | 0.0083 |
| MinHA    | 47 | 1.221 | 0.323 | 0.0063 |
| TMinHA   | 47 | 0.459 | 0.800 | 0.0500 |
| MaxHA    | 47 | 2.189 | 0.072 | 0.0042 |
| TMaxHA   | 47 | 1.135 | 0.300 | 0.0056 |
| HC       | 47 | 2.818 | 0.019 | 0.0038 |
| TTFE     | 47 | 0.746 | 0.635 | 0.0250 |

No significant values using sequential Bonferroni correction (Rice, 1989)

Figure 8 describes individual variation of the two timing variables not directly related to anatomy, and the individual variation in the number of hyoid cycles per feeding event. The average length of gape cycle (LGC) varied from 29.19 to 47.26 ms, and the average time of the total feeding event (TTFE) varied from 272.4 to 725.6 ms, with 1.0 to 2.5 hyoid cycles (HC) per feeding event. The number of HC, the LGC, and the TTFE did not vary significantly between salamanders. The coefficient of variation shows LGC to be highly stereotypical, and HC and TTFE exhibit the same degree of variability, and are not stereotypical (Table II).

Figure 9 describes the individual variation found within the anatomical variables of the feeding cycle. The average MBE varied from 8.91 to 17.10% of the total head length, the average MHD ranged from 18.09 to 27.16% of the total head length, and the average MGD varied from 7.30 to 17.26% of the total head

length. MHD varied between more salamanders than any other variable tested, and MBE and MGD revealed a small amount of variation between individuals as shown by the Tukey post hoc test (Table III). The coefficients of variation for all three morphological variables are all below 0.30, thus showing strong stereotypy.

Table II. Summary of Kinematic Variables

| Variable        | Mean (SD)     | CV    | df | F     | p                | p crit |
|-----------------|---------------|-------|----|-------|------------------|--------|
| LGC (ms)        | 38.3 (5.8)    | 0.22* | 47 | 3.897 | <b>0.003</b>     | 0.0045 |
| MGD (%HL)       | 12.7 (4.7)    | 0.37* | 47 | 4.56  | <b>0.001</b>     | 0.0042 |
| TMGD (ms)       | 20.2 (12.9)   | 0.64  | 47 | 1.473 | 0.205            | 0.0250 |
| MBE (%HL)       | 13.1 (3.6)    | 0.27* | 39 | 3.141 | 0.012            | 0.0050 |
| TMBE (ms)       | 43.5 (28.3)   | 0.65  | 39 | 1.538 | 0.19             | 0.0167 |
| MHD (%HL)       | 21.6 (4.3)    | 0.20* | 47 | 6.597 | <b>&lt;0.000</b> | 0.0038 |
| TMHD (ms)       | 58.6 (39.7)   | 0.68  | 47 | 2.08  | 0.068            | 0.0071 |
| MinHA (degrees) | -18.0 (7.1)   | 0.39* | 47 | 2.317 | 0.044            | 0.0063 |
| TMinHA (ms)     | 59.7 (73.4)   | 1.23  | 47 | 1.526 | 0.186            | 0.0125 |
| MaxHA (degrees) | 4.7 (9.4)     | 2.02  | 47 | 1.947 | 0.087            | 0.0100 |
| TMaxHA (ms)     | 85.3 (54.1)   | 0.63  | 47 | 0.434 | 0.875            | 0.0500 |
| HC              | 1.8 (1.1)     | 0.60  | 47 | 1.964 | 0.085            | 0.0083 |
| TTFE (ms)       | 441.3 (255.9) | 0.58  | 47 | 2.888 | 0.015            | 0.0056 |

Single-factor ANOVA. Table wide significant values shown in bold type, sequential Bonferroni correction used to determine significance (Rice, 1989). CV, coefficient of variation; stereotypical variables are denoted by an asterisk.

Figure 10 describes the individual variation of the head angle during the feeding cycle. The average MinHA during a feeding event varied from  $-25.55^{\circ}$  to  $-15.68^{\circ}$ , and the average MaxHA during a feeding event varied from  $-4.46^{\circ}$  to  $11.84^{\circ}$ . For each salamander, the MinHA roughly corresponds with the MaxHA, with LA having both the lowest MinHA and MaxHA. Neither variable showed significant variation among individuals (Table II). MinHA has a low coefficient of variation, though is not as strongly stereotypical as LGC or the anatomical variables.

Table III. Tukey post hoc analysis

| Variable | Individual             |                      |                          |                          |                          |                          |
|----------|------------------------|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|          | A                      | B                    | C                        | D                        | E                        | H                        |
| GC       | 33.36 <sup>(1,2)</sup> | 29.19 <sup>(1)</sup> | 36.84 <sup>(1,2,3)</sup> | 37.53 <sup>(1,2,3)</sup> | 47.26 <sup>(3)</sup>     | 43.78 <sup>(2,3)</sup>   |
| MHD      | 18.48 <sup>(1,2)</sup> | 17.94 <sup>(1)</sup> | 24.66 <sup>(3)</sup>     | 17.58 <sup>(1)</sup>     | 20.44 <sup>(1,2,3)</sup> | 24.16 <sup>(2,3)</sup>   |
| MGD      | 9.68 <sup>(1,2)</sup>  | 7.30 <sup>(1)</sup>  | 17.26 <sup>(3)</sup>     | 11.39 <sup>(1,2,3)</sup> | 13.28 <sup>(1,2,3)</sup> | 13.11 <sup>(1,2,3)</sup> |

Values are means, df=47, except MBE df=39. Means that are not statistically different ( $p>0.05$ ) share a number

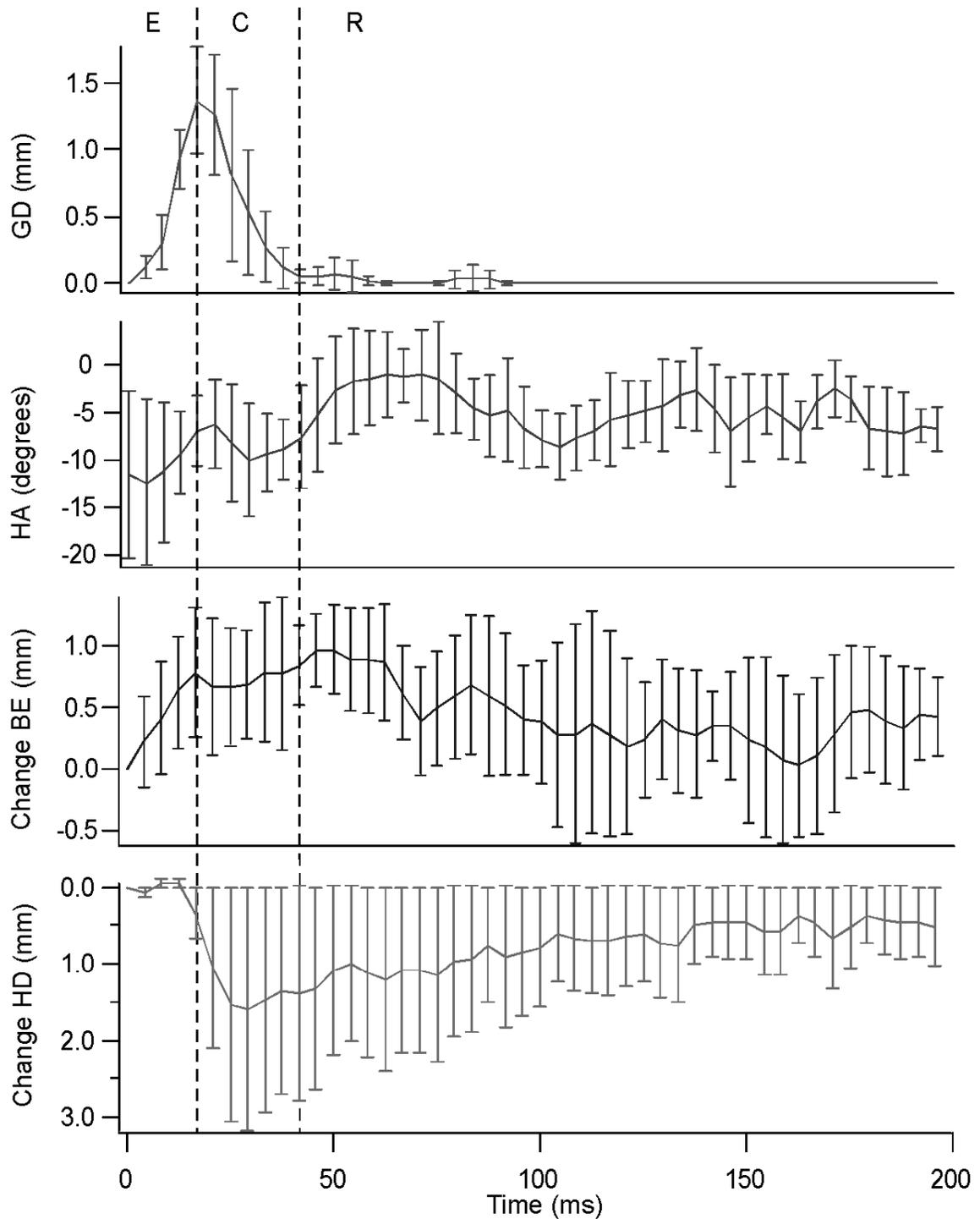


Figure 6. Average kinematic profiles for feeding events in one *Eurycea spelaea*. Lines connect average values, error bars represent  $\pm$  one standard deviation of six trials. Notice gape distance (GD) follows a bell shaped curve, though the compression phase is slightly longer than the expansive phase. Head angle (HA) follows GD during the initial suction event. Buccal expansion (BE) and hyoid depression (HD) are greatest after peak GD. E, expansive phase; C, compressive phase; R, recovery phase.

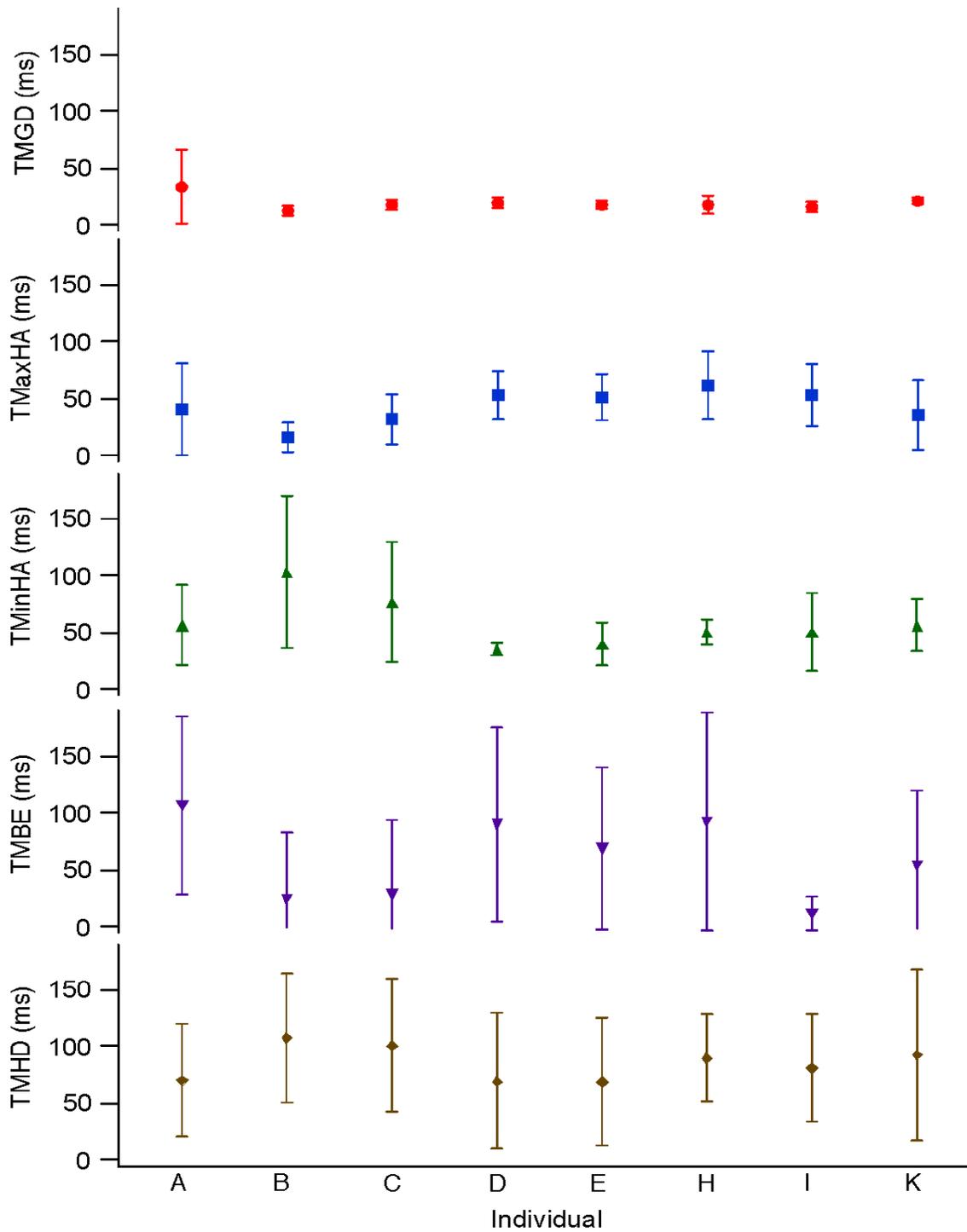


Figure 7. Individual variation in the timing variables of the feeding cycle. Eight specimens of *Eurycea spelaea* were filmed feeding on black worms. Time to maximum gape distance (TMGD), head angle (TMaxHA), buccal expansion (TMBE), hyoid depression (TMHD), and minimum head angle (TMinHA) were calculated. Values represent mean $\pm$  one standard deviation, six trials for each individual except buccal expansion (five trials).

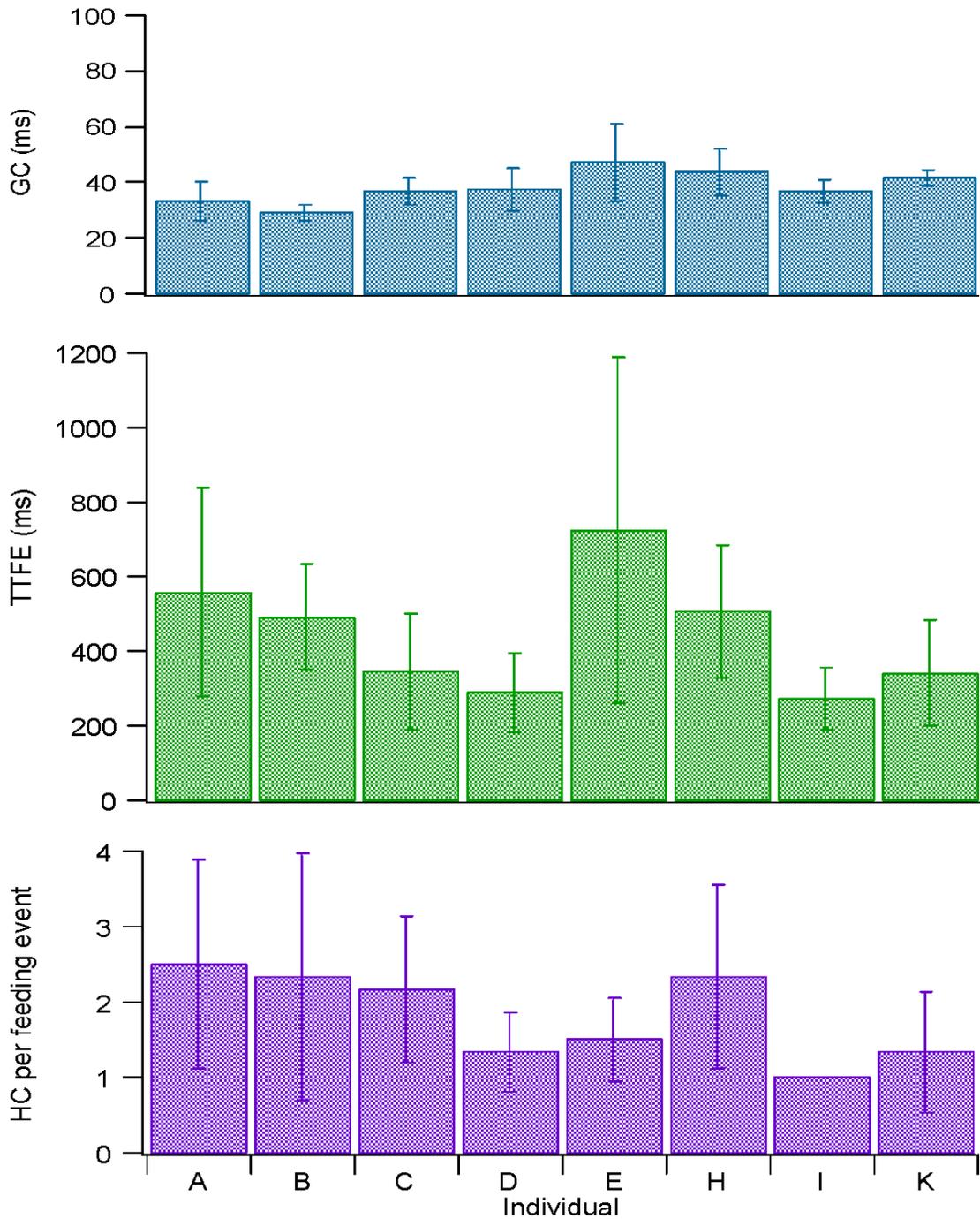


Figure 8. Individual variation in gape cycle, total time of feeding event, and hyoid cycles per feeding event. Eight *Eurycea spelaea* were filmed using inertial suction feeding to capture black worms. The time of the gape cycle (GC), total time of the feeding event, and number of hyoid cycles per feeding event were recorded. Note the variability between individual animals during the TTFE. Values represent means  $\pm$  one standard deviation, six trials for each individual except buccal expansion (five trials).

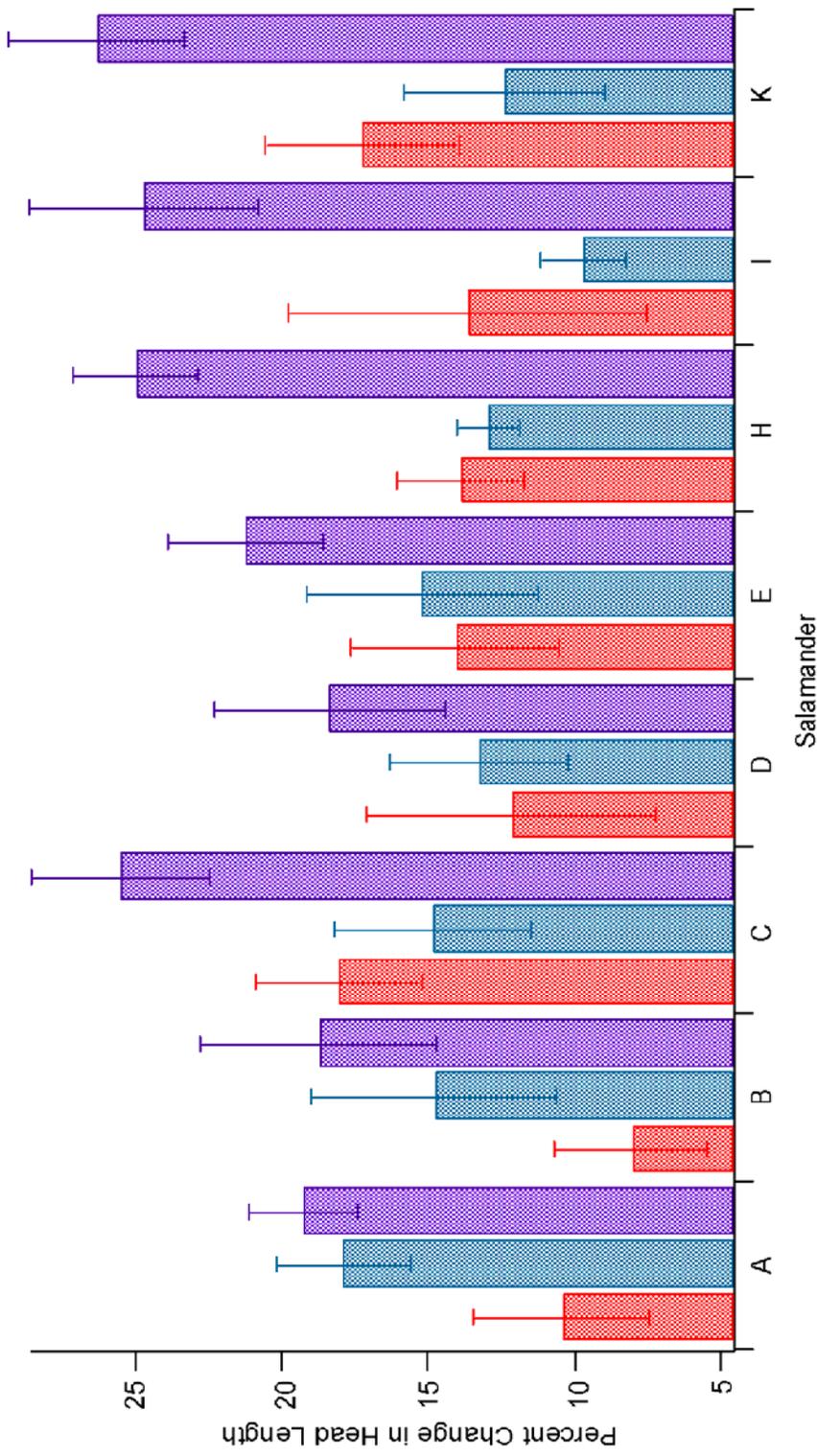


Figure 9. Individual variation in the anatomical variables of the feeding cycle. The average distance change in MGD (red), MBE (blue), and MHD (purple). Eight specimens of *Eurycea spelaea* were filmed suction feeding on black worms. The distances were calculated per feeding event and normalized based on head length to allow for comparison between specimens. Error bars represent +/- one standard deviation, six trials for each individual except buccal expansion (five trials).

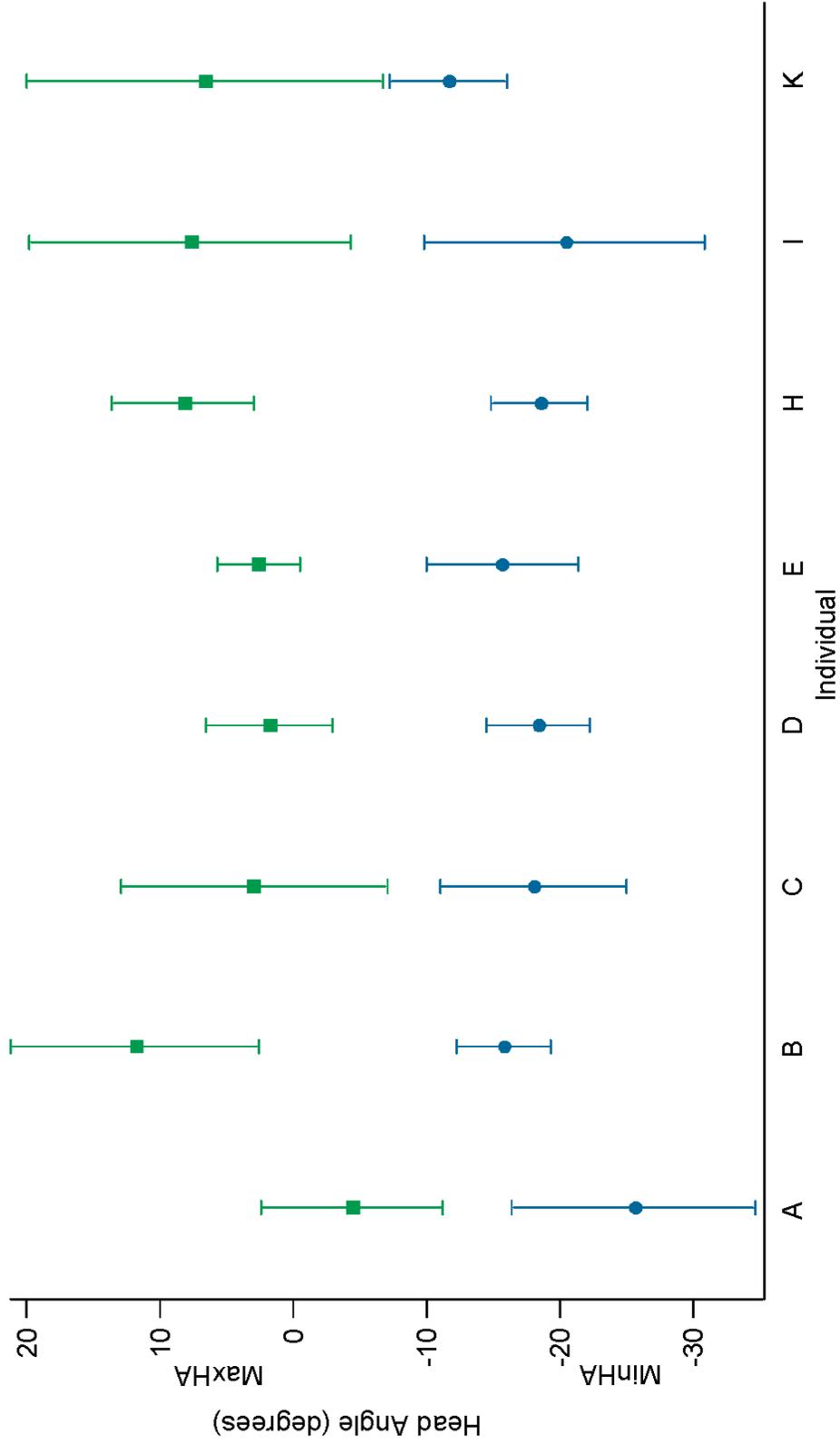


Figure 10. Individual variation in head angle variables of the feeding cycle. Eight *Eurycea spelaea* were filmed using inertial suction feeding to capture black worms. Note the variability between each salamander for the minimum (blue) and maximum (green) head angle per feeding event. Error bars represent +/- one standard deviation, six trials for each variable except buccal expansion (five trials).

## DISCUSSION

*Eurycea spelaea* follows the typical feeding pattern of other larval salamanders, utilizing inertial suction created from buccal expansion. The salamander approaches the prey item, the mouth opens and the hyoid is retracted, drawing water and the prey into the buccal cavity by creating negative pressure. The mouth closes before maximum hyoid depression, which then slowly returns to resting position as water is expelled from the gill slits. The movements of the head mostly mirror the movement of the jaws during the gape cycle.

### ***Gape Profile in Salamanders***

*Gape Cycle.* Plethodontid salamanders follow a typical gape cycle, which generally looks like a bell-shaped curve, though the time to close the mouth after full peak takes slightly longer than the amount of time to open the mouth to full peak (Lauder 1985, Deban and Marks 2002). *E. spelaea* follows the trend (Figure 10). The grotto salamander gape cycle lasts around 38 ms, which is well within the range of other plethodontids (16-70 ms; Deban and Marks 2002). *Ambystoma*, *Siren*, and *Amphiuma*, which are larger salamanders, have longer gape cycles (59.0, 61.0, and 72.0 ms, respectively; Reilly and Lauder 1990). *Necturus*, *Cryptobranchus*, and *Dicamptodon* have gape cycles more similar to that of *E. spelaea* (51.0, 53.3, and 54.4 ms, respectively; Reilly and Lauder 1990). Deban and Marks (2002) suggest that gape cycle duration is dependent on linear body size, that is, the longer the body, the longer the gape cycle, which follows

scaling principles that the duration of movement scales in direct proportion to linear measures of body size (Hill 1950). However, results from Reilly and Lauder (1990) initially seem to suggest that gape cycle duration is not dependent on body size, as *Cryptobranchus* and *Necturus* have a short gape cycle duration compared to body size.

Plethodontids generally also exhibit a strong hyobranchial depression following the end of the gape cycle (Deban and Marks 2002), and *E. spelaea* displays this behavior. The negative pressure created by depressing the hyoid allows the prey to move towards the back of the mouth, and when the hyoid returns to its normal position, the prey is moved into the pharynx. Peak hyoid depression occurs after peak gape, as more water is brought into the mouth and the salamander must compensate by decreasing the pressure even more to continue the flow of water through the buccal cavity.

The gape distance, buccal expansion, and hyoid depression distance were reported as a percentage of head length. The hyoid depression is always greater than gape distance and buccal expansion. Interestingly, occasionally the buccal expansion is greater than gape distance. The salamanders are able to draw the prey into the mouth through a small gape distance, which may aid in forming a circular gape, enabling the water to be directed into the mouth more easily, rather than being deflected out the sides of the mouth. The prey item, *Lumbriculus variegates*, is a thin, yet long, worm. The prey offered were 2 cm in length, up to twice the head length. Thus, it is reasonable that the salamander laterally expanded the buccal cavity to the same extent it expanded

dorsoventrally. Greater expansion of the buccal cavity will lead to a more negative pressure within the buccal cavity, leading to a flow of water into the mouth that is strong enough to overcome the inertia of the prey item (Gans 1969).

Most plethodontids have jaw patterns which are mirrored by head movements during suction feeding (Deban and Marks 2002). The head elevates with respect to the vertebral column while the mouth is opening and lowers again as the mouth closes. The grotto salamander follows this pattern during the gape cycle, but once the mouth is closed the head may move around and no longer follows the jaw movements (see Figure 6). During suction feeding in a laboratory environment, *E. spelaea* typically did not stay firmly planted on the bottom of the tank. The salamanders sometimes would lift up into the water column during the first hyoid depression and would not return to settle on the bottom until the end of the feeding cycle. This behavior may have prevented the salamanders from following the typical head movement of other plethodontid salamanders, closely mirroring the jaw movements. Additionally, the grotto salamanders started the feeding cycle with the snout almost always close to the ground (see Figure 4), causing the absolute value of the head angle to be high at the start of the feeding cycle. The salamanders seldom returned to that head angle at the end of the feeding cycle, typically hovering around -5 degrees. This behavior corresponds with the feeding habits of other salamanders in that the salamander will dip its head to perform a sniff test before consuming the prey item (Roth 1987). After the feeding event, it would be useful for the animal to be able to watch for predators

such as crayfish (Brandon 1971a, Petranka 1998) or additional prey items and no longer needs to have its snout to the ground.

The total time of the feeding event was measured from time zero until the salamander returned to rest. Lauder (1985) defined the recovery phase as the end of the compressive phase until the cranial bones returned to rest. I delimited the recovery phase to end when the whole body of the salamander came to rest on the substrate. This definition does not work for fish for obvious reasons, and would only apply to tetrapods. This information should be included in future kinematic studies of all amphibians to determine how long it takes for the animal to be able to focus on actions other than feeding, such as watching for predators. This time measurement gives more insight into the ecology of the animal, and thus is able to integrate kinematics more fully with life history.

*Hyoid Cycles.* It was not uncommon for *Eurycea spelaea* to use multiple hyoid cycles during a single feeding event. It is unlikely that the grotto salamander is the only salamander to use more than one hyoid depression cycle; however; no other studies have numerically reported this behavior, making interspecies comparison impossible. *E. spelaea* used additional hyoid cycles to draw the prey item more fully into the mouth if the prey was incompletely captured. However, the numbers of additional hyoid cycles were not all accounted for by incomplete prey capture. Thus, it is likely *E. spelaea* used the additional hyoid cycles to move the prey from the buccal cavity to the pharynx as well.

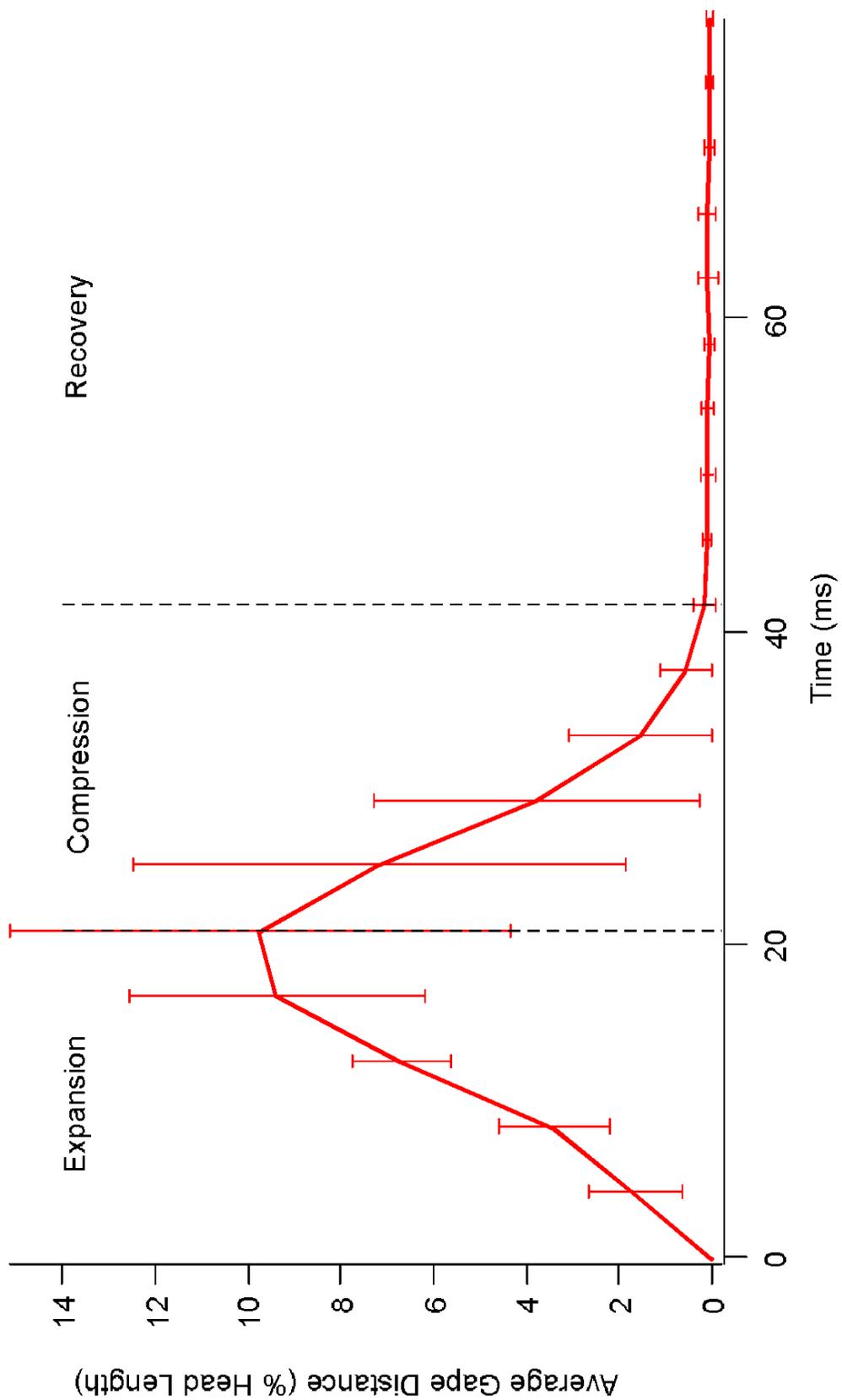


Figure 11. Average gape cycle for *Eurycea spelaea*. Eight specimens were filmed feeding on black worms using inertial feeding. The dashed lines represent breaks between the feeding stages. Error bars represent +/- one standard deviation, six trials for each animal were measured.

## **Stereotypy**

Stereotypical feeding has been explored in many amphibians (see Deban *et al* 2001 for a review). Despite the amount of research that has been conducted, a mathematical definition has only been proposed recently as a standard for measuring the degree of stereotypy. Many researchers measure stereotypy by comparing the feedings between prey types (e.g. Shaffer and Lauder 1985, Reilly and Lauder 1990) or comparing across ontogeny (Reilly 1995). These patterns of discernment do not fully capture the nature of the definition of stereotypy as proposed by Wainwright *et al* (2008). In order for a behavior to be determined stereotypical, the behavior must have little variance under the same set of laboratory conditions. Thus, you cannot compare one prey type to another because then you are looking at the ability of the animal to vary between prey items (flexibility) rather than stereotypy. Wainwright *et al* (2008) follows Gerhardt's (1991) proposal that stereotypy is a continuum rather than a set standard, and is measured from stereotypical to variable. Thus, while the coefficient of variation is used to measure variability within a species, there is no absolute value to determine which variables are definitely stereotypical and which ones are definitely variable.

To make things more understandable for future researchers, I needed to define stereotypy for the grotto salamander. While it is important to realize that stereotypy exists along a continuum, it is also imperative to define standards so comparisons can be made across taxa. I have defined characteristics to be stereotypical if the coefficient of variation is less than 0.50, and all greater values

to be variable. Additionally, it is important to note that the entire feeding cycle does not have to be stereotypical, but rather that some individual characteristics of the feeding cycle can be stereotypical while others can be variable.

*E. spelaea* is stereotypical for five characteristics, and variable for eight. Three of the stereotypical characteristics (MGD, MHD, and MBE) are anatomical variables and thus are limited by the anatomy of the animal. Given that the prey item was held constant, it follows that the salamander would need to open the mouth, depress the hyoid, and expand the buccal cavity about the same amount during each feeding trial. These characteristics are controlled by the muscles and bones, which are limited in their ability to move and contract. I would also hypothesize that these anatomical characteristics are not flexible because of their limitations. The LGC is strongly stereotypical as well. The short duration of the gape cycle prohibits the use of feedforward control (Deban *et al* 2001), causing the LGC to remain constant for all feedings. LGC is limited by the ability of the salamander to react to the situation. Because the gape cycle is so short, the salamander cannot modulate its behavior once it has been initiated. Thus, I hypothesize that LGC is also not flexible during inertial suction feeding events. One other characteristic is stereotypical: MinHA. It can also be argued that MinHA is limited by the anatomy of the salamander, as the salamander need only lower its head so far in order to be in close contact with the prey item. If the prey item is in the water column rather than on the substrate, the salamander will not need to lower its head as far to fixate on it. Thus, I hypothesize that MinHA is flexible. Additionally, as in the case of falling guano, the salamander may have

its head raised before consuming the prey. Thus, the salamander would be flexible for MinHA. It could be argued that MinHA would not be flexible because MinHA does not always occur at the start of the gape cycle. TMinHA was not stereotypical, which means that MinHA occurred at different times during the feeding event. However, this timing variability also means that MinHA did not have to occur for the salamander to swallow the prey item, thus MinHA would be flexible because it is not directly tied to a specific aspect of the feeding event.

### ***Comparative suction feeding***

*Pisces*. Suction feeding is exhibited by several other vertebrate taxa. Ray-finned fishes (Actinopterygii) have been studied extensively (e.g., Lauder 1982, Gillis and Lauder 1995, Ferry-Graham and Lauder 2001). Inertial suction feeding in fish occurs in four stages: preparatory, expansive, compressive, and recovery (see Lauder 1985). The expansive and compressive phases comprise the gape cycle. The gape distance profile of the blue gill sunfish, *Lepomus macrochirus*, is similar to that of *E. spelaea*. The gape cycle begins with a small amount of deviation around the mean values, but the deviation increases as the feeding cycle progresses (Figure 11; Gillis and Lauder 1995).

Syngnathid fishes, however, only have three stages: preparatory, expansive, and recovery (Bergert and Wainwright 1997), though it seems the fish must compress the buccal cavity to allow water to be expelled. The recovery for syngnathids is quite long, lasting over 1000 ms in some cases, while actual prey capture (gape cycle) is quite fast, averaging 6-8 ms (Bergert and Wainwright

1997). The linear length of the syngnathids is quite long compared to the gape cycle and is addressed below. *E. spelaea* has three phases: expansive, compressive, and recovery, though it is not possible to determine if it has a preparatory phase unless velocity or buccal pressure measurements are taken.

Basal shark suction feeding kinematics have been described as well (Edmonds *et al* 2001). The horn shark, *Heterodontus francisci*, uses inertial suction to obtain bottom-dwelling prey, including prey attached to the substrate. To mimic these conditions, the researchers attached squid pieces to the substrate for some of the trials. Like *E. spelaea*, if the initial hyoid cycle was not completely successful, the horn shark would employ additional hyoid cycles until the prey was loosened and captured. This behavior was augmented by leveraging the body and pulling at the prey item. As the prey for *E. spelaea* was not attached, it did not need to leverage the body to complete the prey capture event, and was able to draw the entire prey item into its buccal cavity with additional hyoid cycles.

The nurse shark, *Ginglymostoma cirratum*, also follows the typical inertial suction feeding pattern (Motta *et al* 2002). No preparatory stage was detectable with the variables tested, as in *E. spelaea*, though velocity measurements should be taken before a full conclusion is reached (Motta *et al* 2002). *G. cirratum* typically captures and transports the prey item into the pharynx with one hyoid depression. The grotto salamander is also capable of completing a feeding event with one hyoid depression, which could mean that additional *E. spelaea*

hyoid cycles are indeed for prey transport when the prey is completely drawn into the buccal cavity with one hyoid cycle.

*Testudinines.* Some aquatic turtles also employ inertial suction feeding during aquatic prey capture, while less specialized turtles use ram feeding (Lemell *et al* 2002, Lauder and Prendergast 1992, Summers *et al* 1998). The mata mata, *Chelus fimbriatus*, uses suction feeding and follows a pattern similar to that of previously studied fish and salamanders, though it uses a bidirectional flow system (Lemell *et al* 2002). Transformed salamanders also must use a bidirectional flow system because they no longer have gill slits to allow the water to exit at the posterior of the buccal cavity. Transformed Plethodontid salamanders (i.e. *Eurycea* and *Desmognathus*) do not suction feed, but rather use terrestrial feeding mechanisms in the water (Schwenk and Wake 1993, Deban and Marks 2002). During metamorphosis, the floor of the buccal cavity is rearranged to allow for tongue prehension, preventing the salamander from being able to create the negative buccal pressure needed for inertial suction feeding (Wake 1982, Roth and Wake 1985, Deban 1997). Observations of *E. spelaea* suggest that while they are transforming, they do not feed, which in turn suggests that the grotto salamander does not suction feed as an adult. *C. fimbriatus* is able to suction feed with a bidirectional flow system because the floor of the buccal cavity is designed to support negative pressure. This turtle has one of the largest hyoid apparatus in aquatic feeding turtles, which allows the buccal cavity to take in more water than other aquatic feeding turtles (Lemell *et al* 2002).

*Anura*. One adult anuran, *Hymenochirus boettgeri*, is known exclusively to feed using inertial suction: most adults rely on other methods such as jaw or tongue prehension to feed aquatically (Deban *et al* 2001, O'Reilly *et al* 2002). *H. boettgeri*, the African dwarf frog, follows the typical suction feeding behavior of other lower vertebrates (Dean 2003). To begin the feeding event, the frog lunges towards the prey item, streamlining its body by moving the forelimbs posteriorly. After ingesting the prey, a recoil action occurs in which *H. boettgeri* moves its hind limbs laterally and rostrally to create backward motion, and its forelimbs are moved anterodorsally in a wide arc, until the frog comes to rest with its fore- and hind limbs on the substrate. This pipid frog has large webbed fingers with great surface area which would act as paddles; rostral movement of the hind limbs thus causes backward movement of the frog. Comparatively, *E. spelaea* has small hind limbs and feet, and would not be able to create such an effective movement with the hind limbs. The grotto salamander also did not lunge towards its prey, but rather walked close enough to consume it using inertial suction. This movement would seem to eliminate the need for the salamander to generate backward movement after prey capture. However, it could be hypothesized that the protraction of the hyoid could have created enough force to cause the salamander to lose purchase on the substrate. Without an anterior view of the salamander, I was unable to analyze the fore- and hind limbs when the animals left the substrate. Further study is warranted to determine if *E. spelaea* is using a

recoil behavior similar to that of *H. boettgeri* to return to the substrate or if gravity causes the downward motion.

The salamanders tested here were collected from a spring, and were found between rocks and pebbles. In the field the rocks may have prevented the animal from lifting up into the water column, so the total feeding cycle would not have lasted as long. Additionally, the rocks would have provided cover from predators and allowed larval *E. spelaea* to exhibit sit-and-wait predator behavior. It was not possible to replicate the field environment in the lab because the salamander would not have been visible to the cameras.

The expansion of the buccal cavity was measured as a lateral distance in this experiment. *E. spelaea* increases the volume of the buccal cavity through horizontal and vertical expansion, rather than the lateral compression and vertical expansion common in ray-finned fish (Lauder 1985). It should be noted that ventral measurements in these fish have been taken, but they followed the movement of the opercula, and thus the volume of the opercular cavity rather than the buccal cavity (e.g. Lauder 1982, Gillis and Lauder 1955, Ferry-Graham and Lauder 2001). The difference in buccal expansion mechanisms implies that the salamander draws a wider column of water into the mouth than ray-finned fish. Pressure measurements should be taken to determine if the salamander is able to generate a more negative environment within the mouth based on its expansion mechanisms. Greater negative pressure could account for the movement of the salamander into the water column during some trials. Dean (2003) did not measure the horizontal buccal expansion in *H. boettgeri* so I am

unable to confirm if the horizontal expansion is present across amphibians. Additionally, other species of salamander were not measured in the same way.

Buccal expansion measurements are generally limited to hyoid depression measurements, and the horizontal dimension will be able to add more insight into the inertial suction feeding mechanisms of aquatic animals, as it has for the African catfish, *Clarias gariepinus* (Wassenbergh *et al* 2005). When the horizontal expansion is measured, combined with X-ray video to measure the volume of the buccal cavity, researchers are able to model the hydrodynamics of inertial suction feeding event. While mathematical modeling is beyond the scope of this project, data presented here will make it more feasible for future work on *Eurycea spelaea*.

*Mammalia*. Even some mammals use inertial suction feeding. Similar to fish, bearded seals, *Erignathus barbatus*, sperm whales, *Kogia* sp., and pilot whales, *Globicephala melas*, exhibit four feeding phases: preparatory, jaw opening, gular depression, and jaw closing (Marshall *et al* 2008, Bloodworth and Marshall 2005, and Werth 2000, respectively). Notably, *G. melas* did not have a closed mouth during the preparatory phase in all feedings. Lauder's (1985) definition of the preparatory phase includes a decrease of the volume of water during the preparatory phase. Without buccal pressure measurements, it is impossible to know if the whale is decreasing the volume of water in the buccal cavity during the preparatory stage. Thus, the preparatory phase is included in the gape cycle for these whales, but not for *Kogia* sp. or *E. barbatus* because gape cycle

generally only consists of the expansion and compression phases. Mammals use a much longer gape cycle than amphibians or fish: 540 ms in *E. barbatus* (Marshall *et al* 1008), 230 ms in *Kogia* sp. (Bloodworth and Marshall 2005), and 650 ms in *G. melas* (Werth 2000) compared to 38 ms in *E. spelaea*. Once more organisms are included (linear size was recorded in the text for animals included), it becomes obvious that there is a linear correlation between body size and gape cycle length (Table IV;  $R^2=0.960$ ), even though some animals have remarkably short gape cycles compared to body size. These findings corroborate with Hill (1951) in that the duration of a movement is directly proportional to the linear dimensions of organisms. Thus, the mammals have a slower gape cycle than the salamanders.

### **Conclusions**

Grotto salamander larvae follow a typical inertial suction feeding process. The hyoid is depressed, expanding the buccal cavity, creating enough negative pressure to draw water, along with the prey item, into the buccal cavity. *E. spelaea* may use several hyoid cycles and also lift up into the water column during a feeding event. Further research is needed to determine exactly how the extra hyoid cycles are used and what causes the salamander to lose purchase on the substrate. It is hypothesized that adult *E. spelaea* feeds aquatically using jaw or tongue prehension, but as no one has been able to get this salamander to eat a natural diet in captivity (Fenolio, per. comm.), this hypothesis remains untested.

Current methods of determining stereotypy are vague at best. I have followed the methods set out by Wainwright (2008), and despite the continuum nature of stereotypy, a general standard should be used to diffuse confusion in future research. The method I have adopted here includes defining characteristics of feeding as stereotypical or variable rather than a conclusion about the event as a whole, as other have done (e.g. Shaffer and Lauder 1985, Reilly 1995). Additionally, I have set the critical value for the coefficient of variation at 0.50: all characteristics less than this value are stereotypical, and all characteristics above this value are considered variable. These definitions will allow future researchers to compare stereotypy between species more easily.

Future studies on the grotto salamander should include pressure and velocity measurements, prey type variation, and metamorphosis. I hypothesize that the larvae will behave similarly to other plethodontids, but there is much to be learned from the transformed individuals. It will be interesting to discover how the salamanders feed in the wild and if, as has been suggested, they are capable of significant tongue protrusion (Roth 1987). If the salamander is able to feed terrestrially with the tongue (rather than biting), the implications for the sensory mechanisms will be substantial. All other salamanders using tongue protrusion rely heavily on sight to feed, and if *E. spelaea* is able to accomplish this task with olfaction, the way we look at the sensory system in salamanders will change significantly.

Previous work on grotto salamanders has been limited to morphology, vision, and ecology. These observations are the first in feeding behavior for

troglobitic plethodontids. *E. spelaea* fixes on prey using vision while a larva, but the feeding mechanism must change when it becomes an adult because vision is no longer an option. Additionally, because the hyoid rearranges extensively during metamorphosis (Deban and Marks 2002), adults cannot feed using inertial suction. Thus, this unique species behaves unlike any other salamander as *Gyrinophilus subterraneus* is unable to use vision as larvae because the eyes begin to degenerate well before metamorphosis (Besharse and Holsinger 1977). Perhaps *E. spelaea* retains vision as larvae because so many of them are found in springs where vision is useful, rather than in caves. This salamander also has been observed eating bat guano (Fenolio 2006), which is unusual for a species of an order thought to be carnivorous (reviewed in Deban *et al* 2001). My observations will set the foundation for many interesting studies to come on a salamander that continues to baffle scientists with its intriguing behaviors.

Table IV. Gape Cycle Duration

| Animal     | Species  | Gape Cycle (ms) | Avg Body Size (cm) |
|------------|--|-----------------|--------------------|
| Bony Fish  | <i>Lepomis macrochirus</i> <sup>a</sup>                              | 66              | 15*                |
| Bony Fish  | <i>Hippocampus erectus</i> , <i>Syngnathus floridae</i> <sup>b</sup> | 7               | 10                 |
| Shark      | <i>Heterodontus francisci</i> <sup>c</sup>                           | 125             | 63                 |
| Shark      | <i>Ginglymostoma cirratum</i> <sup>d</sup>                           | 100             | 78                 |
| Salamander | <i>Eurycea spelaea</i>   | 38              | 7                  |
| Frog       | <i>Hymenochirus boettgeri</i> <sup>e</sup>                           | 50              | 3.5                |
| Mammal     | <i>Globicephala melas</i> <sup>f</sup>                               | 650             | 319                |
| Mammal     | <i>Kogia</i> sp. <sup>g</sup>  | 470             | 180                |

Linear regression,  $R^2=0.960$ , \*SL not TL

<sup>a</sup>Gillis & Lauder 1995, <sup>b</sup>Bergert and Wainwright 1997, <sup>c</sup>Edmonds *et al* 2001,  
<sup>d</sup>Motta *et al* 2002, <sup>e</sup>Dean 2003, <sup>f</sup>Werth 2000, <sup>g</sup>Bloodworth and Marshall 2005

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## APPENDIX

### ***Vision versus olfaction in larval feeding***

At the onset of this project, I also wished to determine if *E. spelaea* use olfaction and/or vision to locate prey items. I hypothesized that this salamander, similar to others (Roth 1987), relies solely on vision to locate prey when vision is an option. Noticeably, the salamanders exhibited learning behavior, and preliminary findings corroborate vision as a key sense in locating prey.

## INTRODUCTION

### ***Amphibian Olfaction***

The olfactory system has similar features across all vertebrates, and the vomeronasal system, the accessory olfactory system, evolved as an adaptation to aquatic life, though its function has not been fully established in all vertebrates (Eisthen 1997, 2000).

Amphibians use chemoreception for many different behaviors, including avoiding predators (Sullivan *et al* 2005), reproduction (eg. Houck *et al* 1998), homing (eg. Madison 1969), individual recognition (eg. Madison 1975, Dawley 1984), feeding (Deban *et al* 2001), and territorial defense (eg. Mathis 1990, Mathis *et al* 1998). Most salamanders have both an olfactory system and a vomeronasal system (Eisthen 1997, Heatwole and Dawley 1998), though the vomeronasal system is thought to have been lost in the proteid family (Eisthen 2000). These systems have receptor cells, sustentacular cells to secrete mucus

(Zielinski *et al* 1988) and provide support, and basal cells to replace damaged receptors (Simmons and Getchell 1981). In the larval stage of terrestrial salamanders, the nasal epithelium is a folded sheet in the main nasal chamber, with sensory epithelium lining the grooves and nonsensory epithelium lining the ridges (Farbman and Gesteland 1974, Eisthen *et al* 1994). Adult terrestrial salamanders have a flat sheet of epithelium lining the main nasal chamber composed of both sensory and nonsensory epithelium (Dawley and Bass 1988). This system enables the salamander to locate prey in the absence of light (Deban *et al* 2001). *E. spelaea* probably relies heavily on olfaction to locate and capture prey.

Adult plethodontid salamanders also have nasolabial grooves which act as capillaries to direct fluid into the nasal passages (Brown 1968). As Brown (1968) showed in *Ensatina eschscholtzii*, the fluid is moved directly over the vomeronasal organ after entering the nasal passages. As salamanders exhibit nose-tapping behaviors, it is possible that the nasolabial grooves direct fluid from the environment to the vomeronasal organ, aiding in territory finding, and reproduction and courtship behaviors (Brown 1968). In combination with nose-tapping behavior, these grooves may work similarly in adult *E. spelaea* as a mechanism to locate prey with the lack of functional eyes.

## METHODS

### ***Olfaction Procedure***

The specimens were placed in a 2 gallon aquarium filled with dechlorinated, room temperature water. Two thirds of the aquarium was divided longitudinally into two parts using Plexiglas held in place by silicon adhesive, creating a y-shape. In the remaining third of the aquarium, the salamander was positioned inside the Apoxie® Clay structure and allowed to habituate for approximately five minutes. The specimen was then given a right-left preference test by placing *Lumbriculus variegates* inside a plastic tubing capsule. The capsule was closed with modeling clay. One clear capsule containing a live worm was placed on both sides of the divider at the same time. The structure containing the salamander was closed until both capsules were positioned properly. The salamander was then allowed to choose which worm to attempt to eat, thus determining if there was a preference for the right side of the aquarium or the left side of the aquarium. This procedure was repeated ten times for each specimen. After the right-left preference test showed no preference for all animals, an olfaction test was given. The aquarium had the same setup but this time one of the worms was not visible to the salamander. Instead, the prey item was lightly homogenized before being placed inside the capsule. Additionally, the capsule was covered in lightly homogenized prey as well before submerging the capsule in water. The Apoxie® Clay structure was closed off for two minutes to allow the chemicals of the prey item to diffuse to the place where the subject was located. This time was determined using dye to determine how long it would

take for the chemicals to diffuse, as seen in Sullivan *et al* (2000). Once the structure was opened, the salamander was able to choose between live visible prey and the lightly homogenized prey item. The salamander was given ten minutes to make this decision, and a decision was considered made when the salamander approached the capsule and fixated on it. This procedure was repeated ten times for each animal, randomly placing the visible and the olfaction capsules. The aquarium was thoroughly cleansed between each trial.

## **RESULTS AND DISCUSSION**

Of the five salamanders tested, only one completed the entire experiment. This salamander chose the right side of the tank five times, and the left side of the tank five times, exhibiting no right or left preference. During the olfaction portion of the experiment, the salamander chose the arm with the live prey during all ten trials. Thus, my results from this salamander corroborate Roth's (1987) findings that vision will predominate over other senses during prey capture when vision is possible.

Three salamanders completed six trials of the right left preference test, and one salamander completed one trial of that test. When these salamanders were tested again, they did not make a choice between arms, and instead stayed within the neutral zone of the tank. The prey items were always live and active, and clearly visible to the salamander. However, the salamanders were unable to eat the prey item because it was encapsulated. I hypothesize that the salamanders learned that the prey item was unattainable and therefore the

salamander did not attempt to capture the worm. Additionally, during the preference test, the specimens that made a choice always approached the capsule and fixated with its eyes on the prey item. Many times an unsuccessful feeding attempt was made, visible to the researcher by a clear hyoid depression when the salamander was close to the capsule. Thus, *E. spelaea* was able to orient to the prey without sensing it chemically; when available, vision is the dominant sense guiding feeding behavior.

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Born: 27 January 1982, Atlanta, Georgia, USA

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#### EDUCATION

- 2009 Master's of Science, Graduate College of Arts and Sciences, Wake Forest University. Graduate advisor: Miriam A. Ashley-Ross, Ph.D. Thesis: "Feeding kinematics of the grotto salamander, *Eurycea spelaea*"
- 2004 Bachelor's of Science, *cum laude*, College of Liberal Arts, Mercer University. Major: Environmental Science. Minor: Biology.

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- 6.2007-7.2009 Laboratory Technician II, Department of Plastic and Reconstructive Surgery, Wake Forest University Health Sciences
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- 2004 (spring) Lab Coordinator, Department of Environmental Science, Mercer University
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- 2006 Society for Integrative and Comparative Biology
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