

THE KINEMATICS OF PREY CAPTURE AND THE MECHANISM OF TONGUE PROTRACTION IN THE GREEN TREE FROG *HYLA CINEREA*

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Summary

Prey capture was studied in the green tree frog (*Hyla cinerea*) before and after denervation of either the m. genioglossus or m. submentalis using high-speed videography and kinematic analysis. The prey capture behavior and extent of tongue protraction of several members of the subfamilies Hyalinae, Pelodyadinae and Phyllomedusinae were also studied. Results show that the m. genioglossus is necessary to produce complete tongue protraction and that the m. submentalis is necessary for mandibular bending, but not necessary for complete tongue protraction in *Hyla cinerea*. The tongue of *Hyla cinerea* resembles the weakly protrusible tongues of the archaeobatrachian frogs *Ascaphus* and *Discoglossus* more than the highly protrusible tongues of other neobatrachians, such as *Rana* or *Bufo*. A weakly protrusible tongue is present in the subfamilies Hyalinae and Pelodyadinae, and a highly protrusible tongue is present in the subfamily Phyllomedusinae. These results suggest that hyline and pelodyadine hylids have retained the ancestral anuran tongue morphology and that highly protrusible tongues have evolved once within the family Hylidae, in the subfamily Phyllomedusinae.

Introduction

Studies of prey capture behavior in frogs (Emerson, 1977; Gans and Gorniak, 1982*a,b*; Nishikawa and Roth, 1991; Regal and Gans, 1976) have led to the formulation of several models of tongue protraction. These models ascribe roles in protraction to various muscles, including the mm. genioglossus medialis and basalis, the m. submentalis, the mm. geniohyoideus medialis and lateralis, and the m. sternohyoideus (Emerson, 1977; Gans and Gorniak, 1982*a,b*; Nishikawa and Roth, 1991; Regal and Gans, 1976).

Recent studies of tongue protraction in frogs have focused primarily on two

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muscles, the m. genioglossus and the m. submentalis. Using anatomy, electromyography and high-speed ciné photography, Gans and Gorniak (1982a,b) developed a model of tongue protraction for *Bufo marinus*, a neobatrachian with a highly protrusible tongue. In the Gans and Gorniak (1982a,b) model, the m. genioglossus medialis stiffens the tongue into a rod, while the m. genioglossus basalis forms a wedge at the base of this stiffened rod. The fibers of the paired m. genioglossus medialis originate on the mandibular symphysis and run rostro-caudally to fan out and insert into the tongue pad, interdigitating with fibers of the m. hyoglossus. The m. submentalis is a small, unpaired, ellipsoid muscle oriented transversely across the tips of the mandibles, just posterior to the mentomeckelian bones. In this model it acts on the m. genioglossus basalis to catapult the stiffened tongue out of the mouth. The m. submentalis contributes further to this catapult action by flexing the mandibles downwards at the mentomeckelian joint, thus lowering the base of the stiffened tongue. The mm. genioglossus basalis and medialis and the m. submentalis are all necessary for normal tongue protraction in the Gans and Gorniak (1982a,b) model.

Nishikawa and Roth (1991) tested the generalization of the Gans and Gorniak (1982a,b) model by denervating separately the m. genioglossus and m. submentalis in *Discoglossus pictus*, an archaeobatrachian with a weakly protrusible tongue. They used high-speed videography to analyze the kinematics of prey capture before and after denervation and demonstrated that the m. genioglossus is necessary for tongue protraction, while the m. submentalis is necessary for mandibular bending, but not for tongue protraction. Nishikawa and Roth (1991) proposed a model of tongue protraction similar to that of Regal and Gans (1976) in which the m. genioglossus contracts to thicken and pull the tongue pad forward and over the mandibular symphysis, while the m. submentalis plays no role beyond bending the mandible downwards.

Nishikawa and Roth (1991) determined that the Gans and Gorniak (1982a,b) model of tongue protraction does not apply to *Discoglossus pictus*. However, the model remains to be tested in a neobatrachian taxon, such as *Hyla*, which is more closely related to *Bufo*, for which the model was developed. A photograph by Vences (1988) reveals that *Hyla arborea* possesses not a highly protrusible tongue, as expected for a neobatrachian, but a small, weakly protrusible tongue. Vences' (1988) photograph and the findings of Nishikawa and Roth (1991) suggest that a study of the mechanism of tongue protraction in a less derived neobatrachian such as *Hyla* would contribute greatly to our understanding of the evolutionary transformations of the anuran feeding system.

Here we examine the prey capture behavior and mechanism of tongue protraction in the green tree frog *Hyla cinerea*. Using denervation experiments and high-speed videography, we determine the roles in tongue protraction of the m. submentalis and m. genioglossus with the goal of determining whether either of the above models applies to *Hyla cinerea*. In addition, we describe prey capture behavior and tongue protraction in several other hyliid species and explore the evolutionary implications of our findings.

Materials and methods

Twelve adult individuals of *Hyla cinerea* (snout–vent lengths 3.4–4.8 cm) were obtained from suppliers and maintained on a diet of waxworms (*Galleria* sp.), fruitflies (*Drosophila* sp.), crickets (*Gryllus* sp.) and mealworms (*Tenebrio* sp.). The frogs were assigned randomly to four treatment groups of three frogs each and then videotaped while feeding on waxworm prey. The treatments were: (1) denervation of the m. submentalis; (2) sham denervation of the m. submentalis; (3) denervation of the m. genioglossus; and (4) sham denervation of the m. genioglossus. Three prey capture sequences were obtained for each individual before and after surgery in all treatment groups, except for one individual that received sham denervation of the m. submentalis and was inadvertently killed when only two prey capture sequences had been obtained. Prey capture behavior was videotaped as soon after surgery as possible. An effort was made to videotape the first prey capture attempt of each frog after recovery from anesthesia, so that learned adjustments of behavior would be minimal. Prey capture behavior was also videotaped in six other species of the subfamily Hylinae (*Hyla arenicolor*, $N=4$; *Hyla eximia*, $N=7$; *Smilisca baudini*, $N=1$; *Pternohyla fodiens*, $N=1$; *Osteopilus septentrionalis*, $N=1$; and *Pseudacris triseriata*, $N=4$), one species of the subfamily Pelodyadinae (*Litoria infrafronata*, $N=1$) and two species of the subfamily Phyllomedusinae (*Phyllomedusa tarsius*, $N=1$; and *Pachymedusa dacnicolor*, $N=1$). A variety of prey items was offered, including waxworms, mealworms, crickets, grasshoppers and fruit flies.

Videotaping

The prey capture behavior of *Hyla cinerea* was videotaped at 120 fields per second with a Display Integration Technologies model 660 high-speed video camera with synchronized stroboscopic illumination and Panasonic AG-6300 VCR. A plate of glass covered with a moist paper towel served as the stage, with a grid of 1-cm squares positioned behind it for scaling and aspect ratio correction. The gray level of the backdrop was matched as closely to that of the animal as possible, to maximize the subject's clarity and detail. Videotaping was carried out at room temperature (20–24°C). The subject was positioned on the stage so that a lateral view of the frog was obtained. Waxworms were offered by forceps so that the frog would lunge across the field of view during prey capture. Prey were offered individually with the head of the waxworm facing the frog, about 2–3 cm in front of the frog, and were nudged so that they would move towards the frog and attract its attention. Experimentation with prey position and orientation showed that this technique was the most successful. This method reduced head tilting and turning by the frog during the lunge, as was often observed with other prey orientations. It was often necessary to orient the frog properly on the stage by strategic positioning of the waxworm.

Denervation

Each subject was placed in a shallow dish containing an aqueous solution of

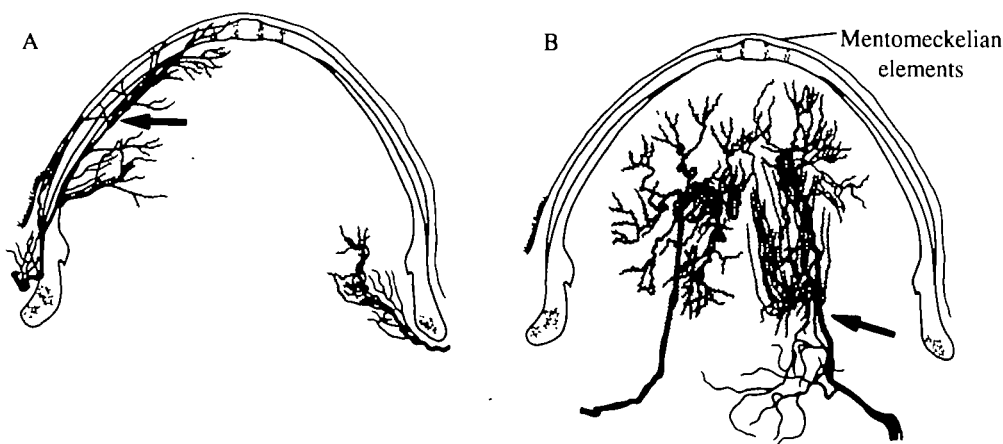


Fig. 1. *Camera lucida* drawing of the lower jaw of *Hyla regilla* stained with Sudan Black B. (A) Left, the ramus mandibularis of the trigeminal nerve. Arrow indicates site of denervation of the m. submentalis. Right, ramus jugularis of the facial nerve. (B) Left, the ramus lingualis of the glossopharyngeal nerve. Right, the ramus hypoglossus of the second spinal nerve. Arrow indicates site of denervation of the m. genioglossus. Note the mentomeckelian elements of the mandibular symphysis. Jaw width, 12.8 mm.

tricaine methanesulfonate (MS222, 1 gram dry mass per liter tap water). The frog remained partially immersed in the solution until breathing stopped, after about 30 min, whereupon it was removed and rinsed with tap water. The anesthetized frog was then placed, ventral side up, on a bed of moist paper towel on the stage of a dissecting microscope. The frog was moistened repeatedly with tap water throughout surgery to prevent desiccation.

The m. submentalis is innervated by the ramus mandibularis of the trigeminal nerve, which carries both sensory and motor fibers (Gaupp, 1896). Denervation of the m. submentalis was performed on the three frogs of treatment group 1. First, the skin along the margin of the lower jaw, just ventral to the eye, was cut with surgical scissors to form a small flap. This flap of skin was then reflected to reveal the spongy mesenchyme below. This tissue was pulled apart with fine forceps to reveal the nerves and blood vessels beneath. The ramus mandibularis (Fig. 1A) was then pulled from the opening in the tissue, the associated blood vessels were teased away from it, and a short (1–2 mm) section of the nerve was removed. The skin flap was then returned to its proper position and glued in place with Nexaband surgical adhesive (cyanoacrylate). This procedure was performed bilaterally, removing both sources of innervation to the m. submentalis. Sham submentalis denervation was performed on three frogs from treatment group 2. Anesthesia and surgery were identical to treatment group 1, but the nerve was not transected after it had been freed from the surrounding tissue.

The m. genioglossus is innervated by the ramus hypoglossus of the second spinal nerve, which carries both sensory and motor fibers (Stuesse *et al.* 1983). Three

frogs from treatment group 3 received surgical denervation of the m. genioglossus. A transverse incision (approximately 5 mm long) was made across the skin of the throat, and the flap was reflected. The underlying interhyoideus muscle was cut parallel to its fibers, directly above the hypoglossal nerve, which was visible through the translucent muscle tissue. The nerve was pulled through this incision, associated blood vessels were teased away, and a short (1–2 mm) section was removed from the nerve. The hypoglossal nerve was transected just proximal to the branch that innervates the m. genioglossus, but distal to the branch innervating the m. geniohyoideus (Fig. 1B). The procedure was performed bilaterally, through the same incision in the throat. The skin was then replaced and glued in place. Anesthesia and surgery were identical for the sham genioglossus denervation (treatment group 4), but the nerve was not transected after it had been dissected free of the surrounding tissue. After each of the above surgical procedures, the frog was rinsed with tap water and returned to its container until recovery, which usually occurred within 1 h.

After all four surgical treatments, prey capture sequences were videotaped for each frog. The frogs were then killed by over-anesthesia in MS222, and denervation was confirmed by dissection, after which they were fixed in 10 % formalin and stored in 70 % ethanol.

Kinematic analysis

The prey capture sequences of *Hyla cinerea* were digitized using a Panasonic AG 6300 VCR and Peak Performance Technologies two-dimensional motion analysis software running on a Rycom 2050 IBM-AT compatible computer. The sequences were digitized from the frame before the frog was first observed to move forward to the frame in which mouth closing was completed. The positions of 10 points on the frog's body were recorded directly from the video monitor for each frame of the feeding sequence (Fig. 2): (1) top of the eye, the lower edge of the upper eyelid; (2) bottom of the eye, the upper edge of the lower eyelid; (3) the naris; (4) tip of the upper jaw, the most anterior point along the lower edge of the upper jaw; (5) middle of the upper jaw, the point along the edge of the upper jaw midway between the tip of the upper jaw and the jaw joint; (6) the jaw joint, the point where the lower and upper jaws meet; (7) middle of the lower jaw, the point along the upper edge of the lower jaw midway between the tip of the lower jaw and the jaw joint; (8) tip of the lower jaw, the most anterior point along the upper edge of the lower jaw; (9) tongue height, the point on the tongue that is the maximum perpendicular distance above the line of the anterior lower jaw; and (10) tongue reach, the point on the tongue that is the maximum perpendicular distance beyond the line that connects the tips of the upper and lower jaws. One point was placed on the prey (11), and another was placed on the background to serve as a stable reference point.

Several angles and distances were calculated from the digitized points. Gape angle was measured as the interior angle formed by the midpoint of the upper jaw (point 5) and the midpoint of the lower jaw (point 7), with the jaw joint (point 6) at

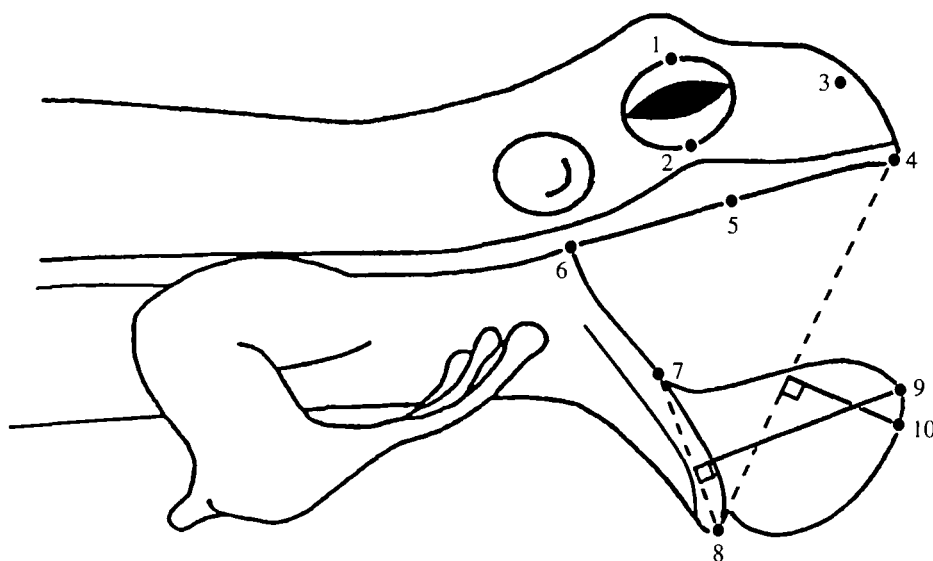


Fig. 2. Points used in kinematic analysis. These points were: (1) top of the eye; (2) bottom of the eye; (3) the naris; (4) tip of the upper jaw; (5) midpoint of the upper jaw; (6) the jaw joint; (7) midpoint of the lower jaw; (8) tip of the lower jaw; (9) tongue height; and (10) tongue reach. Tongue distance measurements are shown as solid lines. Tongue height is measured as the greatest perpendicular distance from line 7–8 to a point (9) on the tongue. Tongue reach is the greatest perpendicular distance from line 4–8 to a point (10) on the tongue.

the vertex (Fig. 2). Mandible angle was measured as the ventral angle formed by the lower jaw tip (point 8) and the jaw joint (point 6), with the midpoint of the lower jaw (point 7) at the vertex (Fig. 2). Distance to prey (point 4 to point 11) and gape distance (point 4 to point 8) were also measured. Two additional angles were measured to calculate tongue height and tongue reach (Fig. 2). Tongue height angle was formed by the midpoint of the lower jaw (point 7) and tongue height (point 9) with the tip of the lower jaw (point 8) at the vertex. Tongue reach angle was formed by the tip of the upper jaw (point 4) and tongue reach (point 10) with the tip of the lower jaw (point 8) at the vertex.

From these angles and distances, true tongue distances were calculated trigonometrically. Tongue height was calculated by multiplying the distance from the tip of lower jaw to tongue height (point 8 to point 9) by the sine of the tongue height angle (7, 8, 9). Tongue reach was calculated by multiplying the distance from lower jaw tip to tongue reach (point 8 to point 10) by the sine of the tongue reach angle (4, 8, 10).

The following additional variables were calculated for each prey capture sequence: (1) maximum gape distance; (2) maximum gape angle; (3) minimum mandible angle; (4) maximum tongue height; and (5) maximum tongue reach.

The times of the following events were recorded relative to the onset of mouth

opening ($t=0$): (1) first forward movement of the frog's body towards the prey; (2) first tongue visibility; (3) prey contact; (4) maximum tongue reach; (5) minimum mandible angle; (6) onset of tongue retraction; (7) maximum gape; (8) maximum displacement of head; (9) onset of mouth closing; (10) completion of tongue retraction; and (11) completion of mouth closing. From these, the following durations were calculated: (1) duration of mouth opening (same as time of event 7 above); (2) duration of tongue protraction (4 minus 2); (3) duration tongue remains at target (6 minus 3); (4) duration of tongue retraction (10 minus 6); (5) duration of mouth closing (11 minus 9); (6) duration mouth is open (same as event 11); and (7) duration of prey capture (11 minus 1). Lunge length was measured as the horizontal distance the upper jaw tip moves from the first frame of the sequence to the time of its maximum horizontal displacement. Overshoot distance is the horizontal distance the upper jaw tip moves from the time of prey contact to the time of its maximum horizontal displacement. Angular velocity of mouth opening and closing were also calculated.

Two variables were analyzed from the prey capture sequences of the other hylid species, as well as for *Bufo marinus* (Nishikawa and Gans, 1992), *Discoglossus pictus* (Nishikawa and Roth, 1991) and *Ascaphus truei* (Nishikawa and Cannatella, 1991): (1) maximum tongue reach, calculated as described above, and (2) lower jaw length, measured as the distance from the jaw joint (point 6) to the tip of the lower jaw (point 8). Relative tongue reach was calculated by dividing maximum tongue reach by lower jaw length. Severe head tilting during prey capture prevented relative tongue reach from being calculated for *Osteopilus septentrionalis*.

Statistical analysis

Selected kinematic variables were compared among treatment groups using an Apple Macintosh IIfx computer and Statview II software. One-way analysis of variance was used to test for significant differences among individuals in kinematic variables before surgery. For each individual, the means of selected kinematic variables were then calculated from three prey capture sequences before and after treatment. Paired t -tests were used to compare the means of kinematic variables for the same individuals before and after treatment. Unpaired t -tests were used to compare the means of kinematic variables for different individuals that received different surgical treatments. For both surgical treatments (m. submentalis and m. genioglossus), three comparisons were made. (1) Paired t -tests (two-tailed, $\alpha=0.05$) were used to compare kinematic variables before and after sham denervation. (2) Paired t -tests (one-tailed, $\alpha=0.05$) were used to compare kinematic variables before and after denervation. (3) Unpaired t -tests (one-tailed, $\alpha=0.05$) were used to compare kinematic variables after sham denervation with those after denervation. In the only treatment group (i.e. genioglossus denervation) that contained sequences of unsuccessful prey capture attempts, two additional comparisons were made. An unpaired t -test (two-tailed, $\alpha=0.05$) was used to compare misses after surgery with captures after surgery, and an unpaired

t-test (one-tailed, $\alpha=0.05$) was used to compare captures before surgery with captures after surgery. One-tailed tests were used when there was an *a priori* expectation about the direction of change in the variables, whereas two-tailed tests were used whenever there was no such *a priori* expectation.

Results

Kinematics of prey capture in Hyla cinerea

The following description of prey capture kinematics in *Hyla cinerea* is based on a sample of 12 individuals and 3 sequences per individual, giving a total of 36 sequences. Only successful prey captures were analyzed. Analysis of variance showed that only maximum gape distance ($F=2.860$, $P=0.0152$) and maximum tongue height ($F=2.895$, $P=0.0143$) differed significantly among individuals. The mean total time of prey capture, from time of first forward movement to completion of mouth closing, was 152 ± 8.1 ms. Values of kinematic variables reported below are averages unless otherwise noted. All times are relative to the onset of mouth opening at $t=0$.

Approach

Hyla cinerea catches prey by lunging forward and protracting the tongue pad. Just prior to initiation of a lunge, the toes of the hind feet are lifted from the substratum and quickly replanted. The lunge begins with the first forward movement of the frog's body towards the prey, which occurs 65 ± 5.0 ms before the onset of mouth opening (Table 1). As the frog approaches the prey, the eyes are partially retracted into the orbits.

Mouth opening and tongue protraction

As the mouth opens, the mandible begins to bend ventrally at the mentomeckelian joint. The tongue first becomes visible nearly simultaneously with the onset of mouth opening, and appears as a small, heart-shaped pad that is protruded 0.08–0.6 cm beyond the jaw tips and is raised 0.4–0.9 cm above the floor of the mouth (Table 1, Fig. 3A). Maximum tongue reach occurs on average at 38 ± 3.3 ms. Occasionally the tongue is protracted and retracted repeatedly during a single prey capture. Maximum gape angle occurs at 57 ± 4.7 ms and is $79\pm 2.2^\circ$. Maximum gape distance is 1.3 ± 0.03 cm.

Prey contact

The frog often contacts the prey before the tongue is fully protracted. Prey contact occurs at 29 ± 2.3 ms, at which time the head is inclined downwards. The tongue remains at the target for 32 ± 3.5 ms before the onset of tongue retraction (Table 1). After prey contact, the lower jaw tip often contacts the substratum and is held in place as the frog continues forwards. This has the effect of further

Table 1. *Minimum, maximum and mean (\pm S.E.) values of kinematic variables during prey capture for all individuals before sham or denervation surgery (N=12 individuals with 3 sequences per individual, for a total of 36 sequences)*

	Minimum	Maximum	Mean \pm S.E.
Time (ms)			
First forward movement	-133	17	-65 \pm 5.0
First tongue visibility	0	8	1 \pm 0.5
Prey contact	8	83	29 \pm 2.3
Maximum tongue reach	8	108	38 \pm 3.3
Minimum mandible angle	8	167	47 \pm 4.7
Onset of tongue retraction	33	142	61 \pm 4.3
Maximum displacement	33	250	82 \pm 8.7
Onset of mouth closing	25	208	85 \pm 6.2
Completion of tongue retraction	58	200	95 \pm 5.2
Completion of mouth closing	92	325	152 \pm 8.1
Duration (ms)			
Mouth opening	17	142	57 \pm 4.7
Tongue protraction	8	108	37 \pm 3.3
Tongue at target	8	117	32 \pm 3.5
Tongue retraction	17	67	34 \pm 2.3
Mouth closing	17	158	68 \pm 4.3
Prey capture	92	325	152 \pm 8.1
Maximum gape angle (degrees)	54	105	79 \pm 2.2
Minimum mandible angle (degrees)	142	167	155 \pm 1.0
Maximum gape distance (cm)	1.0	1.9	1.3 \pm 0.03
Distance to prey (cm)	1.0	5.8	2.5 \pm 0.20
Lunge length (cm)	1.6	6.3	3.3 \pm 0.21
Overshoot distance (cm)	0.05	1.4	0.6 \pm 0.05
Maximum tongue height (cm)	0.4	0.9	0.6 \pm 0.02
Maximum tongue reach (cm)	0.08	0.6	0.3 \pm 0.02
All times are relative to the onset of mouth opening ($t=0$).			

bending the mandibles and enlarging the gape (Fig. 4A). The mandibles bend downwards from an initial angle of approximately 180° to a minimum angle of 155 \pm 1.0°, which occurs at 47 \pm 4.7 ms (Table 1). Maximum gape occurs at the same time as, or slightly after, minimum mandible angle (Table 1).

Tongue retraction, mouth closing and body recovery

Tongue retraction begins at 61 \pm 4.3 ms and requires 34 \pm 2.3 ms for completion. Mouth closing begins at 85 \pm 6.2 ms and requires 68 \pm 4.3 ms for completion. The frog's body reaches maximum horizontal displacement at 82 \pm 8.7 ms. Distance to prey was 2.5 \pm 0.2 cm. The frogs lunge 3.3 \pm 0.21 cm, overshooting the prey by 0.6 \pm 0.05 cm. The forelimbs are used in the recovery to the original position and are often used to push prey into the mouth after the prey capture is complete. The

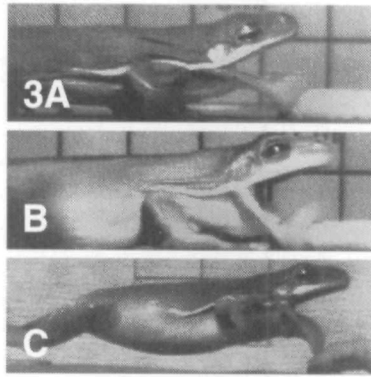


Fig. 3. Tongue protraction in *Hyla cinerea* (A) before surgery, showing bending of the mandibles, (B) after submental denervation, showing the decreased bending of the mandibles, and (C) after genioglossus denervation, showing greatly reduced tongue protraction.

toes of the hind feet usually remain planted on the substratum throughout prey capture, and the frog always returns to its starting position after all but the longest lunges (up to 6.3 cm), in which the hind feet leave the substratum.

Effects of genioglossus denervation

Frogs commonly missed the prey after genioglossus denervation. Five of the nine genioglossus denervation sequences analyzed were unsuccessful capture attempts. Thus, the effects of genioglossus denervation may have been confounded by the effects of capture success. In order to distinguish between the effects of denervation and the influence of capture success, an unpaired *t*-test was used to compare captures with misses after genioglossus denervation. Of the variables reported in Table 2, only gape angle was significantly smaller in misses ($71 \pm 3.2^\circ$) than in captures ($82 \pm 2.8^\circ$) ($t=2.643$, $P=0.0333$) (Fig. 4).

Maximum tongue height and maximum tongue reach were significantly reduced by genioglossus denervation (Table 2, Figs 3C and 4). Maximum tongue height was reduced from 0.60 ± 0.05 to 0.34 ± 0.02 cm ($P=0.0285$), and maximum tongue reach was reduced from 0.27 ± 0.03 to 0.11 ± 0.02 cm ($P=0.0074$). A *t*-test comparing before-denervation captures with after-denervation captures (misses excluded) also revealed that maximum tongue height ($t=3.043$, $P=0.0056$) and tongue reach ($t=3.324$, $P=0.0034$) were significantly reduced after genioglossus

Fig. 4. Selected kinematic profiles (A) before genioglossus denervation and (B) after genioglossus denervation from two prey capture sequences taken from the same individual. The vertical lines in A mark the time of prey contact. In the sequence after denervation, the prey was not contacted. Jaw movements are the vertical positions of the tips of the jaws. Note the extended durations of events after surgery compared with before. Both maximum tongue reach and maximum gape angle are significantly reduced after genioglossus denervation, but mandibular bending is unaffected.

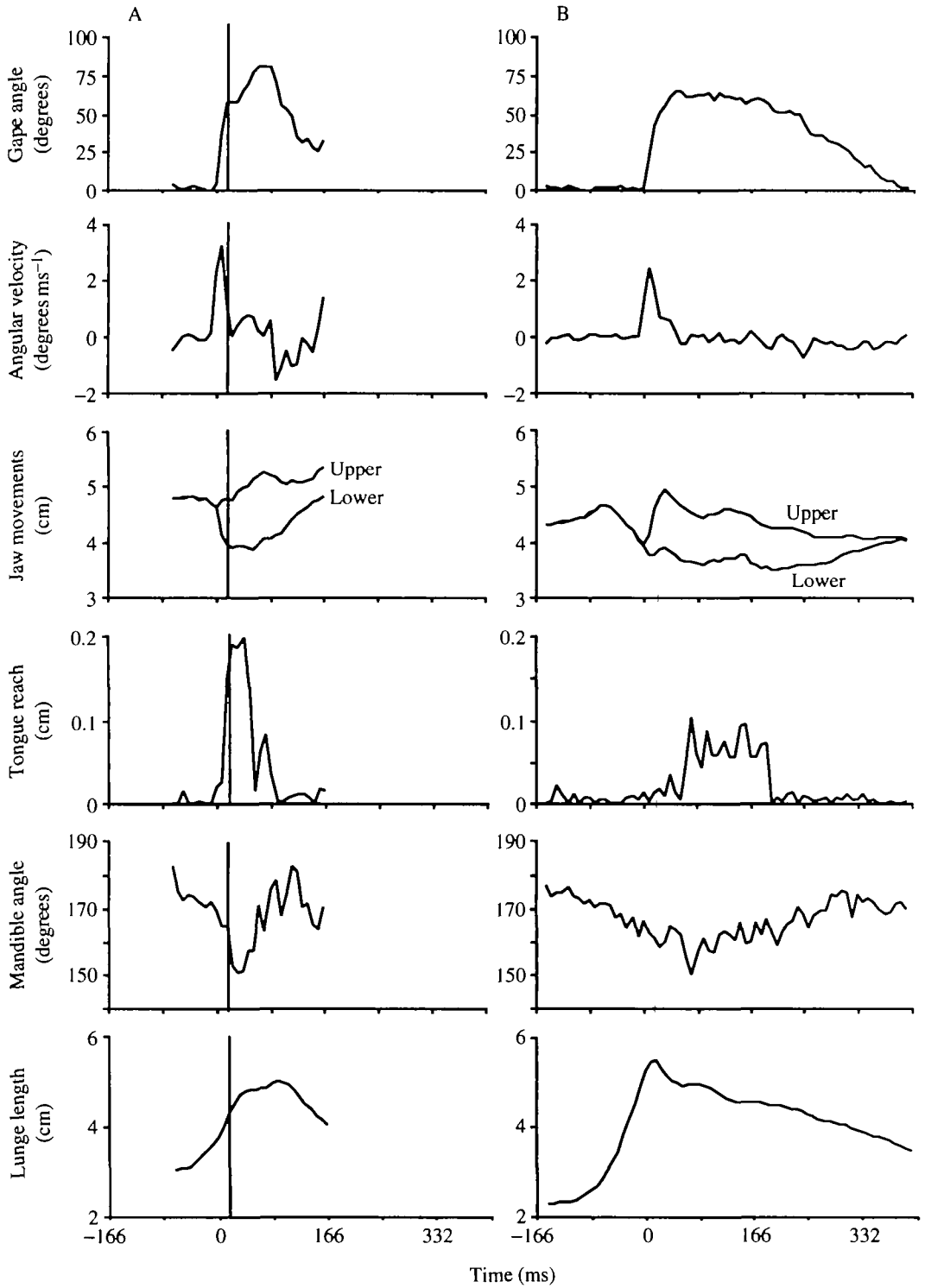


Fig. 4

Table 2. Means and standard errors of selected kinematic variables before genioglossus denervation (BD), before sham genioglossus denervation (BS), after sham genioglossus denervation (AS) and after genioglossus denervation (AD)

	BD	BS	AS	AD	BS vs AS	AS vs AD	BD vs AD
Time (ms)							
First forward movement	-55±7.9	-69±9.3	-84±9.8	-74±20.7	0.3368	0.3956	0.2915
Prey contact	30±4.2	26±3.5	22±2.8	33±8.2	0.6857	0.1374	0.3252
Maximum mandibular bending	45±6.7	61±14.5	41±7.0	59±12.7	0.2567	0.1934	0.1946
Duration (ms)							
Mouth opening	51±6.1	59±9.6	61±8.1	86±25.0	0.9458	0.2451	0.1751
Tongue protraction	43±6.2	28±4.4	30±6.1	81±22.6	0.7418	0.1046	0.1768
Tongue retraction	33±4.2	32±4.5	42±5.9	102±45.1	0.4685	0.1305	0.1235
Mouth closing	69±13.0	68±3.2	55±7.3	209±62.1	0.3392	0.0603	0.0973
Maximum gape angle (degrees)	87±3.7	73±4.7	77±3.2	76±2.9	0.4518	0.4469	0.0386*
Minimum mandible angle (degrees)	157±2.2	152±1.4	155±2.3	157±2.4	0.4984	0.4109	0.4633
Maximum tongue height (cm)	0.60±0.05	0.65±0.02	0.73±0.04	0.34±0.02	0.1587	0.0028*	0.0285*
Maximum tongue reach (cm)	0.27±0.03	0.27±0.04	0.32±0.04	0.11±0.02	0.0519	0.0162*	0.074*

P-values of *t*-tests comparing the treatments.

Asterisks indicate significance at the $\alpha=0.05$ level, $N=3$ individuals per treatment.

Times are given with respect to onset of mouth opening at $t=0$.

denervation. These analyses show that the decrease in maximum tongue height and reach cannot be explained as a result of decreased capture success. The only other change due to genioglossus denervation was a significant decrease in maximum gape angle from $87 \pm 3.7^\circ$ before denervation to $76 \pm 2.9^\circ$ after denervation (Table 2, BD vs AD, Fig. 4). A *t*-test comparing before-denervation captures with after-denervation captures (misses excluded) revealed that maximum gape angle was not significantly different.

There were no significant differences in any kinematic variables before *versus* after sham genioglossus denervation (Table 2, BS vs AS), which shows that neither anesthesia nor surgery affected the kinematics of prey capture. Furthermore, both maximum tongue reach and maximum tongue height were significantly lower after m. genioglossus denervation than after sham denervation. Tongue height was reduced from 0.73 ± 0.04 cm to 0.34 ± 0.02 cm and tongue reach was reduced from 0.32 ± 0.04 cm to 0.11 ± 0.02 cm (Table 2, AS vs AD).

Effects of submentalis denervation

All prey capture attempts after submentalis denervation were successful captures. Maximum gape angle was increased from $70 \pm 3.9^\circ$ before submentalis denervation to $83 \pm 3.7^\circ$ after denervation (Table 3, Fig. 5). Minimum mandible angle increased from $155 \pm 1.3^\circ$ before denervation to $164 \pm 2.6^\circ$ after denervation (Table 3, Figs 3B and 5). Thus, mandibular bending was reduced. No other kinematic variables were significantly affected.

There were no significant differences in any kinematic variables before *versus* after sham submentalis denervation (Table 3, BS vs AS), which shows that neither anesthesia nor surgery affected the kinematics of prey capture. Maximum tongue height and reach were significantly lower after submentalis denervation than after sham submentalis denervation. Tongue height was 0.75 ± 0.07 cm after sham submentalis denervation and 0.52 ± 0.05 cm after submentalis denervation, and tongue reach was 0.32 ± 0.05 cm after sham submentalis denervation and 0.18 ± 0.02 cm after submentalis denervation (Table 3, AS vs AD).

Prey capture in other frogs

The extent of tongue protraction and the prey capture behavior of the hyline hylids and of *Litoria infrafronata* (Pelodyadinae) are similar to that of *Hyla cinerea* (Fig. 6). Prey capture in all the hylids consists of a lunge towards the prey with protraction of the tongue. Relative tongue reach was calculated from tongue reach (TR) and lower jaw length (JL) for each species from the prey capture sequence in which maximum tongue protraction was elicited. The relative tongue reach values are as follows: *Hyla cinerea*, 0.46 (TR=0.56 cm, JL=1.2 cm); *Hyla arenicolor*, 0.17 (TR=0.20 cm, JL=1.2 cm); *Hyla eximia*, 0.28 (TR=0.27 cm, JL=0.97 cm); *Pseudacris triseriata*, 0.30 (TR=0.18 cm, JL=0.60 cm); *Pternohyla fodiens*, 0.21 (TR=0.40 cm, JL=1.9 cm); *Smilisca baudini*, 0.33 (TR=0.59 cm, JL=1.8 cm); *Phyllomedusa tarsius*, 1.5 (TR=2.3 cm, JL=1.5 cm); *Pachymedusa dacnicolor*, 1.5 (TR=3.6 cm, JL=2.4 cm); *Bufo marinus*, 1.9 (TR=4.9 cm,

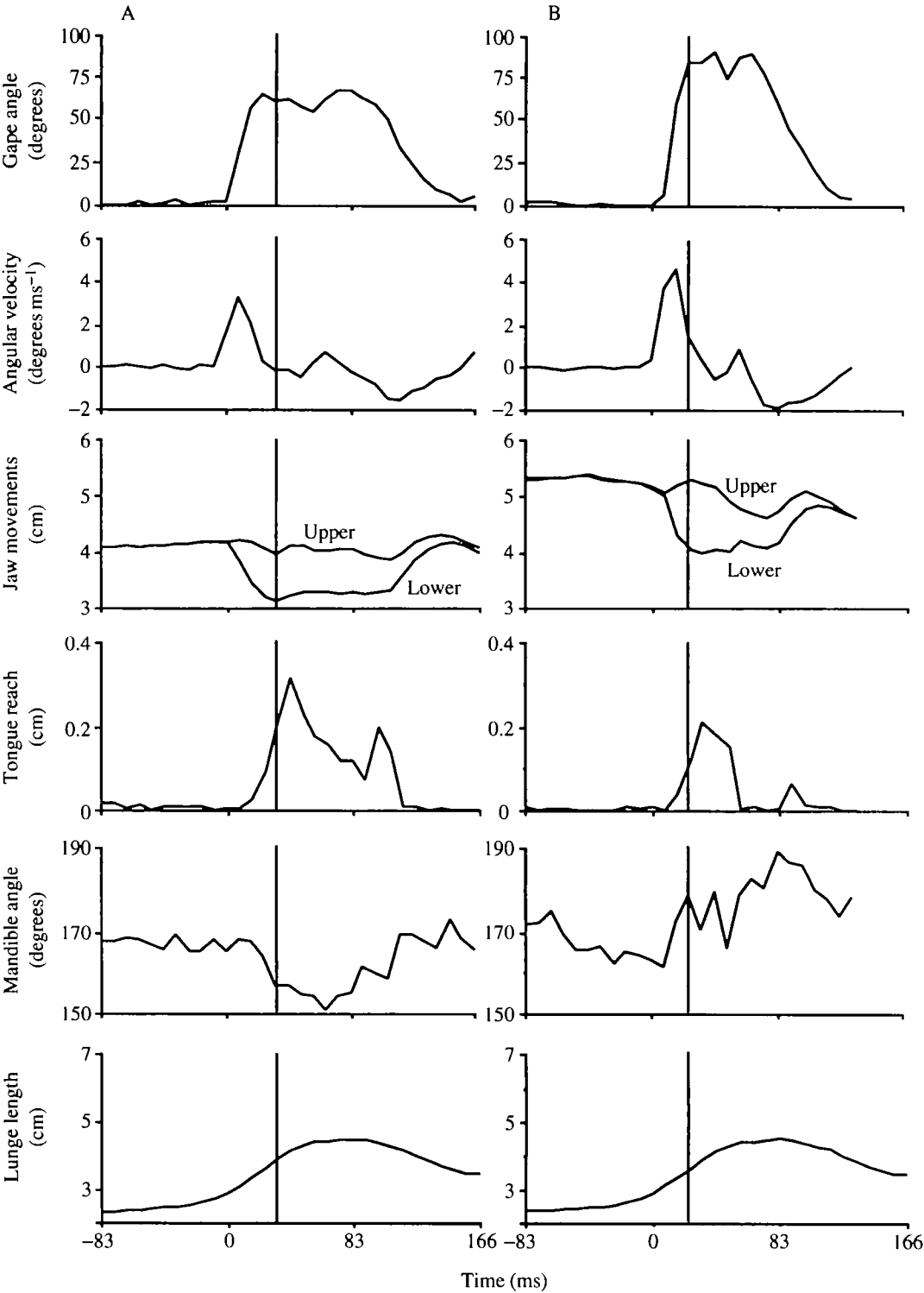


Fig. 5

Fig. 5. Selected kinematic profiles (A) before *m. submentalis* denervation and (B) after *m. submentalis* denervation from two prey capture sequences taken from the same individual. The vertical lines mark the times of prey contact. Jaw movements are the vertical positions of the tips of the jaws. The differences in initial jaw positions are postural. Mandibular bending is reduced significantly and maximum tongue reach is reduced slightly after denervation.

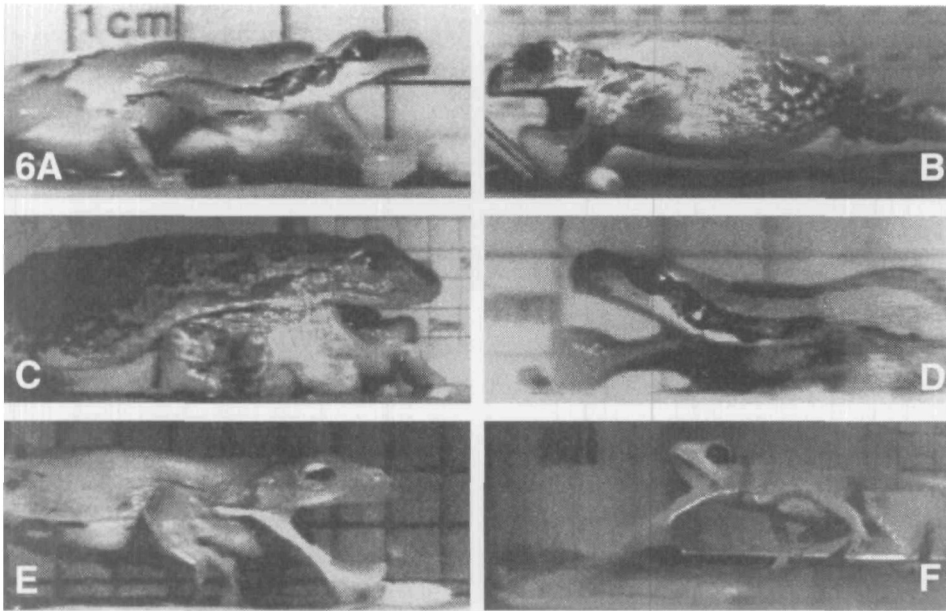


Fig. 6. Frames of sequences showing tongue protraction in (A) *Hyla eximia*, (B) *Smilisca baudini*, (C) *Pternohyla fodiens*, (D) *Pseudacris triseriata*, (E) *Litoria infrafrenata* and (F) *Phyllomedusa tarsius*. Note the long tongue of *Phyllomedusa* and the enlarged tongue base of *Pternohyla*. The tongue of *Pachymedusa dacnicolor* (not shown) resembles that of *Phyllomedusa*, and the tongues of *Hyla arenicolor* and *Osteopilus septentrionalis* (not shown) resemble that of *Hyla eximia*.

JL=2.6 cm); *Discoglossus pictus*, 0.22 (TR=0.24 cm, JL=1.1 cm) and *Ascaphus truei*, 0.50 (TR=0.55 cm, JL=1.1 cm) (Fig. 7). The tongue of *Pternohyla fodiens* differs notably from those of the other hylines, in that it has a more massive base (Fig. 6). The phyllomedusine hylids, *Phyllomedusa tarsius* and *Pachymedusa dacnicolor*, have extremely long tongues which resemble the tongue of *Bufo marinus* more than they do those of the other hylids (Figs 6 and 7).

Discussion

In its prey capture behavior and extent of tongue protraction *Hyla cinerea* resembles the archaeobatrachian frogs *Discoglossus pictus* (Nishikawa and Roth,

Table 3. Means and standard errors of selected kinematic variables before submental denervation (BD), before sham submental denervation (BS), after sham submental denervation (AS) and after submental denervation (AD)

	BD	BS	AS	AD	BS vs AS	AS vs AD	BD vs AD
Time (ms)							
First forward movement	-76±9.3	-62±13.3	-62±6.9	-52±9.5	0.9648	0.2736	0.0723
Prey contact	29±2.4	32±7.4	29±3.1	31±3.3	0.7851	0.3265	0.1127
Maximum mandibular bending	38±4.2	43±8.4	49±6.8	54±8.5	0.6488	0.3596	0.1606
Duration (ms)							
Mouth opening	48±5.5	72±13.5	62±11.6	56±9.3	0.6669	0.2370	0.1127
Tongue protraction	32±3.8	44±9.9	31±3.4	33±7.3	0.3287	0.4267	0.4631
Tongue retraction	35±2.7	36±6.7	41±8.8	30±5.2	0.7577	0.1954	0.2745
Mouth closing	58±6.9	76±9.0	69±8.6	69±13.1	0.2079	0.4784	0.1349
Maximum gape angle (degrees)	70±3.9	85±2.4	80±2.8	83±3.7	0.4927	0.3528	0.0161*
Minimum mandible angle (degrees)	155±1.3	152±2.1	153±12.6	164±2.6	0.6805	0.2806	0.0025*
Maximum tongue height (cm)	0.52±0.03	0.55±0.03	0.75±0.07	0.52±0.05	0.2394	0.0461*	0.5000
Maximum tongue reach (cm)	0.22±0.02	0.26±0.04	0.32±0.05	0.18±0.02	0.4713	0.0405*	0.0553

P-values of *t*-tests comparing the treatments.

Asterisks indicate significance at the $\alpha=0.05$ level, $N=3$ individuals per treatment.

Times are given with respect to onset of mouth opening at $t=0$.

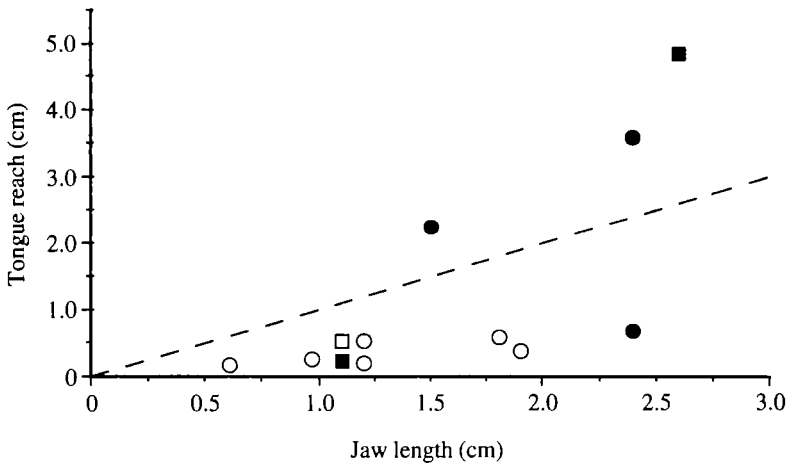


Fig. 7. Maximum tongue reach relative to jaw length. The hylines are shown as open circles, *Litoria infrafrenata* as a shaded circle, and the phyllomedusines as filled circles. *Discoglossus pictus* (filled square), *Ascaphus truei* (open square) and *Bufo marinus* (shaded square) are shown for comparison. The dashed line represents tongue reach equal to jaw length. Note the extremely long tongues of the phyllomedusines and the similarity of the hylines to the archaeobatrachians.

1991) and *Ascaphus truei* (Nishikawa and Cannatella, 1991). All three species exhibit a lunge towards the prey item accompanied by mandibular bending, downward flexion of the head and only slight protraction of the tongue pad beyond the tips of the jaws (Fig. 7). *Hyla cinerea* does, however, differ from the archaeobatrachians in the shape of its tongue, which is triangular in profile and rises up to 9 mm above the floor of the mouth, as a result of a posterior flap of tissue that is free from the mouth floor. The tongues of *Discoglossus pictus* and *Ascaphus truei* are rounded in profile and rise no more than 6 mm above the floor of the mouth (Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991).

The kinematics of mouth opening and closing, tongue protraction and retraction and mandibular bending are similar in all frog species examined so far (Nishikawa and Cannatella, 1991; Nishikawa and Roth, 1991; Nishikawa and Gans, 1992).

Effects of genioglossus denervation

The kinematics of prey capture in *Hyla cinerea* was unaltered by sham genioglossus denervation (Table 2, BS vs AS). Therefore, significant differences in kinematic variables before versus after genioglossus denervation must be the result of hypoglossal nerve transection rather than effects of anesthesia or surgery.

A comparison of selected kinematic variables before and after genioglossus denervation reveals a decrease in maximum gape angle (Table 2, BD vs AD, Fig. 4). Because five of the nine sequences after genioglossus denervation were unsuccessful prey capture attempts, the effects of missing the prey confound the effects of denervation. The *t*-test comparing sequences of successful prey capture

with those of unsuccessful capture attempts after genioglossus denervation reveals that maximum gape angle is significantly smaller in misses than in captures. However, a comparison of captures before denervation with captures after denervation shows no change in maximum gape angle. Although genioglossus denervation has no direct effect on gape angle, it does decrease capture success. This, in turn, produces a decrease in maximum gape angle. This conclusion makes sense, given that maximum gape angle occurs after prey contact (Table 1) and that prey contact enlarges the gape. The gape profiles from two representative sequences from the same individual (Fig. 4) clearly show that gape angle increases after prey contact before genioglossus denervation, but does not increase after denervation when the prey is missed.

Denervation of the m. genioglossus reduces maximum tongue height and maximum tongue reach (Table 2, BD vs AD, Fig. 4). Comparison of captures before denervation with captures after denervation also reveals reductions in maximum tongue height and reach, indicating that the reductions are due directly to genioglossus denervation and are not a result of decreased capture success. A comparison of misses with captures after genioglossus denervation shows no differences in maximum tongue height or reach, indicating that capture success has no effect on the extent of tongue protraction.

While it is tempting to refer to decreases in maximum tongue height and reach after denervation *versus* after sham denervation (Table 2, AS vs AD, Table 3, AS vs AD) as evidence for the effect of denervation, this may be misleading, because these comparisons are between individuals and do not control for the effect of individual variation. In fact, maximum tongue height was shown by a one-way analysis of variance to vary among individuals before surgery.

A muscle is considered necessary for a given movement if denervation of the muscle produces a deficit in the movement. Conversely, it is considered unnecessary if denervation produces no change. A muscle is considered sufficient for a function if denervation of that muscle eliminates the movement, or if all other muscles that could possibly contribute are denervated and no change is produced. Conversely, the muscle is not considered sufficient if other muscles are shown to be necessary. Denervation of the m. genioglossus produces a significant decrease in maximum tongue height and maximum tongue reach (Table 2, BD vs AD, Fig. 4), indicating that the m. genioglossus functions to raise the tongue pad and move it forward in the mouth. The m. genioglossus is therefore necessary for complete tongue protraction in *Hyla cinerea*. It cannot be considered sufficient, however, because tongue protraction was not entirely eliminated and not all tongue muscles were tested for necessity. This result is consistent with the results of Nishikawa and Roth (1991), who found that genioglossus denervation produced a significant decrease in tongue protraction in *Discoglossus pictus*.

Effects of submentalis denervation

The kinematics of prey capture were unaltered by sham submentalis dener-

vation (Table 3, BS vs AS). Therefore, significant differences in kinematic variables before and after submental denervation must be effects of trigeminal nerve transection, rather than effects of anesthesia or surgery.

Denervation of the m. submental produces significant increases in maximum gape angle and minimum mandible angle, but no significant reduction in either maximum tongue height or maximum tongue reach (Table 3, BD vs AD, Fig. 5). Because all prey capture sequences after submental denervation are successful captures, no confounding effects of capture success are present. The increased minimum mandible angle (=decreased bending) is consistent with the Gans and Gorniak (1982a,b) model of tongue protraction in *Bufo marinus*, but the non-significant reductions in maximum tongue height and reach are not. Their model predicts that submental denervation should eliminate tongue protraction. The increase in maximum gape angle may be due to behavioral compensation, in which the frog opens its mouth wider to offset a reduction in gape caused by decreased mandibular bending (see Fig. 5).

The m. submental is necessary for mandibular bending because denervation produces a deficit in bending. It is not sufficient for mandibular bending since not all bending is eliminated (Table 3, Fig. 5). The mm. depressor mandibulae, which have broad insertions on the mandibles and complex fiber orientation, may rotate the mandibles inward and create an additional effect of mandibular bending. The m. submental is neither necessary nor sufficient for tongue protraction, because its denervation produces no significant reduction in maximum tongue height or reach. These results are consistent with the findings of Nishikawa and Roth (1991), who demonstrated that denervation of the m. submental produced a significant decrease in mandibular bending, but no significant decrease in either maximum tongue height or reach in *Discoglossus pictus*. Based on their denervation experiments, Nishikawa and Roth (1991) proposed a model of tongue protraction in which the m. genioglossus contracts, pulling the tongue pad forward towards the mandibular symphysis. Contraction of the m. genioglossus causes the tongue to thicken and thus rise up above the floor of the mouth. Contraction of the m. submental produces mandibular bending, but plays no role in tongue protraction *per se*. This model can be applied to *Hyla cinerea* with one addition: the tongue height of *Hyla cinerea* is further increased by the posterior flap of tissue that is free from the floor of the mouth. This, and the nearly significant reduction in maximum tongue reach after submental denervation, suggests that mandibular bending plays a greater role in the tongue protraction of *Hyla cinerea* than in *Discoglossus pictus*.

Although maximum tongue reach is not significantly reduced by submental denervation and the m. submental cannot be considered necessary for complete tongue protraction in *Hyla cinerea*, the slight reduction in tongue reach can be explained by the interaction of tongue shape with mandibular bending. The rounded tongue of *Discoglossus pictus* rises only 6 mm above the floor of the mouth, so mandibular bending has a negligible effect in rotating the tongue pad beyond the tips of the jaws (Nishikawa and Roth, 1991). The triangular tongue of

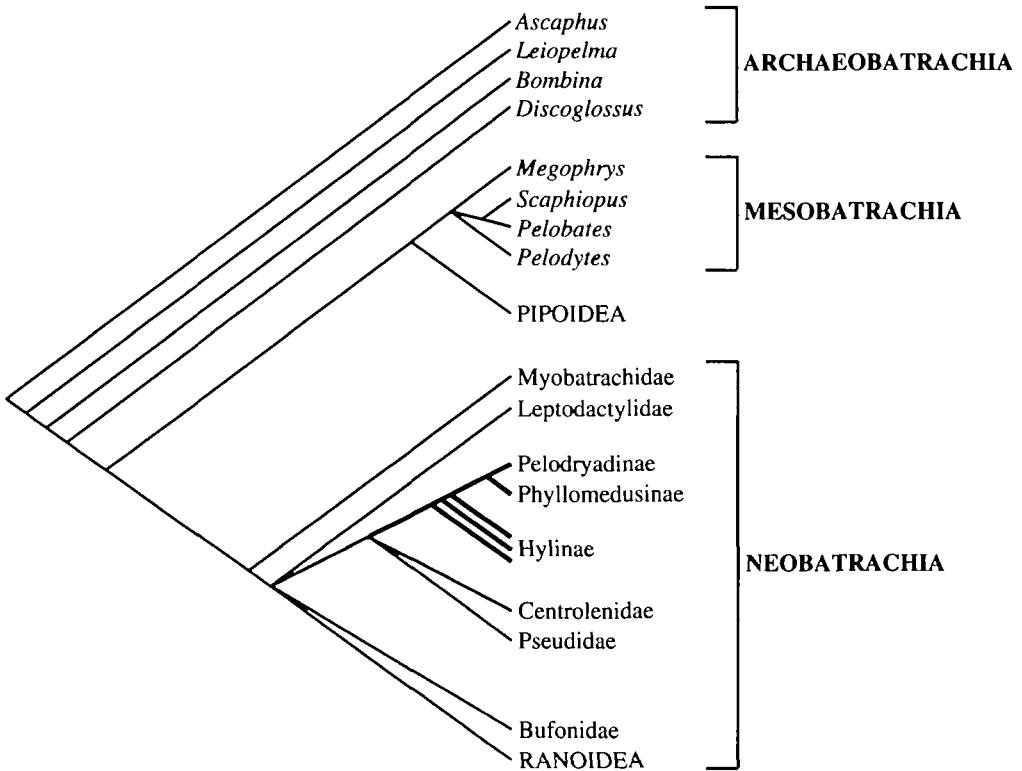


Fig. 8. Simplified phylogeny of the Anura, modified from Duellman and Trueb (1986) and Cannatella (1985). The family Hylidae is shown in bold lines. The subfamily Hylinae is probably polyphyletic (D. C. Cannatella, personal communication).

Hyla cinerea rises up to 9 mm above the floor of the mouth, so mandibular bending may have the effect of rotating the tongue pad beyond the tips of the jaws and contributing to tongue reach. In addition, mandibular bending is greater in *Hyla cinerea* than in *Discoglossus pictus*, contributing to this effect. Although not significant, the measured reduction in tongue reach following submentalis denervation suggests that mandibular bending does play a role.

The mechanism of tongue protraction in *Hyla cinerea* is not consistent with the Gans and Gorniak (1982a,b) model for tongue protraction in *Bufo marinus*, in which the m. genioglossus stiffens the tongue, while the m. submentalis rotates the tongue out of the mouth. In *Hyla cinerea*, the m. genioglossus raises the tongue pad and moves it forward in the mouth, while the m. submentalis plays no significant role, as in *Discoglossus pictus* (Nishikawa and Roth, 1991).

A weakly protrusible tongue and lunging forward with the whole body have been observed in *Ascaphus truei*, the hypothesized sister group to all living frogs (Fig. 8), and these features are considered ancestral for anurans (Nishikawa and Cannatella, 1991). The prey capture behavior of *Hyla cinerea* and a number of

other hylid species described here resembles that of *Ascapheus truei* and another archaeobatrachian, *Discoglossus pictus*. The extent of tongue protraction of all hylines examined and of *Litoria infrafronata* (Fig. 7) resembles that of the archaeobatrachians. However, the extent of tongue protraction of the phyllomedusines resembles that of *Bufo marinus* more than it does that of the other hylids. From these observations we draw three conclusions. First, the family Hylidae has retained the ancestral prey capture behavior of lunging. Second, the subfamilies Hyalinae and Pelodyadinae have slightly modified the ancestral anuran tongue, but retain limited tongue protrusibility. Third, a highly protrusible tongue has evolved within the family Hylidae, in the subfamily Phyllomedusinae.

Three observations support the conclusion that the subfamilies Hyalinae and Pelodyadinae have retained the ancestral tongue rather than secondarily lost highly protrusible tongues. (1) Anderson (1990, in preparation) has observed that *Rana pipiens* uses its highly protrusible tongue and no lunging to capture small prey (waxworms), but lunges and protrudes its tongue only slightly when feeding on larger prey (earthworms). This implies that the long tongue of *Rana pipiens* does not limit its ability to feed on large prey. This is further illustrated by *Rana catesbeiana*, which possesses a long tongue and yet is notorious for taking large prey (Brooks, 1964). (2) Phyllomedusines possess highly protrusible tongues, and yet, like the hylines and the pelodyadines, lunge and take relatively large prey. (3) Nishikawa and Gans (1990, 1992) described a feedback system in *Bufo marinus* that coordinates mouth opening with tongue protraction. This system triggers inhibition of the m. levator mandibulae when the m. genioglossus is activated and may be necessary for tongue protraction in frogs with fast prey capture cycles or long tongues. When the m. genioglossus is denervated in *Bufo marinus*, the toad fails to open its mouth during a prey capture attempt. This effect of genioglossus denervation is not observed in *Hyla cinerea*, implying that it lacks this sensory feedback system (Deban and Nishikawa, 1990), which it would presumably possess if its ancestor possessed a highly protrusible tongue. Thus, it seems unlikely that hylines and pelodyadines have secondarily reduced tongues and more likely that they have retained the ancestral tongue morphology. If we accept that the family Hylidae is monophyletic, and that the subfamily Hyalinae is basal to the other hylids (Fig. 8), we must conclude that the highly protrusible tongue of the subfamily Phyllomedusinae evolved independently within the family Hylidae and is not homologous with that of *Bufo*.

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