

Mechanism of Tongue Protraction During Prey Capture in the Spadefoot Toad *Spea multiplicata* (Anura: Pelobatidae)

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ABSTRACT Recent studies have used muscle denervation experiments to examine the function of muscles during feeding in frogs. By comparing the results of denervation experiments among taxa, it is possible to identify evolutionary changes in muscle function. The purpose of this study was to examine the function of jaw and tongue muscles during prey capture in *Spea multiplicata*, a representative of the superorder Mesobatrachia. All members of this group possess a disjunct hyoid apparatus. We predicted that *Spea* would possess a novel mechanism of tongue protraction on the basis of its hyoid morphology. High-speed video motion analysis and muscle denervation were used to study the feeding behavior and mechanism of tongue protraction in *Spea*. Although *Spea* possesses a relatively long tongue, its feeding behavior is similar to that of short-tongued frogs of similar body size. Denervation of the m. submentalis had no effect on feeding behavior. When the m. geniohyoideus was denervated, the tongue pad was raised and moved forward slightly, but did not leave the mouth. When the m. genioglossus was denervated, the tongue pad was raised slightly, but no forward movement of the tongue occurred. A similar result was obtained after the mm. genioglossus and geniohyoideus were denervated simultaneously. Thus, both the mm. genioglossus and geniohyoideus are necessary for normal tongue protraction in *Spea*. In contrast, only the m. genioglossus is necessary for normal tongue protraction in archaeobatrachians and neobatrachians. We hypothesize that the disjunct hyoid is responsible for the greater role of hyoid movement during feeding in mesobatrachians. © 1995 Wiley-Liss, Inc.

Evolution has produced a bewildering array of morphology and function in organisms. However, relatively little is known about the neuromuscular basis for functional shifts in evolution. On one hand, we might expect that morphological changes would be associated with changes in neuromuscular control. Alternatively, morphological change alone may produce functional shifts, and little or no change may be required in patterns of muscle activation.

One way to study the neuromuscular basis for functional shifts in evolution is to compare patterns of muscle activity among morphologically divergent organisms. Several studies have found that patterns of muscle activity are often conserved across major morphological transitions (Goslow et al., '89; Lauder and Shaffer, '88; Wainwright et al., '89).

An alternative approach to studying functional shifts in evolution is to compare muscle function among morphologically divergent organisms. By comparing the results of muscle denervation experiments among divergent taxa, it is possible to identify evolutionary changes in muscle function. Recent studies have used denervation experiments

to examine the function of jaw and tongue muscles during feeding in frogs (Nishikawa and Roth, '91; Deban and Nishikawa, '92; Nishikawa et al., '92; Ritter and Nishikawa, '95). These studies have identified the function of a number of muscles involved in prey capture in basal frogs, as well as evolutionary transitions among derived neobatrachians. The purpose of this study is to examine the function of jaw and tongue muscle during prey capture in a representative of a previously unstudied lineage of frogs, the mesobatrachians.

The southern spadefoot toad, *Spea multiplicata*, is a member of the superorder Mesobatrachia (Ford and Cannatella, '93), a group containing five families with diverse feeding specializations (Fig. 1). The Mesobatrachia includes the pipoids (families Pipidae and Rhinophrynidae) and pelobatoids (families Pelodytidae, Pelobatidae, and Megophryidae). Mesobatrachians are an es-

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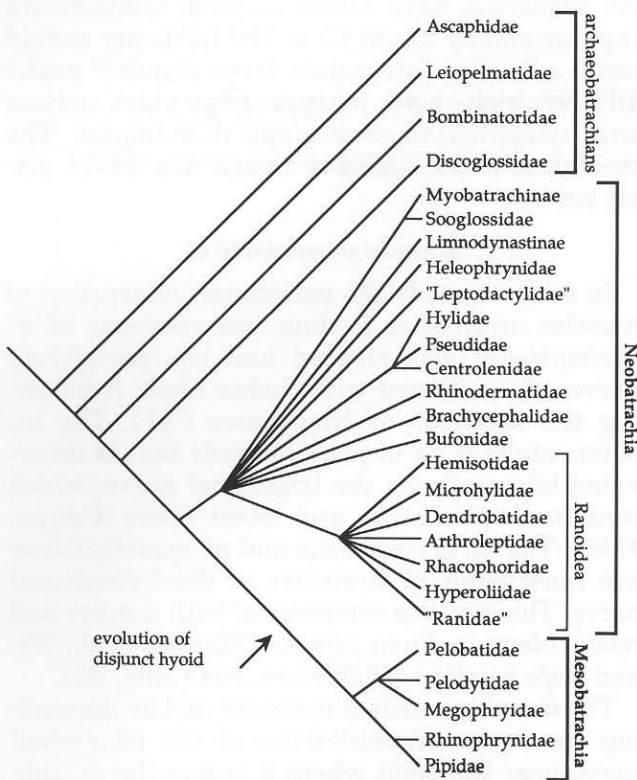


Fig. 1. Phylogeny of the Anura modified from Ford and Cannatella ('93). The arrow indicates the evolution of a disjunct hyoid at the base of the mesobatrachian clade. The "archaeobatrachians" are a grade group that share many plesiomorphic characteristics but are not closely related to each other. The names of families that are probably not monophyletic are enclosed in quotation marks.

pecially interesting group in which to study the mechanism of tongue protraction because they are the only anurans that possess a disjunct hyoid apparatus (Cannatella, '85; Fig. 1). In mesobatrachians, a gap develops in each cornua during metamorphosis (Ridewood, 1897), which frees the hyoid from its attachment to the skull. In all other anurans, the cornua of the hyoid plate are fused to the prootic region of the cranium. In the toad *Bufo marinus* (family Bufonidae), the hyoid appears to play little, if any, role in tongue protraction (Gans and Gorniak, '82a), although cineradiography shows that the hyoid is protracted during feeding (Emerson, '77). We hypothesized that the disjunct hyoid of mesobatrachians might be associated with a unique mechanism of tongue protraction in this group.

Whereas recent studies have described the prey capture behavior of several archaeobatrachian (Nishikawa and Cannatella, '91; Nishikawa and

Roth, '91) and neobatrachian frogs (Anderson, '93; Deban and Nishikawa, '92; Gans and Gorniak, '82a,b), there have been relatively few studies of feeding behavior in mesobatrachians. Prey capture has been studied in the secondarily aquatic pipids *Xenopus laevis* (Avila and Frye, '77, '78) and *Hymenochirus boettgeri* (Sokol, '69), both of which feed only in water and appear to be ram feeders (i.e., they accelerate the head toward the prey) and suction feeders (i.e., they accelerate the prey toward the head), respectively (J.C. O'Reilly, personal communication). Trueb and Gans ('83) studied the feeding apparatus of the terrestrial rhinophrynid *Rhinophrynus dorsalis* anatomically and using muscle stimulation. Based on these studies, they concluded that *Rhinophrynus* may possess a unique tongue protraction mechanism in which the hyoid is involved in tongue protraction.

Although there have been relatively few studies of terrestrial prey capture in pelobatoid frogs, several studies (Magimel-Pelonnier, '24; Regal and Gans, '76; Horton, '82) have reported on the anatomy of the feeding apparatus in mesobatrachians. Based on anatomy, the feeding apparatus of *Pelobates* and *Pelodytes* appears to be similar to that of short-tongued archaeobatrachians such as *Ascaphus* (Magimel-Pelonnier, '24; Horton, '83). A still photo of feeding behavior in *Pelobates fuscus*, published by Vences ('89), also suggests that *Pelobates* possesses a short tongue. In contrast to pelobatids and pelodytids, the Asian leaf frogs (genus *Megophrys*) of the family Megophryidae possess long, ping-pong paddle-shaped tongues (Regal and Gans, '76; Gans et al., '91). The kinematics of terrestrial prey capture and the mechanism of tongue protraction have not been described for any species of pelobatid frog. It remains to be determined whether the North American pelobatids possess short tongues like *Pelobates*, or whether they possess long tongues like *Megophrys*, in which case long tongues may have evolved twice among the Mesobatrachia.

The goals of the present paper are 1) to describe the kinematics of prey capture in *Spea multiplicata*; 2) to compare the results with observations from other frogs; and 3) to use bilateral denervation experiments to test the hypotheses that three different muscles, including the hyoid protractor (m. geniohyoideus), are necessary for normal tongue protraction in *Spea*. Based on hyoid morphology, we predict that the m. geniohyoideus should be necessary for normal tongue protraction in mesobatrachians, but not in archaeobatrachians and neobatrachians.

To test these hypotheses, we performed four experimental treatments: 1) bilateral denervation of the m. submentalis, an unpaired muscle that bends the mandibles downward during feeding in *Discoglossus* (Nishikawa and Roth, '91) and *Hyla* (Deban and Nishikawa, '92), and closes the nares during breathing in *Bufo marinus* (Gans and Pyles, '83); 2) bilateral denervation of the m. genioglossus, which originates near the mandibular symphysis, inserts in the tongue pad, and is the major tongue protractor muscle of *Discoglossus* (Nishikawa and Roth, '91) and *Hyla* (Deban and Nishikawa, '92); 3) bilateral denervation of the m. geniohyoideus, which originates on the hyoid, inserts near the mandibular symphysis (Gans and Gorniak, '82b), and is responsible for protracting the hyoid (Emerson, '77; Gans and Gorniak, '82b); and 4) simultaneous bilateral denervation of the mm. genioglossus and geniohyoideus.

MATERIALS AND METHODS

Adult *Spea multiplicata* were collected from Yavapai County, Arizona, on July 14, 1990. Feeding sequences were videotaped from July 16 through November 29, 1990. In all, 56 successful and 73 unsuccessful capture attempts were videotaped for 11 individuals before surgery (Table 1). For each frog in each treatment group (before vs. after surgery and capture vs. miss), the first sequences in which the long axis of the frog was oriented 90 ± 10 degrees with respect to the camera were digitized and analyzed kinematically. Although we attempted to analyze at least three sequences per treatment, this was not always possible either because too few sequences were videotaped or because some of the videotaped sequences were not digitizable (i.e., the frog was not oriented with the long axis of the body perpendicular to the camera, or some part of the frog's head was obscured from view). A total of 36 successful and 17 unsuccessful feeding sequences was digitized before surgery, whereas 24 successful and nine unsuccessful feeding sequences were digitized after surgical denervation of selected muscles. The total number of sequences videotaped and analyzed kinematically for each individual in each treatment is given in Table 1.

Prior to videotaping, individuals were placed on a stage covered with a moist paper towel and were allowed to habituate for 1–2 min. A 1-cm grid was placed against the rear of the stage. Waxworms (*Galleria* sp., approximately 1 cm in length) were placed 2–4 cm in front of the frog with forceps.

All sequences were filmed at room temperature (approximately 22–23°C) at 120 fields per second using a Display Integration Technologies™ model DIT 660 high-speed, multi-framing video camera with synchronized stroboscopic illumination. The resolution of the digitized image was 36–44 pixels per cm.

Muscle denervation

In order to study the peripheral innervation of muscles involved in feeding, one specimen of *S. multiplicata* was cleared and the peripheral nerves were stained with Sudan black B following the methods of Nishikawa ('87). The m. submentalis is an unpaired muscle that is innervated bilaterally by the trigeminal nerve, which contains both sensory and motor fibers (Gaupp, 1896). The m. genioglossus and m. geniohyoideus are innervated by branches of the hypoglossal nerve. This nerve is composed of both sensory and motor fibers in *Rana pipiens* (Stuesse et al., '83) and *Bufo marinus* (Nishikawa and Gans, '92).

The m. submentalis was denervated by transecting the ramus mandibularis of the trigeminal nerve near the point where it enters the m. submentalis. The m. genioglossus was denervated by transecting the lateral branch of the distal hypoglossal nerve, whereas the m. geniohyoideus was denervated by transecting the medial branch (Fig. 2). For simultaneous denervation of the mm. genioglossus and geniohyoideus, the hypoglossal nerve was transected proximal to the point of bifurcation of the medial and lateral branches. Individuals were anesthetized by immersion in 10% ethanol for approximately 20–25 min, after which the peripheral nerves were exposed and a 1-mm section was removed. After 30–60 min, the frogs recovered from anesthesia and began to feed. Feeding sequences were videotaped both before and after bilateral denervation of either the m. submentalis (n = 3 individuals), m. genioglossus (n = 3 individuals), m. geniohyoideus (n = 2 individuals), or both the m. genioglossus and m. geniohyoideus simultaneously (n = 3 individuals).

Previous studies have shown that frogs learn to compensate for the effects of denervation of the tongue muscles (Innocenti and Nishikawa, '94). Learning begins after about 15–20 trials and continues gradually over the next several weeks. After 3–5 weeks (depending upon the size of the frog), the nerve reinnervates the muscle and tongue protraction increases dramatically, although it never completely returns to normal. Because of the complications caused by learning and

TABLE 1. Total numbers of sequences videotaped (V) and analyzed kinematically (K) for each of the 11 individuals included in the study for all treatments¹

Frog	Before surgery		SM		GH		GG		GH and GG	
	V	K	V	K	V	K	V	K	V	K
1	6C 28M	4C 3M	4C 1M	3C						
2	6C 0M	4C	4C 1M	4C						
3	5C 1M	4C	3C 0M	3C						
4	4C 2M	3C			3C 38M	3C				
5	6C 7M	3C 3M			4C 15M	3C				
6	5C 2M	3C					5C 40M	2C		
7	4C 6M	3C 3M					6C 21M	3C		
8	4C 1M	3C					3C 78M	3C		
9	8C 21M	3C 4M							95M	4M
10	3C 4M	2C 4M							73M	4M
11	5C 1M	4C							2M	1M
Total	56C 73M	36C 17M	11C 2M	10C	7C 53M	6C	14C 139M	8C	170M	9M

¹SM, bilateral denervation of the m. submentalis; GH, bilateral denervation of the m. geniohyoideus; GG, bilateral denervation of the m. genioglossus; GH and GG, bilateral denervation of the mm. geniohyoideus and genioglossus; C, capture; M, miss.

regeneration, it is important to obtain kinematic data as soon as possible after surgery. In the present study, filming sessions began immediately following recovery from anesthesia after surgery. For each frog in each treatment, the first successful, digitizable captures after surgery were digitized, except for the simultaneous mm. genioglossus and geniohyoideus treatment, for which the first digi-

tizable misses were analyzed because the frogs never captured prey after surgery.

In previous studies (Deban and Nishikawa, '92), control surgeries were performed for each denervation treatment. For each control, the frogs received anesthesia and exposure of nerves as for denervation treatments, but the nerves were not transected. The results showed that there were no effects of anesthesia or surgery on feeding kinematics. In the present study, the different denervation treatments were used as each other's controls because the surgical approach was identical for the three hypoglossal nerve transection experiments. Only the nerve branch that was transected differed among these treatments.

Kinematic analysis

Videotapes were analyzed using Peak Performance Technologies™ 2D motion analysis software. The X, Y coordinates of nine points on the head of the frog, as well as a point on the prey and a stationary reference point, were digitized on the video monitor. Kinematic variables were analyzed as in previous studies (Nishikawa and Cannatella, '91; Nishikawa and Roth, '91). The time at which each of the following events occurred was measured relative to the onset of mouth opening ($t = 0$): 1) first forward movement; 2) onset of tongue protraction; 3) minimum mandibular bending; 4) prey contact; 5) maximum tongue protraction; 6) onset of tongue retraction; 7) maximum gape; 8) completion of tongue retraction; 9) maximum forward displacement of upper jaw tip; 10) onset of mouth closing; and 11) completion of mouth closing.

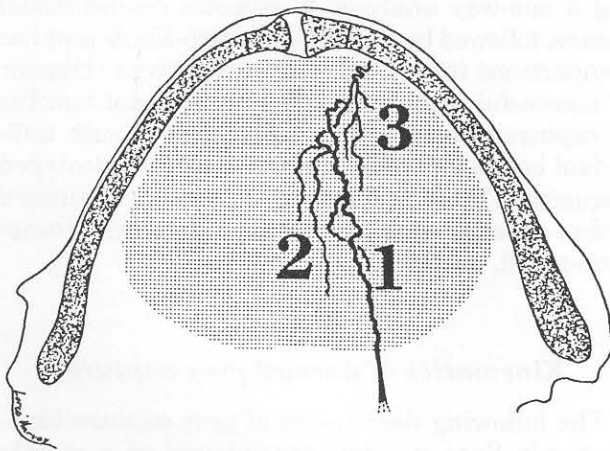


Fig. 2. Camera lucida drawing of the hypoglossal nerve in the tongue of *Spea multiplicata* (jaw width = 20 mm). The tongue was stained with Sudan black B following the methods of Nishikawa ('87). (1) The most proximal branch of the hypoglossal nerve shown here innervates the m. hyoglossus. The hypoglossal nerve bifurcates distally. After the bifurcation, the two medial branches (2) innervate the m. geniohyoideus, and all of the lateral branches (3) innervate the m. genioglossus. The outline of the tongue pad is indicated by stippling.

From these variables, the following variables were calculated as follows: 1) duration of approach = time of maximum forward displacement of upper jaw tip - time of first forward movement; 2) duration of mouth opening = time of maximum gape - onset of mouth opening; 3) duration of tongue protraction = time of maximum tongue protraction - onset of tongue protraction; 4) duration of tongue at target = onset of tongue retraction - time of prey contact; 5) duration of tongue retraction = completion of tongue retraction - onset of tongue retraction; 6) duration of mouth closing = completion of mouth closing - onset of mouth closing; 7) duration of recovery = completion of mouth closing - time of maximum displacement of upper jaw tip; and 8) duration of feeding sequence = completion of mouth closing - time of first forward movement.

In addition, the following variables were calculated from the digitized points: 1) maximum gape angle = maximum angle subtended by the upper jaw tip and the lower jaw tip with the jaw joint at the vertex; 2) minimum mandible angle = minimum angle subtended by the lower jaw tip and the jaw joint with the midpoint of the lower jaw at the vertex (at rest, the minimum mandible angle is approximately 176 degrees); 3) maximum gape distance = maximum distance between the upper jaw tip and the lower jaw tip; 4) maximum tongue reach = maximum distance from the tongue tip to the line formed by the upper and lower jaw tips; 5) maximum relative tongue reach = maximum tongue reach divided by lower jaw length; 6) maximum tongue height = maximum perpendicular distance from the top of the tongue pad to the midpoint of the lower jaw; 7) distance to prey = the distance from the upper jaw tip to the prey before approach (field 1); 8) lunge length = distance between the upper jaw tip at rest and at its maximum forward displacement; and 9) overshoot distance = distance between the upper jaw tip at prey contact and the maximum displacement of the upper jaw tip.

Statistical analysis

Statistical analyses of prey capture kinematics before and after surgery were based on digitized sequences only (Table 1). A one-way analysis of variance was used to examine differences in feeding kinematics among individuals for successful captures before surgery. Paired *t*-tests (one-tailed, $\alpha = 0.05$) on individual means were used to analyze differences in movement kinematics between

captures and misses before surgery for five individuals (Table 1). Misses included sequences in which the prey was not captured, regardless of whether or not the tongue made contact with the prey. Before surgery, the frogs made contact with the prey in 12 of 17 misses, whereas contact was made with the prey in all misses after surgery. Pearson product-moment correlation coefficients were used to test for relationships between kinematic variables and distance to prey for all successful captures before surgery.

To investigate the effects of muscle denervation treatments, data were analyzed using paired *t*-tests (one tailed, $\alpha = 0.05$) on individual means before vs. after surgery. For the mm. submentalis, genioglossus, and geniohyoideus denervation treatments, successful captures before surgery were compared to successful captures after surgery (Table 1). However, none of the frogs was able to feed successfully after simultaneous mm. genioglossus and geniohyoideus denervation (Table 1). For two individuals, misses before surgery were compared to misses after surgery. However, one individual (No. 11, Table 1) never missed the prey before surgery. For this individual, it was necessary to compare successful captures before surgery to misses after surgery.

The effects of the four denervation treatments on maximum relative tongue reach were compared using a one-way analysis of variance on individual means, followed by Student-Newman-Keuls post hoc comparisons ($\alpha = 0.05$). Capture success (number of successful captures divided by the total number of capture attempts) was measured for each individual before and after surgery from all videotaped sequences (Table 1). Capture success was compared before vs. after each treatment using paired *t*-tests (one-tailed, $\alpha = 0.05$).

RESULTS

Kinematics of normal prey capture

The following description of prey capture kinematics in *Spea multiplicata* is based on a sample of 36 successful prey capture sequences from 11 individuals, with two to four sequences per individual (Table 1). All times are relative to the onset of mouth opening ($t = 0$). Values of kinematic variables reported below are averages unless otherwise noted. The mean duration of the feeding sequence was 240 ± 6.1 ms.

Spea multiplicata catches prey by lunging forward, opening the mouth, and protracting the tongue (Figs. 3, 4). The lunge begins before the

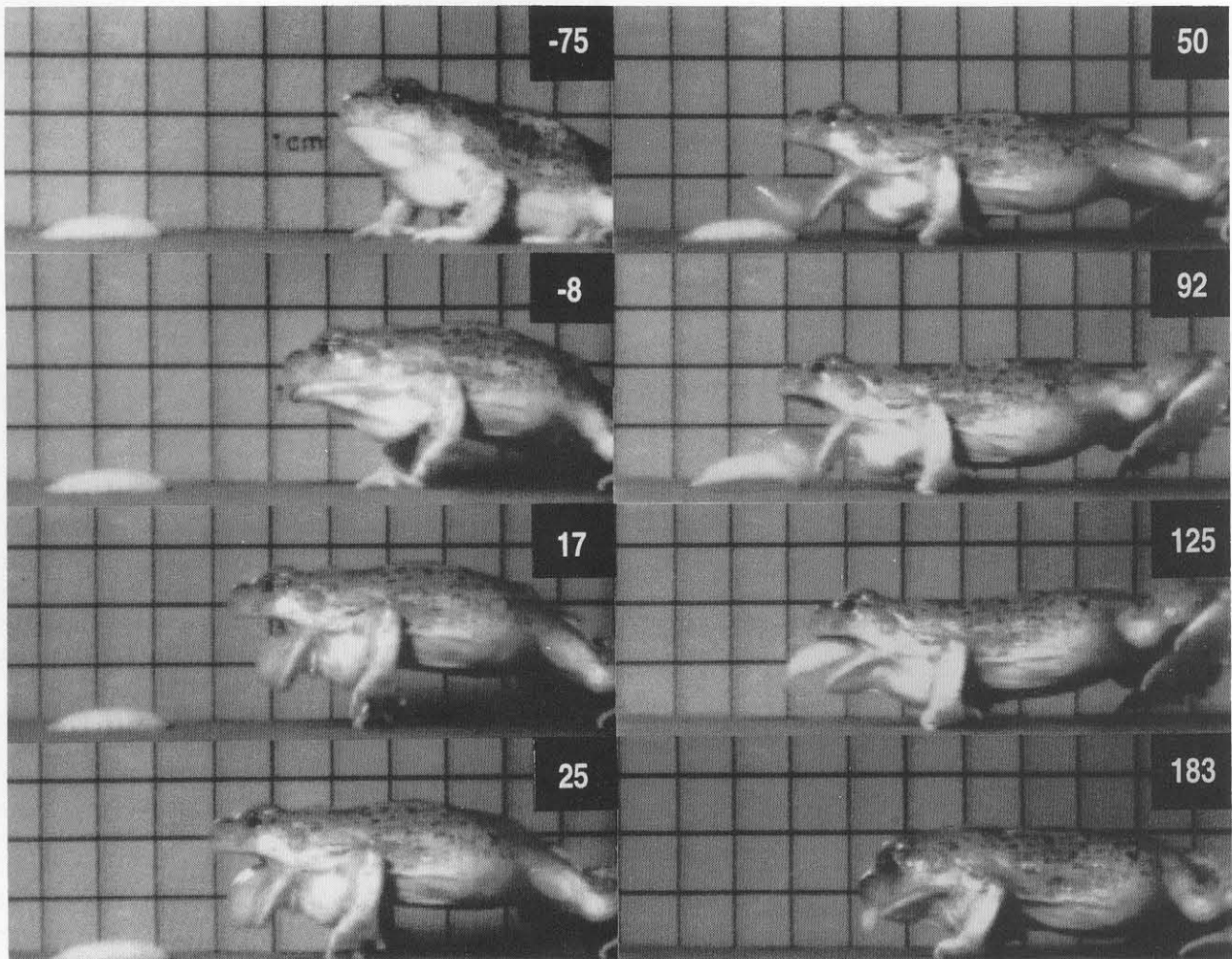


Fig. 3. Selected frames from a normal feeding sequence. Numbers indicate time (ms) relative to the onset of mouth opening ($t = 0$). At -75 ms, the spadefoot toad begins moving forward toward the waxworm larva. Onset of mouth opening and tongue protraction are simultaneous. At 17 ms, the tongue pad has begun to rise in the floor of the mouth. The tongue pad moves forward at 25 ms. The toad contacts the prey at 50 ms, has begun to retract the tongue at 92 ms, has begun to close the mouth at 125 ms, and completes mouth closing at 183 ms.

onset of mouth opening (Table 2). As the frog approaches the prey, the eyes are partially retracted into the orbits (Fig. 3). In anurans generally, movement of the mandibular symphysis relative to the cranium is complicated by the fact that the mandible possesses a movable joint between the mentomeckelian bones and the dentary. Thus, mandibular depression results from a combination of movement of the mandible relative to the cranium (mandibular depression) and movement of the mentomeckelian bones relative to the mandible (mandibular bending). In *Spea*, the lower jaw bends slightly downward at rest (Fig. 3). However, there is no additional bending of the man-

dible at the mentomeckelian joint as the mouth opens (Figs. 3, 4). The minimum mandible angle is never less than 175 degrees (Fig. 4).

The round tongue pad appears in the floor of the mouth shortly after the onset of mouth opening (Fig. 3). The frog contacts the prey with the tongue prior to maximum tongue protraction. At this time, the head is inclined slightly downward. The greatest tongue protraction distance was 0.83 times the lower jaw length. The prey adheres to the sticky tongue, and is transported into the oral cavity by the retraction of the tongue. The jaws then close on the prey, after which the gape cycle is repeated as the prey is transported to the

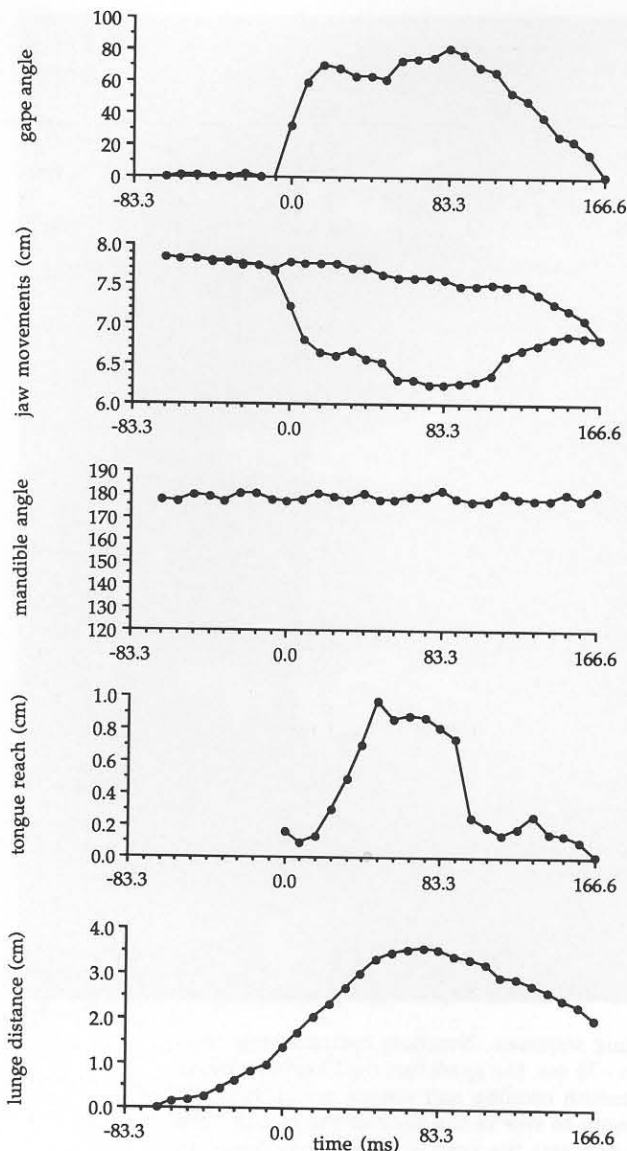


Fig. 4. Kinematic profile for the normal feeding sequence shown in Figure 3. Jaw movements are the vertical positions of the tip of the upper jaw (upper line) and the tip of the lower jaw (lower line). Other variables are defined in the text.

esophagus and finally is swallowed whole. Several transport cycles may be required before deglutition occurs. In three sequences, the tongue was protracted and retracted twice during a single gape cycle. The forelimbs are used during recovery to the original position and are often used to manipulate the prey after prey capture has been completed. The toes of the hind limbs usually remain planted on the substratum throughout the prey capture sequence. The frog always returns to its starting position, except in the longest

lunges, in which the hind feet leave the substratum (Fig. 3).

Several kinematic variables differed significantly among individuals for successful captures before surgery, including the duration of tongue protraction ($F = 3.32$, $P = .0073$), the duration of tongue retraction ($F = 6.50$, $P = .0001$), the duration of mouth closing ($F = 3.32$, $P = .0072$), the duration of recovery ($F = 3.57$, $P = .0047$), and the duration of the feeding sequence ($F = 3.21$, $P = .0086$). Maximum gape angle ($F = 3.16$, $P = .0094$), maximum tongue reach ($F = 4.07$, $P = .0021$), maximum tongue height ($F = 4.12$, $P = .0019$) maximum relative tongue reach ($F = 3.44$, $P = .0059$) and jaw length ($F = 3.03$, $P = .0118$) also varied significantly among individuals. Some but not all of the variation among individuals appears to be due to differences in body size. Maximum gape angle ($r = -0.49$, $P < .01$), minimum mandible angle ($r = 0.38$, $P < .05$), gape distance ($r = 0.39$, $P < .05$) and tongue reach ($r = 0.42$, $P < .05$) were significantly correlated with body size as estimated by jaw length.

Several kinematic variables were correlated significantly with distance to prey. Duration of approach ($r = 0.63$, $P < .01$), duration of the feeding sequence ($r = 0.41$, $P < .05$), and lunge distance ($r = 0.91$, $P < .01$) were positively correlated with distance to prey, whereas duration of mouth opening ($r = -0.4$, $P < .05$) and duration of tongue at target ($r = -0.36$, $P < .05$) were negatively correlated with distance to prey. Maximum tongue reach ($r = 0.39$, $P < .01$) was also positively correlated with prey distance (Fig. 5), which indicates that *S. multiplicata* possesses the ability to modulate its tongue protraction distance in response to prey distance.

Comparisons between successful and unsuccessful prey capture attempts before surgery were performed using paired *t*-tests on individual means ($n = 5$ individuals). Time of maximum tongue protraction ($t = -3.7$, $P = .0104$) and time of maximum forward displacement ($t = -2.24$, $P = .0443$) were delayed in unsuccessful capture attempts compared to successful captures. Duration of tongue protraction ($t = -2.89$, $P = .0224$) was significantly longer in unsuccessful than in successful capture attempts.

Muscle denervation experiments

Denervation of the m. submentalis had no effect on any of the variables except duration of approach, which decreased after surgery (Fig. 6A; Table 3). No significant mandibular bending oc-

TABLE 2. Kinematics of normal feeding behavior before surgery for successful ($n = 36$) and unsuccessful ($n = 17$) capture attempts¹

Variable	Capture		Miss		<i>t</i>	<i>P</i>
	Mean	SE	Mean	SE		
Onset of forward movement (ms)	-56	3.5	-56	5.1	0.55	.3063
Onset of tongue protraction (ms)	6	0.7	6	1.0	-0.98	.1905
Time of prey contact (ms)	44	1.7	53	2.1	-1.43	.1131
Time of max. tongue protraction (ms)	58	2.3	69	2.6	-3.7	.0104
Onset of tongue retraction (ms)	81	2.6	82	2.9	-0.57	.3008
Time of maximum gape (ms)	74	3.5	80	4.7	-0.15	.4444
Completion of tongue retraction (ms)	118	3.7	162	11.4	-1.82	.0719
Time of max. forward displacement (ms)	70	2.0	79	1.3	-2.24	.0443
Onset of mouth closing (ms)	86	3.9	98	4.6	-0.83	.2273
Completion of mouth closing (ms)	184	5.3	198	9.3	-1.02	.1822
Duration of approach (ms)	126	3.4	136	5.8	-1.16	.1561
Duration of mouth opening (ms)	74	3.4	80	4.7	-0.24	.4111
Duration of tongue protraction (ms)	53	2.3	63	2.5	-2.89	.0224
Duration of tongue at target (ms)	37	2.6	31	2.9	0.18	.4328
Duration of tongue retraction (ms)	37	2.6	81	11.9	-1.87	.0673
Duration of mouth closing (ms)	98	5.9	99	8.7	-1.07	.1733
Duration of recovery (ms)	114	4.8	119	8.9	-0.7	.2609
Duration of feeding sequence (ms)	240	6.1	255	11.9	-0.91	.2064
Maximum gape angle (°)	94	1.9	96	2.3	-0.17	.4357
Maximum gape distance (cm)	1.8	0.04	1.8	0.06	-0.08	.4686
Minimum mandible angle (°)	176	0.2	175	0.4	-0.01	.4977
Maximum tongue height (cm)	0.8	0.04	0.8	0.03	0.25	.4065
Maximum tongue reach (cm)	0.7	0.04	0.7	0.06	-0.44	.3423
Maximum relative tongue reach	0.5	0.03	0.6	0.02	-1.87	.0677
Distance to prey (cm)	3.2	0.2	3.6	0.4	-1.29	.134
Overshoot distance (cm)	0.4	0.04	0.4	0.08	-0.42	.3488
Lunge distance (cm)	3.5	0.2	4.0	0.4	-1.45	.1097

¹All times are relative to the onset of mouth opening ($t = 0$). *t*-Values and *P*-values are the results of paired *t*-tests on individual means for the 5 individuals for which captures and misses were digitized before surgery (see Table 1).

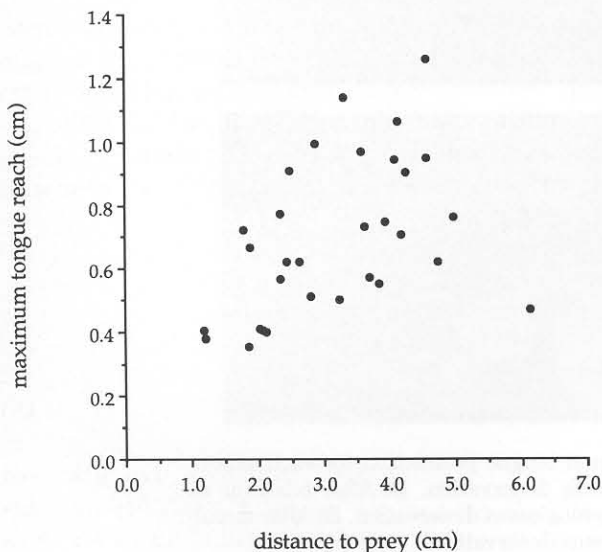


Fig. 5. Maximum tongue reach (cm) vs. distance to prey (cm) for all successful captures before surgery ($n = 36$). In *Spea multiplicata*, tongue protraction distance varies in response to prey distance. This suggests that *Spea* can modulate the amplitude of muscle activity in the tongue protractors, either on the basis of visual information about prey location or on the basis of feedback from mechanoreceptors in the tongue.

curred prior to denervation (mean = $176 \pm 0.2^\circ$ before surgery), and the treatment produced no significant change in mandibular bending (mean = $176 \pm 0.1^\circ$ after surgery). There was no difference in capture success before vs. after surgery (mean capture success = 67% before and 87% after surgery, $t = -0.83$, $P = .2478$).

When the m. geniohyoideus was denervated, the tongue pad was raised in the floor of the mouth and moved forward slightly, but did not leave the mouth (Fig. 6B). Denervation of the m. geniohyoideus significantly decreased both maximum tongue reach and maximum relative tongue reach compared to pretreatment means (Table 4). Maximum tongue reach decreased from 0.82 to 0.31 cm ($t = 35.28$, $P = .0090$), and maximum relative tongue reach was reduced from 0.67 to 0.25 ($t = 8.71$, $P = .0364$). The mean duration of tongue retraction increased from 31 to 61 ms ($t = -11.00$, $P = .0289$). Mean capture success was lower after surgery (14%) than before surgery (56%), but the difference was not significant ($t = 2.46$, $P = .1229$), probably because of the small sample size ($n = 2$ individuals).

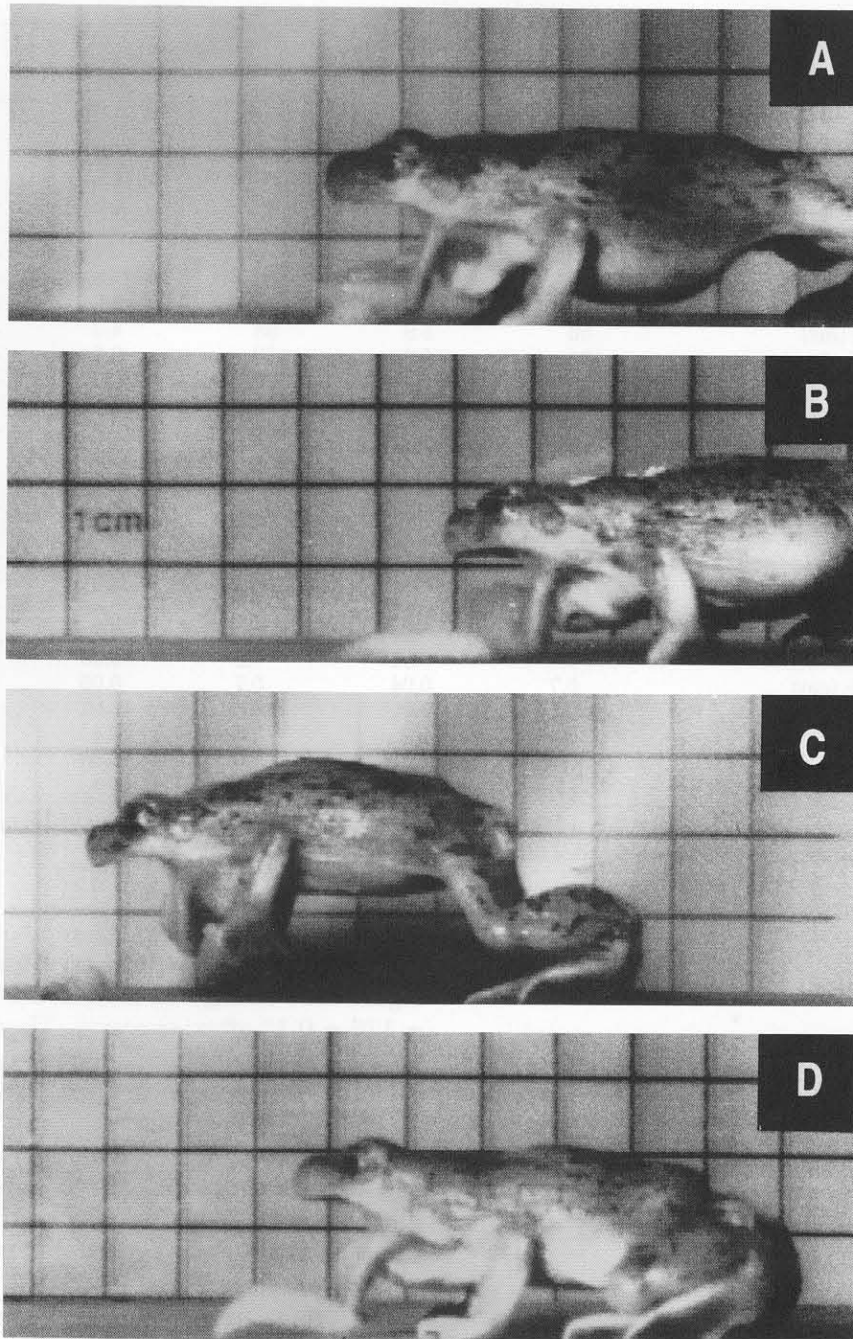


Fig. 6. Selected video frames showing maximum tongue protraction for each experimental treatment. **A:** After bilateral *m. submentalis* denervation. **B:** After bilateral *m. geniohyoideus* denervation. **C:** After bilateral *m. genioglossus* denervation. **D:** After simultaneous bilateral *mm. geniohyoideus* and *genioglossus* denervation.

When the *m. genioglossus* was denervated, the tongue pad was raised slightly above the floor of the mouth, but no forward movement of the tongue occurred (Fig. 6C). Denervation of the *m. genioglossus* produced significant reductions in

maximum tongue height and maximum relative tongue reach compared to pretreatment means (Table 5), with a marginally non-significant reduction in maximum tongue reach. Maximum tongue reach decreased from 0.62 to 0.17 cm ($t = 2.74$, P

TABLE 3. Comparison of feeding kinematics before ($n = 3$ individuals, with 4 sequences per individual) vs. after ($n = 3$ individuals, with 3-4 sequences per individual) denervation of the *m. submentalis* using paired *t*-tests (one-tailed, $\alpha = 0.05$) on individual means¹

Variable	Before		After		<i>t</i>	<i>P</i>
	Mean	SE	Mean	SE		
Duration of approach (ms)	124	5.7	112	6.6	2.94	.0495
Duration of mouth opening (ms)	75	7.7	64	4.8	0.93	.2254
Duration of tongue protraction (ms)	54	4.2	52	3.9	0.29	.4010
Duration of tongue at target (ms)	41	6.0	37	3.8	1.14	.1860
Duration of tongue retraction (ms)	31	1.9	27	2.8	0.99	.2133
Duration of mouth closing (ms)	103	7.6	109	12.5	-0.28	.4022
Duration of recovery (ms)	117	6.5	113	9.7	0.10	.4634
Duration of feeding sequence (ms)	242	7.9	225	8.9	0.80	.2546
Minimum mandible angle (°)	176	0.2	176	0.1	-0.49	.3357
Maximum tongue height (cm)	0.85	0.05	0.81	0.09	0.32	.3896
Maximum tongue reach (cm)	0.63	0.07	0.66	0.09	-0.16	.4443
Maximum relative tongue reach	0.46	0.05	0.47	0.06	-0.08	.4722

¹Only successful captures are included.

= .0557), maximum tongue height decreased from 0.66 to 0.51 cm ($t = 4.67$, $P = .0214$), and maximum relative tongue reach decreased from 0.45 to 0.12 ($t = 3.46$, $P = .0372$). After denervation of the *m. genioglossus*, duration of recovery and duration of the feeding sequence also increased significantly (Table 5). Mean duration of recovery increased from 118 to 229 ms ($t = -3.42$, $P = .0379$), and mean duration of the feeding sequence increased from 242 to 361 ms ($t = -3.92$, $P = .0296$). A small (less than 1°) but statistically significant change was also observed for the minimum mandible angle ($t = 5.00$, $P = .0189$). Capture success was significantly lower ($t = 2.947$, $P = .0492$) after treatment (12%) than before treatment (64%).

After the *mm. genioglossus* and *geniohyoideus* were denervated simultaneously, the tongue was

raised slightly, but did not move forward in the mouth as was observed for the *m. genioglossus* denervation (Fig. 6D). This treatment produced significant reductions in mean maximum tongue height, mean maximum tongue reach, and mean maximum relative tongue reach compared to the pretreatment means (Table 6). Mean maximum tongue height decreased from 0.75 to 0.40 cm ($t = 4.23$, $P = .0259$), mean maximum tongue reach decreased from 0.92 to 0.15 cm ($t = 15.42$, $P = .0021$), and mean maximum relative tongue reach decreased from 0.60 to 0.10 ($t = 15.73$, $P = .0020$). Denervation of both *m. genioglossus* and *m. geniohyoideus* produced significant increases in the means of five duration variables compared to pretreatment means (Table 6): 1) Duration of approach increased from 135 to 147 ms ($t = -8.66$, P

TABLE 4. Comparison of feeding kinematics before ($n = 2$ individuals, with 3 sequences per individual) vs. after ($n = 2$ individuals, with 3 sequences per individual) denervation of the *m. geniohyoideus* using paired *t*-tests (one-tailed, $\alpha = 0.05$) on individual means¹

Variable	Before		After		<i>t</i>	<i>P</i>
	Mean	SE	Mean	SE		
Duration of approach (ms)	124	5.0	149	10.0	-1.29	.2104
Duration of mouth opening (ms)	79	4.7	100	2.1	-3.00	.1024
Duration of tongue protraction (ms)	46	3.46	43	6.9	-1.00	.2500
Duration of tongue at target (ms)	39	3.5	40	6.6	-0.33	.3976
Duration of tongue retraction (ms)	31	4.1	61	4.1	-11.00	.0289
Duration of mouth closing (ms)	87	13.7	104	9.1	-0.86	.2747
Duration of recovery (ms)	108	11.6	142	4.8	-6.00	.0526
Duration of feeding sequence (ms)	235	13.5	275	21.6	-0.94	.2606
Minimum mandible angle (°)	176	0.3	176	0.09	-0.60	.3274
Maximum tongue height (cm)	0.66	0.10	0.53	0.04	2.13	.1397
Maximum tongue reach (cm)	0.82	0.05	0.31	0.03	35.28	.0090
Maximum relative tongue reach	0.67	0.04	0.25	0.02	8.71	.0364

¹Only successful captures are included.

TABLE 5. Comparison of feeding kinematics before ($n = 3$ individuals, with 3 sequences per individual) vs. after ($n = 3$ individuals, with 2-3 sequences per individual) denervation of the *m. genioglossus* using paired *t*-tests (one-tailed, $\alpha = 0.05$) on individual means¹

Variable	Before		After		<i>t</i>	<i>P</i>
	Mean	SE	Mean	SE		
Duration of approach (ms)	123	8.3	132	5.1	-0.58	.3096
Duration of mouth opening (ms)	70	7.1	87	11.7	-2.43	.0678
Duration of tongue protraction (ms)	53	4.6	33	7.6	1.08	.1974
Duration of tongue at target (ms)	37	4.6	32	7.8	0.28	.4017
Duration of tongue retraction (ms)	32	6.0	98	33.5	-1.19	.1782
Duration of mouth closing (ms)	106	18.9	196	25.7	-2.78	.0543
Duration of recovery (ms)	118	13.8	229	29.5	-3.42	.0379
Duration of feeding sequence (ms)	242	19.2	361	28.4	-3.92	.0296
Minimum mandible angle (°)	176	0.1	176	0.05	5.00	.0189
Maximum tongue height (cm)	0.66	0.06	0.51	0.06	4.67	.0214
Maximum tongue reach (cm)	0.62	0.08	0.17	0.02	2.74	.0557
Maximum relative tongue reach	0.45	0.04	0.12	0.02	3.46	.0372

¹Only successful captures are included.

= .0066); 2) duration of tongue retraction increased from 81 to 169 ms ($t = -9.81$, $P = .0051$); 3) duration of mouth closing increased from 105 to 234 ms ($t = -8.29$, $P = .0072$); 4) duration of recovery increased from 119 to 245 ms ($t = -21.17$, $P = .0011$); and 5) duration of the feeding sequence increased from 255 to 395 ms ($t = -15.59$, $P = .0020$). Capture success was significantly lower ($t = 3.082$, $P = .0184$) after simultaneous *m. genioglossus* and *m. genioglossus* denervation (0%) than before treatment (51%).

The denervation treatments differed in their effects on maximum relative tongue reach ($F = 28.905$, $P = .0003$). Maximum relative tongue reach was greatest before surgery and after *m. submental* denervation. The deficit produced by

m. genioglossus denervation alone was significantly greater than the deficit produced by *m. submental* denervation, but significantly less than that produced by *m. genioglossus* denervation or by simultaneous *mm. genioglossus* and *genioglossus* denervation (Fig. 7). The effects of *m. genioglossus* denervation and simultaneous *mm. genioglossus* and *genioglossus* denervation were not significantly different.

DISCUSSION

In general, the feeding behavior of *Spea multiplicata* is similar to that of short-tongued archaebatrachians and neobatrachians of similar body size, such as *Ascaphus truei* (Nishikawa and Cannatella, '91), *Discoglossus pictus* (Nishikawa

TABLE 6. Comparison of feeding kinematics before ($n = 3$ individuals, with 4 sequences per individual) vs. after ($n = 3$ individuals, with 1-4 sequences per individual) denervation of both the *m. genioglossus* and the *m. genioglossus* using paired *t*-tests (one-tailed, $\alpha = 0.05$) on individual means¹

Variable	Before		After		<i>t</i>	<i>P</i>
	Mean	SE	Mean	SE		
Duration of approach (ms)	135	7.7	147	10.4	-8.66	.0066
Duration of mouth opening (ms)	78	6.4	80	9.1	-0.49	.3352
Duration of tongue protraction (ms)	60	4.6	40	5.1	0.88	.2359
Duration of tongue at target (ms)	31	2.8	57	12.9	-1.25	.1692
Duration of tongue retraction (ms)	81	15.7	169	24.0	-9.81	.0051
Duration of mouth closing (ms)	105	12.0	234	18.6	-8.29	.0072
Duration of recovery (ms)	119	12.6	245	17.6	-21.17	.0011
Duration of feeding sequence (ms)	255	17.4	395	23.2	-15.59	.0020
Minimum mandible angle (°)	176	0.1	177	0.1	-2.63	.0594
Maximum tongue height (cm)	0.75	0.02	0.40	0.03	4.22	.0259
Maximum tongue reach (cm)	0.92	0.06	0.15	0.02	15.42	.0021
Maximum relative tongue reach	0.60	0.04	0.10	0.01	15.73	.0020

¹Before surgery, both successful and unsuccessful capture attempts are included, whereas after surgery only unsuccessful capture attempts are included (see Table 1).

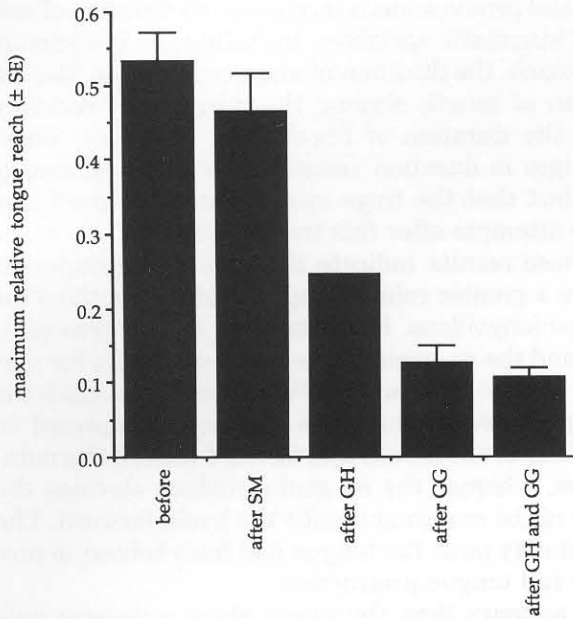


Fig. 7. Maximum relative tongue reach (cm) + 1 SE before surgery and after each of the four experimental treatments. SM, m. submentalis denervation; GH, m. geniohyoideus denervation; GG, m. genioglossus denervation. Error bar after GH is too small to appear above graph. Maximum tongue reach was similar before surgery and after m. submentalis denervation, was reduced after m. geniohyoideus denervation, and was even more reduced after m. genioglossus and both mm. geniohyoideus and genioglossus denervation.

and Roth, '91), and *Hyla cinerea* (Deban and Nishikawa, '92). *Spea* shares several features with short-tongued frogs: 1) The whole body lunges forward during prey capture; 2) the tongue pad is round and broadly attached to the floor of the mouth; and 3) the tongue can be protracted and retracted more than once during a single gape cycle.

The tongue of *Spea*, which can be protracted as much as 83% of lower jaw length, is intermediate in length between that of short-tongued frogs (protraction less than 60% of jaw length) and long-tongued frogs (protraction 100–250% of jaw length). Compared to other mesobatrachians, the tongue of *Spea* is longer than that of *Pelobates* (Vences, '88), but shorter than that of *Megophrys* (Gans et al., '91). Based on the fact that *Pelobates* and *Spea* are members of the family Pelobatidae, and the fact that *Pelodytes* appears to possess a short tongue (Magimel-Pelonnier, '28; Horton, '82), it is likely that long tongues evolved independently in *Spea* and in members of the family Megophryidae (Fig. 1). This hypothesis is supported by the fact that the tongues of *Spea*

(*Scaphiopus*) and *Megophrys* differ greatly in the relative size and shape of the tongue muscles (Regal and Gans, '76).

Studies of the natural diets of spadefoot toads indicate that most species are dietary generalists. The primary prey items in their diets are beetles and termites, whether measured by numbers of individuals consumed or by weight (Dimmitt and Ruibal, '80; Punzo, '91). Other terrestrial pelobatoids, including *Megophrys* and *Leptobrachium*, have larger heads relative to body size than *Spea* and feed primarily on large arthropods (Berry, '65; Emerson, '85).

Most frogs that have been studied, such as *Discoglossus* (Nishikawa and Roth, '91) and *Hyla* (Deban and Nishikawa, '92), protract the tongue the same distance regardless of distance to prey. In contrast, *Spea* possesses the ability to modulate its tongue protraction distance in response to prey distance. *Hemisus marmoratum* (Ritter and Nishikawa, '95) and *Phrynomerus bifasciatus* (Jaeger and Nishikawa, '93) are the only other anuran species that have been shown to possess this ability. These frogs are able to modulate the activity of the tongue protractor muscles in response to prey distance. Whether modulation involves feedforward visual information about prey location (Anderson, '93) or feedback information from mechanoreceptors in the tongue (Matsushima et al., '86) remains to be determined for *Spea*.

Before discussing the results of the denervation experiments, it is first necessary to investigate possible artifacts of anesthesia or surgery that may confound the interpretation of the individual muscle denervation treatments. In the present study, the different denervation treatments served as each other's controls. Because very few changes in feeding kinematics were produced by bilateral denervation of the m. submentalis, it is unlikely that the differences in feeding kinematics produced by the other treatments are general effects of anesthesia or surgery. For the three other treatments, anesthesia and surgery were identical. Any differences among the treatments can only be due to effects of the denervation of specific muscles. The m. genioglossus and m. geniohyoideus denervation treatments shared no effects in common except for a decrease in maximum relative tongue reach. However, m. genioglossus denervation produced a significantly greater deficit in maximum relative tongue reach than did m. geniohyoideus denervation.

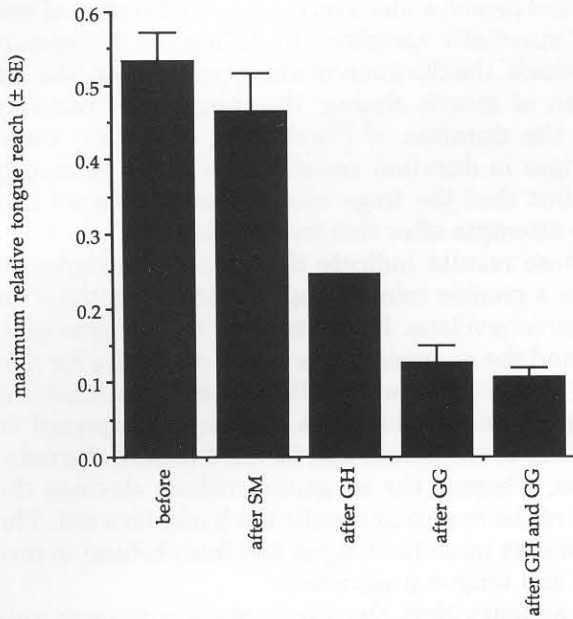


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vation. Thus, none of the changes in feeding kinematics described below are nonspecific artifacts of anesthesia or surgery.

The only significant effect of bilateral denervation of the m. submentalis was a small decrease in the duration of approach. In *Spea*, the mandibles bend slightly downward (approximately 176°) at rest and do not bend downward during tongue protraction, as they do in *Ascaphus* (Nishikawa and Cannatella, '91), *Discoglossus* (Nishikawa and Roth, '91), and *Hyla* (Deban and Nishikawa, '92). Lack of mandibular bending during feeding may be due to fusion of the mentomeckelian bones with the dentaries soon after metamorphosis (Wiens, '89). This likely explains why no differences were observed in feeding behavior before and after denervation of the m. submentalis in *Spea*, whereas this treatment eliminated mandibular bending during feeding in *Hyla* (Deban and Nishikawa, '92) and *Discoglossus* (Nishikawa and Roth, '91).

Denervation of the m. geniohyoideus in *Spea* produced a deficit of tongue protraction in which the tongue pad was raised normally in the floor of the mouth but did not move as far forward as before surgery. In contrast, denervation of the m. genioglossus resulted in a decrease in both tongue height and relative tongue reach. The deficit in tongue protraction produced by m. genioglossus denervation alone was significantly greater than the deficit produced by m. geniohyoideus denervation alone. When the m. genioglossus was denervated, the tongue pad rose only slightly in the floor of the mouth and was no longer protracted beyond the tip of the mandibles. The same result has been obtained in all frogs that have been studied to date. In general, the function of the m. genioglossus during feeding in frogs is to raise the tongue pad and pull it forward toward the mandibular symphysis (Nishikawa and Roth, '91; Deban and Nishikawa, '92). Our results suggest that there has been no change in the function of this muscle in *Spea*.

When the m. genioglossus and the m. geniohyoideus were denervated simultaneously, the tongue pad rose only slightly in the floor of the mouth and no anterior movement was observed. The deficit in tongue protraction produced by simultaneous denervation of these muscles was greater than that produced by denervation of the m. geniohyoideus alone, but similar to that produced by denervation of the m. genioglossus alone (i.e., there was no synergistic effect of denervating the two muscles simultaneously).

Simultaneous denervation of the mm. genioglos-

sus and geniohyoideus increased the duration of several kinematic variables, including the duration of approach, the duration of tongue retraction, the duration of mouth closing, the duration of recovery, and the duration of the feeding sequence. These changes in duration variables are likely related to the fact that the frogs missed the prey in all capture attempts after this treatment.

These results indicate that the m. genioglossus plays a greater role in tongue protraction than the m. geniohyoideus. However, both the m. genioglossus and the m. geniohyoideus are necessary for normal tongue protraction. We hypothesize that the m. genioglossus pulls the tongue pad upward in the floor of the mouth and forward toward the mandibles, whereas the m. geniohyoideus elevates the floor of the mouth and pulls the hyoid forward. The hyoid may push the tongue pad from behind to produce full tongue protraction.

It appears that the hyoid plays a greater role in tongue protraction in mesobatrachians than it does in other frogs due to the unique morphology of the hyoid plate. Mesobatrachian frogs possess hyoids that are not fused to the skull, whereas the cornua of the hyoid plates are fused to the prootic region of the cranium in all other frogs (Cannatella, '85; Ridewood, 1897). Embryological studies in *Pelodytes* show that the cornua are continuous until metamorphosis, during which time a gap develops in the cornua which frees the hyoid plate from its attachment with the skull (Ridewood, 1897). An otic remnant of the cornua is present in all pelobatoids except members of the family Megophryidae, in which it has been lost (Cannatella, '85).

There have been few previous investigations of the role of the hyoid during tongue protraction in frogs. In *Bufo marinus*, cineradiography shows that the hyoid moves anteriorly during feeding (Emerson, '77). However, this movement apparently is not necessary for tongue protraction. Toads were able to feed normally after the hyoid plate was wired to the junction between the xyphoid process and the sternum (Gans and Gorniak, '82a). Based on muscle stimulation experiments, Trueb and Gans ('83) suggested that hyoid protraction plays a role in tongue protraction in *Rhinophrynus dorsalis*, a termite eating mesobatrachian. There are no published reports on the effect of denervation of the m. geniohyoideus during feeding for any other frog species. However, we have observed that *Hyla cinerea* and *Discoglossus pictus* are able to protract their tongues normally after m. geniohyoideus denervation (Nishikawa, unpub-

lished data). Thus, it appears that the m. geniohyoideus is necessary for normal tongue protraction in *Spea*, but not in archaeobatrachians or neobatrachians.

Our data suggest that the disjunct hyoid of mesobatrachians allows a greater range of hyoid movement than occurs in other frogs. This in turn has permitted the m. geniohyoideus to change its function during the course of evolution, from a protractor of the hyoid in basal frogs to a protractor of both hyoid and tongue in mesobatrachians. The fact that another mesobatrachian species, *Rhinophrynus dorsalis*, also appears to use the m. geniohyoideus to protract the tongue during feeding (Trueb and Gans, '83) lends further support to this hypothesis.

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LITERATURE CITED

- Anderson, C.W. (1993) Modulation of feeding behavior in response to prey type in the frog *Rana pipiens*. *J. Exp. Biol.*, 179:1-11.
- Avila, V.L., and P.G. Frye (1977) Feeding behavior in the African clawed frog (*Xenopus laevis* Daudin). *Herpetologica*, 33:152-161.
- Avila, V.L., and P.G. Frye (1978) Feeding behavior of the African clawed frog (*Xenopus laevis* Daudin): (Amphibia, Anura, Pipidae): effect of prey type. *J. Herpetol.*, 12:391-396.
- Berry, P. (1965) The diet of some Singapore Anura (Amphibia). *Proc. Zool. Soc. Lond.*, 144:163-174.
- Cannatella, D.C. (1985) A Phylogeny of Primitive Frogs (Archaeobatrachians). University Microfilms, Ann Arbor, MBA86-08380.
- Deban, S.M., and K.C. Nishikawa (1992) The kinematics of prey capture and the mechanism of tongue protraction in the green tree frog, *Hyla cinerea*. *J. Exp. Biol.*, 170:235-256.
- Dimmitt, M.A., and R. Ruibal (1980) Exploitation of food resources by spadefoot toads (*Scaphiopus*). *Copeia*, 1980: 854-862.
- Emerson, S.B. (1977) Movement of the hyoid in frogs during feeding. *Am. J. Anat.*, 149:115-120.
- Emerson, S.B. (1985) Skull shape in frogs—correlations with diet. *Herpetologica*, 41:177-188.
- Ford, L.S., and D.C. Cannatella (1993) The major clades of frogs. *Herpetol. Monogr.*, 6:118-131.
- Gans, C., and G.C. Gorniak (1982a) How does the toad flip its tongue? Test of two hypotheses. *Science*, 216:1335-1337.
- Gans, C., and G.C. Gorniak (1982b) Functional morphology of lingual protrusion in marine toads (*Bufo marinus*). *Am. J. Anat.*, 163:195-222.
- Gans, C., and R. Pyles (1983) Narial closure in toads: which muscles? *Respir. Physiol.*, 53:215-223.
- Gans, C., K. Nishikawa, and D.C. Cannatella (1991) The frog *Megophrys montana*: specialist in large prey. *Am. Zool.*, 31:52A.
- Gaupp, E. (1896) A. Ecker's und R. Wiedersheim's Anatomie des Frosches, vols 1 and 2. Friedrich Vieweg und Sohn, Braunschweig.
- Goslow, G.E., Jr., K.P. Dial, and F.A. Jenkins (1989) The avian shoulder: an experimental approach. *Am. Zool.*, 29:287-301.
- Horton, P. (1982) Diversity and systematic significance of anuran tongue musculature. *Copeia*, 1982:595-602.
- Innocenti, C.M., and K.C. Nishikawa (1994) Motor learning in toads (*Bufo marinus*) following hypoglossal transection. *Am. Zool.*, 34:56A.
- Jaeger, J.C., and K. Nishikawa (1993) The feeding behavior and tongue aiming ability of the African snake-necked frog, *Phrynomerus bifasciatus*. *J. Arizona-Nevada Acad. Sci.*, 28:59.
- Lauder, G.V., and H.B. Shaffer (1988) Ontogeny of functional design in salamanders (*Ambystoma tigrinum*): Are motor patterns conserved during major morphological transformations? *J. Morphol.*, 197:249-268.
- Magimel-Pelonnier, O. (1924) La langue des amphibiens. Thèse. Fac. Sci. Paris. A. Saugnot et E. Prouillard, Bordeaux.
- Matsushima, T., M. Satou, and K. Ueda (1986) Glossopharyngeal and tectal influences on tongue-muscle motoneurons in the Japanese toad. *Brain Res.*, 365:198-203.
- Nishikawa, K.C. (1987) Staining amphibian peripheral nerves with Sudan black B: progressive vs. regressive methods. *Copeia*, 1987:489-491.
- Nishikawa, K.C., and D.C. Cannatella (1991) Kinematics of prey capture in the tailed frog, *Ascaphus truei* (Anura: Ascaphidae). *Zool. J. Linn. Soc.*, 103:289-307.
- Nishikawa, K.C., and C. Gans (1992) The role of hypoglossal sensory feedback during feeding in the marine toad, *Bufo marinus*. *J. Exp. Zool.*, 264:245-252.
- Nishikawa, K.C., and G. Roth (1991) The mechanism of tongue protraction during prey capture in the frog *Discoglossus pictus*. *J. Exp. Biol.*, 159:217-234.
- Nishikawa, K.C., C.W. Anderson, S.M. Deban, and J.C. O'Reilly (1992) The evolution of neural circuits controlling feeding behavior in frogs. *Brain Behav. Evol.*, 40:125-140.
- Punzo, F. (1991) Feeding ecology of spadefooted toads (*Scaphiopus couchi* and *Spea multiplicata*) in western Texas. *Herpetol. Rev.*, 22:79-80.
- Regal, P.J., and C. Gans (1976) Functional aspects of the evolution of frog tongues. *Evolution*, 30:718-734.
- Ridewood, W.G. (1897) On the structure and development of the hyobranchial skeleton of the parsley-frog (*Pelodytes punctatus*). *Proc. Zool. Soc. Lond.*, 1897: 577-595.
- Ritter, D.A., and K.C. Nishikawa (1995) The kinematics and mechanism of prey capture in the African pig-nosed frog (*Hemismis marmoratum*): the description of a radically divergent anuran tongue. *J. Exp. Biol.*, 198:2025-2040.
- Sokol, O.M. (1969) Feeding in the pipid frog *Hymenochirus boettgeri* (Tornier). *Herpetologica*, 25:9-24.
- Stuessie, S.L., W.L.R. Cruce, and K.S. Powell (1983) Afferent and efferent components of the hypoglossal nerve in the grass frog, *Rana pipiens*. *J. Comp. Neurol.*, 217:432-439.
- Trueb, L., and C. Gans (1983) Feeding specialization of the

Mexican burrowing toad, *Rhinophrynus dorsalis* (Anura: Rhinophrynidae). *J. Zool. Lond.*, 199:189-208.

Vences, M. (1988) Zum Beutefangverhalten der europäischen Amphibien. *Herpetofauna*, 10:6-10.

Wainwright, P.C., C.P. Sanford, S.M. Reilly, and G.V. Lauder

(1989) Evolution of motor patterns: aquatic feeding in salamanders and ray-finned fishes. *Brain Behav. Evol.*, 34:329-341.

Wiens, J. (1989) Ontogeny of the skeleton of *Spea bombifrons* (Anura: Pelobatidae). *J. Morphol.*, 202:29-51.

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LITERATURE CITED

Anderson, C. W. (1988) Mechanisms of feeding behavior in the larval stage of the frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1978) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1979) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1980) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1981) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1982) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1983) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1984) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1985) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1986) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1987) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1988) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1989) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1990) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1991) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1992) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1993) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1994) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1995) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1996) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1997) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1998) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (1999) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2000) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2001) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2002) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2003) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2004) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2005) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2006) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2007) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2008) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2009) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2010) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2011) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2012) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2013) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2014) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2015) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2016) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2017) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2018) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2019) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.

Anderson, C. W., and D. L. (2020) Feeding behavior of the larval frog *Rana temporaria*. *J. Exp. Biol.*, 67:1-12.