

# Activation patterns of the tongue-projector muscle during feeding in the imperial cave salamander *Hydromantes imperialis*

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## Summary

Salamanders of the genus *Hydromantes* project their tongues the greatest distance of any amphibian to capture prey, up to 80% of body length or approximately 6 cm in an adult individual. During tongue projection on distant prey, the tongue is shot ballistically and the tongue skeleton leaves the body of the salamander entirely. We investigated an aspect of the motor control of this remarkable behavior by examining electromyographic patterns within different regions of the tongue-projector muscle, the subarcualis rectus (SAR). SAR activation is strongly modulated, and features of this modulation can be predicted by tongue-projection distance (i.e. prey distance). The strap-like buccal portion of the SAR is always activated first and for the longest duration, compared to any other region. It is in a position to transmit force generated by the posterior SAR to the floor

of the mouth, where it originates. The posterior SAR encompasses and applies force to the epibranchial of the tongue skeleton, and its activation pattern gradually changes from a posterior-to-anterior wave of activation onset during short-distance projection to an all-at-once pattern during the most extreme long-distance (ballistic) projection. The duration of activity and EMG area of each recorded region of the SAR increase with increasing prey distance, showing greater muscle recruitment during long-distance projection. No effect of prey-capture success was observed in the EMG patterns, indicating that SAR activation is controlled in a feed-forward manner.

Key words: electromyography, salamander, *Hydromantes imperialis*, modulation, feeding, tongue.

## Introduction

Terrestrial amphibians capture their prey using their tongues (Duellman and Trueb, 1986), and members of the salamander family Plethodontidae are the most extreme in distance and speed of tongue protraction (Deban, 2002). Among plethodontids, the bolitoglossine genus *Hydromantes* can project the tongue the farthest, up to 80% of body length, and *Hydromantes* and other bolitoglossine plethodontids can project their tongues using a ballistic mechanism in which the tongue is shot with sufficient velocity to reach the target under its own momentum (Deban et al., 1997).

In ballistic tongue projection, the tongue skeleton to which the tongue pad is attached is shot completely from the mouth of the salamander. To accomplish this extraordinary feat, the primary tongue projector muscles, the bilaterally paired subarcualis rectus (SAR), must not be directly attached to the elongated epibranchials, which they encompass. They must also have a morphology and activation pattern that allows them to apply force rapidly enough to accelerate the tongue to high velocity in short distance. Previous research on *Hydromantes* has revealed that the muscle fibers of each SAR wrap in a complex arrangement, circumferentially, around the tapered epibranchial cartilage (Lombard and Wake, 1977) and that the epibranchial is free to evacuate the muscle entirely (Deban et al., 1997).

Articulating cartilaginous elements make up the tongue skeleton of terrestrial salamanders. The single basibranchial lies medially in the floor of the mouth and supports the tongue pad. On each side, a first and second ceratobranchial articulates with the caudal half of the basibranchial. In *Hydromantes*, the basibranchial is approximately twice the length of the ceratobranchials. An epibranchial articulates with the caudal ends of the first and second ceratobranchials. The epibranchial in *Hydromantes* and bolitoglossines is greatly elongated and extends over the shoulder and some distance down the trunk. In *Hydromantes*, the epibranchial is the longest of any salamander, and tapers from rostral to caudal along its entire length (Lombard and Wake, 1977).

During tongue projection, the tongue skeleton folds medially, becoming a compact projectile as it is pulled and squeezed forward relative to the ceratohyals. In bolitoglossines, the tongue skeleton is free from the projection muscles and can be projected completely from the mouth in a ballistic fashion. It is tethered to the body of the salamander by a bundle of tissue that includes the retractor muscles (i.e. the rectus cervicis profundus, RCP), blood vessels, nerves and a connective tissue sheath.

The SAR is the primary tongue-protraction muscle; it

originates broadly along the rostralateral edge of the ceratohyal and travels posteriorly to the epibranchial. The rostral portion (the subarcualis rectus anterior, SARA) has a fiber orientation that can pull the tongue skeleton rostral relative to the ceratohyal, and meets the caudal portion near the joint formed by the ceratobranchials and the epibranchial. The caudal portion of the SAR (subarcualis rectus posterior, SARP) forms an elongate sheath around the epibranchial with a complex segmented arrangement (Fig. 1) of short muscle fibers (less than 1 mm long). This arrangement has been proposed to exert radial forces during contraction that squeeze the tapered epibranchial rostrally and thus project the tongue from the mouth (Lombard and Wake, 1977; Deban et al., 1997). The posterior portion of the SAR in *Hydromantes* and other bolitoglossines is elongated compared to other taxa, encompassing the entire elongated epibranchial and the caudal ends of the ceratobranchials, but not directly attaching to those elements. The arrangement of muscle fibers of the SARP and the tapered shape of the epibranchial together suggest that the SARP can squeeze on the epibranchial anywhere along its length to push the tongue skeleton rostrally.

Tongue retraction is accomplished by the RCP, which is a long strap-like muscle that originates on the pelvis and inserts into the tongue pad. A portion of the RCP lies in a loop in the throat just in front of the heart when the tongue is at rest in the mouth in bolitoglossines; the extra length accommodates long-distance tongue projection. Electromyographic activity patterns of the RCP were reported previously (Deban and Dicke, 1999) in *Hydromantes supramontis*, a closely related species, and are not examined in this study.

Previous research into the activation pattern of the SAR has been limited to electromyographic (EMG) recordings from a single electrode placed at the center of the muscle (Deban and Dicke, 1999). Given the variation in activation pattern observed, and the anatomical complexity of the SAR, a more detailed examination of its activation patterns is necessary to understand its operation.

The objective of the present study is to determine the pattern of activation of different regions of the SAR during tongue projection in *Hydromantes*. We describe the temporal and spatial pattern of activation using 3–4 recording sites arrayed along the length of the SAR, and we examine the effects of prey distance and prey-capture success on the pattern of activation. These data are used to test three specific hypotheses regarding the motor control of tongue projection.

The first hypothesis is that the SAR shows a high degree of variation such that different regions are activated at different times and for different durations. The muscle is elongated, like the epibranchial, and its morphology and muscle fiber orientation suggest that motor units are arrayed lengthwise such that it can apply force to the epibranchial anywhere along its length. We hypothesize that it will be activated differentially depending on the extent of tongue projection, with more of the muscle being recruited as greater tongue projection is required. Previous electromyographic study of *Hydromantes*, using one recording site in the SAR, has shown

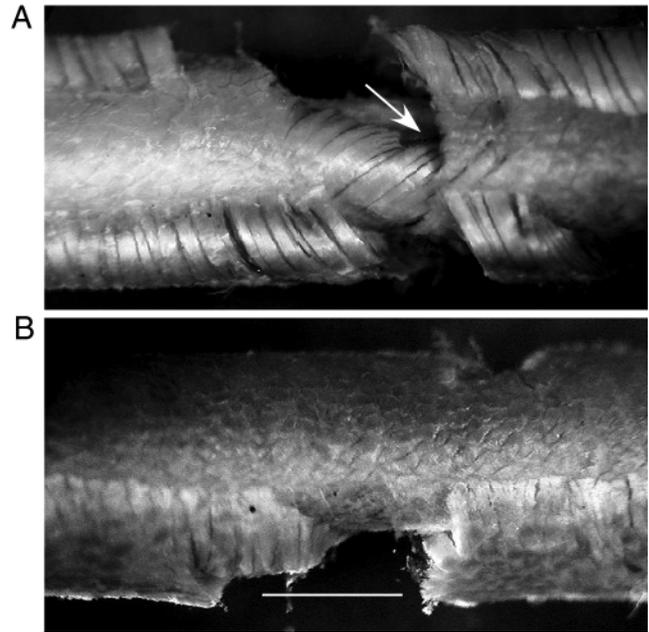


Fig. 1. Photograph of a section of the subarcualis rectus posterior (SARP) muscle of *Hydromantes imperialis* from two perspectives (A) medial, (B) lateral. The portion of the muscle in the center of each photo has been partially dissected to illustrate the complex arrangement of muscle fibers and the position of the epibranchial cartilage (arrow) in the lumen. Scale bar, 1 mm.

that the muscle is activated prior to its antagonist, the tongue retractor RCP, and that the relative onset time and duration of activation of these two muscles are strongly modulated in response to prey distance (Deban and Dicke, 1999). We expect that this modulation extends to recruitment within the SAR.

The second hypothesis, related to the first, is that ballistic and non-ballistic projection will display discrete muscle activity patterns. In ballistic projection, the tongue skeleton is shot completely from the SAR muscle and from the body of the salamander, and it is hence beyond the ability of the SAR to exert force upon it in the later stages of projection. In non-ballistic projection, the tongue skeleton is pulled and squeezed forward by the SAR, but does not leave the SAR, providing an opportunity for coordination of tongue movement by coactivity of the SAR and its antagonist, the RCP (tongue retractor). This pattern of decreasing coactivity with increasing prey distance has been observed in *Hydromantes* (Deban and Dicke, 1999). The greater role for momentum and the lack of coactivity in ballistic projection leads us to predict that the activity pattern of the SAR will differ in these two modes of tongue projection. Chameleons use ballistic projection by means of a similar mechanism and the tongue accelerator muscle is activated all at once (Wainwright and Bennett, 1992a). We expect this pattern during ballistic projection in the SAR of *Hydromantes* as well. In non-ballistic projection, we expect that the SAR will be activated partially, and the duration and extent of activity will increase with increasing distance of tongue projection.

The third hypothesis we tested is that SAR activation pattern will show no effect of prey-capture success (i.e. misses *versus* captures). From a motor control standpoint, this is important because it would indicate whether tongue projection is controlled using sensory feedback or using a feedforward mechanism of motor planning (*sensu* Marsden et al., 1984; Pearson, 1993). Previously, *Hydromantes* and other species of plethodontid salamanders showed almost no effect of prey capture success on the timing of activation of the SAR (one recording site), the RCP, and the depressor mandibulae (Deban and Dicke, 1997). Additionally, transecting the sensory nerves to the tongue had no effect on feeding kinematics in another plethodontid species (Deban, 1997). Based on these previous results, we expect that motor patterns within the SAR will not be influenced by capture success, and that activation of the SAR will therefore be accomplished *via* feedforward control.

The goals of this project were thus to determine the electromyographic (EMG) pattern(s) within the primary tongue protractor muscle, the SAR, in a ballistic-tongued salamander, *Hydromantes imperialis*, and to test three hypotheses: (1) the SAR shows regional variation in the timing and duration of activation, strongly influenced by tongue-projection distance, (2) ballistic and non-ballistic projection are accomplished with discrete activation patterns of the SAR and (3) tongue projection (both ballistic and non-ballistic) is controlled *via* feedforward rather than feedback mechanisms, that is, SAR activation patterns are not influenced by capture success.

### Materials and methods

Individuals of *Hydromantes imperialis* Stefani 1969 collected from Sardinia, Italy, were housed individually in plastic boxes lined with moist paper towels and were maintained on a diet of crickets and house flies. Individuals that fed readily under observation were selected for electromyographic (EMG) recordings. A total of 73 feeding attempts on crickets (approx. 1 cm in length) were recorded from five individuals (70–83 mm snout–vent length). *Hydromantes* was chosen because it has the longest-distance tongue projection, up to 80% of body length, of any genus of plethodontid salamander, and its tongue-projector muscle is the longest and most easily instrumented.

#### Morphology

The main tongue-protractor muscle, the SAR, was chosen for EMG recordings. To determine the approximate tongue-projection distance at which the epibranchial leaves the posterior SAR (SARP), we cleared and double stained (for bone and cartilage) a preserved specimen of *Hydromantes imperialis* with a snout–vent length of 73 mm. We measured the length of the tongue skeleton at 35 mm and the distance from the tongue tip to the rostral margin of the jaws at 20 mm when the epibranchial tips were aligned with the posterior tips of the lower jaws. This position for the tongue skeleton approximates the position it is in during tongue projection when the epibranchial tips are at the rostral edge of the SARP

muscles. Tongue projection beyond this distance of 20 mm relies on the momentum of the tongue skeleton (i.e. ballistic projection) because the SARP muscles can no longer exert force upon the epibranchials.

#### Electrode implantation

Formvar-coated nichrome wire of 25  $\mu\text{m}$  uncoated (38  $\mu\text{m}$  coated) diameter (A-M Systems #761500, Sequim, WA, USA) was used to construct bipolar patch electrodes. Electrodes were made by twisting together two strands of wire approximately 60 cm long, which were then threaded through a 3 mm $\times$ 3 mm piece of silicone rubber in the shape of either a half cylinder (cut from tubing) or a flat square. Insulation was removed from approximately 2 mm of both wires on the inner surface of the electrode. Dipole orientation of the cylindrical patch electrodes was perpendicular to the long axis of the half-cylinder, and thus was parallel to the outermost muscle fibers of the SAR upon implantation.

Prior to electrode implantation, salamanders were anesthetized by immersion in a buffered 2% aqueous solution of MS-222 (3-aminobenzoic acid ethyl ester; Sigma) for 10–30 min. Electrodes were implanted through three or four small incisions in the skin, at the surface of the muscles. An electrode was placed against the anterior portion of the SAR in two individuals through an incision in the skin of the throat. In all five individuals, three patch electrodes were placed against the surface of the right SAR, with the concave surface of the electrode cradling a portion of the cylindrical muscle. Electrodes were positioned with approximately equal spacing along the SAR muscle, with the anterior electrode placed at the level of the gular fold. The SAR has been observed to move rostrally during tongue projection, but because the electrodes were not attached to the muscle, the muscle was free to move relative to them. This arrangement was necessary given the small size and delicate nature of the muscle, which prohibited electrode implantation directly into the muscle. The posterior electrode was positioned several mm rostral to the caudal tip of the SAR to accommodate muscle movement (up to 0.5 cm, see below) and to remain in contact with the muscle throughout tongue projection and retraction. The middle electrode was positioned approximately midway between the anterior and posterior electrodes, such that electrodes were spaced about 7 mm apart (Fig. 2).

Incisions were closed with silk suture. Electrode leads were glued together with modeling glue and attached to the skin of the back with suture to prevent them from being pulled loose. The ends of the leads were soldered to an 8-pin connector, which was plugged directly into the preamplifier.

#### Electromyography

Salamanders fed readily after recovery from anesthesia. Recordings were made within 3 days of recovery, after which electrode positions and spacing were confirmed surgically. A total of 73 feedings (43 captures, 30 misses) were recorded. Numbers of recorded captures (and misses) for each individual were 8 (2), 18 (16), 12 (2), 4 (7) and 1 (3).

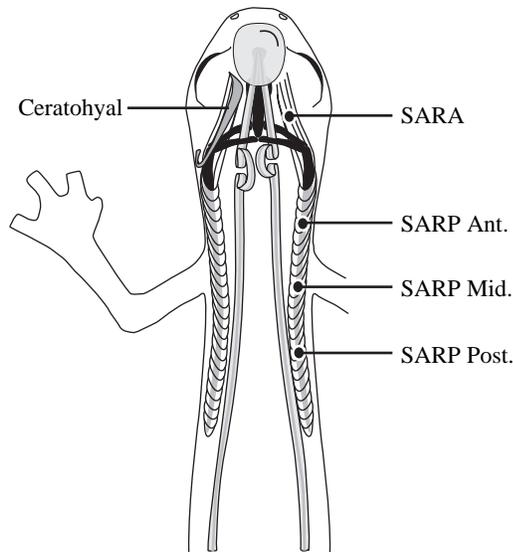


Fig. 2. Diagram of the tongue projection system of *Hydromantes*, with the tongue at rest in the mouth, showing the positions of the EMG recording sites on the subarcualis rectus (SAR) muscle. The SAR originates on the ceratohyal (dark gray), which is shown on the left of the diagram. Also shown are the tongue skeleton (black) and the tongue retractor muscle, the rectus cervicis profundus (gray striped). Ant., anterior; Mid., middle; Post., posterior.

Electromyographic signals were amplified 1000 times by a custom-built differential preamplifier-amplifier. Signals were recorded on an Instrutech (Port Washington, New York, USA) CRC VR-100A digital recorder connected to a JVC HR-S700 video cassette recorder, from which they were captured at a rate of 2000 samples  $s^{-1}$  using a National Instruments data acquisition card and LabVIEW 4.0 (National Instruments, Austin, TX, USA) software running in Windows 98. The raw signals were filtered in LabVIEW to remove 50 Hz line noise, other noise, and low-frequency movement artifacts. Filtered signals were then rectified for analysis.

Each salamander was permitted unrestrained movement in a 20 cm $\times$ 20 cm plastic box with a grounded substrate of moist graph paper while EMG recordings were made. Live crickets were presented to the salamander by dropping them on the substrate at varying distances in front of the salamander. Strikes were recorded as either a successful capture or a miss. The distance from the salamander to the prey at the time of the salamander's strike was estimated, using the graph paper grid, to the nearest 5 mm.

To correlate tongue movement and EMG activity, 10 feedings from two individuals were videotaped in dorsal view at 60 Hz during EMG recording. Video and EMG were synchronized with an LED in the video field and a pulse on one channel of the EMG recording. In these individuals, the SARP and epibranchial contralateral to the instrumented muscle were exposed by pulling the free posterior end through a small incision at the shoulder. The muscle was kept moist by application of Ringer's solution. Because the muscle and the

contained cartilage are normally covered only by skin and are unattached posteriorly, this procedure is unlikely to have affected their function or movement. Tongue projection and prey-capture performance were not noticeably altered by this procedure. Rostrocaudal movement of the SAR and the epibranchial within the translucent muscle were observed in this way. These were the same two individuals that had an electrode on the SARA, and the combined video and EMG data were used to determine the timing of activation at this site relative to the most posterior site in the SARP and relative to SAR and tongue movements.

Five measurements were made from the EMG burst associated with the prey-capture strike for each electrode: (1) time of the onset of activity, the time at which activity exceeded background noise levels by twofold for at least 10 ms, (2) time of the offset of activity, the time at which activity dropped below two times background noise levels for at least 10 ms, (3) burst area, the integrated area under the curve between times 1 and 2, (4) time of peak activity, the starting time of the 10 ms period between times 1 and 2 with the greatest integrated area and (5) peak amplitude, the average amplitude of the 10 ms period in (4).

Twelve timing variables were calculated from these five measurements: (1) duration of activity at each electrode, the onset time minus the offset time (three variables), (2) anterior–posterior relative duration, the duration of the posterior electrode minus the duration of the anterior electrode, (3) anterior–middle relative duration, (4) middle–posterior relative duration, (5) anterior–posterior relative onset, the onset time of the posterior electrode minus the onset time of the anterior electrode, (6) anterior–middle relative onset, (7) middle–posterior relative onset, (8) anterior–posterior relative peak, the time of peak activity of the posterior electrode minus the time of peak activity of the anterior electrode, (9) anterior–middle relative peak and (10) middle–posterior relative peak.

#### *Statistical analyses*

Statistical analyses were performed to examine effects of individual, electrode position, capture success and initial prey distance. Analyses included 18 variables: burst duration for each of the three electrodes (three variables), burst area for each electrode (three variables), peak amplitude for each electrode (three variables), relative durations between pairs of electrodes (three variables), relative onset times between pairs of electrodes (three variables), and relative peak times between pairs of electrodes (three variables). These comparisons were conducted on all trials in all individuals.

Three statistical analyses were conducted. First, to test the hypotheses that individual, prey distance and capture success influence EMG patterns within the SAR, we conducted an analysis of covariance (ANCOVA) examining the effects of individual and capture success, with prey distance as a covariate, on each of the 18 variables individually. Two-way interactions (individual  $\times$  capture, prey distance  $\times$  capture and individual  $\times$  prey distance) were also examined, but because

none were significant they were removed from the model to increase statistical power. The 'individual' term in the analyses accounted for differences in both the electromyographic responses of the salamanders and the properties of the electrodes (e.g. variation in sensitivity and exact position along the SAR) when comparing individuals. The  $P \leq 0.05$  significance level was adjusted in the ANCOVA using the simultaneous Bonferroni correction for experiment-wide error (Sokal and Rohlf, 1995) to a level of  $P \leq 0.0028$ . The ANCOVA was also run with only the 43 feedings in which the prey was captured with individual and prey distance terms in the model.

In the second analysis, burst duration, burst area and relative onset variables (all trials) were plotted against initial prey distance and least-squares regression lines were fitted, to visualize the effects of prey distance on each of the variables independently.

Third, *t*-tests were conducted on the two individuals with electrodes in the SARA together and separately to test the hypothesis that the time of onset, time of peak activity and duration of activity were significantly different from those of the posterior recording site (SARP Post.). One-sample tests were used to determine if relative time of onset, relative time of peak activity and relative duration averages were significantly different from zero, to test the hypotheses that the activity onset and peak were reached at different times, and that the duration of activity varied, along the length of the SARP.

Statistical analyses were performed on an Apple Macintosh PowerBook G4 using StatView software version 5.0. The sums of squares of the ANCOVAs were calculated by the reduction technique (Searle, 1971). The algorithms used can be found in the *StatView Reference* 1998, SAS Institute Inc.

## Results

During a feeding behavior, the salamander oriented to the prey, often approached it, fixated on the prey visually and then snapped at it. The mouth opened and the tongue was protracted from the mouth during the snap. The range of distances over which prey were captured was 0 cm (i.e. prey at the mouth of the salamander) to 5 cm, averaging  $2.3 \pm 0.14$  cm (mean  $\pm$  S.E.M.). Once the tongue made contact with the prey, the tongue with the attached prey was immediately retracted into the mouth, and the mouth was closed. Tongue movements during feedings on nearby crickets (a few cm from the salamander's head) were noticeably slower than during long distance feedings. The entire prey capture behavior was usually completed within 150 ms.

The buccal SARA site was the first to show activation during feeding, and the site remained active until prey contact. After the SARA, the posterior SARP site was activated, followed by the middle SARP site, then the anterior SARP site. The relative onset times between sites diminished with increasing prey distance, until, at maximum tongue projection distance the sites were activated nearly simultaneously. The duration of activation at all sites also increased with prey distance, but the

SARP sites were deactivated well before the SARA site was deactivated.

### *Buccal SARA activity compared to SARP activity*

In the two individuals in which the SARP was exposed during feeding, the SARP was observed to move rostrally by  $0.31 \pm 0.06$  cm (0–0.5 cm), following the onset of activity in the SARA by  $110 \pm 21$  ms (23–186 ms). The tongue first appeared at the mouth  $123 \pm 13$  ms (80–180 ms) after the onset of activation in the posterior electrode of the SARP, and  $107 \pm 16$  ms (66–188 ms) after activity began at the anterior electrode of the SARP. Tongue appearance occurred  $2 \pm 13$  ms after (58 ms before to 40 ms after) activity ended in the SARA, with short distance feedings (0.5–1.5 cm) showing activity in the SARA even after the tongue reached the prey.

The hypothesis that the buccal portion of the SAR (the SARA) is activated separately from the posterior portion was tested with one-sample *t*-tests (two-tailed). The *t*-tests revealed that activity was detected in the SARA electrode significantly earlier ( $42 \pm 4$  ms) than in the most posterior SARP electrode in both individuals together ( $t=11.529$  and  $P < 0.0001$ ), and in each individual separately ( $42 \pm 5$  ms;  $t=9.042$ ;  $P < 0.0001$  and  $43 \pm 6$  ms;  $t=7.018$ ;  $P=0.0059$ ). The timing of peak activity, however, was not significantly different between the SARA and the posterior SARP recording site for both individuals ( $t=-0.440$ ;  $P=0.6664$ ) or for one individual ( $t=0.815$ ;  $P=0.4341$ ), but was significantly different for the other individual ( $t=-4.061$ ;  $P=0.0269$ ), with the peak occurring  $28 \pm 7$  ms later in the SARA. Duration of activity was significantly longer at the SARA site than in the posterior SARP site, in both individuals ( $80 \pm 8$  ms longer;  $t=10.401$ ;  $P < 0.0001$ ) and in each individual separately ( $86 \pm 8$  ms;  $t=10.357$ ;  $P < 0.0001$  and  $61 \pm 15$  ms;  $t=4.001$ ;  $P=0.0280$ ). The anterior site of the SARP was active the latest, on average, and the time of deactivation of the SARA was later than the time of deactivation of the anterior SARP for one of the two individuals ( $t=5.669$  and  $P=0.0002$ ).

### *EMG variation among individuals*

The ANCOVAs revealed significant effects of individual in nine of the 18 variables (Table 1). Effects of 'individual' in the analysis account for both effects due to variation among the individual salamanders and random effects due to variation in electrode characteristics. Therefore, the details of which pair-wise individual differences are driving the overall effect of individual are not informative and are not discussed further.

### *Posterior-to-anterior activation within the SARP*

Average onset time, peak time and duration of the SAR muscle were significantly variable along its length. All relative onset and relative duration averages were significantly different from zero, indicating the recording sites were activated at different times and for different durations (Table 2). The posterior recording site showed the earliest activity, defined as  $t=0$  ms, followed by the middle site ( $t=9 \pm 1$  ms,  $-6$  to 43 ms), followed by the anterior site ( $t=17 \pm 2$  ms,  $-9$

Table 1. Means and standard errors of 18 EMG variables in *Hydromantes imperialis* with results of ANCOVA examining effects of individual, capture success, with prey distance as a covariate

	Mean	S.E.M.	Individual		Capture success		Prey distance	
			F	P	F	P	F	P
SAR area (mV)								
Posterior	1.747	0.069	4.290	0.0038	0.472	0.4946	8.185	0.0057
Middle	4.506	0.248	4.666	0.0023*	0.072	0.7897	62.206	<0.0001*
Anterior	4.452	0.344	38.657	<0.0001*	1.099	0.2983	88.005	<0.0001*
SAR peak amplitude (mV)								
Posterior	0.190	0.008	4.004	0.0058	0.120	0.7301	0.780	0.3803
Middle	0.557	0.022	6.385	0.0002*	0.065	0.7997	1.557	0.2166
Anterior	0.634	0.040	31.261	<0.0001*	0.282	0.5974	9.562	0.0029
SAR duration (s)								
Posterior	0.115	0.004	6.417	0.0002*	4.947	0.0296	37.307	<0.0001*
Middle	0.106	0.004	3.228	0.0177	3.037	0.0861	64.784	<0.0001*
Anterior	0.085	0.004	2.292	0.0689	1.854	0.1781	111.174	<0.0001*
Relative duration (s)								
Mid-post	0.009	0.002	2.157	0.0837	0.035	0.8527	14.820	0.0003*
Ant-mid	0.020	0.002	5.843	0.0004*	0.445	0.5069	2.300	0.1342
Ant-post	0.029	0.003	5.506	0.0007*	0.552	0.4603	20.020	<0.0001*
Relative onset (s)								
Mid-post	0.009	0.001	0.390	0.8153	1.537	0.2195	35.336	<0.0001*
Ant-mid	0.008	0.001	3.197	0.0185	3.488	0.0663	43.587	<0.0001*
Ant-post	0.017	0.002	0.258	0.9038	0.061	0.8053	57.779	<0.0001*
Relative peak (s)								
Mid-post	0.000	0.002	5.125	0.0012*	1.166	0.2842	6.132	0.0159
Ant-mid	0.003	0.002	0.814	0.5206	0.055	0.8146	0.523	0.4723
Ant-post	0.003	0.003	4.772	0.0019*	1.089	0.3006	6.321	0.0144

\*Significant at Bonferroni-adjusted  $P \leq 0.0028$ , otherwise not significant.  
Degrees of freedom are 65 for denominator, and 4, 1 and 1 for numerator of individual, capture success and prey distance, respectively.  
Ant, anterior; Mid, middle; Post, posterior.

to 50 ms), indicating that the SARP was activated in a posterior-to-anterior (i.e. caudal-to-cranial) wave. The posterior site showed the longest duration of activity ( $115 \pm 4$  ms, 53–194 ms), followed by the middle site ( $106 \pm 4$  ms, 29–193 ms), and the anterior site ( $85 \pm 4$  ms, 27–169 ms). None of the relative peak variables were significantly different from zero, indicating that different regions of the SARP reached peak activation at essentially the same time.

#### Effects of capture success and prey distance on SARP activation

Of the 73 feeding attempts for which EMGs were recorded, 30 were strikes in which the prey was not captured. The ANCOVA which included a 'capture success' effect revealed that missing the prey had no significant influence on any of the 18 EMG variables (Table 1).

Unlike capture success, variation in prey distance had a

significant effect on several of the variables, including durations, areas, relative durations and relative onsets. Amplitudes and relative peak variables were not affected significantly (Table 1). None of the interaction terms in the ANCOVA were significant for any variable, including the 'prey distance  $\times$  capture success' effect. This indicates that prey distance had no differential effect on the EMG patterns in captures *versus* misses.

EMG area increased significantly with increasing prey distance at the middle site ( $F=62.206$ ;  $P<0.0001$ ) and the anterior site ( $F=88.005$ ;  $P<0.0001$ ), but area from the posterior electrode did not ( $F=8.185$ ;  $P=0.0057$ ) (Table 1; Fig. 3Ai–iii). All three sites, posterior ( $F=37.307$ ;  $P<0.0001$ ), middle ( $F=64.784$ ;  $P<0.0001$ ) and anterior ( $F=111.174$ ;  $P<0.0001$ ), displayed an increase in the duration of activity with increasing prey distance.

The relative duration of activity of the posterior site decreased with increasing prey distance compared to both the

middle ( $F=14.820$ ;  $P=0.0003$ ) and anterior sites ( $F=20.020$ ;  $P<0.0001$ ), due to the fact that the duration of activity at the posterior site increased at a slower rate than the other sites with increasing prey distance (Table 1; Figs 3Ci–iii, 4Ai–iii). The relative duration of the middle and anterior sites did not show an effect of prey distance ( $F=2.300$ ;  $P=0.1342$ ). Relative onset variables were all affected significantly ( $P<0.0001$ ) by prey distance, such that there was less delay in activation between recording sites as prey distance increased (Table 1; Figs 4Bi–iii, 5). Relative peak, on the other hand, showed no significant influence of variation in prey distance (Fig. 4Ci–iii).

**Discussion**

*Feeding behavior*

Both the video recordings of *Hydromantes* and unaided visual observations of feedings revealed that the salamanders were modulating their movements during the prey-capture strike, particularly tongue-projection distance. Modulation of prey-capture behavior has been observed previously in many groups of salamanders (Erdman and Cundall, 1984; Shaffer and Lauder, 1985; Miller and Larsen, 1990; Reilly and Lauder, 1989, 1992; Elwood and Cundall, 1994), including

Table 2. Means, degrees of freedom, and results of one-sample t-tests with 95% confidence intervals for nine EMG variables in *Hydromantes imperialis*

	Mean	t	P	95% confidence intervals	
				Lower	Upper
Relative duration (s)					
Mid-post	0.009	3.961	0.0002*	0.005	0.014
Ant-mid	0.020	8.426	<0.0001*	0.016	0.025
Ant-post	0.029	10.394	<0.0001*	0.024	0.035
Relative onset (s)					
Mid-post	0.009	6.564	<0.0001*	0.006	0.012
Ant-mid	0.008	10.445	<0.0001*	0.006	0.009
Ant-post	0.017	9.121	<0.0001*	0.013	0.020
Relative peak (s)					
Mid-post	0.000	0.094	0.9251	-0.005	0.005
Ant-mid	0.003	1.857	0.0674	-0.0002	0.006
Ant-post	0.003	1.079	0.2842	-0.003	0.009

\* Confidence intervals do not encompass zero, therefore mean is significantly different from zero. Degrees of freedom are 72.  
Ant, anterior; Mid, middle; Post, posterior.

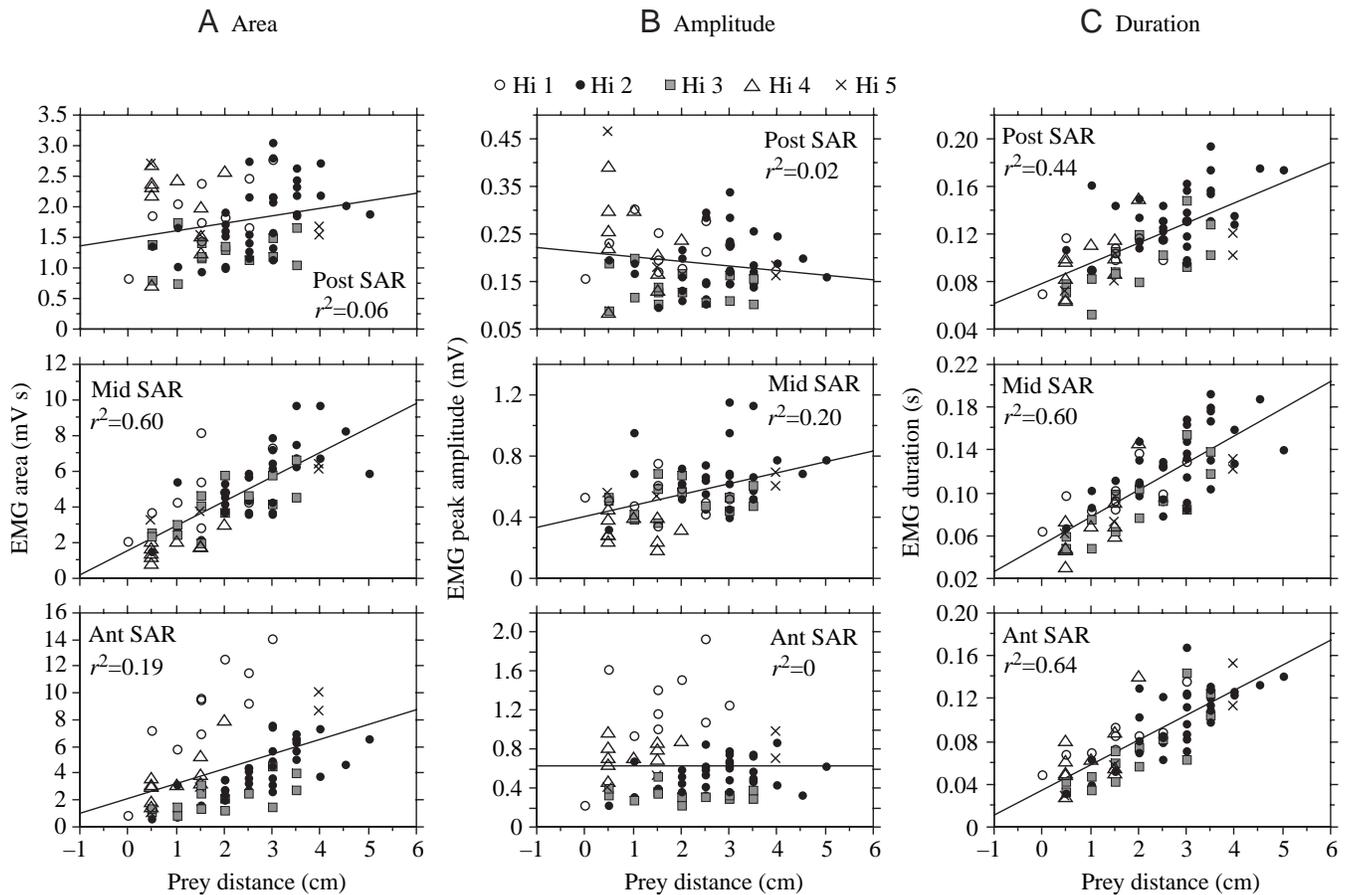


Fig. 3. Values of nine EMG variables plotted against prey distance for all recorded feeding events in five individuals of *Hydromantes imperialis* (Hi 1–5, labeled as different symbols), with least-squares regression lines and coefficients of determination. EMG area (A) and amplitude (B) show weak positive correlations with prey distance compared to EMG duration (C). Post, Posterior; Mid, middle; Ant, anterior.

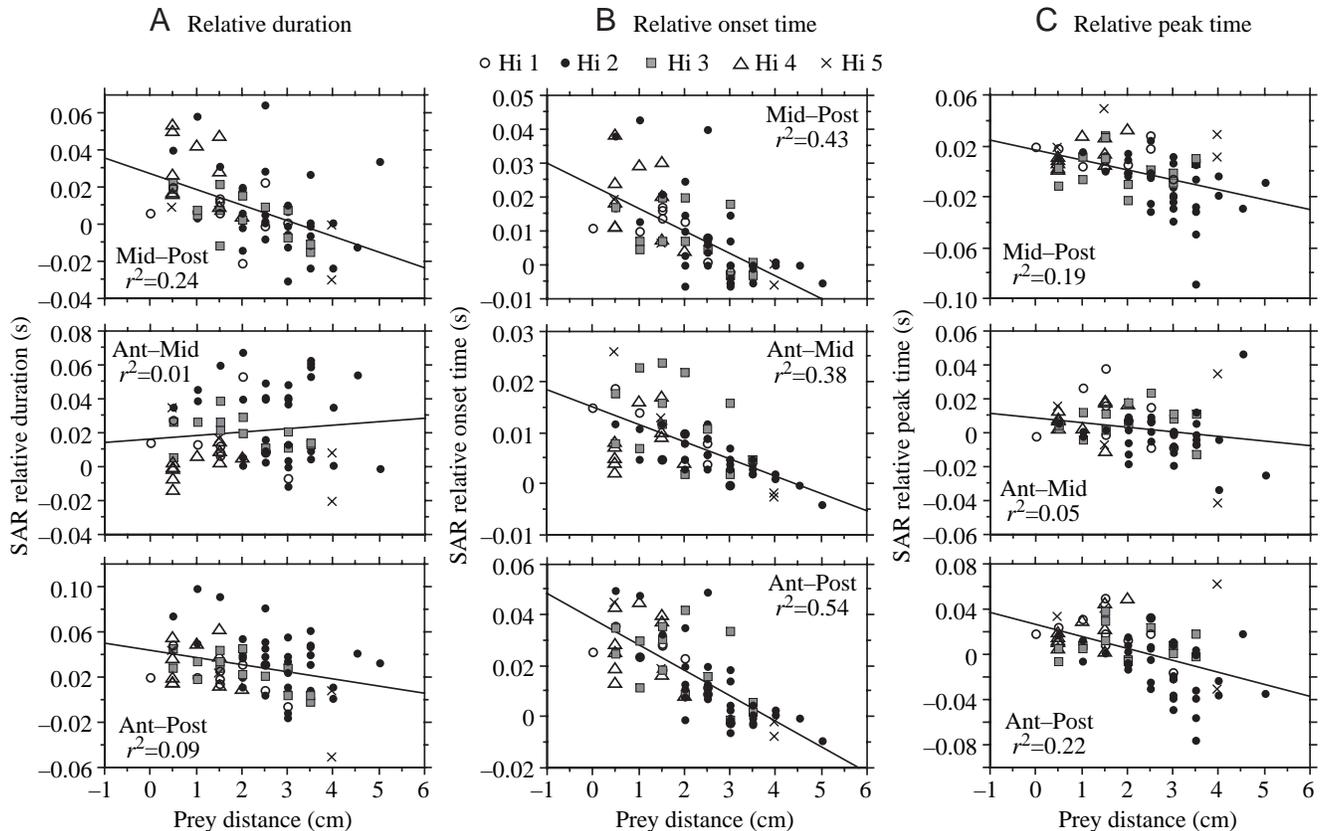


Fig. 4. Values of nine EMG variables plotted against prey distance for all recorded feeding events in five individuals of *Hydromantes imperialis* (Hi 1–5, labeled as different symbols), with least-squares regression lines and coefficients of determination. Relative duration (A) and relative peak times (C) show weak negative correlations with prey distance compared to relative onset times (B), which clearly shows how the SARP activation is piecemeal at short prey distances and all at once at greater prey distances. Mid–Post, middle–posterior; Ant–Mid, anterior–middle; Ant–Post, anterior–posterior.

plethodontid salamanders (Larsen and Beneski, 1988; Maglia and Pyles, 1995; Deban, 1997), which have extremely fast and long-distance tongue protraction. Bolitoglossine plethodontids were previously thought to be highly stereotyped in their feeding movements, based on morphology and on EMG and force recordings in *Bolitoglossa occidentalis* (Thexton et al., 1977). However, the results of this study and of Deban and Dicke (1999), show that *Hydromantes* displays a high degree of modulation in EMG patterns, revealing that stereotypy is not a general phenomenon among bolitoglossines. Further research on additional bolitoglossine taxa is necessary to determine the generality of these results *versus* those of Thexton et al. (1977).

#### Buccal SARA versus SARP activation

The SARA is activated significantly earlier than the SARP, and its activity overlaps extensively with that of the SARP. This result supports the hypothesis that the SAR is divided into two functional components: the anterior SAR, which pulls the tongue skeleton forward relative to the lower jaw and stabilizes the entire SAR against the ‘recoil’ forces generated when the second component, the SARP, squeezes the tongue skeleton forward and out of the mouth. In animals that were videotaped during feeding, the SARP was observed moving rostrally prior

to the epibranchial evacuating the muscle, which is consistent with the activation of the SARA prior to activation of the SARP. In addition, the SARA is innervated in a closely related species, *Hydromantes italicus*, by a separate branch of the glossopharyngeal nerve (cranial nerve IX) from the SARP (G. Westhoff, personal communication), lending anatomical support to the hypothesis that this portion is capable of being controlled separately from the SARP.

Consistent with a stabilizing role for the SARA are three additional pieces of evidence. First, the SARA mechanically connects the SARP to the ceratohyal (Lombard and Wake, 1977; Deban et al., 1997) and therefore must transmit any force generated by the SARP to the ceratohyal; thus, it must be active during and prior to activation of the SARP, so as to transmit and not absorb the energy generated by the SARP. Second, the SARA is active earlier than the SARP and for a longer duration, completely encompassing the time that the SARP is active (Fig. 5). Third, the SARA contains slow tonic as well as fast twitch muscle fibers in this portion of the SAR in *Hydromantes italicus*; in the SARP, only fast twitch fibers have been found (Dicke et al., 1995).

The extensive overlap in EMG activity of the SARA and SARP suggests that the SARA may also play a role in

accelerating the tongue, synergistically with the SARP, which is consistent with the presence of fast fibers in both parts of the muscle (Dicke et al., 1995). Co-activation of these two parts of the SAR muscle may contribute additively to the acceleration of the tongue skeleton, with the SARA pulling the SARP and enclosed tongue skeleton rostrally relative to the ceratohyals, and the SARP concurrently squeezing the epibranchial rostrally.

#### No effect of capture success on SAR activation

The lack of an effect of capture success on any of the EMG variables is consistent with previous results in *Hydromantes supramontis* (Deban and Dicke, 1999), in which muscle activity pattern was independent of capture success and the motor program was therefore not influenced by sensory feedback. Prey capture kinematics in another plethodontid salamander, *Ensatina eschscholtzii*, were unchanged when sensory feedback was prevented by lingual nerve transection (Deban, 1997). These salamanders thus appear to modulate their feeding behavior by changes in motor pattern, but in a feed-forward manner using motor programming (Marsden et al., 1984; Pearson, 1993; Deban et al., 2001) as opposed to using sensory or proprioceptive feedback during the strike.

#### Diverse activation patterns within the SARP

Both sequential and simultaneous activation of different regions of the SARP were observed in *Hydromantes*. The average activity pattern of the SARP (with an average prey distance of  $2.3 \pm 0.14$  cm) is a posterior-to-anterior wave of activation and a decreasing duration of activity from posterior to anterior. The SARP is thus activated regionally to exert force first on the posterior tip of the epibranchial, and subsequently on more rostral portions of the epibranchial. Because the epibranchial moves rostrally when the SARP squeezes it, the posterior tip of the SARP must be activated first, because the epibranchial soon evacuates this portion of the muscle, even in modest tongue protraction.

Sequential activation of the SARP occurs during feedings in which the tongue is projected short distances (i.e. a few cm), but the pattern changes when the tongue is projected farther. The delay in activation of segments of the SARP decreases with increasing prey distance, as do the differences in the regional durations of activity. Thus, the longest-distance tongue projections are accomplished by simultaneous activation of the entire SARP. Absolute durations of activity at all sites on the SARP also increase with prey distance. This pattern indicates that the SARP exerts force simultaneously for a longer period of time along the entire length of the epibranchial when the tongue is shot to its maximum extent. These changes are not abrupt, as we hypothesized, but gradual, suggesting that the biomechanical transition from non-ballistic to ballistic projection is not discrete.

These different patterns of activation make sense in light of observations of *Hydromantes* in which short-distance tongue projection is relatively slow and precise (even appearing 'leisurely'), and the tongue is placed accurately on the prey and

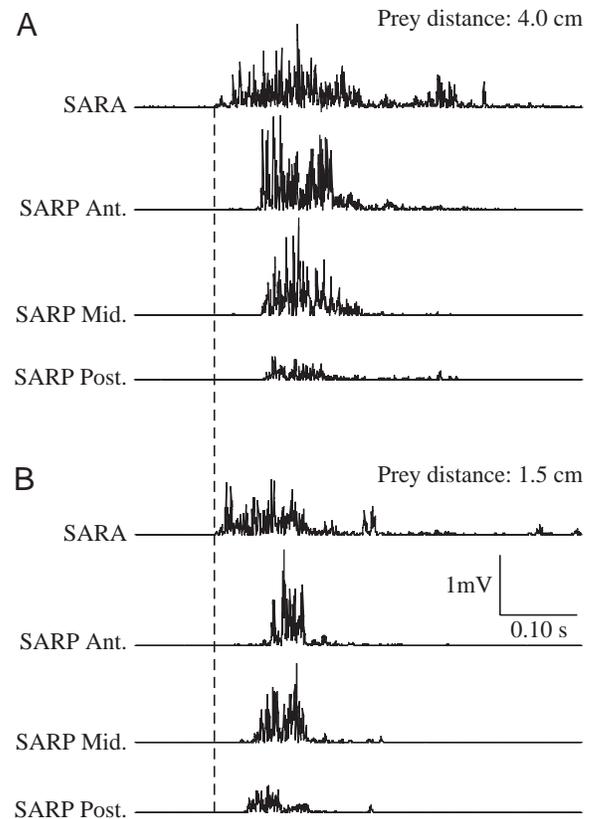


Fig. 5. Sample EMG traces from two feedings in the same individual of *Hydromantes imperialis*, illustrating the different activation patterns observed during feedings at distant prey (A) and near prey (B). Note the differences in total EMG area, duration and relative onset times of the different recording sites on the SARP. The vertical broken line indicates the onset of activation of the anterior SARP (SARA). SARP, posterior SARP.

rarely overshoots it (this study and Deban and Dicke, 1999). In short-distance feedings, tongue movement must be braked and its direction of travel must be reversed soon after tongue protraction begins, by co-contraction of the antagonistic rectus cervicis profundus (RCP) muscles (Deban and Dicke, 1999). Applying less projection force for a briefer duration when antagonistic muscles are soon to be activated to reverse the tongue trajectory is a sensible motor control strategy. Sequential and orderly longitudinal activation of the SARP may also smooth force transmission to the tongue skeleton and, combined with modulated RCP activity, may permit more precise control of tongue-projection force, distance and velocity in short-distance feedings.

Long distance projection (i.e. greater than about 3 cm), on the other hand, requires that the tongue skeleton be launched ballistically (i.e. the epibranchial evacuating both the SARP and the mouth completely and the tongue traveling to the prey under its own momentum; see Deban et al., 1997). Ballistic projection can only be accomplished by high accelerations and velocities of the tongue skeleton, which require that the entire SARP exert force simultaneously along the length of the epibranchial, rather than piecemeal as in feedings at shorter

distances. This conclusion is supported by the increase in the duration and area of EMG activity with increasing prey distance (Fig. 3). The faster movement of the epibranchial associated with longer distance tongue projection requires that the activation bursts along the length of the SARP ‘bunch up’ and overlap more extensively in time. By contracting in its entirety, rather than piecemeal, the SARP exerts maximal force upon the epibranchial. By increasing its duration of contraction (made possible in *Hydromantes* by the lengthy epibranchial) the SARP imparts the greatest impulse (force  $\times$  time) to the tongue skeleton, which is critical for rapid acceleration of the tongue. Also critical for maximum acceleration of the tongue is the lack of any activity in the antagonistic RCP during projection, a pattern that has previously been observed (Deban and Dicke, 1999).

Separate motor units within a muscle are necessary to produce a pattern of regional activation such as that displayed by the SARA and SARP of *Hydromantes*. The motor units of the SARP are probably arranged longitudinally, based on the longitudinal progression of muscle activation. Although the spatial arrangement of muscle fibers is unusual and complex in the SARP, the arrangement of motor units is not functionally unusual for a vertebrate muscle. In the cat sartorius muscle, for example, the motor units extend from origin to insertion (Smits et al., 1994) and therefore each motor unit can contribute to joint torque. Likewise, in the SARP, each putative motor unit (i.e. each longitudinal ‘segment’) can exert force separately upon the epibranchial and, because the epibranchial is tapered, each can contribute to tongue protraction.

The tapered epibranchial can serve an additional function in tongue projection, by altering the relationship between SARP contraction and movement of the tongue tip. Because the muscle fibers of the SARP are short and are arranged mostly in parallel, there are relatively few sarcomeres in series. The SARP as a whole is therefore suited to high force production and low velocity of contraction (Josephson, 1975). Yet, tongue projection is extremely rapid. The gently tapered epibranchial (which the SARP squeezes) reduces the mechanical advantage of the muscle by an amount that depends on the local slope of epibranchial taper. The high-force, low-velocity contraction of the SAR is thus converted to low-force, high-velocity movement of the tongue skeleton. This effect is exaggerated by the medial folding of the tongue skeleton, which thrusts the basibranchial and the attached tongue pad forward faster than the epibranchial moves. The strain rate of the SARP fibers, therefore, may not need to be especially high to produce extremely fast tongue projection.

#### *Comparison to other systems*

No previous studies have examined regional activation of tongue-projector muscles in salamanders, making it impossible to compare the results from *Hydromantes* to other taxa. We suspect that the results will be similar in other salamanders with ballistic tongues. Within the Plethodontidae, ballistic tongue projection has evolved at least twice (independently in the Bolitoglossini and the Hemidactyliini). The morphology of

the tongue system is highly variable among the hundreds of species of plethodontids, providing many opportunities for future comparative evolutionary research in this group.

Like bolitoglossine salamanders, chameleons are also capable of ballistic tongue projection, but their tongue-projection system is reversed compared to salamanders. The tongue-accelerator muscle leaves the mouth with the tongue, and the cartilage it squeezes stays in the body. The accelerator muscle (analogous to the SARP of bolitoglossines) resides inside the tongue pad and is activated approximately 300 ms before the tongue leaves the mouth, remaining active until then (Wainwright and Bennett, 1992a). The delay between activation and projection has been explained as the time required for the cylindrical accelerator muscle to elongate so that it reaches the tip of the entoglossal cartilage (Wainwright and Bennett, 1992b). Once the muscle reaches the tip, it squeezes itself off the entoglossus and thus launches the tongue pad to which it is attached. The tongue pad does not accelerate prior to reaching the entoglossal tip, because the entoglossus is parallel-sided and the squeezing force produced by the muscle produces no net propulsive force. An alternative explanation for the 300 ms activation-projection delay is that the accelerator muscle changes shape and thereby stretches elastic structures within it (de Groot and van Leeuwen, 2004). When the accelerator slides over the end of the entoglossus, the elastic structures recoil, releasing the stored potential energy as kinetic energy and thereby amplifying the power output (i.e. the rate of energy release) of the accelerator muscle. This mechanism can explain the extremely high power output observed during tongue projection that cannot be attributed to direct muscular action (de Groot and van Leeuwen, 2004).

The delay between muscle activation and tongue projection in *Hydromantes* is less than that of the chameleon, mean  $123 \pm 13$  ms (80–180 ms). Nonetheless, given the smaller size and consequently more rapid movements of the salamanders compared to the chameleons, this delay is sufficiently long to implicate elastic energy storage and release, and to raise the possibility of power amplification. Simultaneous activation of the entire SARP in long-distance feedings is similar to the pattern of simultaneous activation of different regions of the accelerator muscle of the chameleon (Wainwright and Bennett, 1992a), further suggesting biomechanical similarities between these two taxa. In the chameleon, the latch that prevents the tongue from protruding while the accelerator muscle changes shape is the parallel-sided shape of the entoglossus, and the elastic structures are collagenous sheaths that reside in the lumen of the accelerator muscle (de Groot and van Leeuwen, 2004). If elastic energy storage and release were also operating in *Hydromantes*, as has been suggested (van Leeuwen et al., 2000), both a latch and elastic structures would also be required. In *Hydromantes*, the latch cannot be the epibranchial itself because it is tapered over its entire length, rather than parallel sided. The data presented here (particularly the  $>100$  ms activation–projection delay) suggest either an elastic mechanism of tongue projection in *Hydromantes*, or, alternatively, a pre-loading mechanism in which shortening of

the muscle fibers is prevented until optimum tension is achieved. To distinguish between these possibilities, future research will need to determine if power amplification is occurring, as in the chameleon. If so, the next step would be to clearly identify morphological structures that can operate as a spring and a latch.

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### References

- Deban, S. M.** (1997). Modulation of prey-capture behavior in the plethodontid salamander *Ensatina eschscholtzii*. *J. Exp. Biol.* **200**, 1951-1964.
- Deban, S. M.** (2002). Constraint and convergence in the evolution of salamander feeding. In *Vertebrate Biomechanics and Evolution* (ed. J.-P. Gasc, A. Casinos and V. L. Bels), pp. 163-180. Oxford: BIOS Scientific Publishers.
- Deban, S. M. and Dicke, U.** (1999). Motor control of tongue movement during prey capture in plethodontid salamanders. *J. Exp. Biol.* **202**, 3699-3714.
- Deban, S. M., Wake, D. B. and Roth, G.** (1997). Salamander with a ballistic tongue. *Nature* **389**, 27-28.
- Deban, S. M., O'Reilly, J. C. and Nishikawa, K. C.** (2001). The evolution of the motor control of feeding in amphibians. *Am. Zool.* **41**, 1280-1298.
- de Groot, J. H. and van Leeuwen, J. L.** (2004). Evidence for an elastic projection mechanism in the chameleon tongue. *Proc. R. Soc. Lond. B* **271**, 761-770.
- Dicke, U., Mühlenbrock-Lenter, S. and Roth, G.** (1995). Fiber types of muscles of the feeding apparatus in plethodontid salamanders. In *Proceedings of the 23rd Göttingen Neurobiology Conference*; Vol. II (ed. N. Elsner and R. Menzel), p. 227. Stuttgart, New York: Georg Thieme.
- Duellman, W. E. and Trueb, L.** (1986). *Biology of Amphibians*. New York: McGraw-Hill.
- Elwood, J. R. L. and Cundall, D.** (1994). Morphology and behavior of the feeding apparatus in *Cryptobranchius alleganiensis* (Amphibia: Caudata). *J. Morphol.* **220**, 47-70.
- Erdman, S. and Cundall, D.** (1984). The feeding apparatus of the salamander *Amphiuma tridactylum*: morphology and behavior. *J. Morphol.* **181**, 175-204.
- Josephson, R. K.** (1975). Extensive and intensive factors determining the performance of striated muscle. *J. Exp. Zool.* **194**, 135-154.
- Larsen, J. H., Jr. and Beneski, J. T., Jr.** (1988). Quantitative analysis of feeding kinematics in dusky salamanders (*Desmognathus*). *Can. J. Zool.* **66**, 1309-1317.
- Lombard, R. E. and Wake, D. B.** (1976). Tongue evolution in the lungless salamanders, Family Plethodontidae. I. Introduction, theory and a general model of dynamics. *J. Morphol.* **148**, 265-286.
- Lombard, R. E. and Wake, D. B.** (1977). Tongue evolution in the lungless salamanders, Family Plethodontidae. II. Function and evolutionary diversity. *J. Morphol.* **153**, 39-80.
- Maglia, A. M. and Pyles, R. A.** (1995). Modulation of prey-capture behavior in *Plethodon cinereus* (Green) (Amphibia: Caudata). *J. Exp. Zool.* **272**, 167-183.
- Marsden, C. D., Rothwell, J. C. and Day, B. L.** (1984). The use of peripheral feedback in the control of movement. *Trends Neurosci.* **7**, 253-258.
- Miller, B. T. and Larsen, J. H., Jr.** (1990). Comparative kinematics of terrestrial prey capture in salamanders and newts (Amphibia: Urodela: Salamandridae). *J. Exp. Zool.* **256**, 135-153.
- Pearson, K. G.** (1993). Common principles of motor control in vertebrates and invertebrates. *Ann. Rev. Neurosci.* **16**, 265-297.
- Reilly, S. M. and Lauder, G. V.** (1989). Physiological bases of feeding behavior in salamanders: do motor patterns vary with prey type? *J. Exp. Biol.* **141**, 343-358.
- Reilly, S. M. and Lauder, G. V.** (1992). Morphology, behavior, and evolution: comparative kinematics of aquatic feeding in salamanders. *Brain Behav. Evol.* **40**, 182-196.
- Searle, S. R.** (1971). *Linear Models*. John Wiley & Sons, New York.
- Shaffer, H. B. and Lauder, G. V.** (1985). Patterns of variation in aquatic ambystomatid salamanders: kinematics of the feeding mechanism. *Evolution* **39**, 83-92.
- Smits, E., Rose, P. K., Gordon, T. and Richmond, F. J. R.** (1994). Organization of single motor units in feline sartorius. *J. Neurophys.* **72**, 1885-1896.
- Sokal, R. R. and Rohlf, F. J.** (1995). *Biometry* (third edition). New York: W. H. Freeman and Company.
- Thexton, A. J., Wake, D. B. and Wake, M. H.** (1977). Tongue function in the salamander *Bolitoglossa occidentalis*. *Arch. Oral Biol.* **22**, 361-366.
- van Leeuwen, J. L., De Groot, J. H. and Kier, W. M.** (2000). Evolutionary mechanics of protrusible tentacles and tongues. *Neth. J. Zool.* **50**, 113-139.
- Wainwright, P. C. and Bennett, A. F.** (1992a). The mechanism of tongue projection in chameleons. 1. Electromyographic tests of functional hypotheses. *J. Exp. Biol.* **168**, 1-21.
- Wainwright, P. C. and Bennett, A. F.** (1992b). The Mechanism of tongue projection in chameleons. 2. Role of shape change in a muscular hydrostat. *J. Exp. Biol.* **168**, 23-40.