THE KINEMATICS AND MECHANISM OF PREY CAPTURE IN THE AFRICAN PIG-NOSED FROG (*HEMISUS MARMORATUM*): DESCRIPTION OF A RADICALLY DIVERGENT ANURAN TONGUE

DALE RITTER* AND KIISA NISHIKAWA

Physiology and Functional Morphology Group, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA

Summary

High-speed videography and muscle denervation experiments were used to quantify the feeding kinematics of *Hemisus marmoratum* and to test hypotheses of muscle function. The feeding behavior of *H. marmoratum*, which feeds on ants and termites, differs radically from that of other frogs that have been studied. During feeding in *H. marmoratum*, the tongue 'telescopes' straight out of the mouth, as opposed to the 'flipping' tongue trajectory observed in most other frogs. At the time of prey contact, two lateral lobes of tissue at the tongue tip envelop the prey. These lateral lobes are capable of applying significant pulling forces to the prey and the tongue is, therefore, described as prehensile. The trajectory of the tongue can be adjusted throughout protraction so that the frog can 'aim' its tongue in all three dimensions; distance, azimuth and elevation. Bilateral denervation of the genioglossus muscles results in a complete lack of tongue protraction, indicating that the genioglossus muscle is the main tongue protractor in *H. marmoratum*, as in other frogs. Thus, *H. marmoratum* provides strong evidence of functional conservatism of the genioglossus muscle within anurans. Bilateral denervation of the hyoglossus muscle indicates that although the hyoglossus is involved in several aspects of normal tongue retraction, including the prehensile capability of the tongue tip, it is not necessary for tongue retraction. Unilateral denervation of the genioglossus muscle causes significant deviation of the tongue towards the denervated side, providing evidence for a mechanism of lateral tongue aiming. On the basis of the kinematics of prey capture, the anatomy of the tongue and the results of the denervation experiments, we propose that *H. marmoratum* uses a hydraulic mechanism to protract its tongue.

Key words: denervation, frog feeding, *Hemisus marmoratum*, hydraulic, hydrostatic, kinematics, prehensile, tongue.

Introduction

Recent studies of feeding behavior in frogs have documented great diversity at some levels of organization, as well as pointing out areas of apparent conservatism (Nishikawa *et al.* 1992; Anderson, 1993; Anderson and Nishikawa, 1993). One characteristic that exhibits diversity among species is tongue length. It appears that frog species with long tongues have evolved convergently from short-tongued ancestors in several independent lineages (Nishikawa *et al.* 1992). Long tongues appear to have evolved to serve a variety of different purposes, including feeding on large prey (Gans *et al.* 1991) as well as feeding on elusive prey (Gray and Nishikawa, 1995).

Although tongue length exhibits great variability, the mechanism of tongue protraction apparently does not (Nishikawa *et al.* 1992). In all frogs that have been studied, protraction is produced by the genioglossus muscle, which 'flips' the tongue over the mandibles and out of the mouth.

During the early phase of protraction, the tongue is stiffened and rotated over the mandible by muscular contraction of the genioglossus (Gans and Gorniak, 1982*b*). The tongue is then carried out of the mouth and towards the prey by inertia imparted to the tongue during rotation over the mandibles.

Expansion of the database of anuran feeding studies increases our knowledge of the evolution of this system by documenting its diversity as well as testing the applicability of general statements about the feeding system, which is important, since these general statements enable general predictions to be made. In this regard, organisms that deviate substantially from the normal condition are of special interest. This study describes the feeding behavior of such an apparently deviant animal.

The African pig-nosed frog *Hemisus marmoratum* belongs to the morphologically divergent family Hemisotidae, which

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*Present address: Department of Ecology and Evolutionary Biology, Box G, Walter Hall, Brown University, Providence, RI 02912, USA.

contains a single genus and eight species (Frost, 1985) and is currently placed in the Ranoidea (Ford and Cannatella, 1993). These are small (males average 32 mm and females average 47 mm in snout–vent length), rotund frogs that inhabit the savannas of Africa, both north and south of the rain forest (Noble, 1924). *H. marmoratum* is believed to live within termite nests and is known to feed on only ants and termites (Noble, 1924). Hemisotids have diverged morphologically from other ranoids in that they lack a sternum and possess a heavily ossified, bullet-shaped skull believed to be used in head-first digging (Ford and Cannatella, 1993).

A kinematic analysis of prey capture was undertaken because initial observations suggested that *H. marmoratum* displays tongue movements that differ radically from those of all frogs previously studied. Data obtained from *H. marmoratum* allow comparisons of its feeding kinematics with those of other species. In addition, the apparently divergent tongue movements of *H. marmoratum* provide an opportunity to address questions of plasticity and conservation of muscle function across a morphological and behavioral transition. As has recently been pointed out regarding tests of neuromuscular conservatism (Smith, 1994), only when some aspect of a neuromotor system changes (e.g. morphology, kinematics or behavior) should we expect other aspects of the system to change as well. Therefore, a hypothesis of neuromuscular conservation is more rigorously tested when there is an *a priori* expectation of change. *H. marmoratum* is especially well suited for a test of the conservation of function in frog lingual muscles because its feeding kinematics differ from those of frogs with flipping tongues, and this provides us with an expectation of change in other aspects of the system.

The mechanism of tongue movement in this species was investigated by performing tests of muscle function. These tests were accomplished by surgically denervating the extrinsic muscles of the tongue. The tongue of anurans is typically composed of two muscles, the genioglossus and the hyoglossus (Regal and Gans, 1976). Both of these are strap muscles, with fibers that are oriented parallel to the long axis of the tongue. Previous studies indicate that the genioglossus muscle is the major protractor of the tongue and its denervation consistently leads to a significant deficit in tongue protraction (Nishikawa and Roth, 1991; Deban and Nishikawa, 1992; Smith and Nishikawa, 1991; Nishikawa and Gans, 1990). Although the function of the hyoglossus muscle has not been tested using denervation experiments, it is believed to be the main retractor of the tongue (Gans and Gorniak, 1982*a,b*). While these are not the only muscles involved in feeding, they are the only muscles that insert in the tongue and can directly affect movements of the tongue relative to the lower jaw. Therefore, we began denervation experiments with the hypotheses that the genioglossus is the main protractor of the tongue and that the hyoglossus is the main retractor of the tongue. On the basis of kinematic analyses, lingual anatomy and muscle denervation experiments, we propose a mechanism of tongue protraction for this species.

Materials and methods

Adult *Hemisus marmoratum* were obtained from Glades Herp, Fort Myers, Florida, USA. Ten individuals, ranging in snout–vent length (SVL) from 2.5 to 3.5 cm, were used for kinematic analyses and muscle denervation experiments. The animals were housed individually in clear plastic containers on moist paper towels and were fed locally obtained termites twice a week. The animals were kept at room temperature (21–23 °C). All of the animals fed well and appeared to be in good health. Table 1 lists the SVLs of individuals used in this study, as well as the number of digitized feeding trials per individual and per treatment. Two animals were cleared and stained with Sudan Black, in order to visualize the peripheral nerves of the lower jaw (Nishikawa, 1987). One animal was used for dissection. The tongue and lower jaw were dissected to determine whether *H. marmoratum* exhibits unique morphological attributes that might be involved in the

Table 1. *Numbers of sequences analyzed for each of the 10 individuals included in the study, before surgery, and for all treatments*

Individual (SVL in cm)	Before surgery	BH	BG	UG, right	UG, left
1 (2.5)	4C, 4M	4C			
2 (3.5)	4C, 4M	4C			
3 (2.7)	4C, 4M	4C			
4 (2.5)	4C, 4M		4M		
5 (2.9)	4C, 4M		4M		
6 (2.5)	4C, 4M		4M		
7 (3.1)	4C, 4M			4C, 4M	
8 (2.6)	4C, 4M			4C, 4M	
9 (3.0)	4C, 4M				4C, 4M
10 (2.5)	4C, 4M				4C, 4M

C, capture; M, miss.

Treatments are: BH, bilateral denervation of the m. hyoglossus; BG, bilateral denervation of the m. genioglossus; UG, unilateral denervation of the m. genioglossus.

mechanism of tongue movement. A more detailed anatomical study (incorporating histological techniques) is beyond the scope of this paper.

Videography

Feeding sequences were videotaped with a Display Integration Technologies model DIT 660 high-speed video camera using synchronized stroboscopic illumination. The frogs were videotaped at 120 fields s^{-1} as they fed, unrestrained, on a flat acrylic stage covered with a damp paper towel. A grid of 1 cm squares was used to calculate scaling factors and aspect ratios. Termites used as prey items were placed in front of the frogs with forceps, 1–3 at a time. Feeding trials chosen for analysis were those in which the animal was oriented with the long axis of its body perpendicular to the camera ($\pm10\,^{\circ}$). This was determined qualitatively by evaluating the position of the near-side eye of the frog and the far-side upper and lower jaw. Departures from a lateral view were obvious because of the short, wide head of this species. Feeding sequences to be analyzed were chosen to include the greatest range of variation in the distance of the prey from the frog. Feeding trials were videotaped at room temperature (approximately 21–23 $^{\circ}$C).

Muscle denervation

Three treatments were performed: bilateral hyoglossus denervation, bilateral genioglossus denervation and unilateral genioglossus denervation. Frogs were anesthetized by immersion in 0.1 % tricaine methanesulfonate (MS222) for 30 min. The animals were then placed on the stage of a dissecting microscope, and all but the lower jaw of the animal was covered with moist paper toweling to avoid desiccation. Small incisions were made in the skin of the lower jaw overlying the muscle and nerve branch of interest. Branches of the hypoglossal nerve innervate both the genioglossus and hyoglossus muscles (Fig. 1). The nerve was isolated close to the point where it entered the muscle of interest, dissected free of surrounding connective tissue and blood vessels, and transected. A 1–2 mm length of the nerve was excised to retard regeneration. The incision was closed with Nexaband surgical adhesive. The frogs recovered from anesthesia within a few hours, and initial feeding attempts were videotaped to obtain post-denervation feeding data. The animals were then killed by over-anesthesia in MS222, and the denervations were verified by dissection.

Kinematic analyses

Feeding sequences were analyzed using Peak Performance Technologies 2D motion-analysis software, running on an IBM-compatible computer. For each field of the feeding sequence, the *x,y*-coordinates of the prey item, a non-moving reference point, and eight points on the frog were digitized (Fig. 2).

From these digitized points, variables were calculated that describe the timing, duration and magnitude of several kinematic events. Timing and duration variables are reported
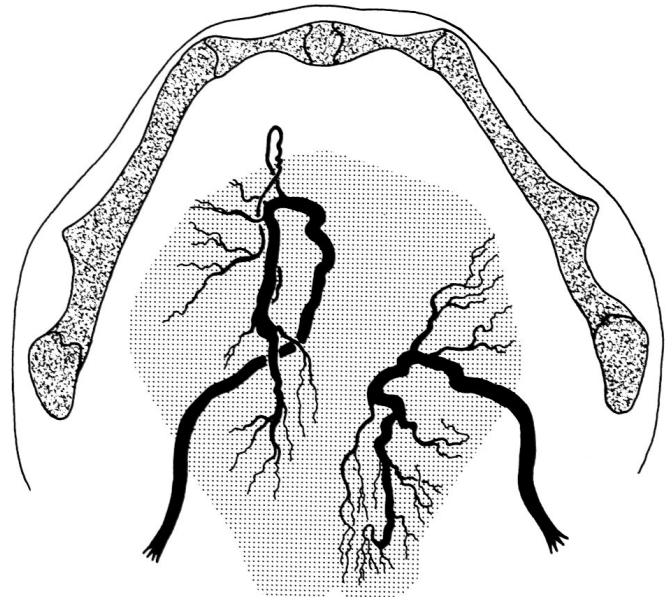

Fig. 1. *Camera lucida* drawing of the peripheral nerves in the lower jaw of *Hemisus marmoratum* stained with Sudan Black. The approximate position of the tongue pad is stippled. This is a dorsal view, showing the hypoglossal nerve (left) and glossopharyngeal nerve (right). Jaw width is 9 mm.

in milliseconds (ms). All timing variables are reported relative to the onset of mouth opening at time zero and are as follows: (1) time of first tongue visibility, the frame in which the tongue is first seen rising from the floor of the mouth; (2) time of maximum gape, the frame in which maximum gape (described below) occurs; (3) time of prey contact, the frame in which the tongue is first seen to make contact with the prey; (4) time of maximum tongue protraction, the frame in which the ventral tongue tip is farthest away from the lower jaw tip; (5) time of onset of tongue retraction, the first frame in which the tongue is moving back towards the frog; (6) time of completion of tongue retraction, the time when the tongue is fully retracted
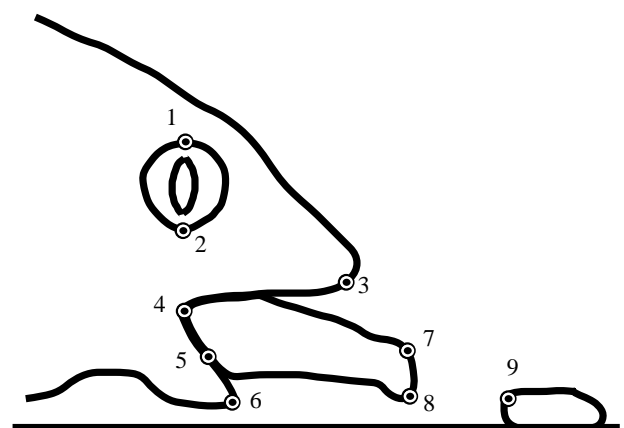

Fig. 2. Points used in kinematic analysis. These are as follows: (1) top of the eye; (2) bottom of the eye; (3) upper jaw tip; (4) jaw joint; (5) mid-point of the lower jaw; (6) lower jaw tip; (7) dorsal tongue tip; (8) ventral tongue tip and (9) prey.

(i.e. no longer visible); (7) time of onset of mouth closing, the time when the lower jaw begins rapid closure (determined from the gape profile); and (8) time of completion of mouth closing, the frame in which the mouth is first seen to be fully closed.

The following duration variables were calculated from the timing variables: (1) duration of mouth opening, the time between the onset of mouth opening (time=0) and the time of maximum gape; (2) duration of tongue protraction, the time between first tongue visibility and maximum tongue protraction; (3) duration of tongue retraction; (4) duration of mouth closing; (5) duration of tongue at target, the time between prey contact and the onset of tongue retraction; and (6) total time the mouth is open.

Three variables that describe the magnitude of certain kinematic events were also calculated: (1) maximum gape angle (in degrees) is the angle formed by points 3 and 6, with point 4 at the vertex (Fig. 2); (2) initial prey distance is the distance (in mm), at $t=0$, between the tip of the lower jaw and the prey item (points 6 and 9 in Fig. 2); and (3) maximum tongue reach is the greatest distance (in mm) in each feeding sequence between the tip of the lower jaw and the ventral tongue tip (points 6 and 8 in Fig. 2).

Results of the bilateral hyoglossus denervation treatment showed that two other variables were needed to quantify the effects of this treatment: (1) overshoot (in mm), which is the total horizontal distance traversed by the dorsal tongue tip between the time of prey contact and the onset of tongue retraction and (2) sum of vertical tongue deviation (in mm), quantifying the deviation of the trajectory of the dorsal tongue tip during retraction relative to the trajectory during protraction. We included this variable after watching the videotaped feeding trials and noting that the tongue trajectory appeared grossly different after denervation of the hyoglossus, in that the tongue did not telescope straight back into the mouth, but appeared to arch upwards during retraction. During both protraction and retraction, the dorsal tongue tip is assigned x,y-coordinates for each video frame as it is digitized. First, the x,y-coordinates of the upper jaw tip are subtracted from the x,y-coordinates of the dorsal tongue tip, so that movements of the entire animal are not included in movements of the tongue tip. Each frame of retraction is then matched with a frame of protraction by finding x (horizontal) values of the dorsal tongue tip that are most similar. This gives pairs of video frames that are 'homologous' in their x-coordinates, one from protraction and one from retraction. For each homologous protraction/retraction pair, the difference in y (vertical) values is then calculated as: vertical tongue deviation = retraction y value minus protraction y value. The values of vertical tongue deviation from all homologous protraction/retraction pairs are then summed for each feeding trial. The closer the retraction trajectory is to the protraction trajectory, the smaller will be the value of this variable. Positive values indicate that the tongue trajectory during retraction is higher (i.e. has greater y values at homologous x values) than the trajectory during protraction.

Statistical analyses

For normal captures, least-squares regressions were performed for all variables *versus* initial prey distance in order to determine which variables were affected significantly by initial prey distance. Because many of the variables covaried with initial prey distance, we used an analysis of covariance (ANCOVA), full interaction model, to compare normal captures with normal misses and to test the effects of all three denervation treatments. Initial prey distance was the covariate for all these analyses. Non-significant interaction terms involving the covariate were removed from the model to increase statistical power.

A two-way ANCOVA was used to compare captures and misses before surgery. The main effects were individual (random effect) and success (capture *versus* miss, fixed effect). The success F-test was performed with the individual $\times$ success mean square as the denominator. Duration of tongue at target was not included in this analysis, because it cannot be calculated for misses.

Two-way ANCOVAs were also performed to test for effects of the denervation treatments. Separate analyses were performed for the bilateral hyoglossus and bilateral genioglossus denervation treatments. Left- and right-side unilateral genioglossus denervation data were pooled for analysis, but separate analyses were performed for captures and misses. The main effects in the denervation analyses were individual (random effect) and treatment (before *versus* after, fixed effect). The treatment F-test was performed with the individual $\times$ treatment mean square as the denominator. These comparisons were made using only the duration (excluding total time the mouth is open) and magnitude variables, because many of the timing variables incorporate others that occur earlier in the feeding sequence. The Bonferroni correction was used to adjust significance levels to account for experiment-wide error. Analyses were performed using Superanova software (version 1.1) running on a Macintosh IIci computer.

Results

Anatomy

The muscles of the tongue are remarkably simple in *H. marmoratum* (Fig. 3). The genioglossus and hyoglossus are the only extrinsic muscles that insert in the tongue, and they retain their usual longitudinal orientation. The fibers of these two muscles do not interdigitate in the tongue pad. There are no radially or circumferentially oriented muscle fibers within the tongue, i.e. there are no intrinsic tongue muscles. The hyoglossus is well fasciculated, and one fascicle extends all the way to the base of the bifid tongue tip. The two halves of the genioglossus originate separately from the mentomeckelian bones, and there is a well-defined sinus between the points of origin. The two halves of the genioglossus muscle continue into the lobes of the tongue tip and extend into them dorsally. There is an extensive sinus between the genioglossus and hyoglossus muscles, which is continuous with the sinus
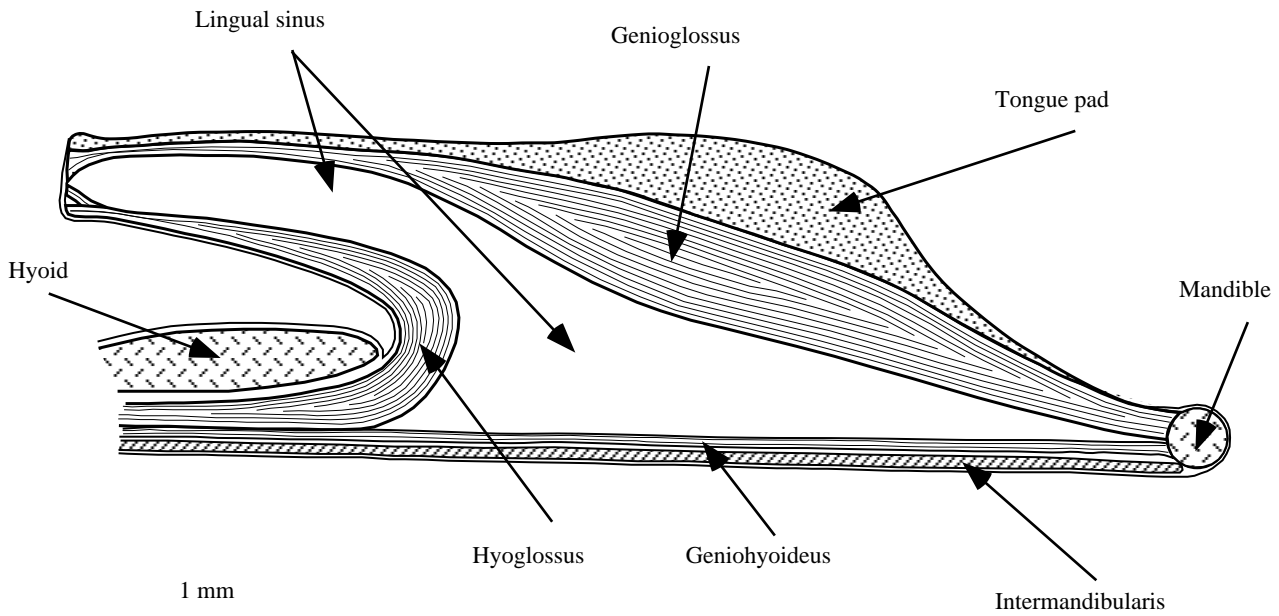
Fig. 3. Schematic mid-sagittal view of the tongue and lower jaw musculature of *Hemisus marmoratum*. Anterior is to the right; dorsal is towards the top of the page. There are no intrinsic tongue muscles, and the genioglossus and hyoglossus fibers are longitudinally oriented and, thus, the arrangement of the genioglossus and hyoglossus muscles is similar to that of other frogs. One difference between *H. marmoratum* and other frogs is that in *H. marmoratum* the genioglossus and hyoglossus muscles do not interdigitate in the tongue pad. This lack of interdigitation contributes to the existence of a discrete sinus between the genioglossus and hyoglossus muscles, forming a broad space in the floor of the mouth and extending all the way into the tongue tip.

between the origins of the two halves of the genioglossus muscle. A relatively thick pad of connective tissue covers the dorsal surface of the tongue and is in direct contact with the dorsal surface of the genioglossus muscle. The hyoid skeleton and associated musculature of *H. marmoratum* do not differ appreciably from those of other frogs.

Normal feeding kinematics

Of the variables analyzed, nine exhibited a significant correlation with initial prey distance ($P<0.01$) and seven did not ($P>0.05$). All significant correlations were positive (Table 2).

In a typical, successful prey capture sequence, there is very little or no body movement of the frog when it feeds (i.e. there is no forward lunge). Instead, movements are restricted to the lower jaw and the tongue. The lower jaw is both depressed and retracted during mouth opening, and as it swings open the tongue begins to rise from the floor of the buccal cavity (32 ms, Fig. 4). There is some rotation (clockwise in Fig. 4) of the tongue in the buccal cavity during mouth opening so that the tongue tip faces towards the prey. Soon after the tongue becomes visible, maximum gape occurs. There may be a small amount of head lifting during mouth opening, which is variable both between and within individuals.

Instead of rotating over the mandible, the tongue telescopes out of the mouth throughout protraction, on a trajectory more or less straight towards the prey (between 64 and 192 ms, Fig. 4). The duration of tongue protraction, the time of prey contact, the time of maximum tongue protraction and maximum tongue reach were all correlated with initial prey distance (Table 2). The farther away the prey, the longer it takes the tongue to reach it. The average speed of the tongue tip during protraction was $14\,\mathrm{cm\,s^{-1}}$, and the maximum tongue reach recorded during this study was 9.0 mm, equal to three times the length of the lower jaw.

Initiation of tongue retraction always occurred in the frame immediately following prey contact, so that the measured duration of tongue at target was equal to the time interval between two video fields. This is an overestimate because the duration of this variable is less than 8 ms. There was a significant correlation between the time of onset of tongue retraction and initial prey distance (Table 2). Once retraction began, however, the duration of tongue retraction was independent of initial prey distance. The time of completion of tongue retraction was also correlated with initial prey distance, because of the inclusion of tongue protraction times in this variable.

Usually, the prey was completely enveloped by the tongue and therefore not visible during retraction (e.g. 224 ms, Fig. 4). In dorsal view, two fleshy, lateral lobes of tissue are visible at the tongue tip (Fig. 5), and these partially or wholly envelop the prey at the time of contact and throughout retraction of the tongue so that, from a lateral view, the prey seems to disappear. In addition, the tongue tip was observed to pull forcefully on the prey. Fig. 6 shows a prey capture sequence during which the posterior portion of the termite was firmly held by the

Table 2. *Kinematics of normal feeding behavior for successful capture attempts*

Variable	Mean	S.E.M.	
A			
Time of first tongue visibility (ms)	30	2	
Time of maximum gape (ms)	68	3	
Duration of mouth opening (ms)	68	3	
Duration of tongue retraction (ms)	45	2	
Duration of mouth closing (ms)	34	1	
Duration of tongue at target (ms)	8*	<1	
Maximum gape angle (degrees)	79	1	
	r	Minimum	Maximum
B			
Time of prey contact (ms)	0.69	88	264
Time of maximum tongue protraction (ms)	0.68	72	248
Time of onset of tongue retraction (ms)	0.68	96	272
Time of completion of tongue retraction (ms)	0.68	128	328
Time of onset of mouth closing (ms)	0.67	96	312
Time of completion of mouth closing (ms)	0.67	160	360
Duration of tongue protraction (ms)	0.68	72	248
Total time the mouth is open (ms)	0.67	160	360
Maximum tongue reach (mm)	0.72	2	9

N=10 individuals, with four trials per individual for a total of 40 feeding sequences.

A. Means and standard errors are given for variables showing no significant correlation with initial prey distance.

B. Product-moment correlation coefficients (r) and minimum and maximum values for those variables that show a significant correlation with initial prey distance (all $P<0.001$).

*This is the shortest time interval measurable given our sampling rate and is therefore an underestimate of this variable.

forceps. The tongue made contact with the prey (168 ms) and, although the prey was held by the forceps, the anterior portion of the termite was successfully transported to the oral cavity. The tongue tip imparted sufficient tensile force to tear the termite in half. For this reason, we describe the tongue tip as prehensile.

The dorsal view (Fig. 5) shows another unusual feature of the tongue of *H. marmoratum*, in that the animal is able to adjust the azimuth of the tongue relative to the head (i.e. *H. marmoratum* can aim its tongue laterally). During protraction, the tongue is able to veer left or right to bring the tongue tip into contact with the prey. The tongue does not simply come out of the mouth at an angle, but can be seen to curve towards one side or the other (Fig. 5).

Both the time of onset of mouth closing and the time of completion of mouth closing were correlated with initial prey distance (Table 2). Again, these significant correlations with prey distance were due to correlations between initial prey distance and tongue protraction variables that occurred earlier in the feeding sequence. The duration of mouth closing was not affected by prey distance. Finally, the total time the mouth is open was correlated with initial prey distance.

Normal captures versus *normal misses*

During normal feeding, misses resulted from the prey being too far away for the tongue to reach. Throughout the study, we never observed a normal feeding sequence in which prey

contact was not followed by prey ingestion. The results of the analysis of covariance showed some significant differences for all model effects (Table 3). Variables associated with mouth opening and closing, retraction of the tongue and maximum gape were not significantly affected by capture success (Table 3). Significant effects due to success (capture *versus* miss) occurred in two variables associated with tongue protraction: the duration of tongue protraction and maximum tongue reach (Table 3). Misses were characterized by a maximal tongue protraction effort, so that values of these two variables were greater in misses than in captures. These same two variables were affected significantly by the covariate, initial prey distance. This was in keeping with the results of the regressions carried out on the pooled normal captures data (Table 2). A significant treatment × individual interaction was also found for duration of tongue protraction and maximum tongue reach.

Five of the six variables differed significantly among individuals (Table 3). Significant among-individual variation is present to some degree in all analyses and is probably due to differences in body size and to the propensity of animals to attempt capture of distant prey items.

Bilateral hyoglossus denervation

A comparison of feeding kinematics before and after bilateral hyoglossus denervation was restricted to successful prey capture sequences only, because unsuccessful feeding
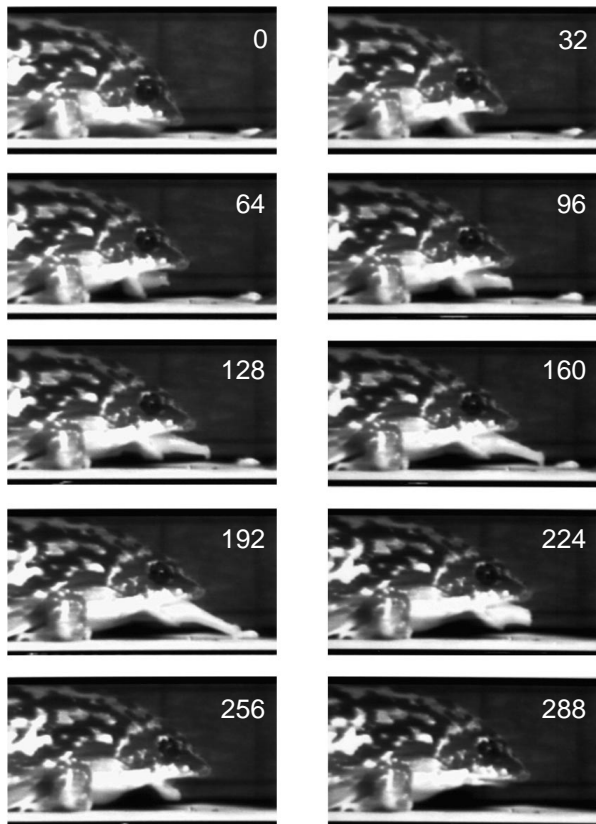
Fig. 4. Video frames taken from a normal feeding sequence. The prey item, a termite, is in the lower right-hand corner. A 1 cm grid may be seen in the background. Time, in milliseconds, is given in the upper right-hand corner of each frame. Time 0 is the frame in which the mouth first begins to open.
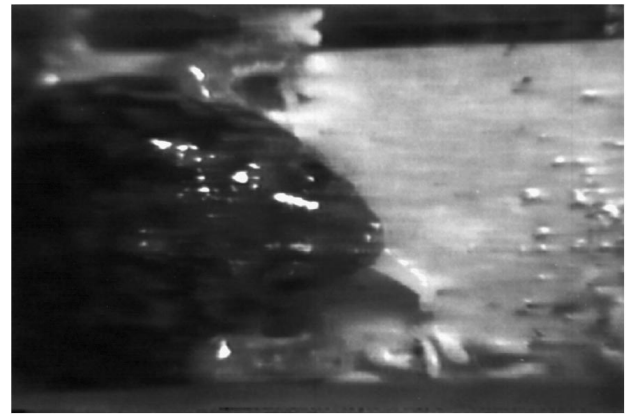

Fig. 5. Video frames taken from a normal feeding sequence in dorsal view. In the upper frame, notice the bending of the tongue to the right of the frog and the lateral lobes of tissue at the tongue tip. In the lower frame, notice that the lateral lobes have closed around the termite.

attempts were qualitatively different after denervation (discussed below). Duration of tongue at target was included in this comparison because we were comparing captures under both conditions. Two additional variables, overshoot and the sum of vertical tongue deviation, were also included in the analysis of this treatment.

Analysis of covariance resulted in significant treatment effects on overshoot and on the sum of vertical tongue deviation (Table 4). Values of overshoot for the normal feeding trials averaged -0.9 ± 0.2 mm and increased significantly to 0.7 ± 0.1 mm after bilateral hyoglossus denervation ($P<0.001$). Mean values for the sum of vertical tongue deviation also increased significantly after denervation, from a normal value of -1.1 ± 0.4 mm to a post-denervation value of 5.4 ± 1.0 mm ($P<0.001$). Thus, following bilateral denervation of the hyoglossus muscles, the trajectory of the tongue arched upwards during retraction relative to the protraction trajectory (Fig. 7). Significant treatment $\times$ individual interaction terms were observed for duration of tongue protraction and duration of tongue retraction.

There were also qualitative differences in feeding trials after hyoglossus denervation that were not apparent from the

quantitative analysis (Fig. 8). First, and most significant, was the loss of the ability of the tongue tip to envelop the prey. Although the frogs could feed successfully after hyoglossus denervation, the prey was not lost from sight during tongue retraction, as was observed in the majority of the normal feeding trials (e.g. Fig. 4). The prey appeared to adhere to the tongue solely due to wet adhesion and often fell off the tongue during retraction. Only in feeding trials after hyoglossus denervation was the termite–tongue interface clearly visible (e.g. 296 ms in Fig. 8). Furthermore, only after hyoglossus denervation were feeding trials observed in which the tongue made contact with the prey and failed to bring the prey into the mouth (the prey may be seen falling to the substratum at 328 ms in Fig. 8). This new class of unsuccessful prey capture attempt is the reason we chose to analyze the effects of hyoglossus denervation only with successful prey capture data.

Deglutition was also adversely affected by hyoglossus denervation. During normal feeding, frogs often ate many termites in sequence, with less than 1 s between prey capture events. After hyoglossus denervation, the animals were seen to swallow forcefully several times after prey capture, suggesting that the prey was not correctly positioned within the oral cavity for successful deglutition. In some feeding attempts after
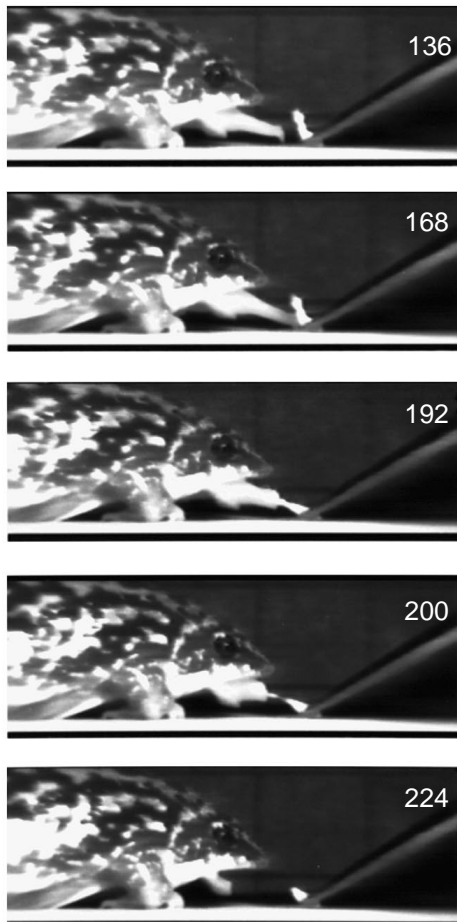
Fig. 6. Video frames taken from a normal feeding sequence to illustrate the pulling force that the tongue tip is capable of applying to the prey. Time, in milliseconds, is given in the upper right-hand corner of each frame, with time 0 being the frame in which the mouth begins to open. In this feeding sequence, the posterior portion of the termite is held firmly by forceps. Prey contact is made at 168 ms, tensile force is seen to be exerted on the termite's body at 192 ms, and finally the exoskeleton fails at 200 ms, and the termite's head is removed.

hyoglossus denervation, a previously ingested termite could be seen still adhering to the tongue.

Bilateral genioglossus denervation

After bilateral genioglossus denervation, the tongue was no longer protracted (Fig. 9) and since this meant frogs were never able to capture the prey, only unsuccessful feeding attempts could be analyzed. Therefore, statistical analysis was restricted to normal and post-denervation misses (Table 5). The lack of tongue protraction after denervation made it impossible to analyze the following variables: duration of tongue protraction, duration of tongue at target and duration of tongue retraction. Of the four variables that were analyzed, only maximum tongue reach showed a significant effect due to bilateral genioglossus denervation (Table 5). The mouth opened and closed as usual, and the magnitude of maximum gape did not show significant change (Table 5). The covariate, initial prey distance, significantly affected duration of mouth closing.

Unilateral genioglossus denervation

To evaluate the effects of unilateral genioglossus denervation on feeding kinematics, successful prey capture sequences before and after denervation were analyzed separately from unsuccessful prey capture sequences. Unilateral genioglossus denervation had no significant effect on any of the duration or magnitude variables during successful prey capture sequences (Table 6). As long as prey contact was made, the feeding sequence was both quantitatively and qualitatively similar when comparing normal with post-denervation trials. Results do show a significant treatment $\times$ individual interaction term for one variable, maximum gape.

In contrast to successful prey capture attempts, there were striking differences when the frogs missed the prey after unilateral genioglossus denervation, compared with normal misses. The tongue began to come out of the mouth more or less normally, but if prey contact was not made early in the feeding attempt, the tongue curved off sharply towards the denervated side (Fig. 10). In all cases, the tongue deviated

Table 3. *ANCOVA (covariate is initial prey distance) testing the effect of prey capture success (captures* versus *misses)*

Variable	Individual		Success		Initial prey distance		Success × prey distance	
	F	P	F	P	F	P	F	P
Duration of mouth opening (ms)	3.2	0.004*	0.6	0.810	0.0	0.8734	0.1	0.7792
Duration of tongue protraction (ms)	3.1	0.004*	21.4	0.001*	16.2	<0.001**	14.9	<0.001**
Duration of tongue retraction (ms)	2.9	0.006*	2.7	0.134	2.2	0.147	0.3	0.603
Duration of mouth closing (ms)	2.7	0.010	2.1	0.186	1.6	0.216	2.6	0.116
Maximum gape (degrees)	5.4	<0.001**	3.2	0.108	1.6	0.210	5.3	0.024
Maximum tongue reach (mm)	10.8	<0.001**	22.7	0.001**	32.8	<0.001**	18.1	<0.001**

$N=10$ individuals, four successful and four unsuccessful trials per individual.

*Significant at $P \leqslant 0.05$; **significant at $P \leqslant 0.01$, after simultaneous Bonferroni adjustments.

Table 4. *ANCOVA (with initial prey distance as the covariate) testing the effect of bilateral hyoglossus denervation*

Variable	Individual		Prey distance		Initial treatment		Treatment × individual	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Duration of mouth opening (ms)	3.7	0.0460	0.3	0.5737	2.0	0.290	2.3	0.1331
Duration of tongue protraction (ms)	15.1	<0.001**	21.4	0.001*	2.7	0.244	10.1	0.001**
Duration of tongue retraction (ms)	12.3	<0.001**	0.6	0.436	<0.1	0.933	6.7	0.007*
Duration of mouth closing (ms)	0.6	0.539	0.1	0.730	16.9	0.054	0.6	0.560
Duration of tongue at target (ms)	1.1	0.363	0.1	0.739	37.8	0.026	0.9	0.422
Maximum gape (degrees)	0.4	0.711	0.7	0.418	12.2	0.073	1.3	0.308
Maximum tongue reach (mm)	2.9	0.080	22.3	<0.001**	0.1	0.833	4.2	0.034
Overshoot (mm)	1.6	0.240	5.46	0.032	32.9	<0.001**	0.5	0.621
Sum of vertical tongue deviation (mm)	3.5	0.054	<0.1	0.986	45.3	<0.001**	4.9	0.021

Only successful prey capture sequences are compared. $N=3$ individuals, with four pre- and four post-denervation feeding trials per individual.

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$, after simultaneous Bonferroni adjustments.

towards the denervated side. In extreme cases, the frog slapped itself in the eye with its tongue, which required a bending angle of more than 180°.

For unsuccessful capture attempts, several of the kinematic variables were affected significantly by unilateral genioglossus denervation (Table 7; Fig. 11). Both duration of tongue retraction and duration of mouth closing were longer after denervation. This was due to the fact that the tongue was in a different configuration (i.e. wrapped around the side of the frog's head) at the onset of retraction. Tongue reach was reduced after unilateral denervation due to curvature during protraction (Table 7; Fig. 11A). Maximum gape angle was larger after denervation (Table 7; Fig. 11B).

Discussion

We begin the discussion by comparing the gross anatomy of the tongue and lower jaw of *H. marmoratum* with the basic condition seen in other frogs. The feeding behavior of *H. marmoratum* is then compared with that of other frogs, in order to point out similarities and differences in the feeding of this species relative to others. Next, we consider the evolution of tongue aiming in frogs, a particularly well-developed attribute of *H. marmoratum*. Results of the denervation experiments are then considered and hypotheses of muscle function are evaluated. Based on the anatomy of the tongue, the kinematics of feeding and results of the denervation experiments, a hypothesis of the mechanism of tongue movements is proposed.

Anatomy

In general, the tongue of *H. marmoratum* is very similar to that of other frogs. The fibers of both extrinsic muscles are oriented parallel to the long axis of the tongue and, although they are well fasciculated, they are not subdivided into separate

elements. As in frogs generally, there is a large sinus between the genioglossus and hyoglossus muscles, but in *H. marmoratum* there is little or no interdigitation of the fibers of the genioglossus and hyoglossus in the tongue pad (Fig. 3), a feature that is shared with microhylids (Horton, 1982). *H. marmoratum* differs from other frogs in having separate origins for the right and left genioglossus. Other unique features include a thick layer of connective tissue dorsal to the
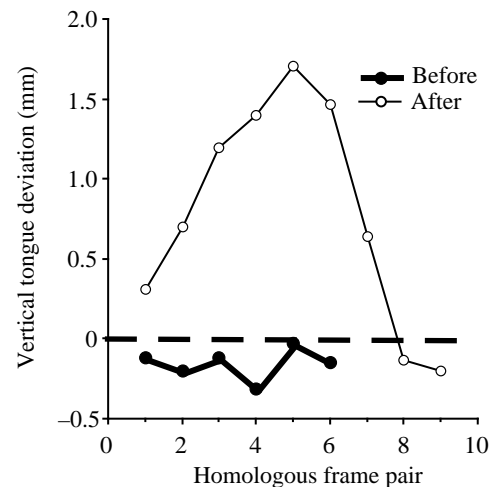

Fig. 7. Graphs of vertical tongue deviation, before and after bilateral hyoglossus denervation. These are data from two successful prey captures, from the same individual. Initial prey distance for the normal trial was 4 mm, and for the post-denervation trial was 3 mm. Negative values for vertical tongue deviation result when the retraction *y*-coordinate is lower than the protraction *y*-coordinate, and positive values result when the retraction *y*-coordinate is higher than the protraction *y*-coordinate. Values close to zero reflect similar protraction and retraction trajectories of the tongue tip. The bell-shaped curve formed by the vertical tongue deviation values after denervation reflects the upward arching trajectory of the tongue tip during retraction.

genioglossus muscle and a fascicle of the hyoglossus muscle that passes between the left and right halves of the genioglossus muscle and inserts between the lateral lobes at the tip of the tongue.

Kinematics

Throughout this section, comparisons will be made with published values from other frog feeding studies. In order to reduce redundancies in citations, those studies are listed below. The range of snout–vent lengths (SVL) of the individuals used in these studies is listed after the species names to facilitate comparison. Unless otherwise noted, all comparisons are made from the following studies: *Ascaphus truei*, 3.4–4.1 cm SVL (Nishikawa and Cannatella, 1991), *Bufo marinus*, 10–15 cm SVL (K. C. Nishikawa and C. Gans, unpublished data), *Discoglossus pictus*, 3.2–4.1 cm SVL (Nishikawa and Roth,

1991), and *Hyla cinerea*, 3.4–4.8 cm SVL (Deban and Nishikawa, 1992)

In general, the jaw movements of *H. marmoratum* are similar to those of other frogs. The mean maximum gape angle is 79 °, which puts it in the mid-range of reported values in the other species (range 71–97 °). The duration of mouth opening averages 68 ms in *H. marmoratum* and is also in the mid-range for the other four species (range 37–78 ms). The main difference in jaw movements is that *H. marmoratum* retracts its lower jaw tip during mouth opening. This does not happen in the other species because the head is elevated as the lower jaw swings open, so that the trajectory of the lower jaw tip is restricted to the vertical plane. Retraction of the lower jaw occurs in *H. marmoratum* because the head is not elevated, so the lower jaw rotates downwards past the vertical, into a retracted position.

Relative to other frogs, *H. marmoratum* protracts its tongue extremely slowly, especially for a frog with such a relatively long tongue (up to three times lower jaw length). Velocity of the tongue tip during protraction in *H. marmoratum* is $14 \, \text{cm} \, \text{s}^{-1}$, very similar to the value of $15 \, \text{cm} \, \text{s}^{-1}$ seen in *Discoglossus pictus* (whose tongue length is less than lower
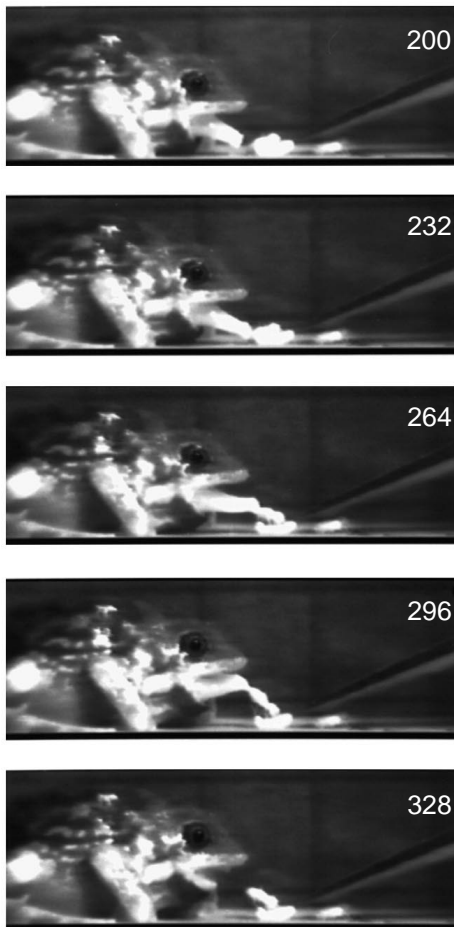

Fig. 8. Video frames taken from a feeding attempt after bilateral denervation of the hyoglossus muscle. Time, in milliseconds, is given in the upper right-hand corner of each frame, with time 0 being the frame in which the mouth first begins to open. A 1 cm grid is visible in the background. Notice that the tongue tip does not envelop the termite. The tongue does not telescope back into the mouth as in normal sequences, but instead describes an upward arching trajectory during retraction. Although prey contact is made, the termite can be seen falling back to the substratum at 328 ms.
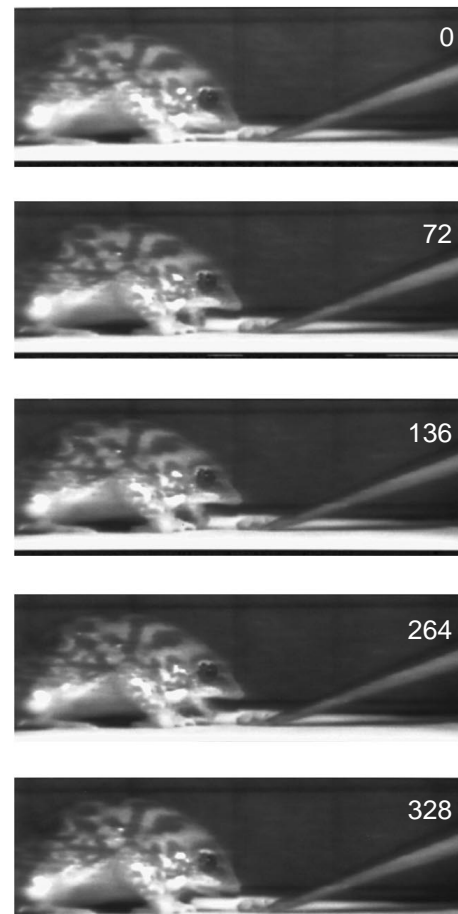

Fig. 9. Video frames taken from a feeding sequence after bilateral denervation of the genioglossus muscle. Time, in milliseconds, is given in the upper right-hand corner of each frame. Although the mouth opens and closes normally, the tongue is not protracted.

Table 5. *ANCOVA (with initial prey distance as the covariate) testing the effect of bilateral genioglossus denervation*

Variable	Individual		Initial prey distance		Treatment	
	F	P	F	P	F	P
Duration of mouth opening (ms)	1.3	0.302	0.2	0.649	<0.1	0.876
Duration of mouth closing (ms)	2.9	0.084	12.2	0.003*	12.7	0.0703
Maximum gape (degrees)	13.8	<0.001**	0.1	0.935	6.6	0.124
Maximum tongue reach (mm)	2.1	0.155	0.5	0.482	193.0	0.005*

Only unsuccessful prey capture sequences are compared.
$N=3$ individuals, with four pre- and four post-denervation feeding trials per individual.
*Significant at $P\leqslant0.05$; **significant at $P\leqslant0.01$, after simultaneous Bonferroni adjustments.

jaw length). The mean velocity of the tongue tip during protraction in *Bufo marinus*, a long-tongued species, is $270\,cm\,s^{-1}$. The duration of tongue protraction in *H. marmoratum* varies from 72 to 248 ms and is significantly correlated with the initial distance between the frog and the prey (Table 2). Mean values for duration of tongue protraction in the four species above range from 35 to 55 ms, and none shows a significant correlation with initial prey distance.

Once the tongue has reached the prey, *H. marmoratum* is quicker to begin retraction than the other species, suggesting fine control of tongue movements. Duration of tongue at target ranges from 17 to 42 ms in other species but in *H. marmoratum* the mean value is less than 8 ms. This rapid onset of retraction does not appear to be a simple consequence of the small size of *H. marmoratum*, because the frog with the second shortest time (17 ms) is *Bufo marinus*, which is several times larger than *H. marmoratum*. The results reported here demonstrate that there is a close relationship between prey contact and the onset of tongue retraction, suggesting that the lingual withdrawal reflex (Matsushima *et al.* 1988) may be especially well developed in *H. marmoratum*.

Although *H. marmoratum* initiates tongue retraction more rapidly than the other species, the duration of retraction is similar to previously reported values. *Ascaphus truei*, *Discoglossus pictus* and *Hyla cinerea*, all of which are similar in size to *Hemisus*, exhibit tongue retraction durations of 28–38 ms, making the retraction time of 45 ms in *Hemisus* only slightly longer.

Tongue aiming

H. marmoratum exhibits the greatest precision of tongue movements yet observed in any frog. 'Precision' here refers to the ability of *H. marmoratum* to control the trajectory of the tongue throughout the course of protraction in three dimensions. Correlations between initial prey distance and two protraction variables (i.e. time of prey contact, maximum tongue reach) indicate that the horizontal trajectory is capable of fine adjustment, and overhead views demonstrated that *H. marmoratum* can control the azimuth of tongue protraction relative to the head. Furthermore, during many feeding trials, the ventral tongue tip periodically touched the substratum and was then lifted clear and protracted further, which indicates that *H. marmoratum* is capable of adjusting the vertical trajectory of its tongue as well. *H. marmoratum* is the first frog known to be capable of adjusting the trajectory of its tongue in all three dimensions.

Table 6. *ANCOVA (with initial prey distance as the covariate) testing the effect of unilateral genioglossus denervation*

Variable	Individual		Initial prey distance		Treatment		Treatment × individual	
	F	P	F	P	F	P	F	P
Duration of mouth opening (ms)	5.2	0.007	1.3	0.271	16.6	0.027	0.1	0.966
Duration of tongue protraction (ms)	5.9	0.004*	16.5	<0.001**	2.7	0.201	1.0	0.425
Duration of tongue retraction (ms)	1.5	0.231	2.0	0.169	1.7	0.279	1.3	0.302
Duration of mouth closing (ms)	1.0	0.399	<0.1	0.908	32.7	0.011	0.3	0.795
Duration of tongue at target (ms)	1.0	0.422	1.0	0.330	0.2	0.721	1.2	0.326
Maximum gape (degrees)	26.1	<0.001**	1.1	0.311	0.6	0.488	8.9	<0.001**
Maximum tongue reach (mm)	14.0	<0.001**	26.4	<0.001**	9.5	0.054	3.5	0.033

Only successful prey capture sequences are compared.
$N=4$ individuals (two left side, two right side), with four pre- and four post-denervation feeding trials per individual.
*Significant at $P\leqslant0.05$; **significant at $P\leqslant0.01$, after simultaneous Bonferroni adjustments.
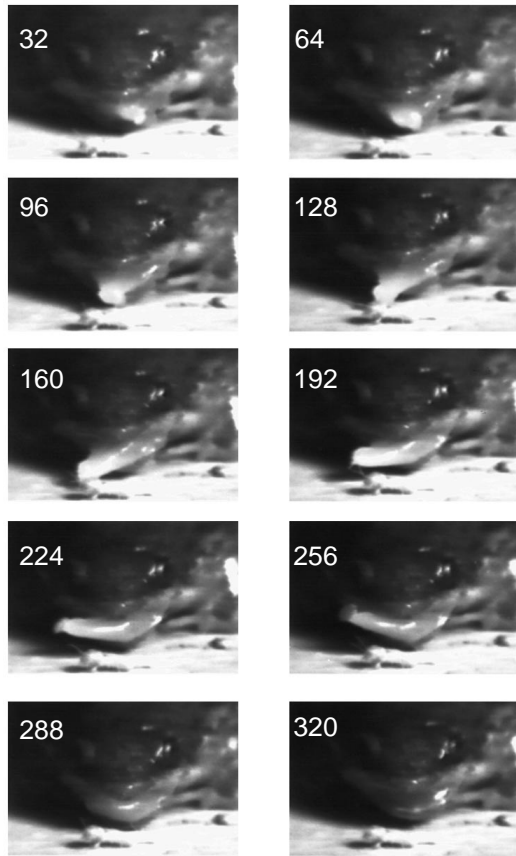
Fig. 10. Video frames taken from a head-on view, from an unsuccessful prey capture attempt after unilateral denervation of the right genioglossus muscle. Time, in milliseconds, is given in the lower right-hand corner of each frame. As the tongue is protracted, it begins to deviate towards the denervated side and is oriented at 90° to the long axis of the frog at 224 ms. The back of the left lateral flap of the tongue tip is visible at 256 ms.

The enhanced precision of tongue protraction in *H. marmoratum* is accompanied by a reduction in speed of protraction, but speed is unlikely to be critical in capturing termites. It is possible that increased precision is simply a corollary of increased protraction time; more adjustments are possible as the duration of movement increases. However, the increased precision of protraction, as well as the rapid onset of retraction, may be important in the ecology of *H. marmoratum* since termites that are in a group, or are moving along a tunnel, can be 'picked off' with precision without alerting other termites to the presence of the frog. Low light levels within a termite mound may make it necessary for *H. marmoratum* to feel for termites during protraction if it cannot see them. In such a situation, a rapid response to prey contact may be important, so that prey do not have time to respond to the presence of the tongue. The idea that these frogs are feeling for termites is supported by the comparison between normal captures and misses. If the tongue does not come into contact with prey, it continues to be protruded to its maximum length (Table 3). These data also suggest that the onset of tongue retraction in *H. marmoratum* is initiated by a tactile cue.

Previous studies suggest that frogs primitively are unable to aim their tongues relative to their heads in any dimension (i.e. distance, azimuth or elevation). Tongue length was not correlated with distance to prey in the archaeobatrachians *Ascaphus truei* (Nishikawa and Cannatella, 1991) and *Discoglossus pictus* (Nishikawa and Roth, 1991) or in a short-tongued neobatrachian (*Hyla cinerea*; Deban and Nishikawa, 1992). Furthermore, these frogs were never observed to aim the tongue relative to the head in terms of azimuth or elevation, although they do orient the head relative to the body. A long-tongued mesobatrachian, *Spea multiplicata*, is the only frog other than *H. marmoratum* that is known to modulate the distance of tongue protraction in response to prey distance (R. Smith and K. C. Nishikawa, unpublished data), but *Spea multiplicata* has never been observed to aim the tongue in either azimuth or elevation. Like *H. marmoratum*, microhylids generally possess the ability to control the azimuth of tongue movements relative to the head (Jaeger and Nishikawa, 1993). Microhylids are also similar to *H. marmoratum* in lacking interdigitation of the hyoglossus and genioglossus muscles in the tongue pad (Horton, 1982). These similarities may indicate close phylogenetic affinities between microhylids and *H. marmoratum*, or they may have evolved independently. Only *H. marmoratum* is known to control the elevation of the tongue relative to the head.

Tongue aiming in frogs offers an ideal model system in which to study the neural control of movement, especially as only two pairs of muscles (genioglossus and hyoglossus) are directly involved in moving the tongue relative to the head. Distance of tongue protraction could be controlled by varying the duration (Gottlieb *et al.* 1989) or the amplitude of contraction of tongue protractor muscles, or by varying the relative onset time of the tongue retractors (J. S. Liaw, A. Weerasuriya and M. A. Arbib, in preparation), or by some combination of the above. Although bilateral denervation of the retractors did not significantly increase maximum tongue reach, overshoot distance was significantly increased (Table 4). Thus, variation in the duration or amplitude of tongue protractor activity is likely to account for the majority of the correlation between tongue protraction distance and prey distance, while retractor activity may be responsible for the initial onset of tongue retraction.

The experiments involving unilateral denervation of the genioglossus muscles suggest that the azimuth of tongue movements is controlled by varying the relative activity (i.e. amplitude or duration) of the tongue protractors on the right and left sides, with recruitment being relatively greater on the side opposite to the direction of movement. Although the mechanical links between muscle contraction and tongue bending need further elucidation, the range of azimuthal control of the tongue approaches 360°. The elevation of the tongue tip is probably controlled by varying the relative recruitment of the protractors and retractors, with greater recruitment of the protractors tending to pull or push the tongue upwards and greater recruitment of the retractors pulling downwards on the tongue. This hypothesis is supported by the

Table 7. *ANCOVA (with initial prey distance as the covariate) testing the effect of unilateral genioglossus denervation*

Variable	Individual		Initial prey distance		Treatment	
	F	P	F	P	F	P
Duration of mouth opening (ms)	6.4	0.003*	0.1	0.717	5.1	0.015
Duration of tongue protraction (ms)	3.5	0.031	<0.1	0.824	3.0	0.069
Duration of tongue retraction (ms)	1.3	0.299	0.1	0.786	9.6	<0.001**
Duration of mouth closing (ms)	4.0	0.020	<0.1	0.873	15.0	<0.001**
Maximum gape (degrees)	0.4	0.753	2.6	0.122	9.4	0.001**
Maximum tongue reach (mm)	3.0	0.049	1.1	0.297	31.2	<0.001**

Only unsuccessful prey capture sequences are compared.

N=4 individuals (two left side, two right side), with four pre- and four post-denervation feeding trials per individual.

*Significant at $P \leqslant 0.05$; **significant at $P \leqslant 0.01$, after simultaneous Bonferroni adjustments.

observation that, when the retractors are bilaterally denervated, the tongue arcs upwards during retraction instead of following a relatively straight path back to the mouth. Elevational movements of the tongue are more restricted than azimuthal movements, in that they appear to be limited to less than 90°. The relative simplicity of the tongue musculature and the evolutionary diversity of aiming ability among species make frogs an excellent model system for further exploration of the biomechanical and neural bases of goal-directed movements.

Denervation experiments

The muscle denervation experiments tested not only muscle function in *H. marmoratum* specifically, but are also a test of conservation of muscle function in frogs as a group. One could argue that *H. marmoratum* supplies an especially strong test (*sensu* Smith, 1994) because its tongue movements differ markedly from those of other frogs. Historically, physiologists have looked to extreme cases to find the most general, conserved characteristics of a given system. *H. marmoratum* is an extreme example of the kinematic variation seen in anuran lingual protrusion.

Differences in kinematic variables before and after surgery could be due to the denervation of the muscles, to artifactual effects of anesthesia or surgery, or to some combination of these effects. However, the different denervation treatments showed no common effect, and, therefore, a general effect of anesthesia or surgery cannot account for the observed results. Furthermore, Deban and Nishikawa (1992) found no effect of sham surgeries, and we have used the same anesthesia and protocols. We therefore believe that the observed effects of the denervation experiments are caused by inactivation of the denervated muscle.

Following bilateral genioglossus denervation, the mouth opens normally and neither the time course nor magnitude of the gape differs from those of normal unsuccessful prey capture trials. However, since the tongue does not come out of the mouth, the genioglossus muscle is necessary for tongue protraction. The same result has been obtained in *Discoglossus pictus* (Nishikawa and Roth, 1991) and *Hyla cinerea* (Deban

and Nishikawa, 1992). It is noteworthy that *H. marmoratum* uses the same muscles to protract the tongue as other frogs, although it exhibits radically different tongue movements.
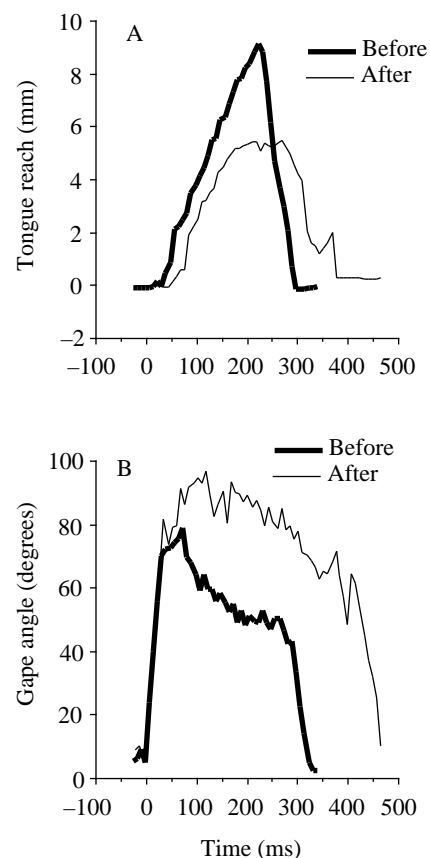

Fig. 11. Kinematic profiles from the same individual comparing unsuccessful prey capture attempts before and after unilateral denervation of the left genioglossus muscle. Initial prey distance was 8 mm for both the normal and the post-denervation trials. Bold lines show normal data, thin lines show post-denervation data. The graphs exemplify two of the significant treatment effects of unilateral genioglossus denervation: a shortened tongue reach (A) and an increased maximum gape (B).

After unilateral genioglossus denervation, the tongue of *H. marmoratum* deviates (sometimes more than 180˚) towards the denervated side. This result suggests that asymmetrical contraction of the genioglossus muscles may be involved in lateral aiming of the tongue. A similar phenomenon occurs in humans. When humans suffer a unilateral paralysis of the hypoglossal nerve, their tongue, upon protrusion, deviates towards the paralyzed side (Oppenheim, 1911). Although mammalian tongues are more complex because they contain intrinsic tongue muscles, the similarity of effect is noteworthy, especially given the phylogenetic distance between anurans and mammals. A different result is seen after unilateral genioglossus denervation in *Bufo marinus* (K. C. Nishikawa, unpublished data) and *Rana pipiens* (C. W. Anderson, unpublished data). In these species, unilateral genioglossus denervation results in a reduced amplitude of tongue protraction, but there is no lateral deviation of the tongue.

We began the denervation experiments with the hypothesis that the hyoglossus is the main retractor of the tongue. However, the tongue remains capable of retraction after bilateral denervation of the hyoglossus muscle, although the trajectory is altered (see below). The statistical analysis indicates that hyoglossus denervation results in significant differences in retraction kinematics. Before denervation, the tongue has already retracted approximately 1 mm, on average, between the time of prey contact and the video frame immediately following prey contact. Following denervation, the tongue continues to protract after prey contact is made. The most extreme effect was seen in post-denervation feeding trials in which prey contact actually resulted in the prey being knocked away from the frog. The difference in overshoot length is related to duration of tongue at target, which increased from less than 8 ms before surgery to 24 ms after surgery (although this difference was not significant after the Bonferroni adjustment, Table 4). Bilateral hyoglossus denervation also affected the trajectory of the tongue during retraction. In normal trials, the retraction trajectory is lower in elevation than the protraction trajectory. After hypoglossal denervation, it is significantly higher than the protraction trajectory (Fig. 7).

There are also qualitative differences after the hyoglossus muscle is denervated (Fig. 8). Among these are a loss of normal tongue tip function, a decrease in success of prey capture and difficulty in deglutition. Decreased prey capture success appears to be due to several factors. The altered trajectory of the tongue tip contributes to reduced prey capture success, as we often witnessed prey dislodged from the tongue tip because it struck the upper jaw tip during retraction (e.g. Fig. 8). However, the most significant factor in producing the decreased success of prey capture is the loss of the ability of the tongue tip to envelop the prey. The prey is no longer 'held' by the adduction of the lateral lobes of the tongue tips, but apparently adheres to the tongue solely due to weak wet adhesion.

The anatomy of the hyoglossus and the results of its denervation indicate that this muscle is involved in the prehensile function of the tongue tip. There is a well-defined fascicle of the hyoglossus muscle, oriented parallel to the long axis of the tongue, that inserts at the inner base of the lateral lobes of tissue (Fig. 3). Contraction of this muscle fascicle at the onset of retraction would pull the inner base of the lateral lobes towards the frog and pull the lobes of tissue towards one another (adduct them). Adduction of the lateral lobes, so that they come into contact with the prey, may enhance the 'hold' of the tongue on the prey through two mechanisms. Envelopment of the prey by the tongue tip increases the surface area of the tongue in contact with the prey, resulting in a corresponding increase in wet adhesion. The lateral lobes may also be forcefully adducted, so that the tongue tip grasps the termite by way of opposing forces.

An obvious question is how the tongue undergoes retraction after hyoglossus denervation. We believe that relaxation of the genioglossus muscle, in concert with recoil of elastic elements within the tongue, causes the tongue to be passively retracted into the mouth. In contrast to *H. marmoratum*, bilateral hyoglossus denervation in *Bombina orientalis*, *Bufo marinus* and *Phrynomerus bifasciatus* results in obvious deficits in tongue retraction (T. Tso, unpublished data). In these species, the denervated animals bite their tongues after failing to retract them fully.

Hypothesized mechanism of tongue protraction

Consideration of the gross anatomy of the tongue, the kinematics of prey capture and the results of the denervation experiments suggests that a hydrostatic mechanism may be involved in tongue protraction. Two observations, in particular, support this hypothesis: (1) the tongue is not flipped out of the mouth, but instead telescopes out of and back into the mouth following a relatively straight trajectory; and (2) unilateral denervation of the genioglossus muscle causes the tongue to bend through an angle of more than 180˚ towards the denervated side if prey contact is not made early in the feeding attempt. Given the absence of intrinsic tongue muscles in *H. marmoratum* and the fact that the fibers of the genioglossus muscle are oriented parallel to the long axis of the tongue, it is difficult to explain these observations on the basis of the pulling action of the genioglossus muscle alone, and we propose that a hydrostatic mechanism is involved.

In a typical muscular hydrostat, contraction of transverse, radial or circumferential muscles causes elongation by decreasing cross-sectional area. Contraction of longitudinal muscles causes shortening, with a concomitant increase in cross section (Kier and Smith, 1985). The tongues of many vertebrates, including lizards (Smith, 1984) and mammals (Kier and Smith, 1985), are examples of muscular hydrostats. In these animals, the tongue is composed of both extrinsic longitudinal muscles that retract the tongue and intrinsic circumferential muscles that protract it (Smith, 1984). *H. marmoratum*, like all other frogs that have been studied to date, possesses a tongue that is composed only of two pairs of extrinsic longitudinal muscles, the genioglossus and hyoglossus (Regal and Gans, 1976; Horton, 1982). This raises

the question of how frogs are able to protract their tongues without any intrinsic circumferential muscles.

In most frogs, the tongue is flipped over the mandible during protraction by contraction of the genioglossus muscle, which originates near the mandibular symphysis and inserts in the tongue pad (Gans and Gorniak, 1982*a*,*b*). Contraction of the genioglossus muscle elevates the tongue pad and pulls it forward towards the mandible. In frogs with short tongues, mechanical pulling of the genioglossus is sufficient to protract the tongue. In frogs with long tongues, sufficient inertia must be imparted to the tongue by contraction of the genioglossus muscle to allow the tongue to rotate over the mandibles and pass out of the mouth.

In *H. marmoratum*, it is unlikely that sufficient inertia is imparted to the tongue by mechanical pulling of the genioglossus, because the speed of protraction is so slow ($14\,\mathrm{cm\,s^{-1}}$). In contrast to other frogs, the tongue of *H. marmoratum* telescopes in and out of the mouth, rather than being flipped (Figs 4, 6, 8). The mechanism of tongue lengthening is likely to be hydrostatic because there are no muscles in the tongue that can lengthen it by mechanical pulling. However, the absence of transverse, radial or circumferential muscles makes a conventional muscular hydrostat model unlikely.

Instead, we hypothesize that tongue lengthening is due to a hydraulic mechanism, in which fluid is pumped into the tongue from some other location. Hydraulic systems are relatively common among invertebrates (Kier, 1988). The tube foot of echinoderms is a classic example of a hydraulic system (Wainwright *et al.* 1976). Like the tongue of *H. marmoratum*, the tube feet of echinoderms are composed only of longitudinal muscles that are responsible for retracting the tube foot. The tube foot is protracted when fluid is pumped into it by contraction of radial muscles in the wall of the ampullae.

An interesting difference between the tube feet of echinoderms and the tongue of *H. marmoratum* is that tube feet bend towards the active side (Wainwright *et al.* 1976), whereas the tongue of *H. marmoratum* bends towards the inactive side. It appears that bending is caused by differential pressure within the tongue, with lower pressure on the denervated side than on the intact side. Bending towards the denervated side would tend to equalize the pressure within the tongue.

The denervation experiments indicate that the genioglossus muscle plays a major role in tongue protraction. However, the source of the fluid that is moved into the tongue by contraction of the genioglossus muscle remains to be identified. It is possible that fluid is moved from the sinus at the base of the tongue into the sinus between the genioglossus and hyoglossus muscles, perhaps by contraction of the proximal genioglossus. The movement of fluid into the tongue would cause the tongue to elongate and would inflate the lateral lobes at the tongue tip (Fig. 4). This hydraulic mechanism, if correct, would represent a novel mechanism of tongue protraction in vertebrates. Further work on tongue anatomy, including a detailed histological analysis of sinuses and connective tissue, is needed to test this hypothesis.

The kinematics of feeding is perhaps the most divergent aspect of the feeding system of *H. marmoratum*. The telescoping, straight-line trajectory of the tongue, the prehensile tongue tip and the ability of this species to modulate the trajectory of the tongue in all dimensions throughout protraction appear to be unique to *H. marmoratum*. In contrast, the gross anatomy of the tongue and associated buccal musculature is similar to the usual condition seen in frogs. It is possible, however, that a more detailed anatomical investigation will reveal differences that explain more fully how tongue protraction is effected. It seems likely that novel connective tissue structures and/or orientations within the tongue may contribute to the divergent feeding kinematics observed in this frog.

Results of the muscle denervation experiments indicate a mixture of both conserved and derived functions for the genioglossus and hyoglossus muscles. The primitive role of the genioglossus muscle, protraction of the tongue, has been conserved, although the mechanism of tongue protraction appears to have changed from mechanical pulling to hydraulic inflation. The function of the genioglossus muscle has also been elaborated in *Hemisus*, resulting in the ability to aim the tongue laterally relative to the head. The function of the hyoglossus in this species has changed considerably from its primitive role, that of tongue retraction. In fact, *H. marmoratum* is capable of retracting its tongue without the hyoglossus muscle, although retraction is both quantitatively and qualitatively different after denervation of the hyoglossus. A derived aspect of the hyoglossus appears to be its role in adducting the lateral lobes of the tongue tip, giving the tongue tip its prehensile grasping ability. The hyoglossus also alters the trajectory of the tongue tip during retraction, a role that is probably a relic of the primitive function of this muscle.

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