Experimental Morphology of the Feeding Mechanism in Salamanders

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ABSTRACT   The subarcualis rectus I muscle (SAR) in the feeding mechanism of four tiger salamanders (*Ambystoma tigrinum*) was removed early in ontogeny and these individuals were allowed to complete metamorphosis. This procedure resulted in postmetamorphic tiger salamanders which differed from control individuals in the size (and thus force generating capacity) of the SAR muscle. The experimental manipulation of muscle ontogeny allowed a test of previous hypotheses of SAR function in postmetamorphic individuals. Multivariate analysis of variance for kinematic variables measured from high-speed video records of feeding revealed that experimentally modified tiger salamanders did not protract the hyobranchial apparatus or project the tongue from the mouth during feeding. Removal of the SAR muscle resulted in significantly reduced hyobranchial elevation in the buccal cavity and reduced maximum tongue projection distance.

Studies in functional anatomy often proceed by describing correlations between the morphology and function of phenotypic features. For example, inferences of muscle function may be made by correlating movements of bones with the timing and amplitude of muscle electrical activity (e.g., Basmajian and De Luca, '85; Gans, '80; Loeb and Gans, '86). Although several authors have indicated that such correlative analyses may be misleading and may not provide evidence of true causal relationships (Gans, '80, Lauder and Reilly, '88; Lauder and Shaffer, '85; Loeb and Gans, '86; Nishikawa and Gans, '90), in practice most studies have focused on correlated measurements of bone movement and muscle activity patterns to understand musculoskeletal function.

Conducting experimental modifications of animal design also has been frequently used in the analysis of functional anatomy (e.g., Gans and Gorniak, '82; Jayne and Bennett, '89; Jenkins, '74; Lauder, '79, '86; Lauder and Reilly, '88; Liem, '70; Nishikawa and Roth, 1991; Webb, '71, '73) and has contributed important insights into animal function. By directly modifying muscle insertions, cutting nerves, removing bones, or adding mass to an animal, functional hypotheses may be tested in ways that are impossible by observation and description alone.

In this paper we employ an ontogenetic manipulation of musculoskeletal design to investigate the functional morphology of the feeding system in salamanders. By removing a key muscle of the feeding mechanism (the subarcualis rectus I) early in ontogeny, a direct experimental test of previous hypotheses of feeding function can be made.

BACKGROUND

The subarcualis rectus I (SAR) muscle in post-metamorphic tiger salamanders (*Ambystoma tigrinum*) extends from the distal end of the first ceratobranchial bone of the hyobranchial apparatus anteriorly to the ceratohyal element of the hyoid arch (Fig. 1B). In larvae, the SAR muscle is much smaller (Reilly and Lauder, '90a) and extends from the proximal end of the first ceratobranchial to the junction between the ceratohyal and hypohyal (Fig. 1A). During metamorphosis, the branchiohyoideus muscle degenerates and the SAR muscle changes its mass and orientation so that after metamorphosis the SAR assumes the position held by the larval branchiohyoideus (Lauder and Reilly, '90; Reilly and Lauder, '90a). In normal metamorphosis the branchiohyoideus muscle is the last larval feature to fully transform and it often takes several weeks to completely disappear (Reilly and Lauder, '90a). Panels A to B in Figure 1 illustrate the normal metamorphic
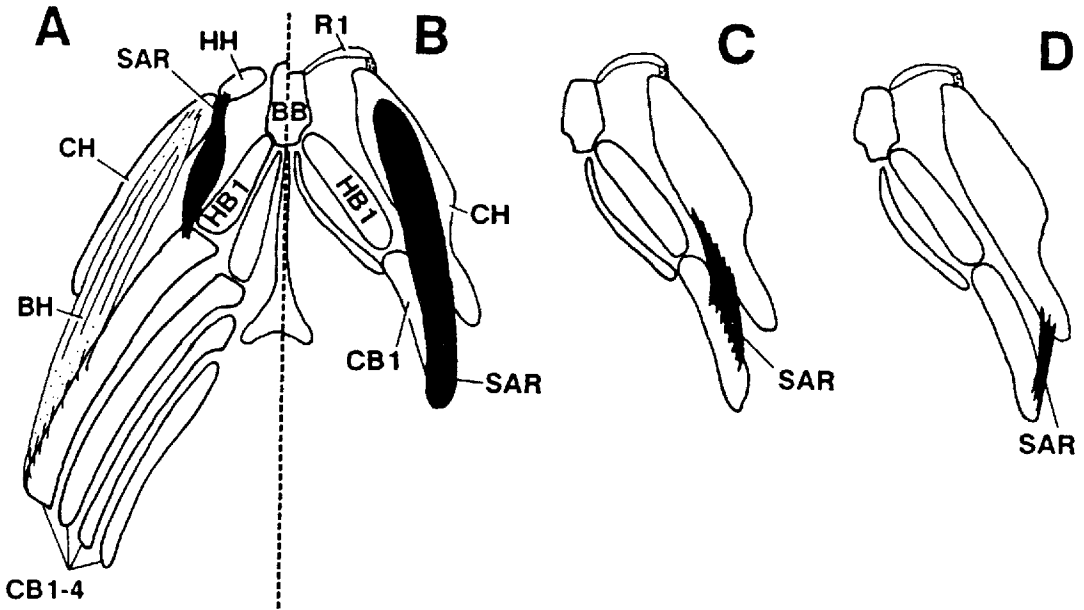
Fig. 1. Metamorphic remodeling of the hyobranchial apparatus and subarcualis rectus 1 muscle in *A. tigrinum.* **A:** The larval hyoid arch is a long rod composed of the ceratohyal (CH) and hypohyal (HH) elements, which attach to the medial basibranchial (BB). Two anterior branchial arches attaching to the basibranchial are composed of ceratobranchial (CB) and hypobranchial (HB) elements and the two posterior branchial arches have only free floating ceratobranchials. In larvae, two muscles extend between the hyoid and first branchial arches. The small subarcualis rectus 1 (SAR) and the larger branchiohyoideus (BH). **B:** During normal metamorphosis (A to B) the hypohyal becomes the rodlike Radial 1 (R1) connecting the basibranchial to the ceratohyal, which has become spatulate and shorter. The first branchial arch shortens and second hypobranchial is the only remnant of the last three branchial arches. The BH muscle is reabsorbed and the SAR enlarges and moves down both arches to insert broadly on the CH anteriorly and along the anterolateral aspect and tip of the CB1 posteriorly. **C:** One experimental animal had about 20 fibers of the SAR extending between the opposing edges of the CB and CH. **D:** Two experimental animals had 3–4 SAR fibers extending between the CB and CH near their tips.

changes that occur in the hyobranchial apparatus and SAR muscle.

The function of the SAR in tongue projection by salamanders has been of interest to biologists for well over a century. Rusconi (1854) was the first to describe larval and transformed head musculature in salamanders and to examine changes that occur in the gill apparatus at metamorphosis. The accuracy and extent of his observations are remarkable, considering the methods available to him. He proposed that the SAR functioned to dilate the back of the mouth cavity through the abduction of the first branchial arch (which it apparently does during breathing and swallowing). Drüner ('02, '04) in his detailed works on the cranial morphology of salamanders, described the metamorphosis of the SAR muscle and proposed a mechanism by which the first branchial arch slides past the hyoid arch via action of the SAR

muscles to protract the hyobranchial apparatus during tongue projection. Although Drüner did not actually observe feeding behaviors, he was able to establish that electrical stimulation of the SAR in decerebrate animals could produce this movement. Drüner's model, which has been fit to a mathematical tractrix model to describe the derived, long-distance tongue projection dynamics of the Family Plethodontidae (Lombard and Wake, '76; Severtsov, '71), remains our best understanding of the mechanical basis of hyobranchial protraction in salamanders. More recently, Reilly and Lauder ('89, '90c) used quantitative analyses of electromyographical and kinematic patterns during tongue projection and muscle stimulation experiments to develop a kinetic vector model for the combination of muscular forces necessary to protract the hyobranchial apparatus during tongue projection in *A. tigrinum.* This model

proposes that the gular muscles elevate the hyobranchial apparatus above the lower jaw as the SAR muscles on each side contract to protract the hyobranchial apparatus antero-dorsally into the oral cavity.

In this paper we use an experimental manipulation to examine the functional role of the SAR during tongue projection in the tiger salamander, *A. tigrinum.* We removed the SAR muscles on each side of the head before metamorphosis, allowed metamorphosis to proceed, and thus generated fully transformed individuals effectively lacking SAR muscles. Comparisons of the kinematics of tongue projection in these animals to normal (control) tiger salamanders demonstrate the role of the SAR in hyobranchial protraction.

MATERIALS AND METHODS
Animals

Nine large *A. tigrinum* larvae obtained from a commercial supplier (C.D. Sullivan Co., Nashville, TN) were divided into control (n = 5) and experimental (n = 4) groups of similar body size (morphological measurements for each specimen are given in Table 1). Each of the experimental larvae were anesthetized by immersion in a solution of tricaine methanesulfonate (MS-222: 1 g/l) for about 30 minutes and the subarcualis rectus 1 muscles (SAR) were removed. In larval salamanders, the small SAR muscle is externally visible and can be easily removed (in less than a minute) by directly cutting its origin and insertion through the thin skin on the ventral surface of the hyobranchial apparatus. A 2 mm slit was made in the thin skin covering the SAR and the muscle was removed by cutting its tendinous posterior attachment to ceratobranchial 1 and peeling its

anterior tendon from the ceratohyal/hypohyal junction on the hyoid arch (Fig. 1A). Care was taken to cut the posterior tendon as close to the ceratobranchial as possible and to remove all muscle fibers that were visible. The control animals were anesthetized, measured, and allowed to recover. Further modifications were not done on control animals because of the possibility of injuring the SAR muscle. This muscle is located directly beneath a thin sheet of connective tissue, and sham operations would not be possible without disrupting the integrity of the muscle. It is important to note that because the SAR muscle in larvae is directly visible through the thin covering connective tissue sheet, little surgical trauma was experienced by the experimental animals beyond that associated with direct removal of the SAR muscle (trauma that cannot be mimicked in control animals). Upon recovery from anesthesia the *A. tigrinum* larvae were then induced to metamorphose by moving them from 10°C water-filled aquaria on a 12:12 photoperiod to plastic shoe boxes with paper towels at 20°C with constant light. These containers were tilted and filled with just enough water to cover the larvae at one end. After two to six weeks gill resorption began indicating the onset of metamorphosis. After 10 weeks all nine specimens had completed external metamorphosis (gill slits, gular fold, toe webbing and tail fin gone, terrestrial color pattern present) and began feeding on worm pieces. The animals were fed every other day for two weeks or less between the end of metamorphosis and filming; thus, they had little opportunity for learned compensation mechanisms or neural rearrangements to occur in terrestrial feeding. Experimental animals were ineffective in

TABLE 1. *Morphometric characteristics (in mm or g) of nine* A. tigrinum *used in this study*[1]

Variable	Experimentals				Controls				
	1	2	3	4[2]	1	2	3	4	5
Snout-vent length	114.0	119.0	114.0	127.0	113.0	113.0	131.0	106.0	126.0
Head width	27.5	25.5	26.5	28.5	27.5	24.5	25.0	25.5	26.5
Snout-axilla length	41.0	47.0	42.5	47.0	45.5	43.5	48.0	43.5	46.0
Ceratobranchial 1 length	7.0	6.0	8.0	10.0	10.0	8.0	7.0	8.0	5.5
Hypobranchial 1 length	9.0	9.5	10.0	10.0	10.5	8.0	11.0	9.5	9.5
SAR length	4.0	7.0	4.5	20.0	23.5	18.0	17.5	22.5	19.0
Body mass	64.6	64.1	67.5	87.6	69.4	78.3	68.1	57.3	68.6
Geniohyoideus mass	0.045	0.040	0.039	0.048	0.044	0.046	0.047	0.036	0.043
Subarcualis rectus I mass	0.002	0.007	0.004	0.044	0.082	0.064	0.036	0.050	0.046
Hyobranchial condition (Fig. 1)	D	C	D	B[2]	B	B	B	B	B

[1]Experimental (subarcualis rectus muscles removed before metamorphosis) and control (untreated) animals were measured after metamorphosis on the day that feedings were video taped.
[2]Experimental animal that regenerated SAR muscle

capturing prey by tongue projection and fed
by grasping prey between the jaws; they had
no apparent difficulty in transporting and
swallowing prey.

The SAR muscle was also removed bilater-
ally in two *Ambystoma mexicanum* which
were allowed to recover from anesthesia and
were maintained post-operatively in an aquar-
ium for 12 weeks. *A. mexicanum* do not natu-
rally metamorphose and these animals were
used as a test for possible regeneration of
SAR muscles due simply to the passage of
time.

High-speed video analysis

Twelve weeks after SAR removal terres-
trial prey capture by each individual was
video taped at 200 fields per second using a
NAC HSV-400 high-speed video system with
two synchronized strobes. Tiger salamanders
were filmed in lateral view in an 8 liter glass
aquarium with a background grid of 1 cm
squares. In order to elicit maximal tongue
projection, salamanders were enticed to strike
at prey held just beyond the reach of normal
tongue projection by offering them 3 cm long
pieces of nightcrawler (*Lumbricus*) presented
about 2–3 cm directly in front of the jaws
with a pair of forceps. Only long-distance
misses were analyzed because of the strong
likelihood that successful strikes would not
be reflective of maximum tongue projection
distance: In successful feedings the tongue
contacts the prey prior to maximum possible
extension (differences between control and
experimental groups could easily be obscured
by the shorter prey distance during success-
ful feedings). From many strikes filmed from
each individual, ten video sequences (the best
lateral views of misses in good focus) for each
individual were analyzed field by field using a
computer image analysis system. For each
strike sequence, field zero (or time 0) was
defined as the field preceding the first field in
which the mouth began to open before the
strike.

To illustrate graphically the general kine-
matic effects of the SAR removal treatment
throughout a feeding, seven kinematic vari-
ables were digitized from one control and one
experimental individual: Head angle was mea-
sured as the angle between the anteroventral
tip of the upper jaw and two landmark points
on the body. This variable indicates the eleva-
tion of the tip of the upper jaw relative to the
body. Mouth opening was quantified by gape
distance, which is the distance between the
anteroventral tip of the upper jaw and the
anterodorsal tip of the lower jaw. These two
points were distinct on the video fields and
the line between them defines the plane of
the gape. Lower jaw angle or depression of
the lower jaw relative to the body was mea-
sured as the angle between a line defining the
dorsal margin of the lower jaw and two land-
mark points on the body. Hyoid depression
was measured at the level of the angle of the
jaws as a vertical distance from the buccal
area to a landmark near the angle of the jaws.

Tongue movements were quantified by
three variables. Tongue projection distance
was measured from the anterior edge of the
tongue to the plane of the gape. It is negative
until the tip of the tongue breaks the plane of
the gape. Two tongue height measures were
digitized as perpendicular distances above a
line along the dorsal margin of the lower jaw:
Tongue bulge height was measured to the
highest point of the tongue bulge above the
lower jaw, and hyoid elevation to the highest
point just behind the tongue pad. Tongue
bulge height reflects the elevation of the
tongue above the lower jaw owing to both
hyobranchial protraction and forward flip-
ping of the tongue pad. Hyoid elevation indi-
cates the elevation of the basibranchial ele-
ment of the hyobranchial apparatus above
the lower jaw. During normal tongue projec-
tion the basibranchial is seen as a small pro-
trusion behind the tongue mass (see Fig. 2),
but in the experimental animals the basibran-
chial was often not visible because of insuffi-
cient tongue projection. In these cases the
hyoid elevation variable was measured as the
height of the tongue at the angle of the jaws
and thus is an overestimate of the actual
elevation of the hyobranchial apparatus dur-
ing tongue projection.

To compare statistically tongue projection
kinematics in the nine tiger salamanders, a
series of kinematic variables were measured
from video sequences that quantify the posi-
tion of the tongue, hyoid, and gape during
feeding. Ten strike sequences from each ani-
mal were analyzed (for a total of 90 feedings).
Several variables were measured from the
video field in which maximum tongue projec-
tion occurred. These included peak tongue
projection distance, peak hyoid elevation,
peak tongue bulge height, and peak gape
distance, as described above, and peak hyoid
protraction measured from the plane of the
gape to the basibranchial dimple located just
behind the tongue mass. These variables were
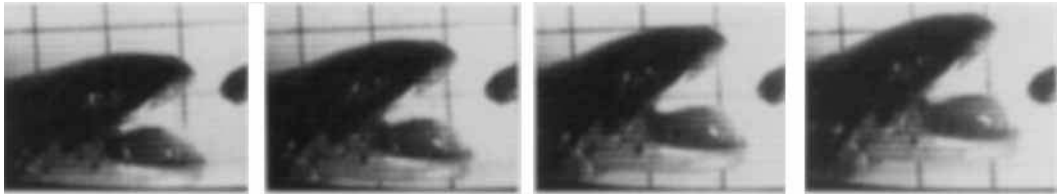chosen to quantify the elevation and forward

Control (SAR +)
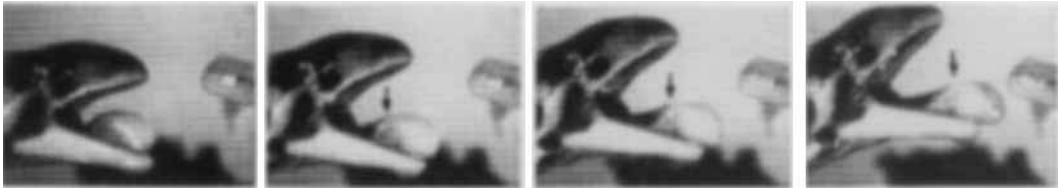
20ms   **25ms**   **30ms**   **35ms**

Experimental (SAR -)

Fig. 2. Video fields depicting average tongue projection in *A. tigrinum* in a normally metamorphosed individual (**top panel:** peak tongue projection = 2.7 mm) and in an individual with the subarcualis rectus I muscle experimentally removed (**lower panel:** peak tongue projection = −1.1 mm) while striking at a worm. Time (in milliseconds) is indicated from the onset of mouth opening. Arrows point to the basibranchial element of the hyobranchial apparatus, indicating the maximum extent of hyobranchial protraction during tongue projection. The distance of this point above the lower jaw and from plane of the gape indicates peak hyoid elevation and protraction distance, respectively. Tongue bulge height is the distance from the lower jaw to the highest point of the tongue bulge. Note that the SAR− individual (lower panel) has a normal tongue bulge by 35 ms but does not protract the hyobranchial apparatus toward the plane of the gape.

protraction of the hyobranchial apparatus, as well as the overall projection of the tongue resulting from combined hyobranchial protraction and flipping of the tongue mass. As mentioned above, because the basibranchial dimple was often not visible in the experimental animals, peak hyoid protraction as measured (often measured at the angle of the jaw) is an overestimate of the amount of hyobranchial protraction. Two timing variables were measured. Time to peak gape was measured as the period from time 0 to the beginning of the plateau in gape distance characteristic of terrestrial feeding in tiger salamanders (Reilly and Lauder, '89). Gape cycle time was the period from time 0 to the mouth closing after the strike. In addition, the maximum single tongue projection distance from each individual was noted.

Morphology

After video taping was completed the nine tiger salamanders and the two *A. mexicanum* were sacrificed by gradual overdose of anes-
thetic (MS-222) and preserved in 10% formalin. Each specimen was weighed and the following morphological measurements were taken. Snout-vent length was measured from the tip of the snout to the anterior end of the vent. Head width was measured just behind the mandibles. Snout-axilla length was measured from the tip of the snout to the rear of the arm where it meets the body. From the hyobranchial apparatus the length of the first ceratobranchial and hypobranchial were taken and the length of the SAR muscle was measured from the extremes of its insertion on the hyobranchial elements. The geniohyoideus muscle, which does not change in mass at metamorphosis (Lauder and Reilly, '90), and the SAR were then carefully removed from the right side of each specimen and weighed. The anterior insertion of the SAR muscle was scraped from the ceratohyal, then the muscle was carefully pulled posteriorly to peel it from the ceratobranchial. Each muscle was weighed three times (to the nearest mg) and an average of the three masses was taken.

The *A. tigrinum* specimens were examined for internal metamorphic condition and the *A. mexicanum* specimens were dissected to inspect hyobranchial morphology 12 weeks after removal of the SAR muscles.

Statistical analyses

Morphological variables and maximum tongue projection (n = 1 for each of 9 individuals) were compared using t-tests. Kinematic variables were compared using nested analysis of variance (ANOVA) with individuals (n = 10 feedings per individual) nested within groups (controls or experimentals) based on SAR condition. To test specifically for differences in hyobranchial protraction and tongue projection kinematics (the a priori hypothesis), a multivariate analysis of variance (MANOVA) was conducted on the three variables quantifying hyoid and tongue movements (tongue projection distance, peak hyoid protraction, and peak hyoid elevation). In analyses of variance the F-value for the treatment effect was calculated using the interaction mean-square as the error term (Sokal and Rohlf, '81). Statistical significance was based on significance levels determined using sequential Bonferroni tests (Holm, '79; Rice, '89). To relate variables to the mass of the SAR muscle, means of kinematic variables and individual values of morphological variables and maximum tongue projection were regressed against SAR mass for each of the nine individuals.

RESULTS

Morphology

Figure 1 depicts normal metamorphic remodeling of the hyobranchial apparatus and SAR muscle (panels A and B) and the metamorphic conditions observed in the experimental animals in which the SAR was removed (panels B–D). Dissection revealed that the five control tiger salamanders had undergone normal and complete metamorphosis (Reilly and Lauder, '90a), as in Figure 1A to 1B. The branchiohyoideus muscle was gone and branchial arches 2–4 and their associated muscles had been reabsorbed. The ceratohyal and first branchial arch had undergone normal metamorphic remodeling, with the SAR inserted over a broad area of the anterior third of the ceratohyal and extending the full length of ceratobranchial 1 to insert around its distal end. Dissection of the experimental specimens revealed that one animal had regenerated the SAR muscle and

was morphologically indistinguishable from the control animals (Table 1, Experimental specimen number 4). Therefore, this individual is considered to be normally metamorphosed.

The remaining three experimental animals were completely metamorphosed, except for a minute remnant (3–4 fibers) of the branchiohyoideus muscle attached to the ceratohyal. It often takes several days for this muscle to completely disappear in recently metamorphosed individuals (Reilly and Lauder, '90a) and this is not considered abnormal. These individuals had practically no SAR muscle (mass = 2–7 mg). One had a sheet of about 20 muscle fibers between the medial opposing edges of the ceratohyal and ceratobranchial 1 (Fig. 1C) and two individuals had three to four SAR fibers between the opposing edges of these elements near their distal tips (Fig. 1D). There was no evidence of abnormal metamorphic development or effects of the SAR removal: Except for the reduced condition of the SAR, these individuals were anatomically indistinguishable from the control animals. Dissection of the two *A. mexicanum* revealed no regeneration of the SAR muscle 12 weeks after surgical removal.

On the basis of morphological condition (Fig. 1) and significant differences in length and mass of the SAR muscle (Tables 1, 2), the nine animals are divided into two groups for purposes of comparison of SAR function. The five control animals and the experimental animal that regenerated its SAR muscle are called the "SAR+" group and the three experimental animals with significantly smaller SAR muscles are called the "SAR−" group (Table 2).

Table 2 shows that body size, size of the hyobranchial components, and the geniohyoideus muscle mass were not significantly different in the SAR+ and SAR− groups, but the length and mass of the SAR muscle were significantly smaller in the SAR− individuals.

Feeding kinematics

Video fields illustrating tongue projection in SAR+ and SAR− individuals are shown in Figure 2. During normal tongue projection considerable protraction of the hyobranchial apparatus toward the plane of the gape occurs as indicated by the movement of the basibranchial element (Fig. 2, arrows, top panel). In addition, simultaneous flipping of the tongue pad from the protracting hyobran-

TABLE 2. Statistics for morphological measurements for the "SAR–" (experimental individuals 1–3) and normal SAR+ (controls plus experimental individual 4) groups of A. tigrinum[1]

Variable	Means + SE			Regressions on SAR mass		
	SAR–	SAR+	P-value	Slope	R	P
Subarcualis rectus mass	0.004 ± 0.001	0.053 ± 0.007	0.001[2]	—	—	—
Subarcualis rectus length	5.2 ± 0.9	20.1 ± 1.0	0.0001[2]	0.451	0.970	0.0001[2]
Body mass	65.4 ± 1.1	71.6 ± 4.2	0.210	0.070	0.495	0.175
Snout-vent length	115.7 ± 1.7	119.3 ± 4.1	0.435	0.006	0.125	0.748
Head width	26.5 ± 0.58	26.3 ± 0.6	0.779	−0.006	0.163	0.674
Snout-axilla length	44.0 ± 2.3	45.6 ± 0.7	0.568	0.017	0.420	0.260
Geniohyoideus mass	0.041 ± 0.002	0.044 ± 0.002	0.371	0.001	0.208	0.591

[1]Group means ± standard errors are presented on the left. Statistical comparisons of the two groups (t-tests, n = 1 per individual) are presented in the center. Regression statistics (log_{10} transformed data) illustrating the relationship of each variable to subarcualis rectus I muscle mass across all nine individuals are on the right. Units are: mass (g) or distance (mm).
[2]Significant based on sequential Bonferroni test (Holm, '79).

chial apparatus results in overall projection of the tongue well beyond the plane of the gape. In SAR– individuals (Fig. 2, lower panel) the hyobranchial apparatus is not protracted forward and although some tongue movement occurs, overall peak tongue projection (at 35 ms) is well inside the plane of the gape.

Kinematic plots of head, jaw, hyoid, and tongue movements illustrating general movement patterns are presented in Figure 3 for a control and a SAR– individual. Head angle, gape distance, lower jaw angle, and hyoid depression profiles have the same basic patterns and differ primarily in the longer time course for the SAR– profiles.

However, tongue projection distance, hyoid elevation and tongue bulge height are all substantially lower for the SAR– individual. Tongue projection distance is 6 mm in this SAR+ individual vs. −1 mm in the SAR– salamander. Hyoid elevation is over 4 times higher in normal tongue projection than when the SAR muscle is absent.

Statistical comparisons of kinematic variables for the SAR– and SAR+ groups are presented in Table 3. Variables describing aspects of the gape cycle (peak gape, time to peak gape, and gape cycle time) were not significantly different in the two groups. Maximum tongue projection, peak hyoid elevation, and tongue bulge height were significantly lower in the SAR– group. In addition, multivariate analysis of the kinematics of hyoid and tongue movements (peak tongue projection, peak hyoid protraction, and peak hyoid elevation) revealed significantly reduced hyoid protraction and tongue projection in the SAR– group (MANOVA: 3, 5 degrees of freedom, Wilks' Lambda = 0.196, P = 0.032).

Effects of body size and SAR mass

Regression statistics indicating the relationships between morphological and kinematic variables and SAR mass are presented in Tables 2 and 3. SAR mass was not significantly correlated with body mass (P = 0.175). Variables describing body size, body mass, mass of the geniohyoideus muscle, mean peak gape distance, and mean time to peak gape were not significantly correlated with the mass of the SAR. All five measures of tongue projection kinematics had significant positive correlations with SAR mass (Table 3, Fig. 4). The mean and maximum peak tongue projection distance variables had nearly identical slopes and correlations with SAR mass.

DISCUSSION

By taking advantage of key morphological changes that occur at metamorphosis in tiger salamanders, we were able to experimentally remove a muscle hypothesized to be the primary protractor of the hyobranchial apparatus during tongue projection (Rusconi, 1854; Drüner, '02; Lombard and Wake, '76; Reilly and Lauder, '89) and quantitatively measure how this muscle affects tongue projection performance after metamorphosis. Manipulation of the ontogeny of the SAR muscle allows us, first, to test a vector model of feeding function (which predicts changed tongue kinematics with SAR ablation), and secondly, to examine correlations between SAR mass and feeding function that would have otherwise been difficult due to the small range of variation in SAR mass among normal adult salamanders of similar body mass (Reilly and Lauder, '90a).

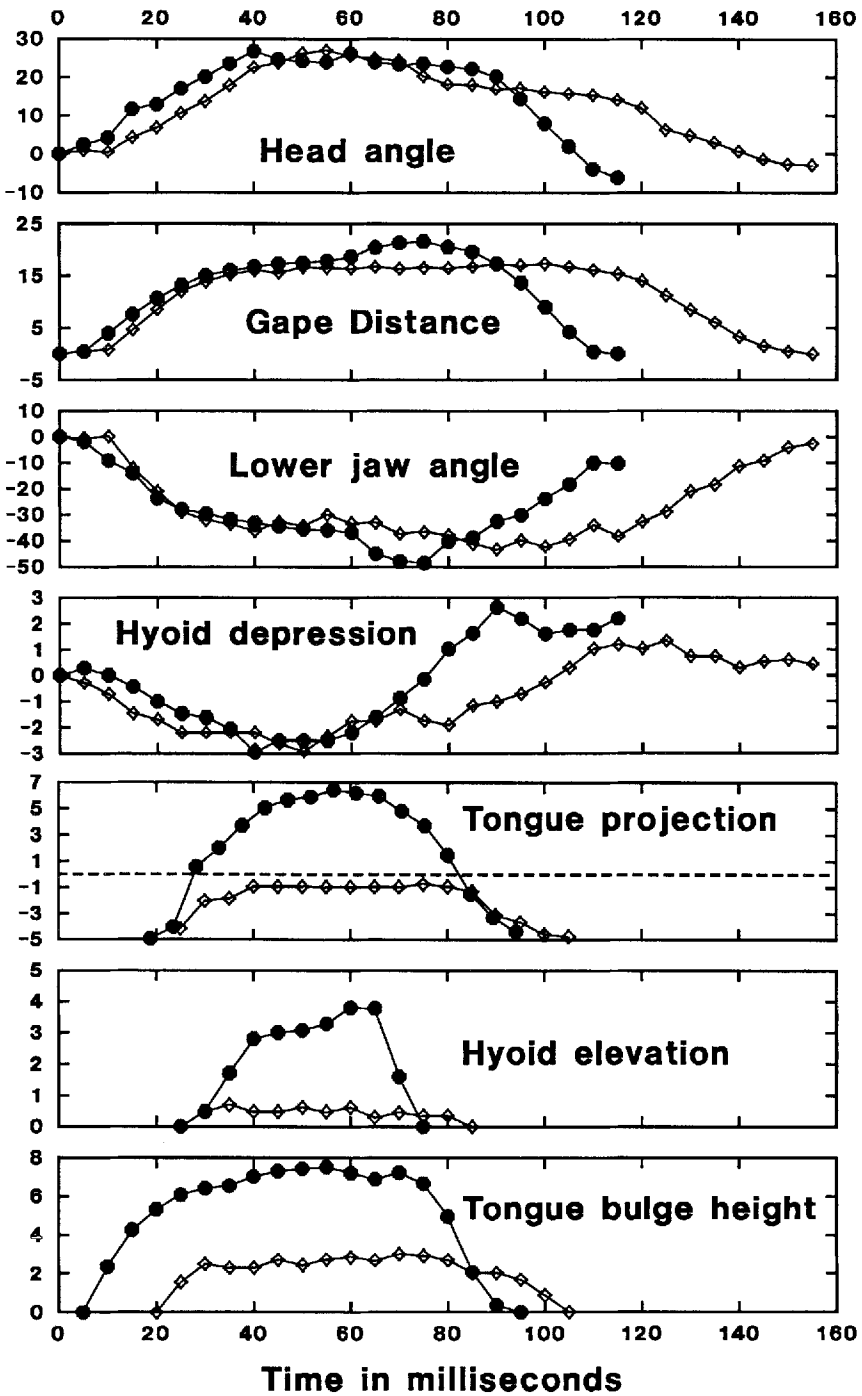In three of four experimental animals, premetamorphic removal of the SAR muscle

Fig. 3. Profiles for seven variables illustrating general tongue projection kinematics for a strike in normal (solid circles) and SAR− (open diamonds) tiger salamanders attempting to feed on worms. Y-axis units are in degrees for angle measurements and millimeters for distance measurements. Note that head and hyoid movements exhibit the same patterns and differ primarily in relative duration associated with a longer gape cycle in the SAR− individual. The SAR− individual did not project the tongue beyond the plane of the gape. Statistical comparisons of tongue projection kinematics are presented in Table 3 and the text.

TABLE 3. Statistics for kinematic variables during tongue projection in A. tigrinum[1]

| | Means + SE | | | Regressions of means on SAR mass | | |
Variable	SAR−	SAR+	P-value	Slope	R	P
Maximum tongue projec-						
tion	−0.9 ± 0.3	3.6 ± 0.8	0.002[2]	0.400	0.850	0.044[2]
Peak tongue projection	−1.6 ± 0.4	2.6 ± 0.9	0.012	0.495	0.812	0.008[2]
Peak hyoid protraction	−9.1 ± 0.8	−4.0 ± 1.3	0.037	1.225	0.817	0.007[2]
Peak hyoid elevation	0.5 ± 0.1	3.2 ± 0.4	0.001[2]	0.669	0.917	0.0001[2]
Tongue bulge height	3.3 ± 0.4	5.7 ± 0.4	0.007[2]	0.200	0.820	0.007[2]
Gape cycle time	129.8 ± 25.4	126.2 ± 6.5	0.854	−0.035	0.222	0.565
Peak gape distance	15.1 ± 1.0	15.2 ± 0.8	0.905	0.001	0.013	0.974
Time to peak gape	36.3 ± 2.9	35.2 ± 1.2	0.658	−0.016	0.240	0.535

[1]Means ± standard errors for the "SAR−" (experimental individuals 1–3) and normal SAR+ (controls plus experimental individual 4) groups are presented on the left. Statistical comparisons of the two groups are presented in the center. Maximum tongue projection (n = 1 per individual) was compared using a t-test. The other kinematic variables (n = 10 feedings from each of nine individuals) were compared using nested ANOVA (see text). Regression statistics ($\log_{10}$ transformed data) illustrating the relationship of each variable mean to subarcualis rectus one muscle mass across all nine individuals are on the right. Units are: distance (mm) or time (ms).
[2]Significant based on sequential Bonferroni test (Holm, '79).

resulted in transformed animals with tiny, essentially non-functional SAR muscles (see below). Complete regeneration of the SAR muscles in one experimental animal probably resulted from incomplete removal of the SAR muscle during surgery that left a few complete larval SAR fibers that were induced to fully regenerate during metamorphosis. The fact that the two A. *mexicanum* had no indication of regeneration of the SAR muscles 12 weeks after they had been surgically removed indicates that time alone does not account for the regeneration.

The SAR− animals, which were otherwise completely metamorphosed and similar in body size, head size, and geniohyoideus muscle mass, had significantly smaller SAR muscles consisting of a few fibers averaging one fourth the length and 7% the mass of these muscles in the SAR+ animals (Tables 1, 2). The distal position of the SAR fibers on ceratobranchial I (Fig. 1) and the fact that we cut (rather th·.n removed) this end of the muscle during removal suggest that these fibers developed from the posterior insertion of the larval SAR that moves from the proximal to the distal end of the first ceratobranchial during metamorphosis. The small number of fibers (mean = 9), short length (mean = 5.16 mm), small mass (mean = 0.004 mg), and limited span between the hyoid and first branchial arches render these muscles biomechanically ineffective in protracting the hyobranchial apparatus.

The experimental manipulation of SAR mass and length (and thus force generating capacity) provides a direct test of the vector model of hyobranchial protraction proposed by Reilly and Lauder ('89, '90c). The model of Reilly and Lauder ('89, '90c) proposes that anterodorsal protraction of the hyobranchial apparatus into the oral cavity during tongue projection results from the summation of two force vectors acting on the medial basibranchial element of the hyobranchial apparatus: The basibranchial is, first, elevated somewhat by the combined vertical forces of the buccal muscles (interhyoideus and intermandibularis muscles) and, secondly, propelled anterodorsally as contraction of the SAR forces the first branchial arch to slide anteriorly over the hyoid arch.

Comparison of the SAR− and SAR+ groups (ANOVA and MANOVA) reveal a significant performance decrement in kinematic variables describing tongue projection. Removal of the SAR decreased maximum tongue projection distance by 4.5 mm (Table 3), so that without the SAR the tip of the tongue never reached the plane of the gape. When considered together (MANOVA), maximum hyoid protraction and elevation, which provides the underlying foundation from which the tongue is flipped, and peak tongue projection distance were significantly lower in the SAR− animals (decreased by 5.0 mm, 2.8 mm, and 4.2 mm, respectively).

Comparisons of the scaling relationships between kinematic performance and SAR mass across all nine individuals illustrate the importance of the SAR in hyobranchial protraction. Each kinematic variable describing hyoid and tongue movements has a significant linear relationship with SAR mass (Table 3, Fig. 4). Both hyoid elevation and protraction were significantly correlated with the mass of the SAR, even though elevational effects of the buccal muscles are equal. Thus, it can be concluded that the SAR functions to produce anterodorsal protraction of the hyo-
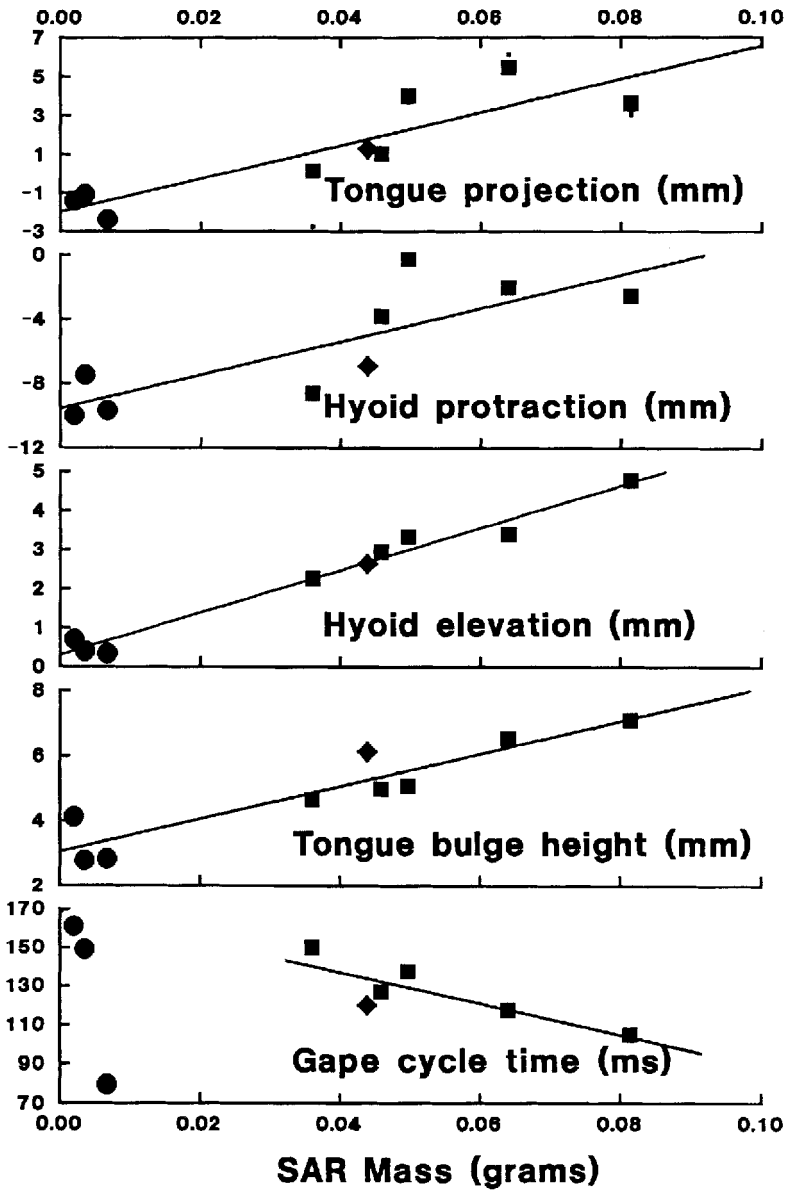
Fig. 4. The relationship between kinematic variables and SAR mass in all nine individuals of *A. tigrinum*. Untransformed mean values of 10 feedings for each of the nine salamanders are plotted versus SAR mass for SAR− (circles) and SAR+ (squares) individuals. The diamond represents the experimental individual that regenerated the SAR muscle. The four tongue projection measures have significant correlations with SAR mass (Table 3). The regression line for gape cycle time is plotted for the six SAR+ individuals only. SAR mass was not significantly correlated with body mass ($P = 0.175$), and body mass effects do not explain the patterns shown here (also see Table 1 for body mass values for each individual).

branchial apparatus during tongue projection and that the mass of the SAR is directly related to the distance the hyoid is protracted. The three animals with a very small SAR did not project the tongue beyond the plane of the gape.

In addition, flipping of the tongue pad from the hyobranchial apparatus is dependent on

amount of hyobranchial protraction. The decrease in hyoid elevation and protraction contributed to a significant decrease in the height of the tongue bulge by 2.5 mm. Although the genioglossus muscles of the tongue pad contract during normal tongue projection (Reilly and Lauder, '90c; see changes in tongue shape during feeding; Fig. 2, top), normal tongue flipping movements did not occur in the SAR− animals because of insufficient protraction of the hyobranchial apparatus (Fig. 2, bottom). The SAR− individuals did not flip the tongue as it bulged dorsally from the hyobranchial apparatus. When the genioglossus muscle lies stretched posteriorly over the incompletely protracted hyobranchial apparatus its contraction (shortening) can only bulge the tongue dorsally. This supports the hypothesis of Reilly and Lauder ('89) that contraction of the genioglossus and hyoglossus muscles act to flip the tongue pad forward only when the base of the tongue is rapidly protracted by movement of the hyobranchial apparatus.

It is interesting that SAR muscle mass is correlated with the duration of the gape cycle (Fig. 4). The six SAR+ salamanders had a significant negative correlation of gape cycle time with SAR muscle mass (Fig. 4, slope = −0.38, R = 0.86, P = 0.03). The inverse relationship between SAR mass and gape cycle time may be merely a consequence of the biomechanical necessity of keeping the gape open while the tongue is moving in and out of the mouth. Based on data from Reilly and Lauder ('90c), gape cycle time is significantly correlated with the time to prey contact by the tongue (R = 0.49, P = 0.04, n = 33). Thus, in normal animals the longer the time taken to project the tongue (due presumably to reduced SAR force generation), the longer the gape must remain open.

One SAR− individual did exhibit an extremely short gape cycle time. Examination of the video records of feeding indicated that this individual lacked a plateau in the gape cycle normally evident in metamorphosed individuals (Fig. 3, gape distance), and may have retained the larval bell-shaped gape profile (Reilly and Lauder, '89; Shaffer and Lauder, '88). We hypothesize that this individual had not yet completed metamorphosis behaviorally and retained a larval gape pattern despite having metamorphosed morphologically.

The results of this manipulative study of SAR function are also of interest in light of previous correlative analyses of the motor pattern of the SAR muscle and feeding kinematics in salamanders. Reilly and Lauder ('90c) showed that the SAR muscle in *Ambystoma tigrinum* exhibits a double burst of electrical activity during the strike. The first burst of activity correlated well with tongue projection during mouth opening, but a second burst occurred during mouth closing and tongue retraction. The presence of this second burst during the retraction phase of the strike, and the presence of a single burst of activity in the SAR during the tongue retraction phase of prey transport (which involves no tongue projection; Reilly and Lauder, '90b, in press) suggest that the SAR does not function only as a major hyobranchial protractor, but also possesses as yet undetermined functions in addition to that demonstrated here.

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