

## Kinetics of Tongue Projection in *Ambystoma tigrinum*: Quantitative Kinematics, Muscle Function, and Evolutionary Hypotheses

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**ABSTRACT** The projectile tongue of caudate amphibians has been studied from many perspectives, yet a quantitative kinetic model of tongue function has not yet been presented for generalized (nonplethodontid) terrestrial salamanders. The purposes of this paper are to describe quantitatively the kinematics of the feeding mechanism and to present a kinetic model for the function of the tongue in the ambystomatid salamander *Ambystoma tigrinum*. Six kinematic variables were quantified from high-speed films of adult *A. tigrinum* feeding on land and in the water. Tongue protrusion reaches its maximum during peak gape, while peak tongue height is reached earlier, 15 ms after the mouth starts to open. Tongue kinematics change considerably during feeding in the water, and the tongue is not protruded past the plane of the gape. Electrical stimulation of the major tongue muscles showed that tongue projection in *A. tigrinum* is the combined result of activity in four muscles: the geniohyoideus, Subarcualis rectus 1, intermandibularis posterior, and interhyoideus. Stimulation of the Subarcualis rectus 1 alone does not cause tongue projection. The kinetic model produced from the kinematic and stimulation data involves both a dorsal vector (the resultant of the Subarcualis rectus 1, intermandibularis posterior, and interhyoideus) and a ventral vector (the geniohyoideus muscle), which sum to produce a resultant anterior vector that directs tongue motion out of the mouth and toward the prey. This model generates numerous testable predictions about tongue function and provides a mechanistic basis for the hypothesis that tongue projection in salamanders evolved from primitive intraoral manipulative action of the hyobranchial apparatus.

The morphology of the tongue projection mechanism in salamanders has been studied for over a century. In its most extreme condition, the tongue is projected up to 80% of the body length out of the mouth to contact prey (Duellman and Trueb, '86). In all salamanders, the tongue is also used after prey capture to hold prey against the roof of the mouth and to manipulate food in the buccal cavity. Lunged terrestrial salamanders such as *Ambystoma tigrinum* have partially protrusible tongues and a buccal pump mechanism that is used in respiration. In lungless salamanders, the tongue and hyobranchium are modified to project the tongue greater

distances and no longer function in respiration (Wake, '82).

Morphological aspects of the tongue projection mechanism in salamanders have been the topic of several studies, and many of these present qualitative hypotheses describing the function of the system. Early discussions of the tongue projection mechanism based on morphological analysis provided detailed anatomical descriptions and proposed functions of the muscles involved (Drüner, '02, '04; Edgeworth, '35; Francis, '34; Krogh and Tanner, '72). Other studies presented interpretations of the functional morphology of salamander feeding mechanisms from com-

parative anatomy and behavioral observations (Lombard and Wake, '76, '77; Ozeti and Wake, '69; Regal, '66; Roth, '76; Wake, '82; Wake and Lombard, '71). Of the few studies using cinematic or electromyographic data in support of functional hypotheses (Dockx and De Vree, '86; Larsen and Guthrie, '75; Severtsov, '71, '72; Thexton et al., '77), none presents a quantitative analysis of the kinematics of tongue projection. Several studies mention using muscle stimulation (Drüner, '02; Eaton, '57; Larsen and Guthrie, '75), but do not report specific stimulation results for individual muscles controlling feeding functions.

Much of the recent work has centered on the most terrestrial group of salamanders, the family Plethodontidae, in which extreme modification of the feeding mechanism has occurred (Lombard and Wake, '86; Wake, '82; Wake et al., '83). The model presented to describe the mechanics of tongue projection in plethodontids involves the rapid medial buckling of hyobranchial elements by the action of a single muscle (the Subarcualis rectus 1) to push the tongue pad out of the mouth (Lombard and Wake, '76). This model is soundly supported in plethodontids by a wealth of morphological (Lombard and Wake, '77, '86) and some functional data (Thexton et al., '77) and basically matches the ideas of the first morphologists to describe the tongues of salamanders (Drüner, '02; Rusconi, 1854). The more recent studies of tongue projection all have followed Regal ('66) in assuming that other salamanders (hynobiids, salamandrids, and ambystomatids) with *Ambystoma tigrinum* as an example, protrude the tongue using the derived mechanical model hypothesized for the plethodontids (Wake, '82). To date, no kinetic model for the mechanics of tongue projection in other salamanders has been presented.

In this paper, we present a new functional model of tongue projection mechanics for *Ambystoma tigrinum* based on quantitative kinematics and muscle stimulation data. This kinetic model is developed from the analyses of observed movements of the tongue and head and the demonstrated actions of major muscles involved in feeding. Electrical activities of the major muscles involved in terrestrial feeding are presented in Lauder and Shaffer ('88) and, thus, are not considered here. This model is applicable to salamanders with generalized terrestrial feeding morphology (ambystomatids, salamandrids,

and hynobiids) and provides testable biomechanical and historical hypotheses for future research.

We emphasize that our data and model apply only to initial prey capture and not to subsequent intraoral manipulations of prey that have been modeled by Bramble and Wake ('85). However, morphological and kinetic aspects of this model do suggest an historical hypothesis that tongue projection in salamanders evolved from modification of a tongue mechanism used to manipulate prey against the roof of the mouth.

## MATERIALS AND METHODS

### Terminology

In our discussion of *Ambystoma tigrinum*, the following terminology will be used to explain the functional components involved in projecting the tongue from the mouth. The time from the resting state until the tongue is completely projected is termed "tongue projection." Tongue projection is composed of three interdependent and temporally overlapping functional phases: "lift," "protraction," and "flip," each involving specific mechanical components of the gular musculature and hyobranchial apparatus. The action of pulling the tongue back into the mouth after the strike is called "retraction." Two terms are used to describe feeding movements and mechanics in tiger salamanders. "Kinematics" is simply the study of movements exclusive of the influences of mass and force, while "kinetics" is the study of all aspects of motion including both kinematics and the mechanical forces affecting motion (Considine, '76).

We use hyobranchial terminology for salamanders that reflects homology with other lower vertebrates (Reilly and Lauder, '88) and the muscle terminology of Duellman and Trueb ('86). The muscle called the "geniohyoideus" in salamanders is not homologous to the muscle of the same name in ray-finned fishes, and, thus, Lauder and Shaffer ('85) used the term "coracomandibularis" for this muscle, following Allis ('17), Edgeworth ('11), and Wiley ('79), to avoid confusion with fishes. In teleost fishes, the geniohyoideus is formed by reorientation of the intermandibularis posterior and interhyoideus, two muscles that still are present in salamanders (Lauder, '80a; Winterbottom, '74). The salamander geniohyoideus (coracomandibularis) is homologous, rather, to the geniothoracis of lungfishes (Bemis and Lauder, '86; Wiley, '79).

We now believe that for reasons of terminological consistency it is better to retain the tetrapod term "geniohyoideus" for tetrapods, while recognizing that the homology of this muscle in plesiomorphic clades is with the geniothoracis (of lungfishes) and with the coracomandibularis (of primitive ray-finned fishes).

#### *Morphology and kinematics*

The morphology of the feeding system was examined on five larvae and nine adult *Ambystoma tigrinum* preserved in 10% formalin and on two larvae and three adult specimens that had been cleared and double-stained for bone and cartilage (Dingerkus and Uhler, '77). Morphological figures were drawn with the aid of a camera lucida on a Zeiss SV-8 binocular dissecting microscope at 8–64 $\times$ .

Two adult *Ambystoma tigrinum mavortium* (SVL 8.1 and 9.9 cm) collected near Limon, Lincoln County, Colorado, were used for kinematic analyses. Filming procedures followed Shaffer and Lauder ('85). Animals were trained to feed under bright lights (1,200 W) and filmed with a Photosonics 16-1PL camera using Kodak 4X Reversal film at 200 frames per second (1/1,200 s shutter speed) in a feeding arena with a background grid.

Individual salamanders were studied feeding both in the water and on land (following the experimental paradigm of Lauder and Shaffer, '88) in order to assess the effect of the environment on the kinematics of tongue projection and to perturb the feeding system to assess the extent to which the tongue projection behavior is an immutable sequence of kinematic events. If tongue projection behavior is a sequence of fixed actions, then the pattern of tongue movement in the aquatic and terrestrial environments is expected to be very similar. Adult *Ambystoma tigrinum* feed naturally both on land and in the water (Burger, '50; Miller and Larsen, '86; Reese, '69; Webb and Roueche, '71).

Aquatic and terrestrial feedings were elicited by presenting a piece of earthworm anterior and slightly above the salamander. Prey were presented in this position for five reasons: 1) From prior experience in feeding these salamanders, we knew that prey positioned anterior and slightly above the animal almost invariably elicited a strong and instant feeding attempt aimed directly at the prey item (Lauder and Shaffer, '85, '86; Shaffer and Lauder, '85). 2) By holding the prey

still and slightly above the animal, we could control prey movement and film feedings that were not confounded by lateral movement of the prey or predator or contact with the substrate. 3) Feeding in the water occurs by suction feeding, and prey are captured from anterior to the head (Lauder and Shaffer, '85, '86). 4) In order to provide a constant feeding situation, we chose to feed the terrestrial animals in the same position as the aquatic animals to avoid confounding feeding position with environment in our statistical comparisons. 5) By getting the salamanders to direct their feeding movements anteriorly, we could repeatedly film feedings that encompass the broadest range of movement of the tongue and hyoid apparatus in order to quantitatively analyze kinematics.

Worm pieces were thus presented to the salamander in the same position for all feedings and were held loosely by forceps to allow the worm to be captured. Procedures for aquatic feedings were identical except that the animals were in water at 17°C. In both terrestrial and aquatic feedings, the animals were standing on the bottom of the aquarium. A total of ten aquatic and ten terrestrial feedings (five feedings from each of two animals in each environment) were used for detailed analysis and digitized. This represents a sample of 160 feedings analyzed by Shaffer and Lauder ('88) who provide a statistical analysis of the extent of variation in aquatic and terrestrial feedings for 13 animals at all metamorphic stages; variability among individuals is thus not considered in detail here, and the error bars given in Figures 3 and 4 illustrate the variation seen in these feedings.

Films of feeding were projected frame by frame onto a Houston Instruments Hipad digitizer (50- $\mu$ m accuracy.), beginning two frames (10 ms) before the mouth opened, and coordinates of digitized points were then sent to a microcomputer for calculation of distances and angles. Ten milliseconds prior to the first detectable increase in gape was taken as the reference point for comparing kinematic sequences. Six variables describing the positions of the head, jaws, and tongue were measured from each of 39 frames, which covered skull bone movement from 10 ms before mouth opening until 30 ms after mouth closing. Angle measurements were taken from each frame, and tongue distance measurements were taken whenever the gape was open and the tongue was visible.

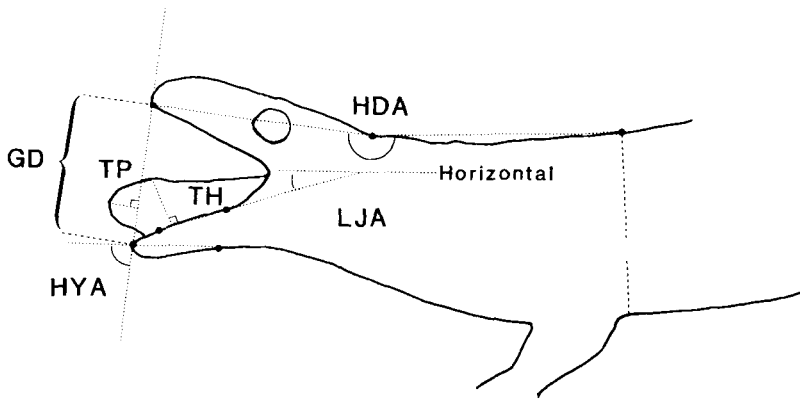


Fig. 1. Schematic view of *Ambystoma tigrinum* with the tongue protruded to show the kinematic variables measured from film frames of feeding. Details of how the variables were measured and interpreted are given in the text. Note that an increase in LJA corresponds to

the lower jaw moving ventrally, whereas an increase in HDA indicates that the head is being elevated dorsally. GD gape distance, HDA head angle, HYA hyoid angle, LJA lower jaw angle, TH tongue height, TP tongue protrusion.

There are a variety of possible ways that movements of the skull could be quantified. Our measurements of head bone movement were chosen specifically to allow comparison with previously published kinematic curves in salamanders and fishes and to maximize the accuracy with which bone motion could be quantified. For example, nearly all previous investigators who have studied lower vertebrates have chosen to represent changes in gape by measuring the distance between points on the jaws, as opposed to measuring the angles of the upper and lower jaw (e.g., Anker, '78; Bemis et al., '83; Bemis and Findeis, '86; Elshoud-Oldenhavé and Osse, '76; Lauder, '79, '80a,b; Lauder and Shaffer, '85; Liem, '70; Osse and Muller, '80; Shaffer and Lauder, '85), and we thus elected to measure gape distance.

Three distance measurements were made from each frame. 1) Gape distance (GD) was measured as the distance between the anteroventral tip of the upper jaw and the anterodorsal tip of the lower jaw (Fig. 1). These two points were distinct on the films and the line through them defines the position of the plane of the gape perpendicular to the body. 2) Tongue height (TH) was defined as the greatest height of the tongue measured perpendicular to the line defining the dorsal margin of the lower jaw (Fig. 1). This variable reflects dorsal movement of the tongue and hyobranchial apparatus relative to the lower jaw. 3) Tongue protrusion (TP) is de-

finied as the perpendicular distance of the anterior tip of the tongue from the plane of the gape (Fig. 1). This distance was defined as positive when the tongue was beyond the plane of the gape and negative when the tongue was within the oral cavity.

Three angle measurements were made from each frame. 1) Hyoid angle (HYA) is measured by the angle between the line indicating the plane of the gape and a line defined by a point on the anterodorsal tip of the lower jaw and one at the ventral tip of the hyobranchial apparatus (Fig. 1). This angle was chosen to provide an indirect indication of ventral excursion of the hyobranchial apparatus, since movements of the individual components of the hyoid and branchial arches are not visible externally. Hyoid angle decreases as the hyoid is elevated (protracted) and increases as it is depressed below the ventral margin of the lower jaw. 2) Head angle (HDA) was measured as the angle between the point on the anteroventral tip of the upper jaw, the point of flexion of the skull on the back, and a point on the back located directly dorsal to the axilla (Fig. 1). An increase in HDA indicates that the head is being bent dorsally on the vertebral column. The error in locating these points gives rise to a 1–2° repeatability error in HDA measurement. 3) Lower jaw angle (LJA) was measured as the angle between the horizontal and a line defining the dorsal margin of the lower jaw (Fig. 1). The hori-

zontal position of the body of the animals filmed did not change (within the limits of our measurement error) during the relatively short duration (190 ms) of the initial prey capture sequence. An increase in LJA indicates that the lower jaw is pivoting ventrally with respect to the horizontal, if the head and body do not move. If the lower jaw is entirely passive and moves with the rest of the head, elevation of the head (increase in HDA) will decrease LJA.

The measurement of LJA, in conjunction with HDA and GD, allows the extent to which gape is a function of cranial elevation, lower jaw depression, or both, to be determined. For example, if the increase in gape were due exclusively to cranial elevation, then HDA should increase while LJA either remains fixed (due to antagonistic activity in the depressor mandibulae and adductor mandibulae muscles) or decreases (due to the mandible being carried posterodorsally by the elevating cranium). If depression of the lower jaw is entirely responsible for the increase in gape, then HDA should stay constant, while LJA increases.

Kinematics were summarized by averaging values for each variable across ten feedings for each of the 39 frames and were plotted with standard errors against time in milliseconds (5 ms/frame). This produced quantitative curves describing the feeding kinematics of tiger salamanders in both aquatic and terrestrial environments. These average kinematic curves were produced by aligning curves for each individual feeding. Ten milliseconds prior to the first visible mouth opening was set as the reference point, and curves were averaged from this point on despite differences in amplitude of movement or duration of the gape cycle. We explicitly decided against standardizing the curves (to 100% of movement amplitude or feeding sequence duration, e.g.) prior to averaging because this 1) masks kinematic variability giving an artificial impression of uniformity among feedings, 2) introduces variability in time samples across feedings (because not all measurements would align at the same *relative* time, and 3) does not allow an assessment of true regions of kinematic consistency within a feeding.

When kinematic curves are averaged in this manner it is important to realize that the *absolute* variability at any point may

increase with time as sequences get progressively out of alignment with each other. If error-bar amplitude for the averaged curves decreases with time (even as amplitude increases), then kinematic stereotypy is strongly indicated. We note that for our kinematic sequences, the alignment procedure had no deleterious effect on variation with time: error bars were often smaller as the means increased, and error bars were as likely to decrease with time as they were to increase (Figs. 3, 4).

A series of electrical stimulation experiments was conducted using six *Ambystoma tigrinum* to test the function of key muscles hypothesized to be involved in tongue protrusion. Resultant information and kinematic data were used to develop a model for the kinetics of tongue projection in tiger salamanders. All animals were anesthetized using buffered tricaine methanesulphonate. The integument and superficial buccal musculature were removed from three of the six experimental animals to assure the exact placement of electrodes and to allow an unobstructed view of muscle actions and the movement of hyobranchial elements. Single monopolar stainless steel electrodes (0.051 mm in diameter) were implanted in opposite ends of each muscle. The bared electrode tips were 0.5 mm long, and electrodes were inserted the same way in each experiment. The electrodes from each muscle were connected to muscle stimulators (Grass S44 and S48) so that equal electrical stimulation was simultaneously delivered to each muscle of a bilateral pair. Synchronization of the two stimulators made it possible to independently activate two or more bilateral muscle pairs simultaneously to assess the combined actions of several muscles. Muscles were given both twitch (single pulse) stimuli with a 2–5 ms duration and a presumably tetanic stimulus (30–50 Hz, 2–5 ms pulse duration, 5–15 V). This produced contractions of bilateral muscle pairs with no visible cross stimulation to other muscles. As single muscle pairs and combinations of different muscles were stimulated, their actions and effects on the head, mandible, hyobranchial apparatus, and tongue were recorded. Each stimulation was repeated several times in each of the six animals, and movements were observed from dorsal, lateral, ventral, and anterior aspects.

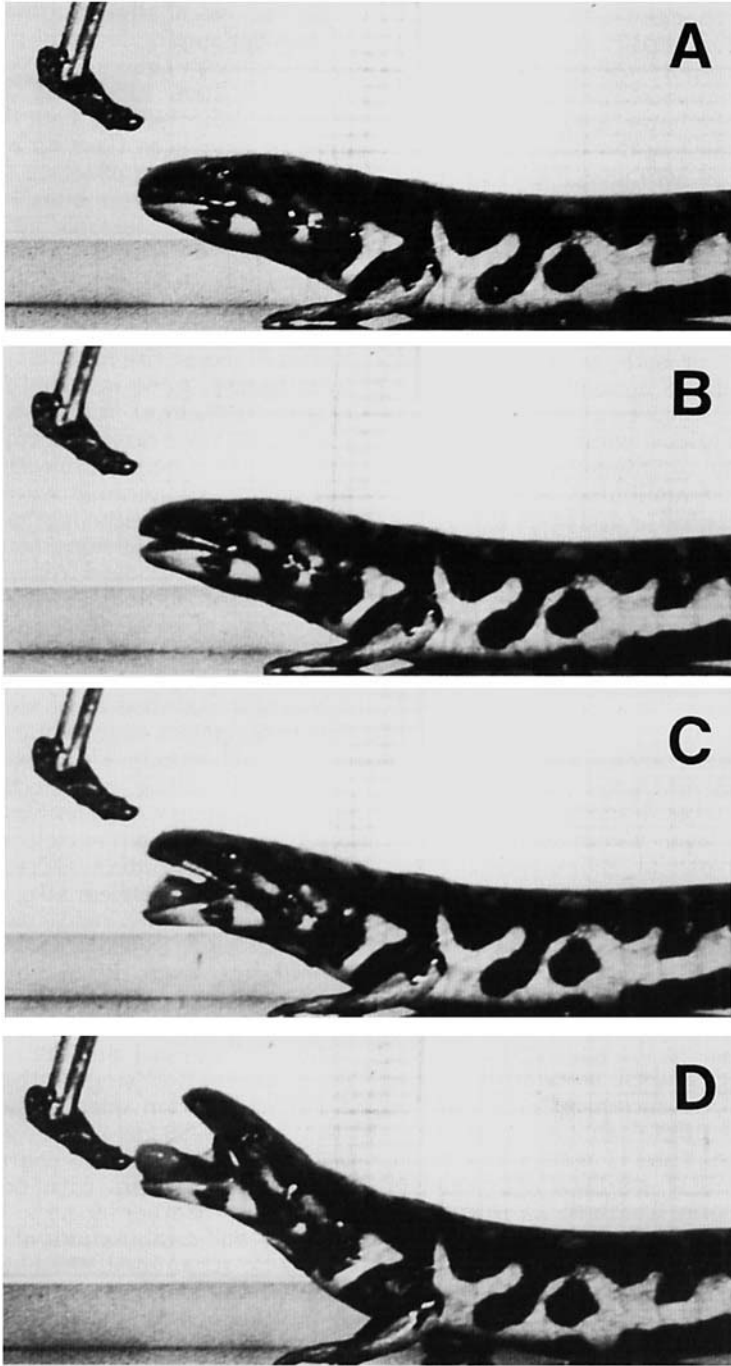


Fig. 2. Frames from high-speed films depicting four stages in tongue projection: **A**, resting condition 5 ms before mouth begins to open; **B**, lift phase 5 ms after mouth opens with gular area raised; **C**, protracted phase 15 ms after the mouth opens with the hyobranchial apparatus protracted; **D**, fully projected and flipped tongue

at approximately 40 ms after the mouth starts to open with the hyobranchial apparatus protracted and tongue pad flipped anteriorly. Note that these frames represent only one feeding sequence, while Figures 3, 4 show means for ten feedings.

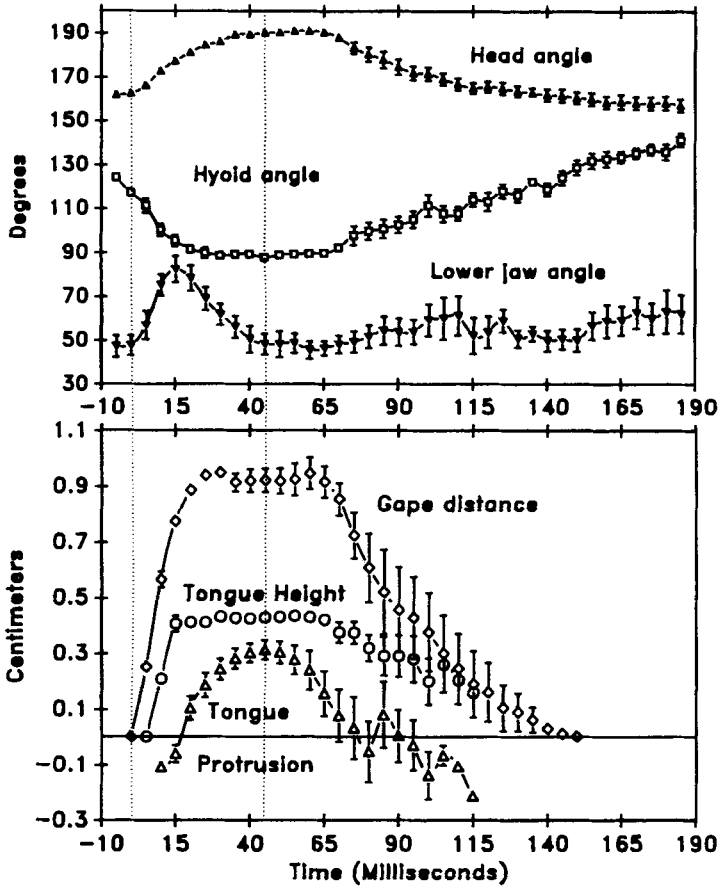


Fig. 3. Quantitative curves for six variables describing the kinematics of terrestrial feeding in *Ambystoma tigrinum*. Symbols represent means ( $\pm$ SE) at 5-ms intervals for variables digitized from ten different feedings of two individuals. Vertical dotted lines indicate time zero and the time of peak tongue protrusion. Where no

error bars are visible, they lie within the width of the plotted symbol. Note the rapid increase to maximum tongue height, and depression and elevation of the lower jaw relative to the horizontal prior to peak tongue protrusion.

## RESULTS

### Quantitative kinematics

#### Terrestrial feedings

Figure 2 shows a typical pattern of movement during terrestrial feeding in *Ambystoma tigrinum*. Each frame depicts one of four major stages seen during the process of tongue projection (resting, lifted, protracted, and flipped). Quantitative kinematics of ten averaged feedings from two individuals are presented in Figure 3. As a reference point for discussion, time zero is defined as the frame preceding the first frame in which the mouth begins to open. The most stereotyped part of the feeding cycle occurs during the

period of tongue projection (Fig. 3): tongue height, protrusion, and gape error bars all decrease in amplitude. The retraction period shows much greater variation, especially in the distance variables that reflect the motion of the tongue on its way back into the mouth (Fig. 3). The gape opens to its widest point in 25 ms as the head is raised and the lower jaw is quickly lowered (Fig. 3). The gape is held at its widest point for 40 ms while the tongue is being projected and then gradually closes for a maximum of 150 ms that the mouth is open. During tongue projection, the hyoid angle decreases over  $30^\circ$ , reflecting the anterodorsal protraction of the hyobranchial apparatus.

From the kinematic distance variables (Fig. 3), several important features of the tongue projection process can be seen. First, the tongue attains its near-maximum height in the first 15 ms of gape opening and remains near its peak excursion, which corresponds to the period of maximum gape, until well into the retraction phase. This indicates that the base of the tongue moves and is held dorsally in the opening mouth during projection.

Second, tongue protrusion proceeds rapidly, breaking the plane of the gape between 15 and 20 ms, well before maximum gape and synchronously with the time that maximum tongue height is reached (Fig. 3: 15 ms). Maximum tongue projection of 3 mm beyond the plane of the gape is reached 45 ms after the start of mouth opening. The tongue again breaks the plane of the gape during retraction 75–80 ms after mouth opening.

Third, coinciding with the opening gape, dorsal tongue movement, and anterior movement of the tongue through the plane of the gape, there is a rapid increase in lower jaw angle (LJA) as the symphysis moves ventrally (Fig. 3). The increase in LJA while head angle (HDA) is also increasing (Fig. 3) indicates that the lower jaw is rotating ventrally about the jaw joint and not being passively carried dorsally. By the time of peak tongue protrusion, the lower jaw has returned to its initial angle (Fig. 3: LJA). The increase in LJA while HDA and gape are also increasing demonstrates that the increase in gape is due to *both* lower jaw depression and cranial elevation: the lower jaw is not held in a fixed absolute position during prey capture, nor is it held fixed with respect to the skull.

#### Aquatic feedings

The kinematics of aquatic feeding strikes by metamorphosed tiger salamanders are compared with terrestrial feedings in Figure 4. The kinematic pattern of tongue projection exhibited in terrestrial situations is not seen when the animals feed on the same prey held in the same relative position in water. During the first 20–35 ms of aquatic feeding, gape distance, hyoid angle, and tongue height closely parallel terrestrial feeding movements. In aquatic feeding, the gape distance is greater but the total time the mouth is open is shorter (100 ms maximum). Tongue height and hyoid angle measurements indicate that early in aquatic feeding there is

dorsal movement of the tongue and hyobranchial apparatus prior to peak gape, but the tongue does not move out of the mouth. Instead, the hyobranchial apparatus begins to move posteroventrally 15 ms prior to aquatic peak gape rather than 5 ms after peak gape seen in terrestrial feeding. Thus, the hyoid is depressed 20 ms earlier during aquatic feeding (Fig. 4). Lower jaw angle also peaks later in aquatic feeding coinciding with the time of buccal depression. As in terrestrial feeding, the initial stages of the feeding behavior are highly stereotyped, with peak gape and hyoid depression having substantially smaller coefficients of variation compared with later or earlier stages.

#### *Morphology of the feeding system*

To facilitate description of the kinetic model of tongue projection in tiger salamanders, the anatomy of the feeding system is described using schematic diagrams of the tongue and associated muscles and skeletal elements reconstructed in three different positions (resting, protracted, and flipped).

Figure 5 is a schematic view of the head of a tiger salamander in a nonfeeding, resting position with the buccal area depressed and the tongue lying in the floor of the mouth (as shown in Fig. 2A). The hyobranchial apparatus consists of the hyoid arch (Fig. 5, vertical hatching) and two branchial arches (Fig. 5, horizontal hatching). The median basibranchial (Fig. 5, bb) provides a platform that supports the tongue pad and is the medial articulation point for the hyobranchial arches. The ceratohyals (Fig. 5, ch) lie above the rest of the hyobranchial apparatus in the floor of the mouth and articulate anteriorly with the ventral basibranchial via the small cartilaginous first radials (Fig. 5, r1). Posteriorly, the ceratohyals are tightly attached to the quadrate bone of the skull by the hyoquadrate ligaments (Fig. 5, hq1). In addition, fascia and the depressor mandibulae secure the last 1–2 mm of the ceratohyals to the quadrate bone. These ligaments and other attachments greatly restrict the movement of the ceratohyals posteriorly (personal observation, Dockx and De Vree, '86). The first radials, through their attachments to the ceratohyals, limit the forward movement of the basibranchial and therefore the entire hyobranchial apparatus. The second radials (Fig. 5, r2) project from the body of the basibranchial dorsolaterally into



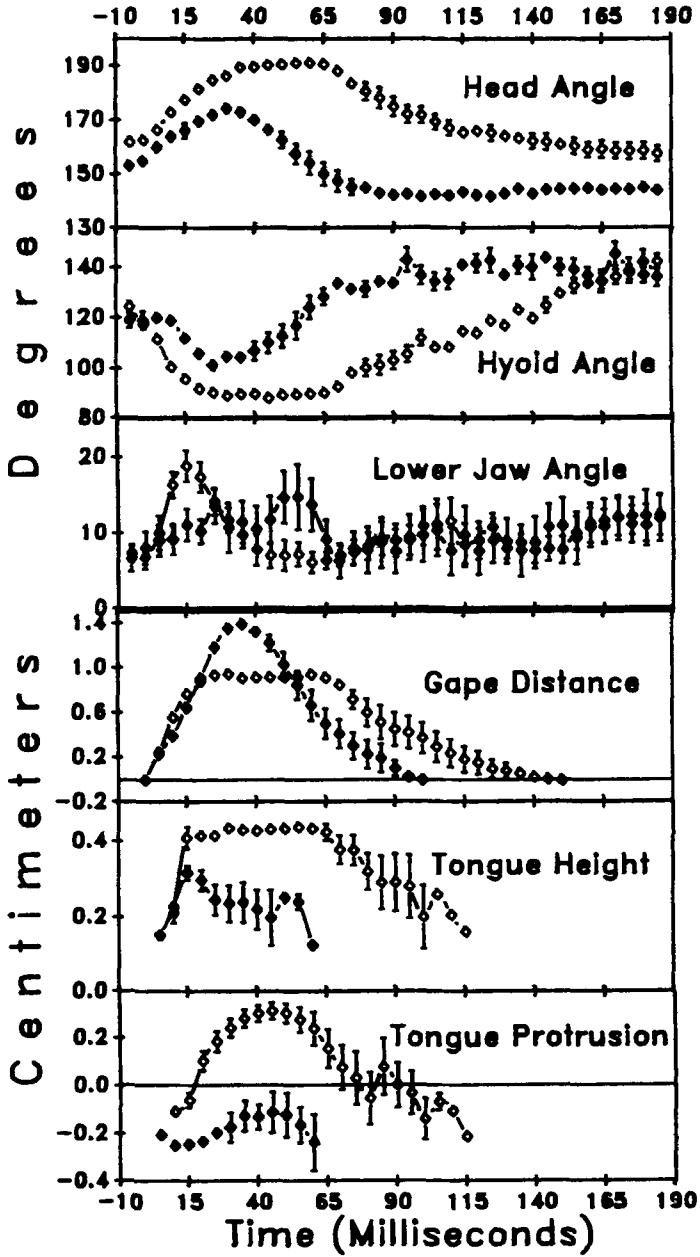


Fig. 4. Comparison of kinematic differences between two adult *Ambystoma tigrinum* feeding on land (open symbols) and in the water (closed symbols); both individuals fed in both environments. Initial dorsal movements of the hyobranchial apparatus and tongue are similar for the first 20 ms in both environments, but they differ considerably thereafter, indicating kine-

matic modulation of tongue function in response to the environment. Note that the plateau phase of the gape profile disappears when feeding occurs in the water and that tongue protrusion never breaks the plane of the gape. The plane of the gape is indicated by the zero line.

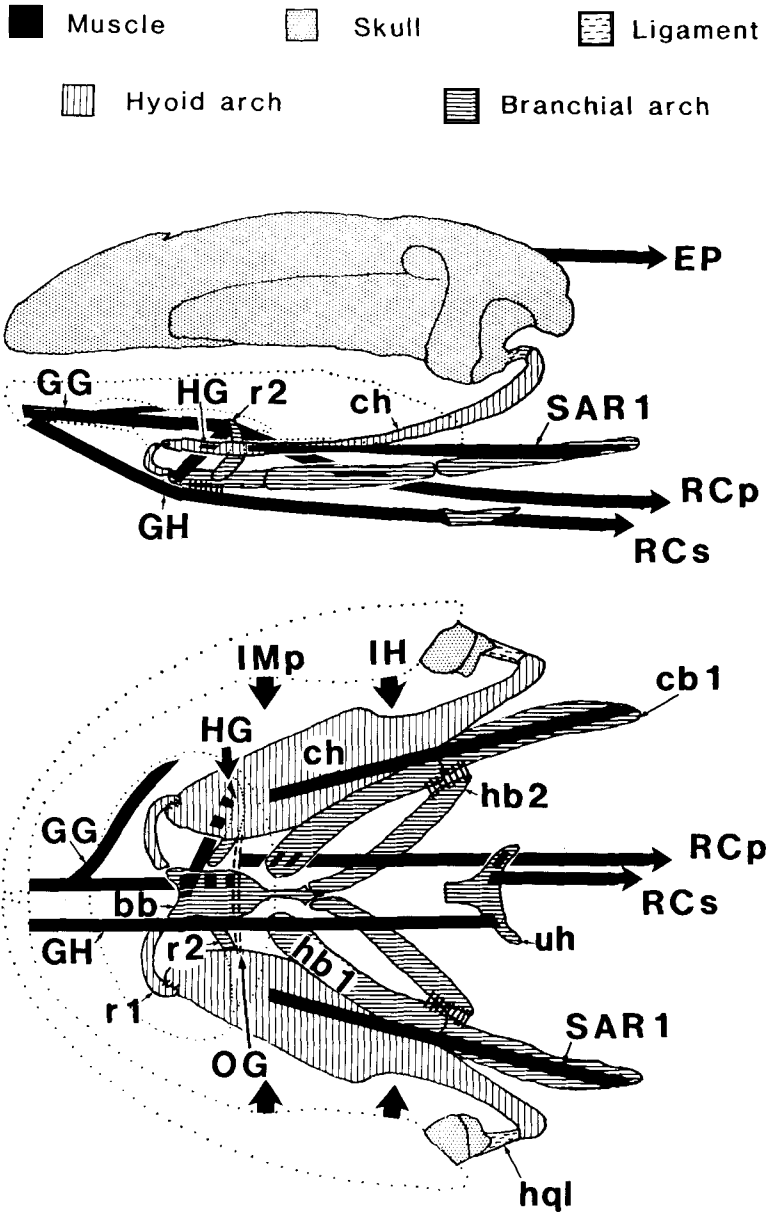


Fig. 5. Schematic diagram of the major functional components of the feeding mechanism in *Ambystoma tigrinum* at rest in lateral (as in Fig. 2A) and ventral views. Thick arrows associated with the intermandibularis posterior and interhyoideus muscles indicate that these muscles span the mandibular rami ventrally and that these muscles are not drawn to allow display of the underlying muscles. Dotted lines indicate the extent of the lower jaw and tongue surface. bb basibranchial, cb

ceratobranchial, ch ceratohyal, EP epaxial muscles, GG genioglossus muscle, GH geniohyoideus muscle, hb hypobranchial, HG hyoglossus muscle, hql hyoquadrate ligament, IH interhyoideus muscle, IMp intermandibularis posterior muscle, og otoglossal cartilage, r radial, RCp rectus cervicis profundus muscle, RCs rectus cervicis superficialis muscle, SAR1 subarcualis rectus 1 muscle, uh urohyal.

the tongue pad. They support the otoglossal cartilage (Fig. 5, og), which is suspended between them dorsal to the basibranchial and forms the posterior margin of the tongue pad. The hypobranchials (Fig. 5, hb1-2) articulate proximally with posterior facets on the basibranchial. Ceratobranchial 1 (Fig. 5, cb1) articulates with the distal end of Hypobranchial 1. The distal end of Hypobranchial 2 is attached to the junction of Hypobranchial 1 and Ceratobranchial 1 by a ligament. The Y-shaped urohyal (Fig. 5, uh) is imbedded in muscle posterior to the basibranchial.

The muscles involved in the feeding system are illustrated by thick lines connecting the major origin and insertion of each muscle and lying along the major axis of action (Fig. 5). The intermandibularis posterior (Fig. 5, IMp) and interhyoideus (Fig. 5, IH) each extend from the mandibles (a few fibers insert on the fixed dorsal portion of the ceratohyal) to meet on a median raphe. Just dorsal to these muscles and parallel to the midline is the geniohyoideus (Fig. 5, GH), which extends from the mental area of the mandible underneath the basibranchial to insert on the lateral flange of the urohyal. A very important aspect of geniohyoideus muscle anatomy is that it has a strong attachment to the basibranchial through fascia and a close association with the genioglossus muscle. Its attachment to the basibranchial allows the geniohyoideus to act in two separate parts or as a whole depending on the movement of its posterior insertion on the urohyal.

There are two muscles in the tongue itself. The genioglossus (Fig. 5, GG) extends from the mental area of the mandible and spreads out to attach directly to the basibranchial and otoglossal cartilage and throughout the tongue pad, forming the bulk of the tongue mass. Weak lateral slips of the genioglossus insert dorsolaterally along the anterior fifth of the ceratohyal. Between the mental insertion and the basibranchial, the genioglossus lies directly dorsal and parallel to the geniohyoideus. The hyoglossus (Fig. 5, HG) is a small muscle that extends between the tips of the otoglossal cartilage and second radials anteriorly to the dorsal surface of the basibranchial.

The rectus cervicis muscle extends from the ventral trunk musculature anteriorly in two parts to the hyobranchial apparatus. The rectus cervicis superficialis (Fig. 5, RCs) inserts on the posterior aspect of the urohyal.

The rectus cervicis profundus (Fig. 5, RCp) runs dorsal to the superficialis, passes ventral to Hypobranchial 2, and dorsal to Hypobranchial 1 to insert lightly on the basibranchial, Hypobranchial 1, but mostly on the otoglossal cartilage in the tongue.

The subarcualis rectus (Fig. 5, SAR1) has a wide origin on the ventral side of the anterior half of the ceratohyal. Its insertion forms an attached sheath around Ceratobranchial 1. Some fibers attach directly to the ventrolateral surface of the ceratobranchial, and many fibers wrap around before inserting at various angles all along the ceratobranchial. The ceratobranchial cannot be pulled from the sheath without tearing subarcualis muscle fibers.

The morphology of the tongue in the protracted position (similar to Fig. 2C) is shown in Figure 6. In this position, the skull has been elevated and the lower jaw has been depressed. The gular area is elevated and the hyobranchial apparatus is fully protracted toward the open gape. In this position, the anterior branchial arch elements (Fig. 6, horizontal hatching) project above the ceratohyals into the tongue pad with the first radials stretched ventrolaterally by the ceratohyals. The basibranchial almost reaches the plane of the gape. The tongue pad protrudes anteriorly just beyond the plane of the gape, but the anterior glandular portion remains on the anterior surface and the second radials project dorsolaterally to suspend the otoglossal cartilage along the rear margin of the tongue. The geniohyoideus, through its connection to the basibranchial, extends vertically from its origin on the mandible, and the urohyal is displaced anteriorly. The posterior tips of the ceratobranchials have moved forward slightly and align with the posterior end of the ceratohyals (compare Fig. 5 to Fig. 6: ch, cb1). Very little medial buckling occurs in the branchial arch.

In the flipped position (Fig. 2D), the tongue has reached its fully projected state (Fig. 7). The basibranchial is rotated ventrally by the first radials, genioglossus, and geniohyoideus, as can be seen when the tongue of a cleared and stained specimen is pulled out of the mouth and in cineradiography during feeding (Dockx and De Vree, '86). The posterior third of the ceratohyal is fixed to the quadrate and cannot move, and the anterior part remains ventral to the tongue in the floor of the mouth. The Subarcualis rectus

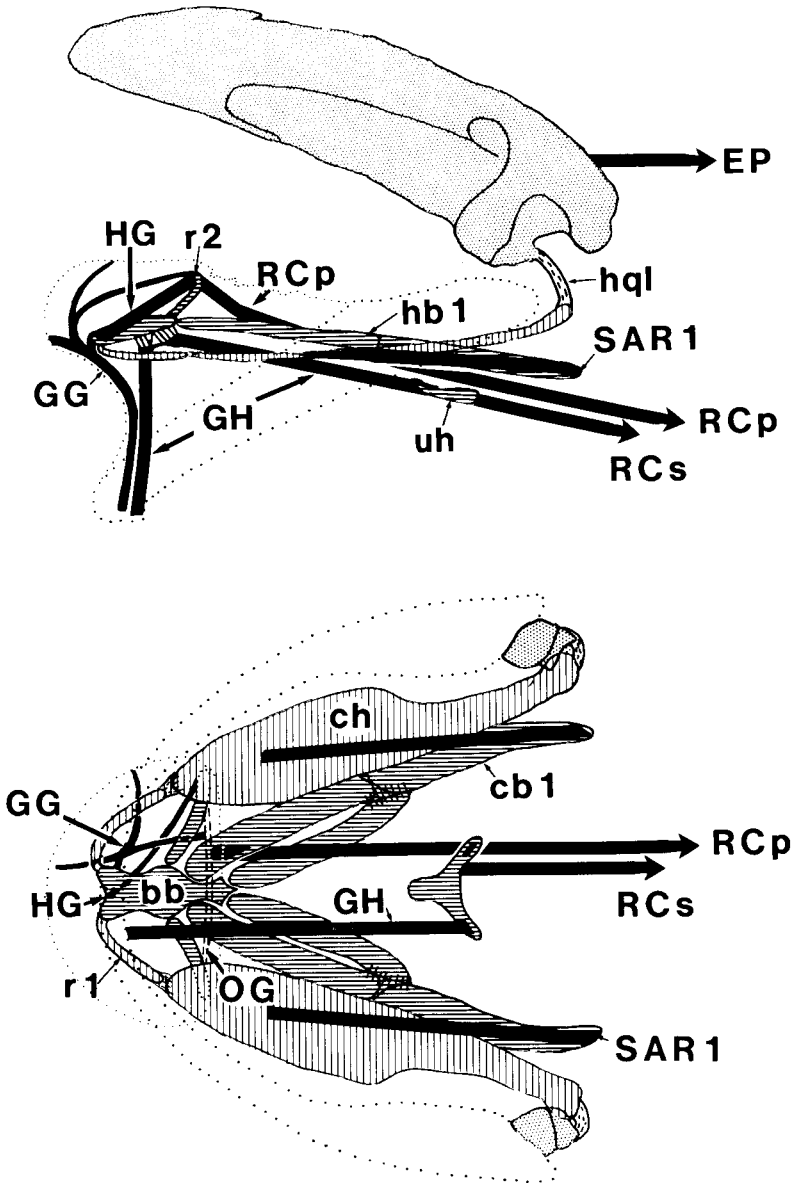


Fig. 6. Schematic diagram of the feeding mechanism in *Ambystoma tigrinum* during tongue projection; the hyobranchial apparatus is lifted and protracted and the

tongue not flipped (as in Fig. 2C). Dotted lines indicate the extent of the lower jaw and tongue surface. See Figure 5 legend for abbreviations.

1, the lateral slips of the genioglossus, and the tissue surrounding the anterior part of the ceratohyal prevent it from moving dorsoanteriorly as far as the tongue and hyobranchial apparatus. The glandular surface

is now directed ventrally, and the otoglossal cartilage and second radials extend anteriorly into the tongue mass. The hyoglossus and genioglossus muscles are presumably fully contracted.

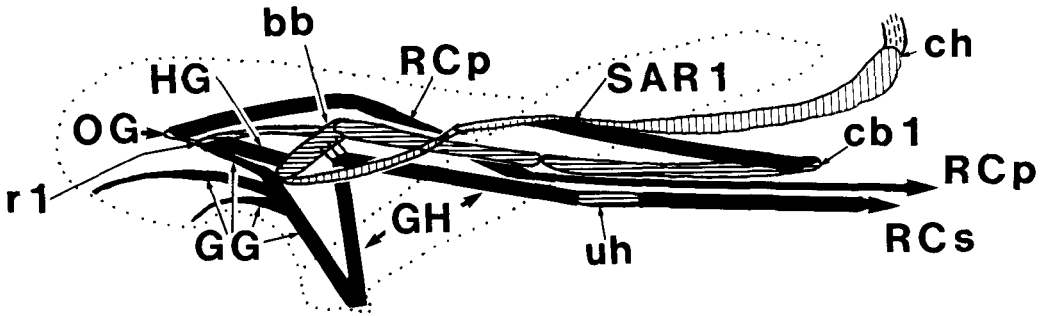


Fig. 7. Schematic diagram of fully projected tongue in *Ambystoma tigrinum* (as in Fig. 2D). Dotted line in-

dicates the extent of the tongue surface. See Figure 5 legend for abbreviations.

### Stimulation experiments

Muscle stimulations were done with the anesthetized animals held in three different positions to ascertain muscle actions and tongue movements with the hyobranchial apparatus, tongue, and jaws in different spatial arrangements. In the first position, the mouth was closed and the gular region was depressed as shown in Figure 5. In the second position, the mouth was held open by raising the head and lowering the jaw with the gular area still depressed. In this position, any muscle that could project the tongue when the mouth was open would be able to when stimulated. In the third position, the mouth was held open and the gular area was raised by holding a probe across the lower jaw under the point of jaw articulation. This simulated the contraction of the intermandibularis posterior and interhyoideus by creating a rigid buccal floor and brought the hypo- and ceratobranchials above the ceratohyal.

We stimulated the Subarcualis rectus 1 and geniohyoideus muscles individually or both simultaneously (Table 1). With the gular area depressed and mouth closed, stimulation of the Subarcualis rectus 1 moved the tips of the ceratobranchials anteriorly, buckled the hyoid apparatus ventrally (not medially), and pushed the basibranchial and tongue mass directly toward the mental symphysis about 1 mm. Slight ventral movement was seen in the ceratohyal and floor of the mouth. With the mouth open and the gular area elevated, the Subarcualis rectus 1 produced a strong anterodorsal movement of the hyobranchial apparatus and tongue

toward the palate. The posterior tips of the ceratobranchials moved about 2 mm anteriorly and 1 mm medially. When the hyobranchial apparatus is depressed (mouth open), the subarcualis rectus pushes the hyobranchial apparatus and tongue toward the mental symphysis, which impedes its forward movement, causing the cerato- and hypobranchials to buckle ventrally.

Stimulation of the geniohyoideus muscle moved the basibranchial, tongue, and urohyal about 1–2 mm toward the mental symphysis (Table 1). From the resting position (Fig. 5), the geniohyoideus pulls the hyobranchial apparatus and tongue anteriorly and slightly dorsally directly toward the mental symphysis. With the mental symphysis being directly anterior to the hyobranchial apparatus, the geniohyoideus pulls the tongue directly anterior toward the symphysis. With the mouth open and the hyobranchial apparatus elevated above the level of the symphysis, the geniohyoideus pulls the tongue anteroventrally, putting a strong anteroventral force on the basibranchial.

Simultaneous stimulation of the geniohyoideus and the Subarcualis rectus 1 produced additive vectorial effects (Table 1). In jaw positions with gular depression, contraction of both muscles moved the tongue and hyobranchial apparatus toward the mental symphysis. In both cases, the gular region bulged ventrally from the buckling of the hypo- and ceratobranchials. When the gular area was elevated, the anterior and dorsal force of the Subarcualis rectus 1 summed with the anterior and ventral force of the geniohyoideus to push the tongue and hyobranchial apparatus anteriorly about 2

TABLE 1. Movements of the tongue and hyobranchial apparatus resulting from electrical stimulation of key tongue projection muscles in *Ambystoma tigrinum*<sup>1</sup>

Muscle	Direction of tongue and hyobranchial movement		
	Mouth closed, buccal floor depressed	Mouth open, buccal floor depressed	Mouth open, buccal floor elevated
SAR1	To symphysis (anterior)	To symphysis (anterior)	Toward vomer (anterior and dorsal)
GH	To symphysis (anterior)	To symphysis (anterior)	To symphysis (anterior and ventral)
SAR1 + GH	To symphysis (anterior)	To symphysis (anterior and dorsal)	Forward to gape (anterior)

<sup>1</sup>Direction of movement is given relative to morphological structures and the axis of the body (in parentheses), SAR1, subarcualis rectus one; GH, geniohyoideus.

mm almost to the plane of the gape. The protracted hyobranchial apparatus pushed the basibranchial into the tongue pad, causing it to bulge anteriorly, but no flipping of the glandular dorsal surface of the tongue occurred. The morphology of the tongue projection mechanism with the hyobranchial apparatus fully protracted (Fig. 6) is a result of the combined contractions of the interhyoideus, intermandibularis posterior, subarcualis rectus, and geniohyoideus.

The genioglossus muscle was stimulated from the dorsal side by electrodes placed near the mental symphysis and near the posterior edge of the tongue pad. The genioglossus moved the tongue toward the symphysis similarly to the geniohyoideus and curled the tongue pad anteriorly. Stimulation of the genioglossus probably also caused the hyoglossus to contract because it is located inside the genioglossus, and the second radials were observed to rotate anteriorly. The genioglossus (and hyoglossus) flipped the tongue pad forward but not to the extent seen in actual feeding (as in Fig. 7). Simultaneous stimulation of the genioglossus, geniohyoideus, and subarcualis rectus caused the protraction of the hyobranchial apparatus (as in Fig. 6) with some flipping of the tongue pad, but complete projection to the extent seen during natural feeding was not reproduced.

#### DISCUSSION Feeding kinetics

Based on kinematics and the observed line of action of individual muscle pairs and combinations of muscles, we present the following hypothesized kinetic model for tongue

projection in *Ambystoma tigrinum*. This model involves the coordinated protraction of the hyobranchial apparatus and simultaneous flipping of the tongue pad. Hyobranchial protraction results from the action of several muscles on the basibranchial or root of the tongue (Fig. 8). First, the intermandibularis posterior and interhyoideus lift the hyobranchial apparatus dorsally so that it can then slide anteriorly over the mandibles (Fig. 8, vector 1). This lifting phase (Fig. 2B) is seen in the rapid decrease in hyoid angle early in protraction (Fig. 3). Without this lifting force vector, forward movement of the hyobranchial apparatus is impeded by the lower jaw and the apparatus buckles ventrally (Table 1). When the lift force (Fig. 8, vector 1) is combined with the force of the Subarcualis rectus 1 (Fig. 8, vector 2), the hyobranchial apparatus moves anterodorsally toward the roof of the mouth (Fig. 8, dorsal vector). Dorsal movement of the tongue is seen in the occurrence of maximum tongue height early in the kinematics of feeding (Figs. 2C, 3) and from the muscle stimulations (Table 1). Similar dorsal movements have been reported in *Desmognathus* by Beneski and Larsen ('87). Strong dorsal movement of the basibranchial during feeding has been shown for tiger salamanders by cineradiography (Dockx and De Vree, '86).

To produce anterior movement of the tongue from this dorsal motion, a ventral force must be applied to the tongue. This is accomplished by the action of the geniohyoideus and genioglossus muscles attaching anteriorly to the lower jaw. The geniohyoideus, contracting between the symphysis and the basibranchial (Fig. 8, ventral vector), pulls

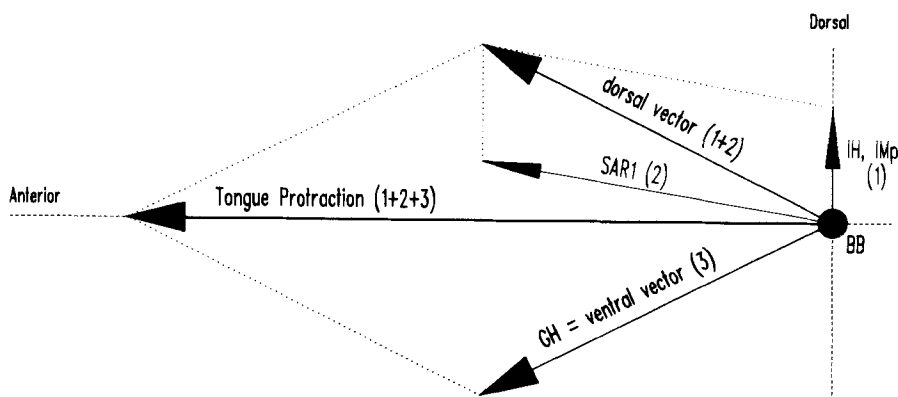


Fig. 8. Model of muscular forces estimated for the protraction of the hyobranchial apparatus during tongue projection in *Ambystoma tigrinum*. The dorsal vector (the resultant of vectors 1 and 2) is the force on the basibranchial (BB) produced by the Subarcualis rectus 1 muscle (vector 2) once the hyobranchial apparatus has been elevated by the combined vertical forces of the IMP and IH muscles (vector 1). The ventral vector (GH) is the force on the basibranchial produced by the genio-

yoideus once the tongue has been elevated and the lower jaw is depressed. The resultant net force of these two vectors on the basibranchial causes the anterior "protraction" of the hyobranchial apparatus (= tongue platform) from which simultaneous flipping of the tongue pad by other muscles projects the tongue out of the mouth. See Figure 5 legend for abbreviations.

the hyobranchial apparatus anteroventrally (Table 1). This action is aided by the dynamic insertions of this muscle on the urohyal and mental symphysis. Forward movement of the urohyal is needed to allow contraction of the anterior part of the geniohyoideus to move the tongue anteroventrally through its attachment to the basibranchial (Fig. 6). Equally important is the dynamic insertion of the geniohyoideus on the symphysis, which rapidly moves ventrally during protraction (Fig. 3). Depending on the position of the symphysis, the geniohyoideus lifts the hyobranchial apparatus, then pulls the tongue anteriorly, and, finally, it pulls the basibranchial anteroventrally. The rapid depression of the lower jaw, which occurs only during protraction, allows the tongue to be pulled ventrally. The combination of this ventral vector (Fig. 8) and the dorsal vector produced by the subarcualis rectus and other muscles (Fig. 8, dorsal vector) results in a net anterior force that protracts the basibranchial anteriorly toward the open gape (Fig. 8, tongue protraction vector; Table 1).

Protraction imparts considerable force and momentum on the tongue pad, bulging it anteriorly and protruding it to the plane of the gape (Figs. 2C, 6; Table 1). The flipping

of the tongue pad after hyobranchial protraction can be seen by comparing the protracted and projected phases of feeding (Fig. 2C,D). Maximum tongue height and the drop in the lower jaw angle reflecting the two force vectors needed for anterior protraction are attained 35 ms before peak tongue protrusion (Fig. 3). From the kinematics, we know that the tongue pad is flipped during projection (Fig. 2). In the stimulation experiments, we were able to protract the hyobranchial apparatus using two muscles or protract and partially flip the tongue when the tongue muscles also were stimulated (Table 1). Therefore, it seems likely that contraction of the genioglossus and hyoglossus muscles flips the tongue at the time when maximum momentum has been imparted on the tongue pad by hyobranchial protraction (Fig. 8, tongue protraction vector). Flipping of the tongue pad occurs simultaneously with tongue protraction, resulting in complete projection of the tongue with the glandular surface of the tongue facing ventrally (Fig. 7).

#### *Comparative functional morphology*

The kinematic data reported here for adult *Ambystoma tigrinum* show several novelties when compared to feeding in other aquatic lower vertebrates and in larval tiger sala-

manders. The gape profile in adult *A. tigrinum* feeding on land has a significant plateau phase of near-constant gape (Fig. 3) during which the tongue is protruded from the mouth. The tongue first crosses the plane of the gape when mouth opening reaches 82% of its maximum value and achieves peak protrusion while the gape is held at its maximum position. During aquatic feeding in ray-finned fishes studied to date, there is never a plateau phase to the gape profile (Elshoud-Oldenhove and Osse, '76; Lauder, '79, '80a,b; Liem, '70; Osse and Muller, '80). Similarly, in larval salamanders, no plateau phase has been reported for the gape profile (Lauder and Shaffer, '85; Shaffer and Lauder, '88), and when adult tiger salamanders feed in the water, the plateau disappears (Fig. 4: gape distance). The plateau in the gape profile of terrestrial feedings is thus correlated with tongue protrusion past the plane of the gape. In the water, adult *A. tigrinum* do not protrude the tongue past the gape.

Similar results to ours were obtained by Bemis and Findeis ('86) who analyzed 19 feedings for ten adults and noted a plateau in the gape profile of *Taricha* that coincided with maximum tongue protrusion. Our results differ, however, from gape profiles of *Salamandra* (Dockx and De Vree, '86) and *Cynops* (Miller and Larsen, 1987), which appear to lack a plateau and show maximum gape occurring instead during tongue retraction. Perhaps the presence or absence of a plateau phase in the gape profile (if substantiated by further research) may be a reflection of family-level differences between the Ambystomatidae and Salamandridae.

The increase in gape that is recorded during feeding (Figs. 3, 4) could be achieved by elevation of the head (with the lower jaw held fixed), by depression of the lower jaw (with the skull held in a constant position), or by a combination of both movements. Our data (Fig. 3) show that both lower jaw depression and head elevation produce the initial rapid increase in gape during the first 15 ms of mouth opening. Then, as peak gape is attained (15–25 ms), the head continues to rise and the lower jaw is elevated. The plateau at peak gape is maintained by the continued increase in head angle and decrease in lower jaw angle to its initial position, then the head angle remains constant. Mouth closing is achieved almost exclusively by a rapid depression of the head.

If the increase in gape were due solely to cranial elevation, lower jaw angle as measured here should either stay the same or decrease (because of the lower jaw being elevated passively with the skull). In fact, the angle of the jaw *increases* markedly as the gape widens. Dockx and De Vree ('86) also illustrate a ventral rotation of the mandible during feeding in *Ambystoma*. These results differ from those stated by Larsen and Guthrie ('75) who indicate that gape increase in *A. tigrinum* occurs by cranial elevation alone with the position of the lower jaw symphysis remaining stationary. However, the jaw movements shown in plate 1 of Larsen and Guthrie ('75) illustrate that the angle of the lower jaw does increase during the strike in a manner that is consistent with the data shown in Figure 3.

The results shown here for *Ambystoma tigrinum* emphasize for terrestrial feedings a point made repeatedly in the literature on aquatic feeding in lower vertebrates: the large epaxial muscles are fundamentally important elements in the feeding mechanism and contribute significantly to causing an increase in gape (Lauder, '79, '80a; Lauder and Shaffer, '85; Shaffer and Lauder, '85, '88). This result is in contrast to Hinderstein ('71) who suggests that the dorsal spinal muscles hold the skull in a fixed position during mouth opening.

If the kinetic model for tongue protrusion in *Ambystoma tigrinum* presented above is corroborated by experimental investigation (discussed below), then the tongue protrusion mechanics in ambystomatid and plethodontid salamanders are not identical. Stimulation of the buccal musculature (intermandibularis, interhyoideus, geniohyoideus, and anterior Subarcualis rectus 1) through the skin in plethodontids produces partial tongue protraction (Eaton, '57) and suggests that, as in our model for *A. tigrinum*, these muscles are important in lifting and partially protracting the hyobranchial apparatus. Bramble and Wake ('85: p. 255) also emphasize the importance of contractions of the buccal floor musculature to prevent ventral buckling of the hyobranchial apparatus. As hypothesized in a series of investigations on the morphology and function of the feeding system in plethodontid salamanders (Lombard and Wake, '76, '77, '86; Roth, '76; Thexton et al., '77; Wake et al., '83; also see Drüner, '02; Severtsov,



'71), the Subarcualis rectus 1 muscle provides the primary motive force projecting the tongue from the mouth. In ambystomatids, while this muscle is a fundamental component of the projection system, it functions in concert with the geniohyoideus (and probably genioglossus) to produce the resultant anterior force mediating hyobranchial protraction. The differences between ambystomatid and plethodontid tongue projection mechanisms appear to be due, in part, to differing structure of the hyobranchial apparatus and the Subarcualis rectus 1 muscle.

In plethodontids, tongue projection is thought to involve a "tractrix" model where the ceratobranchials are folded medially as they are propelled forward by the Subarcualis rectus 1 (Lombard and Wake, '76). This mechanism is dependent on elongate flexible ceratobranchials that can freely bend and slide in and out of the unattached sheath formed by the Subarcualis rectus 1 during projection and retraction. The tractrix model cannot apply to *Ambystoma tigrinum* and other terrestrial salamanders in which the Subarcualis rectus 1 inserts broadly on the distal half of Ceratobranchial 1, preventing it from sliding. Therefore, movement of the hyobranchial apparatus is limited to the longitudinal distance that the Subarcualis rectus 1 shortens when it contracts. In plethodontids, movement of the hyobranchial apparatus involves both this longitudinal contraction distance of the anterior part of the Subarcualis rectus 1 and the distance that Ceratobranchial 1 is squeezed from the bulb of circularly arranged fibers at the posterior end of the Subarcualis rectus 1. Thus, it seems that at least the longitudinal component of Subarcualis rectus 1 action is shared among terrestrial salamanders. In *Ambystoma* and other lunged salamanders, the ceratobranchials are short and rigid with varying amounts of ossification that limit medial buckling necessary for the tractrix model of hyobranchial protraction.

A further difference confounding functional comparisons of Subarcualis rectus 1 action among terrestrial salamanders is that the plethodontids have greatly elongate first ceratobranchials that slide through the long bulb-like Subarcualis rectus 1 muscles. These may be novel developmental additions to the primitive condition seen in *Ambystoma tigrinum*. In *Eurycea bislineata*, the elongate

adult Ceratobranchial 1 forms from a novel proliferation of anterior perichondral cells of the larval element at metamorphosis (Alberch and Gale, '86; Alberch et al., '85). This area of proliferation, or developmental "hot spot," lies under the posterior insertion of the larval Subarcualis rectus 1 (Christopher Rose, personal communication). Development of the adult Ceratobranchial 1 from this hot spot may induce the novel developmental induction of the greatly enlarged and bulb-like posterior part of the Subarcualis rectus 1, seen in plethodontids, as the adult ceratobranchial grows posteriorly through the muscle at metamorphosis. Thus, plethodontids may be characterized by novelties in both the development and function of the tongue projection system not present in ambystomatids.

#### *Metamorphosis of the tongue*

The discussion of feeding kinetics in ambystomatid salamanders is incomplete without considering the transformations (both conserved features and novelties) that occur in the feeding mechanism over metamorphosis.

Several key functional components of the aquatic feeding mechanism are conserved through metamorphosis and play important roles in both aquatic and terrestrial feeding in adults. Hyobranchial elements (ceratohyal, basibranchial, Hypobranchials 1 and 2, and Ceratobranchial 1) and muscles directly involved in suction feeding (buccal depression: rectus cervicis, geniohyoideus; hyoid elevation: intermandibularis posterior, interhyoideus; Lauder and Shaffer, '85) remain intact through metamorphosis, and, thus, adult salamanders are able to suction feed in the water (Lauder and Shaffer, '86). Another function that remains through metamorphosis is the ability to elevate the hyobranchial apparatus (Fig. 3). The muscles that produce buccal elevation in suction feeding also are vital to the tongue projection model since they contribute to the dorsal vector (vector 1, Fig. 8) that lifts the hyobranchial apparatus early in terrestrial feeding (Fig. 3) and probably in prey manipulation (see below).

Novelties also arise in feeding morphology at metamorphosis. A major change in one biomechanical pathway mediating lower jaw depression during feeding is accomplished when the larval mandibulohyoid ligament

becomes the hyoquadrate ligament (Lauder and Shaffer, '88). The change involves a shift of one insertion of the ligament from the lower jaw to the quadrate, while its other insertion moves to the posterior end of the ceratohyal. This secures the posterior end of the ceratohyal tightly behind the point of jaw articulation and allows free dorsoventral movements of the anterior hyobranchial apparatus without coincident movements of the lower jaw (e.g., in buccopharyngeal respiration and aquatic suction feeding by transformed adults). Thus, after metamorphosis, the depressor mandibulae muscles become the primary muscles to produce lower jaw depression.

In another addition at metamorphosis, the adult tongue develops from several new additions and changes in the existing morphology. Most of the tongue pad forms during metamorphosis as the genioglossus, hyoglossus, glandular area, and lingual sinuses develop (Yamasaki, '56). Within the tongue pad, the second radials and otoglossal cartilage form and anterior slips of the rectus cervicis profundus grow forward to insert on the otoglossal cartilage.

The kinematic data obtained from aquatic and terrestrial feedings (Figs. 3, 4) show that these morphological changes produce a terrestrial feeding system that is not fixed in its motor action. Aquatic feedings by metamorphosed individuals show no tongue projection past the plane of the gape, in sharp contrast to terrestrial feedings (Fig. 4). It thus appears that, in contrast to the overall conservatism in the motor pattern of the six jaw and hyoid muscles analyzed across metamorphosis by Lauder and Shaffer ('88), muscles (such as the genioglossus) and functions (such as tongue projection) that appear at metamorphosis are modulated in response to environmental factors.

#### *Tongue function and prey manipulation*

In salamanders, the hyobranchial apparatus generally has three major functions during aquatic feeding by larvae (Lauder and Reilly, '88; Lauder and Shaffer, '85, '86; Wake, '82): to open and close the gill slits (thus modulating intraoral fluid flow), to expand and contract the buccal cavity in suction feeding, and to press the rudimentary tongue against palatal teeth during prey manipulation. During the ontogenetic change to terrestriality, the latter two functions are retained and metamorphic changes in the

hyobranchial apparatus and tongue allow the buccal pump to move air and the tongue to be projected from the mouth to capture prey.

Regal ('66) hypothesized that the projectile tongue evolved from one of manipulative function. We concur, and provide a mechanistic explanation of intraoral prey manipulation and crushing that provides a functional basis for hypothesizing such an historical transition in function. Hypotheses for these functions can be explained on the basis of our model for *Ambystoma tigrinum*.

We hypothesize that the combination of the lift force and the dorsal force of the Subarcualis rectus 1 (Fig. 8., dorsal vector) that occurs early in tongue projection is the same mechanism used to press prey against the vomerine teeth after the prey is brought into the mouth by the retraction of the tongue and the mouth is closed. Note that the dorsal vector results in the tongue being pushed more dorsally than anteriorly and would press the tongue against the opposing arc of vomerine teeth to crush or subdue the prey when the mouth is closed. The functional mechanism to crush prey with the tongue with the dorsal vector requires only that the mouth be held closed by the adductor musculature.

Manipulations to move the prey item posteriorly in the mouth can be produced by combined actions of the dorsal vector to hold the tongue up and the rectus cervicis muscle to retract the hyobranchial apparatus and tongue. Then the tongue could be swung ventrally (by relaxation of the dorsal vector muscles) and then anterodorsally again by reactivating the dorsal vector while the prey item remains partially held in the pharynx or stuck to the vomerine teeth. Thus, a cycle of activity is formed by alternation of activity in dorsal and ventral vector biomechanical systems.

In cases involving large prey items (that fill the mouth and bulge the hyoid and tongue ventrally below the jaw), a mechanism is needed to allow the tongue to slide forward under the prey item. This type of manipulation could be initiated by opening the mouth slightly and thus reactivating the anterior protraction vector (Fig. 8, ventral vector), causing the tongue to slide anteriorly (as in tongue projection), underneath the prey item. Then, as the lower jaw is elevated again, achieving a new grasp on the prey with the jaws, 1) the ventral vector is removed, canceling the net anterior movement of the tongue and 2) the dorsal vector again predom-

inates to push the tongue and prey dorsally against the palate. Once again, the combined actions of the dorsal vector and the rectus cervicis could move the tongue and prey item posteriorly toward the pharynx. This hypothesis implicates movement of the lower jaw symphysis as the key factor controlling manipulative and projectile tongue functions by altering the line of action of the geniohyoideus and genioglossus muscles.

Morphological additions to the tongue at metamorphosis also may allow the tongue pad to be flipped anterodorsally whether the mouth is open or not. When the mouth is closed, hyobranchial protraction pushes the tongue pad against the palate. We hypothesize that the flipping action of the hyoglossus and genioglossus may pull the otoglossal cartilage forward and laterally, shaping it to conform with the shape of the transverse row of vomerine teeth.

#### Predictions

A key value of any model is the ability to make predictions, and the kinetic model proposed in this paper generates testable predictions on three levels.

First, several predictions about causal biomechanical relationships during tongue projection in *Ambystoma tigrinum* can be examined. 1) Our kinetic model can be tested by experimentally removing the force vectors involved and recording the kinematic effects on tongue projection. For example, if the geniohyoideus is cut near the symphysis, thus removing the ventral force vector (Fig. 8), we predict that the tongue would move strongly toward the roof of the mouth, and, if projected, would possess an increased dorsal component. The model would be refuted if this treatment produced no change in feeding kinematics. 2) The kinetic model also could be tested using quantitative electromyography. From our model, we predict that the intermandibularis posterior, interhyoideus, geniohyoideus, and subarcualis rectus will show peak activity at the time when the hyobranchial apparatus is being protracted and that the rectus cervicis is relatively inactive during protraction and at peak activity during the initial retraction.

Second, the model allows testing of the predicted causal bases of differences in kinematics observed between terrestrial and aquatic feedings. Genioglossus and hyoglossus muscle activity is predicted to be modulated with the environment. These muscles

should be inactive during aquatic feedings by metamorphosed individuals, but active during intraoral prey manipulation or terrestrial feeding. This prediction can be tested by quantifying electromyographic activity in the genioglossus and hyoglossus muscles during both aquatic and terrestrial feedings.

Third, the model predicts kinematic and electromyographic patterns used during intraoral prey manipulation. 1) We predict that the electromyographic pattern of the Subarcualis rectus 1, intermandibularis posterior, and interhyoideus muscles will be similar during both the initial strike and dorsal movements of the tongue in intraoral prey manipulation. Actions of these muscles activate the dorsal vector of the kinetic model (Fig. 8) and should function to press prey between the tongue and vomerine teeth. 2) In addition, we predict that the activity pattern of the depressor mandibulae, adductor mandibulae, and geniohyoideus will differ significantly between peak tongue projection and intraoral manipulation, as these muscles modulate the line of action of the ventral vector of the model (Fig. 8).

A final phylogenetic and historical prediction derives from the idea that tongue projection evolved from an intraoral manipulative function. We predict that the motor patterns used to generate the dorsal vector of the model (Fig. 8) are more primitive than those that modulate the ventral vector and should have a more general phylogenetic distribution than do motor patterns associated with the ventral vector. Furthermore, we predict that the ventral vector motor pattern arose at the same phylogenetic level as the morphological specializations at metamorphosis that allow tongue projection. Limitations on both our knowledge of salamander phylogenetic relationships and on the diversity of extant plesiomorphic taxa may make testing this last hypothesis difficult, but we suggest that hypotheses of this nature are integral to extending current research in historical morphology.

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