

## MOTOR CONTROL OF TONGUE MOVEMENT DURING PREY CAPTURE IN PLETHODONTID SALAMANDERS

STEPHEN M. DEBAN<sup>1,2,\*</sup> AND URSULA DICKE<sup>2</sup>

<sup>1</sup>Hanse Institute for Advanced Study, Lehmkuhlenbusch 4, 27753 Delmenhorst, Germany and <sup>2</sup>Brain Research Institute, University of Bremen, 28334 Bremen, Germany

\*e-mail: deban@uni-bremen.de

Accepted 21 September; published on WWW 29 November 1999

### Summary

Four species of salamander of the family Plethodontidae were examined using electromyographic (EMG) recording during prey-capture behavior to test the hypotheses that the tongue retractor, tongue protractor and jaw depressor muscles are activated simultaneously and in a stereotyped pattern, as was found in other salamanders, and to determine whether species with different tongue morphologies and tongue protraction abilities exhibit different motor control strategies. The results show that sequential activation was observed far more frequently than simultaneous activation; the jaw depressor muscle was activated first, followed by the tongue protractor and then the tongue retractor. Species with short, attached tongues (*Desmognathus quadramaculatus* and *Plethodon jordani*) showed simultaneous activation more often than species with long, free tongues (*Pseudotriton ruber* and *Hydromantes supramontis*), which showed strongly non-

simultaneous activation. Most EMG variables showed no effect of prey-capture success, suggesting that sensory feedback is not involved in modulating the motor pattern during the prey-capture strike. *Hydromantes supramontis* was examined for modulation of its motor pattern in response to prey distance, and several EMG variables were found to be positively correlated with tongue protraction distance. The motor pattern of strongly non-simultaneous activation of antagonistic tongue muscles has evolved along with the evolution of long, free tongues in plethodontids. The variable motor patterns observed provide further evidence that amphibian feeding in general is not as highly stereotyped as has been previously thought.

Key words: electromyography, tongue protraction, modulation, feeding, sensory feedback, amphibian, salamander, stereotypy, Plethodontidae.

### Introduction

Studies of the motor control of salamander feeding have found nearly simultaneous activation of feeding muscles, including antagonistic tongue muscles, and a relatively invariant electromyographic pattern within individuals (e.g. Lauder and Shaffer, 1988). This is in contrast to studies of frogs, which show sequential activation of feeding muscles (Roth et al., 1990; Nishikawa and Gans, 1996). The only previous electromyographic study of tongue function in plethodontid salamanders – the salamanders most highly specialized for tongue protraction – found that many muscles involved in tongue and jaw movements in the free-tongued *Bolitoglossa occidentalis* showed nearly simultaneous activation and deactivation and a stereotyped motor pattern, regardless of tongue-projection distance (Thexton et al., 1977). The arrangement of peripheral structures, namely the slack, elongate retractor muscles and the taut protractor muscles, was concluded to be largely responsible for the sequence of tongue protraction and retraction observed, with little modulation by the central nervous system.

In contrast, behavioral studies have demonstrated that other species of plethodontids are capable of modulating their tongue

movements in response to features of the prey (Maglia and Pyles, 1995; Deban, 1997); this modulation can only be accomplished by modulation of the motor pattern, that is, by central nervous system control of the relative timing, duration and/or strength of muscle activation.

To examine these apparent differences among plethodontid species more closely, we made electromyographic recordings from the main tongue protractor and retractor muscles and from a major mouth-opening muscle during feeding in four species with different tongue types. Each species is a representative of one of the four major clades within the family Plethodontidae (Lombard and Wake, 1977, 1986). Plethodontid salamanders are particularly interesting for comparative studies of the motor control of feeding both because of their specialization for speed and the extent of tongue projection compared with other salamanders (Regal, 1966; Lombard and Wake, 1976; Roth, 1987) and because of their great morphological and functional diversity of tongue types (Lombard and Wake, 1977). Among plethodontids, the groups Desmognathinae and Plethodontini retain protrusible tongues with attachments to the lower jaw, while the

Bolitoglossini and most of the Hemidactyliini have lost these attachments and have evolved greater projection distance. The Bolitoglossini, a clade that contains more than half of all salamander species, have evolved true ballistic tongue projection, and some species extend their tongues up to 80% of body length (Deban et al., 1997). This last group displays high speed and accuracy of visual prey localization and of tongue protraction (Roth, 1987).

The present study was conducted with several goals in mind: (1) to examine patterns of feeding behavior and muscle activation during feeding; to determine (2) if muscle activation during feeding is simultaneous or nearly so, particularly activation of tongue protractor and retractor muscles; (3) if there is modulation in the motor pattern within an individual in response to prey distance; (4) if modulation, if it occurs, is accomplished by changes in the timing of muscle activation, strength of activation or both; (5) if sensory feedback through the tongue, which differs in captures and misses, plays a role in modifying EMG patterns; (6) if there are species differences in the general EMG pattern, specifically between species with different tongue types (protrusible *versus* projectile); and to consider (7) how motor patterns and motor control have evolved within the Plethodontidae with the evolution of different tongue types and feeding strategies; and (8) if feeding motor patterns in plethodontids conform to the view that amphibian feeding is highly stereotyped in general.

### Materials and methods

Individuals of four species of plethodontid salamander were used in this study, all of which were collected from wild populations. *Hydromantes supramontis* were collected in Sardinia, Italy, and were housed individually in plastic boxes lined with moist paper towels. *Desmognathus quadramaculatus*, *Pseudotriton ruber* and *Plethodon jordani* were collected in North Carolina, USA, and were housed communally in glass aquaria or individually in plastic boxes. Salamanders were maintained on a diet of crickets and houseflies at 12–15 °C.

Individuals that fed readily under observation were selected for electromyographic (EMG) recordings. In total, 154 feedings on crickets (approximately 1 cm in length) were recorded from five *Hydromantes supramontis* Lanza, Nascetti and Bullini (71–83 mm snout–vent length, SVL), two *Desmognathus quadramaculatus* (Holbrook) (75–77 mm SVL), three *Pseudotriton ruber* (Latreille) (80–82 mm SVL) and three *Plethodon jordani* Blatchley (61–66 mm SVL).

### Morphology

Three feeding muscles were chosen for simultaneous EMG recording on the basis of their importance in tongue and jaw movements. Electrodes were implanted unilaterally in only three muscles, because of the small size of the salamanders and the difficulties in eliciting feeding after implanting more electrodes. Electrodes were implanted in the m. depressor mandibulae posterior (DMP), the m. subarcualis rectus (SAR)

and the m. rectus cervicis profundus (RCP) (Fig. 1). The DMP was chosen as a reference for measuring the timing in activation of the SAR and RCP muscles, which are the main tongue protractor (SAR) and retractor (RCP).

The morphology of the feeding system of plethodontid salamanders is discussed in detail by Lombard and Wake (1977). The morphology relevant to the current study is therefore presented here only briefly.

The tongue skeleton consists of seven articulating cartilaginous elements. The unpaired basibranchial lies medially and supports the tongue pad. On each side, a first and second ceratobranchial articulates with the caudal half of the basibranchial. The epibranchial articulates with the caudal ends of the first and second ceratobranchials. During tongue protraction, the tongue skeleton folds medially and is pulled rostrally relative to the paired ceratohyals, which lie in the floor of the mouth just lateral to the basibranchial when the tongue is at rest. The ceratohyals remain in the floor of the mouth during tongue protraction, while the other elements protrude from the mouth. In the case of *Hydromantes supramontis*, the entire tongue skeleton, including the epibranchials, can be projected completely from the mouth.

The DMP is one of the main mouth-opening muscles, along with the m. depressor mandibulae anterior. The DMP originates broadly on the fascia cephalodorsalis (the dorsolateral surface of the m. dorsalis trunci) and inserts on the prearticular bone of the lower jaw. The DMP swings the lower jaw ventrally when it contracts.

The SAR is the primary tongue protractor muscle; it originates broadly along the rostralateral edge of the ceratohyal in the floor of the mouth and travels caudally to the

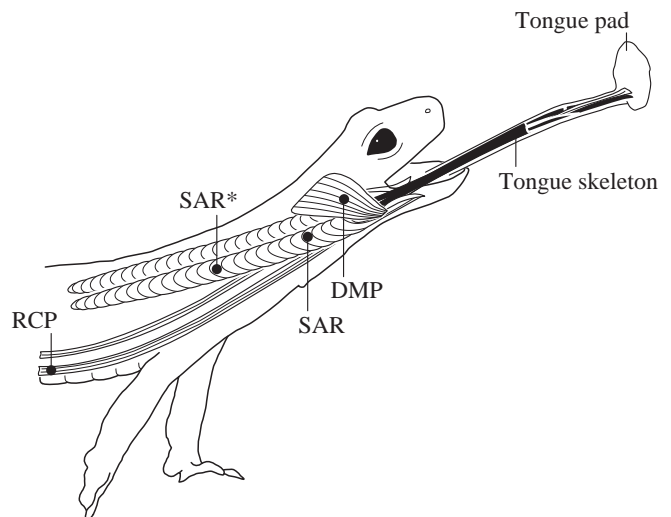


Fig. 1. Diagram of *Hydromantes supramontis* with the tongue partially protracted, showing the muscles examined and the positions of the recording electrodes (filled circles) for all species. SAR\* indicates the position of the electrode in *H. supramontis* only. See text for further explanation. DMP, m. depressor mandibulae posterior; RCP, m. rectus cervicis profundus; SAR, m. subarcualis rectus.

epibranchial of the tongue skeleton. The rostral portion of the SAR is in a position to pull the lingual skeleton rostral relative to the ceratohyal, and joins the caudal portion of the SAR near the junction of the ceratobranchials and the epibranchial. The caudal portion of the SAR has short fibers that wrap around the epibranchial at angles to its long axis; these fibers form an elongate sheath around the epibranchial. The posterior portion of the SAR thus exerts radial forces during contraction which squeeze the tapered epibranchial rostrally relative to the ceratohyal and carry the tongue out of the mouth. The posterior portion of the SAR in *Hydromantes supramontis* is especially elongate compared with that of the other taxa, encompassing the long epibranchial that extends over the shoulder and partially along the trunk.

The RCP is the primary tongue retractor and is continuous with the m. rectus abdominis profundus. The combined muscle (called simply the RCP here) originates on the pelvis and travels rostrally to insert into the tongue pad at the rostral tip of the tongue skeleton. In the bolitoglossine *Hydromantes supramontis*, this muscle lies in a loop in the throat just rostral to the heart, while in the other species the muscle is slack but does not form a loop.

#### Electrode implantation

Bipolar hook and patch electrodes were constructed from Formvar-coated nichrome wire of uncoated 25  $\mu\text{m}$  diameter (38  $\mu\text{m}$  coated) (A-M Systems no. 7615). Hook electrodes were constructed by twisting together two strands of wire approximately 60 cm long and bending the tips back 2 mm from the end. The ends of the hooks were bared to expose 1 mm of the conductive metal surface. Patch electrodes were constructed from similar twisted pairs of wires in which the wires were threaded through a 3 mm  $\times$  3 mm piece of silicone rubber that had been cut from tubing (3 mm outer diameter, 2 mm inner diameter) to form a patch in the form of a half-cylinder. Insulation was removed from approximately 2 mm of wire on the concave surface of the electrode, and the ends of the wire were wrapped around the electrode lead. Patch electrodes were fashioned in two orientations: one with the electrode dipole oriented parallel to the axis of the silicone cylinder, and the other with the electrode dipole oriented perpendicular to the axis.

Salamanders were anesthetized by immersion in an 2% aqueous solution of MS-222 (3-aminobenzoic acid ethyl ester, Sigma) for 10–30 min prior to surgery. Electrodes were implanted through three small incisions in the skin, into or at the surface of three muscles: a hook electrode was implanted in the DMP via a 26 gauge hypodermic needle, and patch electrodes were placed against the surface of the SAR and RCP after the skin and overlying muscles of the body wall had been reflected. The DMP electrode was inserted so that the poles of the electrode lay parallel to the muscle fibers. The SAR electrode was positioned on the SAR muscle just behind the shoulder (at the second costal groove) in *Hydromantes supramontis*, which has the longest SAR, and just caudal to the jaw joint in the other species (Fig. 1). The electrode thus lay

near the middle of the muscle in all species. The RCP electrode was placed against the RCP between the fourth and fifth costal grooves. The SAR and RCP electrodes did not penetrate the muscles, but the metal surfaces of the electrodes made good contact with the muscles. In each, the electrode dipole was oriented along the muscle fibers, using the two patch electrode designs described above. The silicone half-cylinder was used to prevent these electrodes from detecting depolarization of neighboring muscles.

Electrodes were held in place by their hooks in the case of the DMP, and by the concave shape of the silicone tubing and the overlying muscles and skin in the cases of the SAR and RCP. Incisions were closed with silk suture. Electrode leads were glued together with modeling glue and attached to the skin of the back with a loop of silk suture to prevent them from becoming entangled in the salamander's feet or being pulled loose. The ends of the leads were soldered to an eight-pin IC connector, which was then plugged into the preamplifier.

While EMG recordings were being made (described below), each salamander was allowed unrestrained movement within a 20 cm  $\times$  20 cm square plastic box with an electrically grounded substratum of moist paper towels. Live crickets were presented to the salamander by dropping them onto the substratum at varying distances in front of the salamander. Strikes were recorded as either a successful capture or a miss. Prey-capture strikes of two *Hydromantes supramontis* on which the most EMG recordings were made were videotaped at 50 fields  $\text{s}^{-1}$  from above to determine the distance of tongue protraction and the distance between salamander and prey at the start of the strike. Experimentation revealed that tongue length could not reliably be determined at this framing rate for the other taxa.

Salamanders fed readily after recovery from anesthesia and produced good EMG recordings. All EMG recordings were made within 3 days of recovery, after which electrode placement was confirmed surgically. Thirty-eight feedings (32 captures, 6 misses) were recorded in *Plethodon jordani*, 25 feedings (17 captures, 8 misses) in *Pseudotriton ruber*, 57 feedings (23 captures, 34 misses) in *Hydromantes supramontis*, and 34 feedings (17 captures, 17 misses) in *Desmognathus quadramaculatus*. The numbers of recorded captures (and misses) for each individual were 14 (5), 10 (1) and 8 (0) for *Plethodon jordani*, 6 (2), 5 (2), 4 (3) and 2 (1) for *Pseudotriton ruber*, 12 (12), 7 (11), 3 (4), 1 (5) and 0 (2) for *Hydromantes supramontis*, and 10 (13) and 7 (4) for *Desmognathus quadramaculatus*.

#### Electromyography

Electrode leads were connected to a custom-built differential preamplifier–amplifier which amplified the signals 1000 times. Signals were recorded on an Instrutech 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  $\text{s}^{-1}$  using a National Instruments data-acquisition card and LabVIEW 4.0 on an IBM PC-compatible computer. 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.

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

*Hydromantes supramontis* had a more complex burst pattern than the other species in which a burst of high activity was discernible within the period of activity in the SAR and RCP during the strike. For this reason, the above measurements were made both on this main burst and on the entire period of activity and analyzed separately.

From the five measurements above, nine timing variables were calculated: (1) duration of activity of each muscle (SAR, RCP and DMP), the time of offset minus the time of onset; (2) RCP–SAR relative onset, the time of onset of RCP minus the time of onset of SAR; (3) RCP–SAR relative peak, the time of peak activity of RCP minus the time of peak activity of SAR; (4) DMP–SAR relative onset, the time of DMP onset minus the time of SAR onset; (5) DMP–SAR relative peak, the time of DMP peak minus the time of SAR peak; (6) DMP–RCP relative onset, the time of DMP onset minus the time of RCP onset; and (7) DMP–RCP relative peak, the time of DMP peak minus the time of RCP peak.

#### Statistical analyses

Statistical analyses were performed to examine differences among species, within species (i.e. among individuals) and within individuals. Comparisons among species and individuals were made for nine variables: (1) duration of SAR activity; (2) duration of RCP activity; (3) duration of DMP activity; (4) RCP–SAR relative onset; (5) RCP–SAR relative peak; (6) DMP–SAR relative onset; (7) DMP–SAR relative peak; (8) DMP–RCP relative onset; and (9) DMP–RCP relative peak. These comparisons were conducted on all trials in all species using an analysis of variance (ANOVA) with a nested design: species, and individual nested within species. The  $P \leq 0.05$  significance level was adjusted using the simultaneous Bonferroni correction for experiment-wide error (Sokal and Rohlf, 1995) to a level of  $P \leq 0.0056$ . Variables that showed a significant effect of species in the ANOVA were examined in pair-wise comparisons using Games–Howell, Fisher's PLSD and Sheffé's *S post-hoc* tests at the  $P \leq 0.01$  level. A consensus was sought using these three tests that differ in their conservatism and in their sensitivities to the unequal sample sizes in our data.

Each species was also examined individually in an ANOVA comparing individuals and capture success nested within individual to determine the degree of individual variation within species (which may affect species comparisons).

Individual comparisons were made for the same nine variables listed above and at the same significance level of  $P \leq 0.0056$ . Capture success nested within individual was compared for these nine variables and an additional six variables: (1) burst area of SAR; (2) burst area of RCP; (3) burst area of DMP; (4) peak area of SAR; (5) peak area of RCP; and (6) peak area of DMP. Maximum tongue length was included as a covariate in the ANOVA for *Hydromantes supramontis* to account for its effect on EMG variables. In addition, a capture success by individual interaction term was included; it was removed when non-significant on a variable-by-variable basis to increase statistical power. The burst area variables were included only in within-individual comparisons because the electrode configuration was constant within an individual. For these tests with 15 variables, significance levels were adjusted to  $P \leq 0.0033$  using the simultaneous Bonferroni correction. The burst area variables were not included in among-individual comparisons because electrode placement and configuration may affect EMG area and confound comparisons among individuals (and, thus, comparisons among species).

Comparisons within individuals (i.e. among trials) were conducted to test for the effects of capture success on the modulation of muscle activity. *t*-tests were used to compare the means of all 11 variables for successful captures *versus* unsuccessful capture attempts (i.e. misses). Significance levels were adjusted to  $P \leq 0.0045$  for these tests.

In two individuals of *Hydromantes supramontis*, maximum tongue length was recorded during prey capture during EMG recordings. To determine which EMG variables might be affected by the distance of tongue protraction, least-squares regressions were performed on all 11 EMG variables *versus* maximum tongue length. This analysis was conducted twice for each individual, first using variables measured from the entire period of muscle activity during the strike, and then using variables measured from only the main bursts of activity in the SAR and RCP (described above). The simultaneous Bonferroni correction was used to adjust significance levels to  $P \leq 0.0045$  for these tests.

Because previous electromyographic studies of prey capture in salamanders found simultaneous or nearly simultaneous activation of many muscles involved in feeding, we tested the hypothesis that the SAR, RCP and DMP muscles are activated at the same time during prey capture. A one-sample *t*-test was conducted in which the means of RCP–SAR relative onset, DMP–SAR relative onset and DMP–RCP relative onset were compared with zero for each species independently and all species together. The same test was conducted on the relative peak variables to test the hypothesis that muscles reach peak activation simultaneously. Each of these tests used a Bonferroni-corrected significance level of  $P \leq 0.0167$ .

Statistical analyses were performed on an Apple Macintosh PowerBook G3 using StatView software version 5.0 and SuperANOVA software version 1.11. The sums of squares of the ANOVAs were calculated by the reduction technique described by Searle (1971). The reader is referred to the

StatView Reference (1998, SAS Institute Inc.) for the algorithms used.

## Results

### Feeding behavior

All salamanders used tongue protraction to capture prey during these experiments. In a typical feeding event, the salamander oriented to the prey, fixated on the prey visually and then snapped at it. During the snap, which was by far the most rapid stage of the feeding, the mouth opened and the tongue was protracted from the mouth to varying degrees, depending on species. *Hydromantes supramontis* has the longest tongue and captured prey from the greatest range of distances (5–35 mm). *Pseudotriton ruber* has the next longest tongue, and *Desmognathus quadramaculatus* and *Plethodon jordani* have shorter tongues of similar length. Once the tongue contacted the prey, the tongue and adherent prey were immediately retracted into the mouth, and the mouth was closed. Mouth opening and tongue protraction were sometimes accompanied by a forward lunge of the whole body towards the prey in *Pseudotriton ruber*, *D. quadramaculatus* and *Plethodon jordani*, but never in *H. supramontis*. The entire prey-capture behavior was performed rapidly, taking a fraction of a second to complete. In the fastest feedings, the tongue was often not visible to the eye as it usually was in slower feedings or feedings on distant prey (in *H. supramontis*).

### Electromyographic patterns

The pattern of EMG activity for each muscle was typically a large burst followed or preceded by a period of approximately the same duration of lower-amplitude activity (Figs 2, 3). The low-amplitude period was sometimes absent and only the large burst was detected.

During prey capture, the mean duration of SAR activity was  $148 \pm 5$  ms ( $N=154$ ), the mean duration of RCP activity was  $139 \pm 5$  ms and the mean duration of DMP activity was  $151 \pm 5$  ms (means  $\pm$  S.E.M.) (Table 1). The first muscle to be activated of the three recorded was usually the DMP, followed by the SAR and then the RCP. The onset of DMP activity preceded that of SAR by an average of  $8 \pm 2$  ms (mean  $\pm$  S.E.M.,  $N=143$ ), and that of RCP by an average of  $22 \pm 3$  ms. SAR activity preceded RCP activity by an average of  $19 \pm 3$  ms, and the SAR reached its peak of activity  $54 \pm 3$  ms earlier (Tables 2, 3). Thus, there was considerable temporal overlap in the activity of the muscles during prey capture, even for the antagonists SAR (tongue protractor) and RCP (tongue retractor). In general, sequential activation of muscles was observed; however, nearly simultaneous activation of muscles was also observed in some feedings, particularly in *D. quadramaculatus* and *Plethodon jordani*.

The hypothesis that SAR and RCP muscles are activated simultaneously was rejected by a one-sample  $t$ -test ( $t=6.002$ ,  $P<0.0001$ ) when all species were considered together. In each species except *Plethodon jordani* this hypothesis was rejected, indicating that the RCP–SAR relative onset was significantly

different from zero. In *Plethodon jordani*, the mean RCP–SAR relative onset was  $0 \pm 2$  ms ( $N=38$ ), but it reached a maximum of 30 ms. The hypothesis of simultaneous DMP and SAR activation was also rejected in all species considered together ( $t=-4.57$ ,  $P<0.0001$ ) and was rejected for each species except *Pseudotriton ruber*, in which the mean DMP–SAR relative onset was  $4 \pm 6$  ms ( $N=18$ ). Finally, the hypothesis of simultaneous activation of DMP and RCP was rejected by the  $t$ -test for all species together ( $t=-7.72$ ,  $P<0.0001$ ) and for each species except *D. quadramaculatus*, in which the mean DMP–RCP relative onset was  $1 \pm 1$  ms ( $N=34$ ) (Table 2).

The hypothesis that muscles reach peak activity simultaneously was also rejected using a one-sample  $t$ -test for

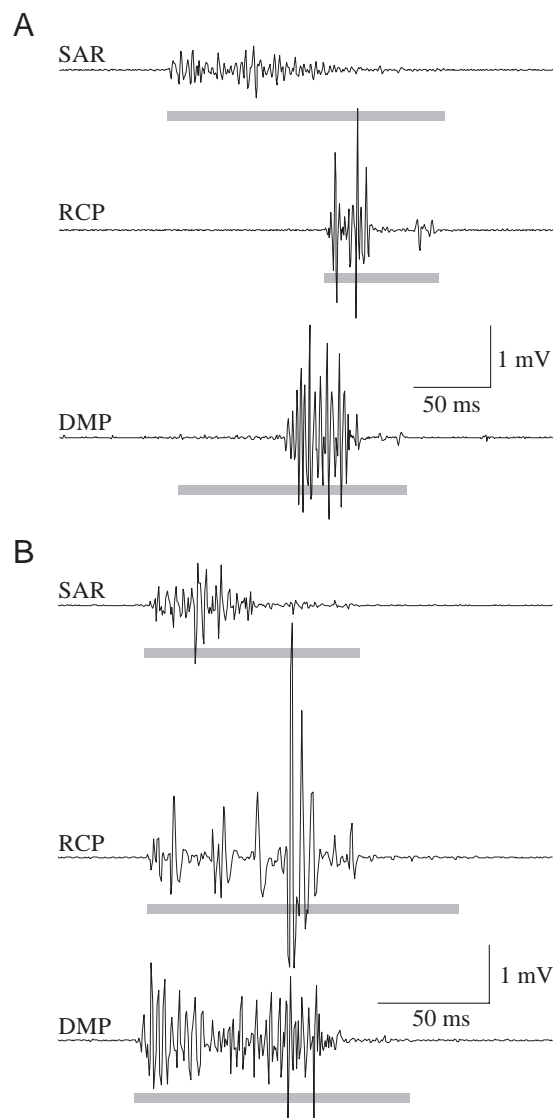


Fig. 2. Representative electromyographic traces showing a 100 ms delay between activation of the tongue protractor (SAR) and retractor (RCP) in a feeding by *Pseudotriton ruber* (A) and nearly simultaneous activation in a feeding by *Plethodon jordani* (B). The gray bars indicate the periods of activity of the muscles. Muscle abbreviations are as in Fig. 1.

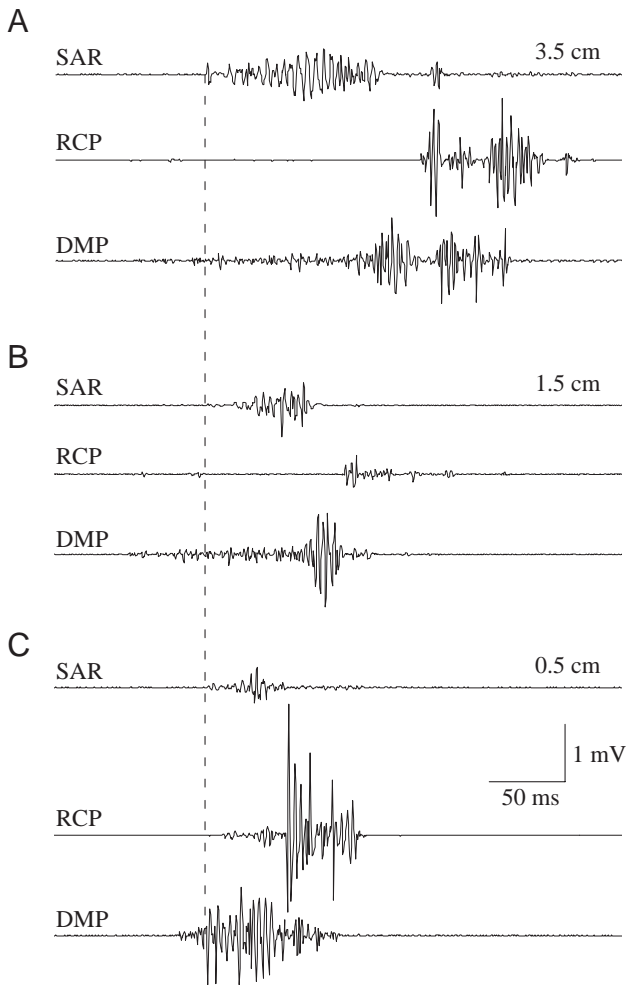


Fig. 3. Representative electromyographic traces from three feedings in the same individual of *Hydromantes supramontis* showing variation in RCP–SAR relative onset as a function of maximum tongue length. Traces are aligned at SAR onset (vertical dashed line). Note the displacement of both RCP and DMP bursts towards the left (earlier in time) and the decrease in SAR amplitude as tongue reach decreases from 3.5 cm (A) to 1.5 cm (B) and 0.5 cm (C). Muscle abbreviations are as in Fig. 1.

all comparisons ( $P < 0.0001$ ) except DMP–SAR relative peak in *Plethodon jordani* and DMP–RCP relative peak in *D. quadramaculatus*. Antagonistic tongue muscles reached peak activity at different times in all comparisons, with RCP reaching peak activity much later ( $54 \pm 3$  ms,  $N = 154$ ) than SAR (Table 3).

The period of peak activity in DMP preceded that of RCP by an average of  $29 \pm 2$  ms for all species taken together ( $N = 143$ ) and preceded RCP peak activity in each species considered separately (Table 3). Peak SAR activity preceded peak DMP activity by an average of  $23 \pm 3$  ms ( $N = 143$ ). Thus, the temporal sequence of peak activity differed from the sequence of activation onset in these three muscles; SAR reached its peak first, followed by DMP and then RCP.

Table 1. Means, standard errors and sample sizes of EMG duration variables for each species of plethodontid salamander

	SAR duration (ms)		RCP duration (ms)		DMP duration (ms)	
	N	Mean	N	Mean	N	Mean
All species	154	148 $\pm$ 5	154	139 $\pm$ 5	143	151 $\pm$ 5
All species <sup>M</sup>	154	111 $\pm$ 3	154	106 $\pm$ 3	143	151 $\pm$ 5
<i>Hydromantes supramontis</i>	57	185 $\pm$ 9	57	174 $\pm$ 10	53	194 $\pm$ 9
<i>H. supramontis</i> <sup>M</sup>	57	86 $\pm$ 4	57	81 $\pm$ 4	53	194 $\pm$ 9
<i>Pseudotriton ruber</i>	25	149 $\pm$ 5	25	105 $\pm$ 10	18	145 $\pm$ 9
<i>Plethodon jordani</i>	38	110 $\pm$ 6	38	123 $\pm$ 6	38	122 $\pm$ 6
<i>Desmognathus quadramaculatus</i>	34	128 $\pm$ 6	34	122 $\pm$ 6	34	118 $\pm$ 6

<sup>M</sup>Variables are measured only from the main burst of SAR and RCP in *Hydromantes supramontis*.

Values are means  $\pm$  S.E.M.

DMP, m. depressor mandibulae posterior; RCP, m. rectus cervicis profundus; SAR, m. subarcualis rectus.

#### Variation among species

The nested ANOVA revealed a significant effect of species in all variables except RCP–SAR relative peak: SAR duration, RCP duration, DMP duration, RCP–SAR relative onset, DMP–SAR relative onset, DMP–SAR relative peak, DMP–RCP relative onset and DMP–RCP relative peak. When only main bursts in *H. supramontis* were included, all variables showed a significant species effect (Table 4).

*H. supramontis* (main bursts) and *Pseudotriton ruber* showed the greatest range and mean for RCP–SAR relative onset, while *D. quadramaculatus* and *Plethodon jordani* showed much smaller range and mean. When entire bursts in SAR and RCP were considered in *H. supramontis*, RCP–SAR relative onset dropped below values for *Pseudotriton ruber*, but still far exceeded values for *Plethodon jordani* and *D. quadramaculatus* (Table 2). Similarly, DMP–RCP relative onset and DMP–SAR relative onset were greatest for *H. supramontis* and *Pseudotriton ruber*, and smallest for *D. quadramaculatus* and *Plethodon jordani* (Fig. 4).

The main differences in relative peak variables were in DMP–RCP and DMP–SAR relative peak, indicating that the RCP and SAR muscles retain similar relative peak values among species, but that these peaks are shifted relative to the peak of activity in DMP. This is particularly evident in *Plethodon jordani* compared with the other species. *Plethodon jordani* stood out from the other species with a very low mean for DMP–SAR relative peak ( $-1 \pm 5$  ms) ( $N = 38$ ), indicating that SAR and DMP reached peak activity at almost the same time. In the other species, SAR peaked approximately 30 ms before DMP (Fig. 5). *D. quadramaculatus* and *Plethodon jordani*

Table 2. Means, standard errors and sample sizes of relative onset variables and results of one-sample t-tests examining the hypothesis that muscles are activated simultaneously

	N	Mean	d.f.	t	P	99 % lower	99 % upper
RCP–SAR relative onset							
<i>Desmognathus</i>	34	3±1	33	3.694	0.0008*	1	4
<i>Hydromantes</i>	57	24±7	56	3.427	0.0011*	5	39
<i>Hydromantes</i> <sup>M</sup>	46	83±5	45	17.923	<0.0001*	71	96
<i>Plethodon</i>	38	0±2	37	-0.166	0.8689 <sup>NS</sup>	-5	4
<i>Pseudotriton</i>	25	61±6	24	10.019	<0.0001*	44	79
All species	154	19±3	153	6.002	<0.0001*	11	27
All species <sup>M</sup>	143	38±4	142	10.296	<0.0001*	28	48
DMP–SAR relative onset							
<i>Desmognathus</i>	34	3±1	33	3.309	0.0023*	1	6
<i>Hydromantes</i>	53	-21±3	52	-6.339	<0.0001*	-29	-12
<i>Hydromantes</i> <sup>M</sup>	53	-29±3	52	-9.778	<0.0001*	-38	-21
<i>Plethodon</i>	38	-5±1	37	-4.327	0.0001*	-9	-2
<i>Pseudotriton</i>	18	4±6	17	0.668	0.5131 <sup>NS</sup>	-13	21
All species	143	-8±2	142	-4.569	<0.0001*	-12	-3
All species <sup>M</sup>	143	-11±2	142	-5.987	<0.0001*	-16	-6
DMP–RCP relative onset							
<i>Desmognathus</i>	34	1±1	33	1.058	0.2975 <sup>NS</sup>	-1	3
<i>Hydromantes</i>	53	-34±5	52	-6.432	<0.0001*	-48	-20
<i>Hydromantes</i> <sup>M</sup>	45	-112±6	44	-20.098	<0.0001*	-127	-97
<i>Plethodon</i>	38	-5±2	37	-3.388	0.0017*	-9	-1
<i>Pseudotriton</i>	18	-69±7	17	-9.792	<0.0001*	-89	-48
All species	143	-22±3	142	-7.720	<0.0001*	-30	-15
All species <sup>M</sup>	135	-48±5	134	-9.876	<0.0001*	-60	-35

99% confidence limits of the mean encompass zero in only three cases.  
 \*Mean value is significantly different from zero (indicating non-simultaneous activation of muscle pair) at  $P \leq 0.0167$ ; NS, not significant.  
<sup>M</sup>Variables are measured only from the main burst of SAR and RCP in *Hydromantes*.  
 Muscle abbreviations and full species names are given in Table 1.

were quite similar in relative onset variables, but differed in relative peak variables (Table 5; Figs 4, 5), in which respect *D. quadramaculatus* resembled *H. supramontis* and *Pseudotriton ruber*.

The species-pair *post-hoc* tests revealed which species differences may have contributed to the species effects detected by the ANOVA. DMP–RCP relative onset differed significantly in every species comparison using all three tests (Games–Howell, Fisher’s PLSD and Sheffé’s *S*), except that *D. quadramaculatus* versus *Plethodon jordani* was significant only for the Games–Howell test, the least conservative test. *H. supramontis* and *Pseudotriton ruber* showed the greatest delay in activation between DMP and RCP, while *D. quadramaculatus* and *Plethodon jordani* showed nearly simultaneous activation of these muscles on average (Tables 2, 5). RCP–SAR relative peak differed the least frequently and only in the comparisons between *H. supramontis* and *Plethodon jordani* and *H. supramontis* (main bursts only) and *D. quadramaculatus*. *H. supramontis* SAR activity peaked an average of 64±6 ms ( $N=57$ ) before RCP peak, compared with 39±5 ms ( $N=38$ ) in *Plethodon jordani* and 52±4 ms ( $N=34$ ) in *D. quadramaculatus*. *Pseudotriton ruber* and *D. quadramaculatus* differed in only two variables, RCP–SAR

relative onset and DMP–RCP relative onset, with delays between activation being greater in *Pseudotriton ruber*. Other species comparisons differed in at least four variables. In general, the results of the three *post-hoc* tests agreed with one another (Table 5).

#### Variation among individuals

The nested ANOVAs for each species revealed significant effects of individual within *Pseudotriton ruber* and *H. supramontis* that were not attributable to differences in capture success, but failed to find any systematic individual variation across all species. *H. supramontis* showed a significant effect of individual in the duration of SAR, RCP and DMP activity as well as DMP–SAR relative onset, when the entire bursts of SAR and RCP were considered, and in the duration of DMP activity, DMP–SAR relative onset and DMP–RCP relative onset, when only the main bursts within SAR and RCP were considered (Table 6). However, when distance to prey was included in the analysis as a covariate, these effects of individual vanished. The effects of maximum tongue length in two individuals of *H. supramontis* were examined in a regression analysis (below). *Pseudotriton ruber* showed significant individual differences in RCP–SAR relative peak

and DMP–SAR relative peak; however, these differences may be influenced by variables not included in the analysis for this species, such as prey distance. *D. quadramaculatus* and *Plethodon jordani* showed no significant effects of individual on any of the EMG variables, indicating less individual variation in their EMG patterns than in those of *H. supramontis* and *Pseudotriton ruber* (Table 6).

#### Variation within individuals

Only *D. quadramaculatus* showed significant effects of capture success in the nested ANOVA: DMP area ( $F=8.47$ ,  $P=0.0012$ ) and DMP peak area ( $F=11.79$ ,  $P=0.0002$ ) were significantly greater in captures than in misses.

When entire bursts of SAR and RCP were analyzed, *H. supramontis* 1 showed a significant positive correlation with

Table 3. Means, standard errors and sample sizes of relative peak variables and results of one-sample t-tests examining the hypothesis that muscles reach peak activation simultaneously

	<i>N</i>	Mean	d.f.	<i>t</i>	<i>P</i>	99% lower	99% upper
RCP–SAR relative peak							
<i>Desmognathus</i>	34	52±4	33	12.560	<0.0001*	41	63
<i>Hydromantes</i>	57	64±6	56	11.026	<0.0001*	48	79
<i>Hydromantes</i> <sup>M</sup>	46	70±6	45	11.848	<0.0001*	54	86
<i>Plethodon</i>	38	39±5	37	7.553	<0.0001*	25	53
<i>Pseudotriton</i>	25	52±5	24	11.626	<0.0001*	40	65
All species	154	54±3	153	18.797	<0.0001*	46	60
All species <sup>M</sup>	143	55±3	142	19.246	<0.0001*	47	62
DMP–SAR relative peak							
<i>Desmognathus</i>	34	39±6	33	6.353	<0.0001*	22	56
<i>Hydromantes</i>	53	28±5	52	5.643	<0.0001*	15	41
<i>Hydromantes</i> <sup>M</sup>	53	31±5	52	6.739	<0.0001*	19	43
<i>Plethodon</i>	38	−1±5	37	−0.324	0.7479 <sup>NS</sup>	−14	11
<i>Pseudotriton</i>	18	33±5	17	6.005	<0.0001*	17	49
All species	143	23±3	142	7.788	<0.0001*	15	31
All species <sup>M</sup>	143	24±3	142	8.341	<0.0001*	17	32
DMP–RCP relative peak							
<i>Desmognathus</i>	34	−13±5	33	−2.413	0.0215 <sup>NS</sup>	−28	2
<i>Hydromantes</i>	53	−35±3	52	−10.704	<0.0001*	−43	−26
<i>Hydromantes</i> <sup>M</sup>	45	−38±4	44	−10.217	<0.0001*	−48	−28
<i>Plethodon</i>	38	−40±5	37	−8.279	<0.0001*	−54	−27
<i>Pseudotriton</i>	18	−20±3	17	−7.617	<0.0001*	−27	−12
All species	143	−29±2	142	−12.290	<0.0001*	−35	−23
All species <sup>M</sup>	135	−30±3	134	−11.901	<0.0001*	−37	−23

99% confidence limits of the mean encompass zero in only two cases.

\*Mean is significantly different from zero (indicating non-simultaneous peak of muscle pair) at  $P \leq 0.0167$ ; NS, not significant.

<sup>M</sup>Variables are measured only from the main burst of SAR and RCP in *Hydromantes*.

Muscle abbreviations and full species names are given in Table 1.

Table 4. Results of ANOVAs examining the effect of species on nine EMG timing variables

	SAR duration	RCP duration	DMP duration	RCP–SAR relative onset	RCP–SAR relative peak	DMP–SAR relative onset	DMP–SAR relative peak	DMP–RCP relative onset	DMP–RCP relative peak
Effect of species									
<i>F</i>	18.9	6.5	18.5	34.5	3.7	6.3	9.7	33.9	7.6
<i>P</i>	<0.0001*	0.0004*	<0.0001*	<0.0001*	0.0140	0.0005*	<0.0001*	<0.0001*	<0.0001*
Effect of species <sup>M</sup>									
<i>F</i>	13.9	6.2	21.8	129.6	5.6	24.2	10.8	182.9	7.5
<i>P</i>	<0.0001*	0.0006*	<0.0001*	<0.0001*	0.0013*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

All but one variable show a significant species effect.

\*Significant at Bonferroni-adjusted  $P \leq 0.0056$ .

<sup>M</sup>Variables calculated from main bursts in SAR and RCP only in *Hydromantes supramontis*.

Muscle abbreviations are given in Table 1.



maximum tongue length in SAR duration, SAR area, RCP duration, DMP duration and DMP area (Fig. 6). *H. supramontis* 2 showed significant positive correlation with maximum tongue length in RCP–SAR relative peak (Table 7). The distance between the salamander and the prey at the start

of the prey-capture strike (i.e. initial prey distance) was also highly positively correlated with maximum tongue length (e.g. *H. supramontis* 1;  $r=0.92$ ,  $P<0.0001$ ) (see Fig. 6).

When only the main bursts of SAR and RCP were analyzed, *H. supramontis* 1 showed significant positive correlation with maximum tongue length in SAR area, SAR duration, RCP–SAR relative onset, DMP area and DMP duration, and *H. supramontis* 2 showed significant positive correlation in

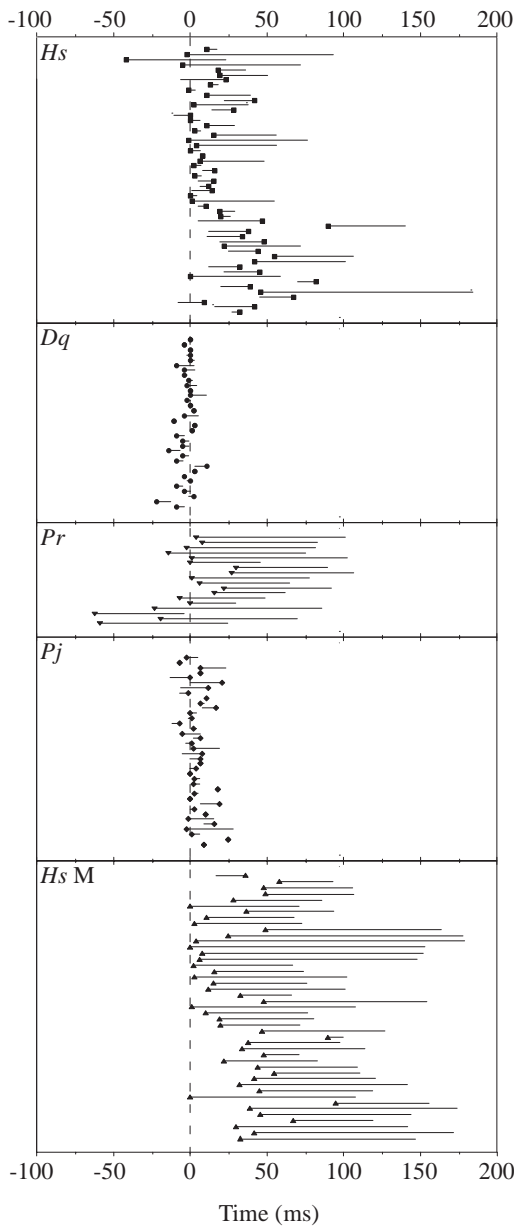


Fig. 4. Times of SAR onset and RCP onset relative to DMP onset (vertical dashed line). SAR onset is the end of the line marked with a symbol and RCP onset is the unmarked end of the line. Note the differences in line length (indicating RCP–SAR relative onset) between the two long-tongued species *Hydromantes supramontis* (*Hs*) and *Pseudotriton ruber* (*Pr*) compared with the short-tongued species *Desmognathus quadramaculatus* (*Dq*) and *Plethodon jordani* (*Pj*). Note also the slight shift to the left of the onset times in *D. quadramaculatus* compared with *Plethodon jordani*. *Hs M* indicates measurements made from the main bursts of activity in *H. supramontis*. Muscle abbreviations are as in Fig. 1.

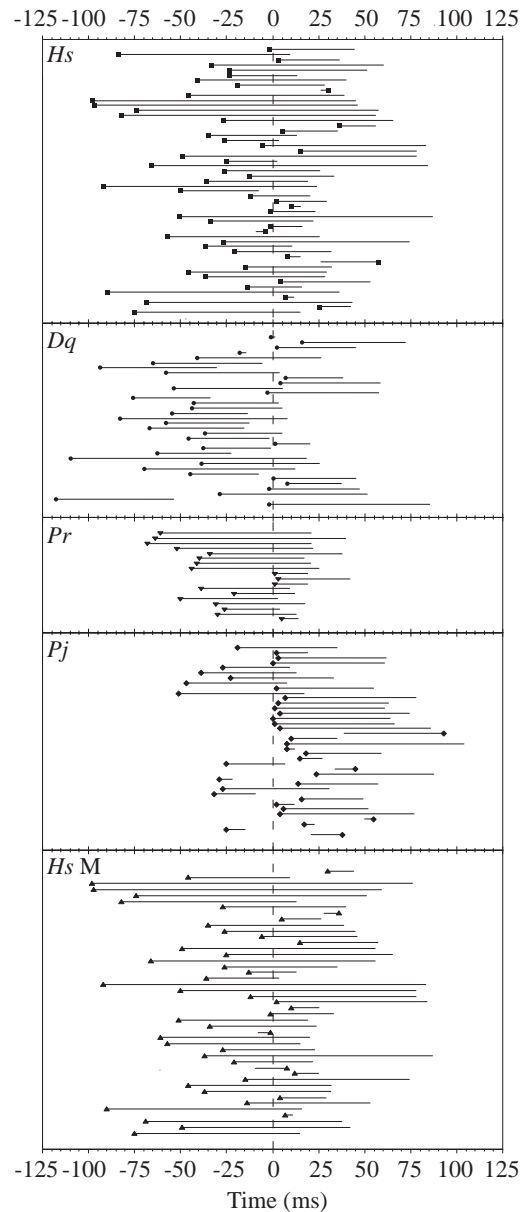


Fig. 5. Times of SAR peak activity and RCP peak activity relative to DMP peak activity (vertical dashed line). SAR peak is the end of the line marked with a symbol and RCP peak is the unmarked end of the line. Note the distinctive pattern in *Plethodon jordani* (*Pj*) in which the SAR and RCP peaks are shifted later in time relative to the DMP peak. *Hs M* indicates measurements made from the main bursts of activity in *Hydromantes supramontis*. Muscle abbreviations are as in Fig. 1. *Hs*, *H. supramontis*; *Dq*, *Desmognathus quadramaculatus*; *Pr*, *Pseudotriton ruber*.

Table 5. Differences in the means of species pairs and results of post-hoc tests performed on the results of species-effect ANOVA

	SAR duration	RCP duration	DMP duration	RCP-SAR relative onset	RCP-SAR relative peak	DMP-SAR relative onset	DMP-SAR relative peak	DMP-RCP relative onset	DMP-RCP relative peak
<i>Hyd versus Pleth</i>	68 GFS	54 GFS	69 GFS	14	23 F	15 GFS	29 GFS	29 GFS	6
<i>Hyd versus Desm</i>	50 GFS	56 GFS	73 GFS	11	10	24 GFS	11	35 GFS	21 GFS
<i>Hyd versus Pseud</i>	28	70 GFS	47 GFS	60 GFS	9	24 GFS	5	35 GFS	15 G
<i>Desm versus Pleth</i>	18	1	4	3	13	9 G	40 GFS	6 G	27 GFS
<i>Desm versus Pseud</i>	21	14	27	70 GFS	1	1	6	70 GFS	7
<i>Pleth versus Pseud</i>	39 GF	16	23	73 GFS	14	9	34 GFS	64 GFS	21 GF
<i>Hyd<sup>M</sup> versus Pleth</i>	23 GF	42 GFS	76 GFS	83 GFS	32 GFS	24 GFS	34 GFS	107 GFS	2
<i>Hyd<sup>M</sup> versus Desm</i>	41 GFS	40 GFS	80 GFS	80 GFS	19 F	32 GFS	6	112 GFS	25 GFS
<i>Hyd<sup>M</sup> versus Pseud</i>	63 GFS	26	54 GFS	10	18	33 GFS	0	43 GFS	18 G

G, F and S indicate significant differences from zero at  $P \leq 0.01$  from Games-Howell, Fisher's PLSD and Sheffé's  $S$  post-hoc tests, respectively.

<sup>M</sup>Variables calculated from main bursts in SAR and RCP only in *Hydromantes supramontis*.

*Hyd*, *Hydromantes supramontis*; *Pleth*, *Plethodon jordani*; *Desm*, *Desmognathus quadramaculatus*; *Pseud*, *Pseudotriton ruber*.

Muscle abbreviations are given in Table 1.

SAR duration, RCP-SAR relative onset, RCP-SAR relative peak, DMP-RCP relative onset and DMP duration (Table 7).

## Discussion

### Feeding behavior

The feeding behavior observed in this study confirms observations made previously of the role of the tongue and jaws in salamander feeding, with the exception of the degree of stereotypy of the feeding movements. It was evident from both the video recordings of *H. supramontis* and from unaided visual observations of feedings in all the species that the salamanders were modulating their movements during the prey-capture strike. While modulation of prey-capture behavior has been observed previously in many groups of salamanders (Erdmann and Cundall, 1984; Shaffer and Lauder, 1985; Miller and Larsen, 1990; Reilly and Lauder, 1989, 1992; Elwood and Cundall, 1994), it has only recently been documented in plethodontid salamanders (Larsen and Beneski, 1988; Maglia and Pyles, 1995; Deban, 1997), which have extremely fast and long-distance tongue protraction. Bolitoglossine plethodontids in particular were predicted to be the most stereotyped of all salamanders on the basis of their morphology and on EMG and force recordings of *Bolitoglossa occidentalis* (Thexton et al., 1977). However, the bolitoglossine *H. supramontis* showed by far the highest degree of modulation among the species examined here, as reflected in both behavior and EMG patterns.

### Electromyographic patterns

Previous electromyographic studies of prey capture in aquatic and terrestrial salamanders, although quite limited in taxonomic scope, have found many muscles of the feeding system to be active nearly simultaneously (i.e. within a few milliseconds of one another) during the prey-capture strike (*Bolitoglossa occidentalis*, Thexton et al., 1977; terrestrial

*Ambystoma tigrinum*, Lauder and Shaffer, 1988; aquatic *Ambystoma*, Reilly and Lauder, 1989). These studies also found that the pattern of muscle activation was relatively invariant within a given individual. The results presented here differ from these previous findings; the activation of tongue muscles was typically not simultaneous, and the relative timing, duration and amplitude of activation shifted within individuals among different feeding events (Tables 2, 3).

Muscle activation was sequential, and the first muscle to become active was the DMP; this activity was associated with mouth opening in preparation for tongue protraction. Next, the SAR was activated for tongue protraction, followed by the RCP for tongue retraction. The short delay between DMP onset and SAR onset indicates a tight association between mouth opening and tongue protraction. These two movements are also closely associated kinematically in salamander feeding generally (Larsen and Guthrie, 1975; Dockx and De Vree, 1986; Larsen et al., 1989; Deban, 1997). Of particular interest is that there is typically a delay in onset between the antagonistic tongue muscles SAR and RCP (RCP-SAR relative onset), a finding that does not agree with previous studies.

In general, peak activity was also sequential rather than simultaneous. The sequence was SAR-DMP-RCP on average, which indicates that the sequence of maximum force production also followed this order. Protraction of the tongue precedes retraction, so it makes sense that peak SAR activity should occur before peak RCP activity. Because peak DMP activity usually fell between the peak activities of the SAR and RCP, the greatest mouth-opening effort probably occurred near this time. This agrees with previously published descriptions of salamander feeding (Roth, 1987; Larsen et al., 1989, 1996; Findeis and Bemis, 1990), in which the mouth is often opened fully and rapidly during tongue retraction to accommodate the prey. The second most frequent temporal sequence was DMP-SAR-RCP, indicating that more forceful mouth opening

Table 6. Results of ANOVAs examining the effect of individual on nine EMG timing variables for each species

	SAR duration		RCP duration		DMP duration		RCP-SAR relative onset		RCP-SAR relative peak		DMP-SAR relative onset		DMP-SAR relative peak		DMP-RCP relative onset		DMP-RCP relative peak	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>Hydromantes</i>	8.8	<0.0001*	4.6	0.0033*	8.4	<0.0001*	0.7	0.5944	2.4	0.0638	5.7	0.0009*	1.4	0.2365	1.4	0.2362	1.2	0.3393
<i>Hydromantes</i> <sup>M</sup>	2.4	0.0713	0.8	0.5086	8.7	<0.0001*	3.1	0.0284	1.3	0.2601	6.1	0.0007*	0.9	0.4662	7.1	0.0003*	0.7	0.5846
<i>Pseudotriton</i>	0.1	0.8890	0.4	0.6760	6.2	0.0140	0.1	0.8739	14.2	0.0007*	5.4	0.0218	13.6	0.0008*	3.0	0.0868	4.0	0.0475
<i>Plethodon</i>	2.8	0.0765	0.6	0.5800	0.1	0.8880	0.3	0.7685	1.0	0.3695	1.8	0.1790	1.0	0.3728	1.4	0.2572	2.3	0.1174
<i>Desmognathus</i>	0.1	0.7175	0.6	0.4482	0.0	0.9645	0.0	0.9363	1.2	0.2821	0.7	0.4181	2.9	0.0964	1.1	0.3044	1.0	0.3236

\*Significant at Bonferroni-adjusted  $P \leq 0.0056$ .

<sup>M</sup>Variables are measured only from the main burst of SAR and RCP in *Hydromantes*.

Muscle abbreviations and full species names are given in Table 1.

Table 7. Correlations between maximum tongue length and fifteen EMG variables for two individuals of *Hydromantes supramontis*

Individual	SAR duration		RCP duration		DMP duration		RCP-SAR relative onset		RCP-SAR relative peak		DMP-SAR relative onset		DMP-SAR relative peak		DMP-RCP relative onset		DMP-RCP relative peak	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
1	0.67	0.0005*	0.70	0.0002*	0.80	<0.0001*	0.23	0.2894	0.26	0.2253	0.01	0.9693	0.30	0.1817	0.20	0.3493	0.08	0.7318
1 <sup>M</sup>	0.77	<0.0001*	0.35	0.1861	0.80	<0.0001*	0.90	<0.0001*	0.14	0.5963	0.08	0.7281	0.51	0.0134	0.63	0.0092	0.13	0.6385
2	0.60	0.0102	0.41	0.1048	0.69	0.0059	0.22	0.3951	0.69	0.0020*	0.13	0.6556	0.51	0.0606	0.38	0.1809	0.42	0.1331
2 <sup>M</sup>	0.77	0.0003*	0.49	0.0775	0.79	0.0009*	0.84	0.0002*	0.77	0.0013*	0.16	0.5903	0.54	0.0445	0.80	0.0011*	0.43	0.1472

Individual	SAR area		SAR peak area		RCP area		RCP peak area		DMP area		DMP peak area	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
1	0.59	0.0028*	0.41	0.0533	0.29	0.1730	0.07	0.7502	0.65	0.0009*	0.52	0.0114
1 <sup>M</sup>	0.59	0.0033*	0.40	0.0584	0.20	0.4682	0.02	0.9423	0.65	0.0009*	0.52	0.0114
2	0.58	0.0144	0.08	0.7556	0.37	0.1448	0.26	0.3051	0.52	0.0563	0.47	0.0878
2 <sup>M</sup>	0.54	0.0256	0.00	0.9911	0.27	0.3498	0.08	0.7888	0.52	0.0563	0.47	0.0878

\*Significant at Bonferroni-adjusted  $P \leq 0.0033$ .

<sup>M</sup>Variables are measured only from the main burst of SAR and RCP.

Muscle abbreviations are given in Table 1.

occurred in preparation for tongue protraction; this matched the sequence of muscle activity onset.

*Simultaneous activation*

The hypotheses that muscles are activated simultaneously or reach peak activity simultaneously during the feeding strike

were rejected by the one-sample *t*-tests when all species were considered together. Simultaneous or nearly simultaneous activation or peak activity, however, was observed in some feedings, particularly in muscles that are involved in temporally coupled movements such as mouth opening and tongue protraction.

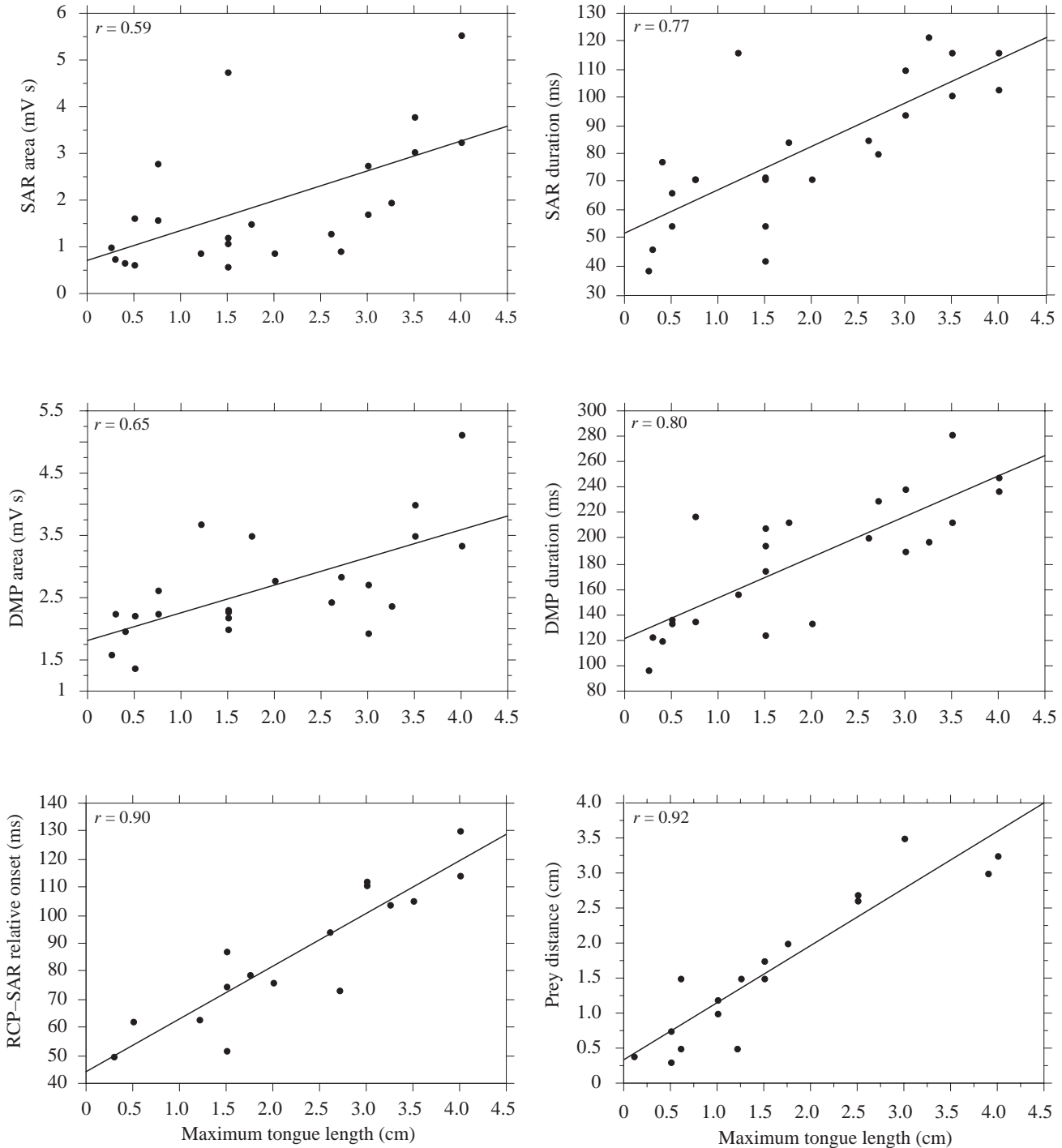


Fig. 6. Electromyographic variables with a significant correlation with maximum tongue length for all feedings of a single individual of *Hydromantes supramontis* (individual 1) in which main bursts of SAR and RCP were analyzed. Note the relationship between maximum tongue length and SAR area and SAR duration, which indicates modulation of protractor activity, and the relationship with prey distance, which indicates accurate placement of the tongue onto the prey. Muscle abbreviations are as in Fig. 1.

The only species that never showed simultaneous activation or simultaneous peak activity was the bolitoglossine *H. supramontis*, which showed highly variable relative onset values. *H. supramontis* differs in this regard from *Bolitoglossa occidentalis*, which showed nearly simultaneous activation of all the muscles examined, including the SAR and RCP, as well as the m. interhyoideus (a superficial transverse throat constrictor) and the m. depressor mandibulae (Thexton et al., 1977). *B. occidentalis* also showed no effect of maximum tongue protraction distance either on the timing of tongue muscle activity (duration or relative onset) or on the integrated area of the muscle bursts. The main activity duration of the muscles, however, did not differ from our findings and ranged from 60 to 90 ms, with a period of lower-amplitude activity, often preceding the main burst, of approximately 150 ms duration.

Thexton et al. (1977) proposed a mechanism in which the peripheral arrangement of muscles, a taut protractor and slack retractor when the tongue is at rest, and the reverse when it is fully projected, was largely responsible for the stereotyped tongue movements they observed, rather than fine control by the central nervous system. Our findings in *H. supramontis* suggest just the reverse, i.e. that the nervous system controls precise placement of the tongue by metering muscle activity; adjustments are made to the relative timing, duration and degree of muscle contraction. One possible explanation for the nearly simultaneous muscle activation observed in *B. occidentalis* is their small size (37–43 mm snout–vent length), which would cause shorter-duration movements than in our larger (71–83 mm) *H. supramontis* specimens and would, consequently, produce closer temporal coupling of muscle activation, particularly of antagonists. This effect has been observed in EMG studies of feeding in bass (*Micropterus salmoides*) of different body sizes (Wainwright and Richard, 1995). Thexton et al. (1977) did not examine the relative timing of peak muscle activities in *B. occidentalis*, which perhaps would have revealed an effect of prey distance, although this would also be expected to be slight, given the small size of the animals and the speed of tongue protraction. Another reason for the conflicting findings in *H. supramontis* and *B. occidentalis* may be that there are biomechanical differences in tongue projection that require different motor control strategies (for example, the much longer tongue of *H. supramontis* relative to body size). It is clear from our results that any model of motor control of tongue movements in plethodontid salamanders must take into account the fact that motor patterns can be altered, permitting a range of movement patterns with a given morphology.

#### Species differences

*D. quadramaculatus* and *Plethodon jordani* showed nearly simultaneous activation of DMP, SAR and RCP most frequently. This is probably related to their limited degree of tongue protraction relative to the other species. Both species have tongue pads that are attached by a genioglossus muscle to the mandible and have tongues that are more limited in

protrusibility than those of *Pseudotriton ruber* and *H. supramontis*, which lack this muscle (Lombard and Wake, 1977). The short range of movement of these tongues compared with those of the free-tongued *H. supramontis* and *Pseudotriton ruber* may explain why the muscle activity onsets are more closely grouped temporally and why the activity of these antagonists overlaps more extensively. Strong overlap and nearly simultaneous activation of SAR and RCP were found in *Ambystoma tigrinum* as well, a species that also possesses a short tongue (Lauder and Shaffer, 1988; Reilly and Lauder, 1990). A similar pattern was also observed in *H. supramontis* during the shortest-distance tongue protractions (Fig. 3), confirming that close protraction–retraction temporal coupling is produced by strongly overlapping antagonistic muscle activity.

*Plethodon jordani* stands out from the other species in showing predominantly a pattern of peak activities in the sequence DMP–SAR–RCP, indicating that the mouth is opened most forcefully at approximately the same time as the most forceful tongue protraction, rather than prior to retraction as in the other species. An explanation for this pattern may be that *Plethodon jordani* has a relatively large tongue pad, compared with that of the other species, that must be flipped over the mandibular symphysis during protraction because of its attachment via the genioglossus muscle to the lower jaw (Lombard and Wake, 1977). In *Pseudotriton ruber* and *H. supramontis*, the small tongue pad lacks a genioglossus muscle and is thrust out along a linear trajectory. *Plethodon jordani* open its mouth wide early in the feeding sequence to accommodate tongue-pad flipping, while *Pseudotriton ruber* and *H. supramontis* protract the tongue while opening the mouth only slightly.

*D. quadramaculatus* also possesses an attached tongue pad, but its tongue pad is smaller than that of *Plethodon jordani* and its DMP–SAR relative peak values are greater than those of *Plethodon jordani* on average, showing that it delays maximum mouth opening until after tongue protraction, much like *H. supramontis* and *Pseudotriton ruber*. Fig. 5 reveals that the differences between *Plethodon jordani* and *D. quadramaculatus* consist mainly of the relative frequencies of the different sequences of peak activity and that both species display a pattern of DMP peak activity close to the SAR peak, as well as a pattern in which the DMP peak occurs later in the strike. The choice of motor pattern may depend on features of the prey, such as size, and on the role of the jaws in prey capture. *D. quadramaculatus* possess very strong jaws and jaw musculature (Schwenk and Wake, 1993) and may preferentially show a later mouth opening in preparation for a powerful bite following tongue retraction.

#### Modulation of tongue projection in *Hydromantes supramontis*

*Hydromantes supramontis* differs from *B. occidentalis* (Thexton et al., 1977) in showing highly variable motor patterns within individuals, in which many features vary as a function of prey distance. The increases in SAR area and SAR duration with increasing maximum tongue length (and prey

distance) indicate that the force and/or duration of SAR contraction is greater in long-distance protraction, which requires more time to complete than short-distance protraction. RCP duration, DMP area and DMP duration also increase with increased protraction distance because tongue retraction requires more time after long-distance protraction, and the mouth is opened during tongue retraction to accommodate the returning tongue and attached prey. These prolonged activities are reflected in the greater values of the area and duration variables. The kinematic relationships between tongue retraction and mouth opening have been documented in previous studies of salamander feeding (e.g. Larsen et al., 1989; Deban, 1997). In response to prey distance, *H. supramontis* thus modulates the strength of muscle contraction (possibly recruitment) and the duration of contraction as measured by the integrated area of the EMG bursts, in addition to modulating the relative onset of activity in the RCP and SAR muscles.

The relationship between RCP–SAR relative onset and maximum tongue length shows that, for each additional centimeter of tongue protraction in *H. supramontis*, RCP–SAR relative onset of the main bursts (i.e. not the entire activity period) increases by 20 ms up to a maximum of 130 ms (Fig. 6). Tongue protraction in *H. supramontis*, from the start of protraction until prey contact, is known to occur in approximately 10 ms (Roth, 1976; S. M. Deban, unpublished results); therefore, the main bursts of activity in the RCP are clearly not associated with braking the tongue at the end of protraction. These bursts are probably associated with the beginning of tongue retraction, when the tongue pad and prey are pulled from the substratum and maximum retraction force is required. Because the tongue is small and light, any force generation in the RCP necessary to brake the tongue (beyond that supplied by the prey and substratum) may not be reflected in increased EMG activity. In contrast, the SAR should contract most forcefully during tongue protraction and should show the greatest EMG activity early, which is exactly what we observed (Fig. 3).

The shallow slope of the correlations between maximum tongue length and RCP–SAR relative onset and relative peak indicates that *H. supramontis* starts retracting the tongue more quickly after it strikes the target during short-distance strikes than during long-distance strikes. Short-distance strikes would therefore seem to be more precisely controlled by antagonistic muscle co-activity than extreme long-distance strikes. This interpretation is supported by high-speed video recordings of feeding in *H. supramontis* (S. M. Deban, unpublished data), which generally show immediate retraction after short-distance strikes and delayed retraction in long-distance strikes.

These results in *H. supramontis* reveal that the central nervous system responds to differences in prey position (and probably size and other features of the prey) by altering motor output (i.e. timing, duration and amplitude), and disclose a greater role for the brain in modulation of feeding than was previously hypothesized for amphibians.

#### *Motor programming and sensory feedback*

The lack of significant effects of prey-capture success on EMG variables indicates that the prey-capture strike is not influenced by sensory feedback in *Pseudotriton ruber*, *Plethodon jordani* and *H. supramontis*. In *D. quadramaculatus*, the greater DMP area and DMP peak area in captures suggests that the mouth is opened more rapidly or to a greater degree in captures than in misses. However, it is not clear whether this effect is due to feedback or 'feedforward' control; faster, more vigorous feedings may simply be more successful than slower feedings. The short strike duration in *D. quadramaculatus* makes modulation of mouth opening by sensory feedback unlikely. Also, in the plethodontid *Ensatina eschscholtzii*, sensory information from the tongue pad via the glossopharyngeal nerve was not involved in the modulation of feeding movements (Deban, 1997). Support was found for the hypothesis that *E. eschscholtzii* was assessing aspects of the prey visually and executing the appropriate movements in response to differences in prey size via motor programming (*sensu* Marsden et al., 1984). The data presented here suggest that sensory feedback plays little or no role in modulating tongue movements in other plethodontid species.

If modification of the motor program by peripheral feedback were to be found in salamander feeding, it would be expected in the latter portion of the strike, i.e. during tongue retraction and just after, when sensory information about tongue position and resistance to retraction have been processed by the central nervous system. The delayed tongue retraction (up to 200 ms) seen in *H. supramontis* in the present study is perhaps modulated to some extent by feedback, perhaps for load compensation (for a discussion of sensory feedback, see Pearson, 1993). In the *Plethodon jordani* examined here, cyclic activity of the SAR and RCP indicative of prey transport often began less than 50 ms after the end of RCP retraction activity in successful captures but not in misses. Sensory information concerning strike success can therefore generate the appropriate motor pattern within the time course of a single feeding event, but certainly not during the projection phase, which takes only approximately 10 ms.

#### *Evolution of motor control in the Plethodontidae*

Phylogenetic relationships within the family Plethodontidae show that projectile tongues have evolved at least twice, once within the Hemidactyliini and once within the Bolitoglossini (Lombard and Wake, 1986). Among these taxa, free tongues (with no genioglossus) have evolved three times independently, once in the Hemidactyliini and twice in the Bolitoglossini (in *Hydromantes* and in the ancestor of the tropical species, supergenus *Bolitoglossa*) (Jackman et al., 1997). Our results show that the motor patterns of *H. supramontis* and *Pseudotriton ruber* are similar to one another, and that those of *D. quadramaculatus* and *Plethodon jordani* are similar to one another, particularly in the relative onset variables (Tables 2, 5). The relationships among the four major clades to which these species belong indicate that the

distinctive motor pattern of *H. supramontis* and *Pseudotriton ruber* must have evolved convergently along with the evolution of long tongues. The motor patterns of *D. quadramaculatus* and *Plethodon jordani* resemble more closely that of outgroup taxa which have been studied, namely species of *Ambystoma* (e.g. Lauder and Shaffer, 1988), and can be considered to be similar to the ancestral state for the Plethodontidae. The longer and variable delay between the onset of protractor and retractor activity in *H. supramontis* and *Pseudotriton ruber* may have evolved concomitantly with the evolution of long tongues in these taxa and the resulting increase in range of motion. In this light, the simultaneous activation of feeding muscles in *B. occidentalis* (Thexton et al., 1977) remains anomalous; bolitoglossine salamanders clearly deserve further study with regard to feeding behavior.

Evolutionary changes in the biomechanics of tongue protraction (from protrusible to projectile tongues) in frogs have necessitated accompanying changes in motor control to maintain coordinated function. These changes appear to have been accomplished by changes in the peripheral nervous system *via* the evolution of sensory feedback mechanisms, rather than in the output of the central pattern generator (Nishikawa et al., 1992; Nishikawa and Gans, 1996). These changes in motor control are required in frogs because the tongue is attached anteriorly to the mandible and moves with it as the mouth opens. To maintain a linear trajectory of the tongue tip, which is observed in the projectile-tongued *Bufo*, precise coordination of mouth opening and tongue rotation is necessary (Anderson and Nishikawa, 1996; Nishikawa and Gans, 1996). In salamanders, tongue movements are not as tightly linked to jaw movements because they rely less on tongue-pad flipping. What linkage exists in generalized salamanders (the genioglossus muscle) is lost completely in free-tongued plethodontids. Therefore, precise jaw-tongue coordination should become less important with the evolution of projectile tongues in plethodontids and we should find no accompanying evolution of feedback mechanisms of the type seen in frogs. Nonetheless, the greater range of motion of a projectile tongue, such as that of *H. supramontis*, may require mechanisms of fine motor control of the tongue, perhaps relying to some extent on sensory or proprioceptive feedback during slower feedings, as opposed to pure motor programming of tongue movement based on visual calculation of prey position.

#### *Stereotypy in amphibian feeding*

Amphibian feeding has long been a major example of stereotyped movement in vertebrates; feeding in the toad *Bufo* has even become a textbook example of the 'fixed action pattern' concept of Lorenz (1937a,b, 1939) (see Ewert, 1989). The fixed action pattern concept has been largely discredited (Zippelius, 1992; Roth and Dicke, 1994); however, the view that amphibian feeding is highly stereotyped has persisted, despite accumulating evidence to the contrary. The results presented here, showing that plethodontid salamanders are capable of modulating their feeding behavior and motor

patterns, provide additional evidence that feeding in amphibians cannot be considered highly stereotyped in general. While relatively few studies on a few taxa of salamanders and frogs have revealed stereotyped feeding movements (Larsen and Guthrie, 1975; Thexton et al., 1977; Gans and Gorniak, 1982; Shaffer and Lauder, 1985), many more studies have shown that feeding behavior in amphibians can be highly plastic (salamanders, Erdman and Cundall, 1984; Shaffer and Lauder, 1985; Larsen and Beneski, 1988; Miller and Larsen, 1990; Reilly and Lauder, 1989, 1992; Elwood and Cundall, 1994; Maglia and Pyles, 1995; Deban, 1997; frogs, Nishikawa and Cannatella, 1991; Deban and Nishikawa, 1992; Nishikawa et al., 1992; Anderson and Nishikawa, 1996; Ritter and Nishikawa, 1995). These observations of behavioral flexibility can be accepted at face value as demonstrating the lack of stereotypy, but studies that show only invariant, apparently stereotyped, behavior have to be interpreted with caution. Experiments in which a single type of prey is presented in a standardized manner to trained animals (often necessary for kinematic analysis) can elicit a limited behavioral response and give a misleading impression of stereotypy (see Galis, 1996). The view that feeding in amphibians is highly stereotyped clearly must be revised to consider the experimental methods and limited taxonomic scope of earlier studies as well as the numerous recent studies on many taxa of amphibians that show a high degree of modulation of feeding movements and, therefore, of motor patterns.

This research was supported by a fellowship to S.M.D. from the Hanse Institute for Advanced Study, Delmenhorst, Germany, and by grant SFB 517 from the Deutsche Forschungsgemeinschaft to U.D. We thank Gerhard Roth and two anonymous reviewers for helpful comments on the manuscript.

#### References

- Anderson, C. W. and Nishikawa, K. C. (1996). The roles of visual and proprioceptive information during motor program choice in frogs. *J. Comp. Physiol. A* **179**, 753–762.
- Deban, S. M. (1997). Modulation of prey-capture behavior in the plethodontid salamander *Ensatina eschscholtzii*. *J. Exp. Biol.* **20**, 1951–1964.
- Deban, S. M. and Nishikawa, K. C. (1992). The kinematics of prey capture and the mechanism of tongue protraction in the green tree frog *Hyla cinerea*. *J. Exp. Biol.* **170**, 235–256.
- Deban, S. M., Wake, D. B. and Roth, G. (1997). Salamander with a ballistic tongue. *Nature* **389**, 27–28.
- Dockx, P. and De Vree, F. (1986). Prey capture and intra-oral food transport in terrestrial salamanders. *Studies in Herpetology*, pp. 521–524.
- Elwood, J. R. L. and Cundall, D. (1994). Morphology and behavior of the feeding apparatus in *Cryptobranchus alleganiensis* (Amphibia: Caudata). *J. Morph.* **220**, 47–70.
- Erdman, S. and Cundall, D. (1984). The feeding apparatus of the salamander *Amphiuma tridactylum*: morphology and behavior. *J. Morph.* **181**, 175–204.
- Ewert, J.-P. (1989). The release of visual behavior in toads: stages

- of parallel/heirarchical information processing. In *Visuomotor Coordination, Amphibians, Comparisons, Models and Robots* (ed. J.-P. Ewert and M.A. Arbib), pp. 39–120. New York: Plenum.
- Findeis, E. K. and Bemis, W. E.** (1990). Functional morphology of tongue projection in *Taricha torosa* (Urodela: Salamandridae). *Zool. J. Linn. Soc.* **99**, 129–157.
- Galis, F.** (1996). The application of functional morphology to evolutionary studies. *Trends Ecol. Evol.* **11**, 124–129.
- Gans, C. and Gorniak, G. C.** (1982). Functional morphology of lingual protrusion in marine toads (*Bufo marinus*). *Am. J. Anat.* **163**, 195–222.
- Jackman, T. R., Applebaum, G. and Wake, D. B.** (1997). Phylogenetic relationships of bolitoglossine salamanders: A demonstration of the effects of combining morphological and molecular data sets. *Mol. Biol. Evol.* **14**, 883–891.
- 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.
- Larsen, J. H., Jr, Beneski, J. T. and Miller, B. T.** (1996). Structure and function of the hyolingual system in *Hynobius* and its bearing on the evolution of prey capture in terrestrial salamanders. *J. Morph.* **227**, 235–248.
- Larsen, J. H., Jr, Beneski, J. T. and Wake, D. B.** (1989). Hyolingual feeding systems of the Plethodontidae: comparative kinematics of prey capture by salamanders with free and attached tongues. *J. Exp. Zool.* **252**, 25–33.
- Larsen, J. H., Jr and Guthrie, D. J.** (1975). The feeding system of terrestrial tiger salamanders (*Ambystoma tigrinum melanostictum* Baird). *J. Morph.* **147**, 137–154.
- Lauder, G. V. and Shaffer, H. B.** (1988). Ontogeny of functional design in tiger salamanders (*Ambystoma tigrinum*): are motor patterns conserved during major morphological transformations? *J. Morph.* **197**, 249–268.
- 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. Morph.* **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. Morph.* **153**, 39–80.
- Lombard, R. E. and Wake, D. B.** (1986). Tongue evolution in the lungless salamanders, Family Plethodontidae. IV. Phylogeny of plethodontid salamanders and the evolution of feeding dynamics. *Syst. Zool.* **35**, 532–551.
- Lorenz, K.** (1937a). Über den Begriff der Instinkthandlung. *Folia Biotheor.* **2**, 17–50.
- Lorenz, K.** (1937b). Über die Bildung des Instinktbegriffes. *Naturwissenschaften* **25**, 289–300.
- Lorenz, K.** (1939). Vergleichende Verhaltensforschung. *Zool. Anz.* **12** (Suppl.), 69–102.
- 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.
- Nishikawa, K. C., Anderson, C. W., Deban, S. M. and O'Reilly, J. C.** (1992). The evolution of neural circuits controlling feeding behavior in frogs. *Brain Behav. Evol.* **40**, 125–140.
- Nishikawa, K. C. and Cannatella, D. C.** (1991). Kinematics of prey capture in the tailed frog *Ascaphus truei* (Anura: Ascaphidae). *Zool. J. Linn. Soc.* **103**, 289–307.
- Nishikawa, K. C. and Gans, C.** (1996). Mechanisms of tongue protraction and narial closure in the marine toad *Bufo marinus*. *J. Exp. Biol.* **199**, 2511–2529.
- Pearson, K. G.** (1993). Common principles of motor control in vertebrates and invertebrates. *Annu. Rev. Neurosci.* **16**, 265–297.
- Regal, P. J.** (1966). Feeding specializations and the classification of terrestrial salamanders. *Evolution* **20**, 392–407.
- 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.** (1990). The strike of the tiger salamander: quantitative electromyography and muscle function during prey capture. *J. Comp. Physiol. A* **167**, 827–839.
- 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.
- Ritter, D. and Nishikawa, K.** (1995). The kinematics and mechanism of prey capture in the African pig-nosed frog (*Hemisus marmoratus*): description of a radically divergent anuran tongue. *J. Exp. Biol.* **198**, 2025–2040.
- Roth, G.** (1976). Experimental analysis of the prey catching behavior of *Hydromantes italicus* Dunn (Amphibia, Plethodontidae). *J. Comp. Physiol.* **109**, 47–58.
- Roth, G.** (1987). *Visual Behavior in Salamanders*. Berlin: Springer.
- Roth, G. and Dicke, U.** (1994). Is fixed action pattern a useful concept? In *Flexibility and Constraint in Behavioral Systems* (ed. R. J. Greenspan and C. P. Kyriacou), pp. 3–14. New York, Brisbane: John Wiley & Sons.
- Roth, G., Nishikawa, K. C., Wake, D. B., Dicke, U. and Matsushima, T.** (1990). Mechanics and neuromorphology of feeding in amphibians. *Neth. J. Zool.* **40**, 115–135.
- Schwenk, K. and Wake, D. B.** (1993). Prey processing in *Leurognathus marmoratus* and the evolution of form and function in desmognathine salamanders (Plethodontidae). *Biol. J. Linn. Soc.* **49**, 141–162.
- Searle, S. R.** (1971). *Linear Models*. New York: John Wiley & Sons.
- Shaffer, H. B. and Lauder, G. V.** (1985). Patterns of variation in aquatic ambystomatid salamanders: kinematics of the feeding mechanism. *Evolution* **39**, 83–92.
- Sokal, R. R. and Rohlf, F. J.** (1995). *Biometry* (third edition). New York: W. H. Freeman & 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.
- Wainwright, P. C. and Richard, B. A.** (1995). Scaling the feeding mechanism of the largemouth bass (*Micropterus salmoides*): motor pattern. *J. Exp. Biol.* **198**, 1161–1171.
- Zippelius, H.-M.** (1992). *Die Vermessene Theorie*. Braunschweig: Vieweg.