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Metamorphosis of the feeding mechanism in tiger salamanders (*Ambystoma tigrinum*): the ontogeny of cranial muscle mass

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(With 6 figures in the text)

Most previous research on metamorphosis of the musculoskeletal system in vertebrates has focused on the transformation of the skeleton. In this paper we focus on the transformation of the muscles of the head during metamorphosis in tiger salamanders (*Ambystoma tigrinum*) in order (1) to provide new data on changes in myology during ontogeny, and (2) to aid in interpreting previous data on the metamorphosis of function in the head of salamanders.

The physiological cross-sectional area of nine head muscles was calculated by measuring fibre angles, fibre lengths, and muscle mass in two samples of tiger salamanders obtained just before and just after metamorphosis. The major mouth-opening muscles (rectus cervicis and depressor mandibulae) exhibit a significant decrease in estimated maximum tetanic tension (MTT) across metamorphosis of about 36%. The jaw-closing muscles (adductor mandibulae internus and externus) and the head-lifting muscles (epaxials) also decrease in MTT but not significantly. The muscles associated with tongue projection during feeding on land (the subarcualis rectus I, geniohyoideus, interhyoideus and intermandibularis) all show a slight increase in MTT at metamorphosis.

Metamorphic transformation of feeding behaviour in *Ambystoma tigrinum* involves changes in performance, the design of skeletal elements, changes in muscle force-generating capability, and changes in hydrodynamic design from unidirectional flow in larvae to bidirectional flow during aquatic feeding after metamorphosis. Although muscle activity patterns during aquatic feeding do not change across metamorphosis, tongue-based terrestrial feeding involves a suite of novel muscle activity patterns, morphological characters acquired at metamorphosis, and a metamorphic increase in the masses of muscles important in tongue projection.

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Introduction

The process of metamorphosis is of special interest to biologists because of the dramatic nature of changes in organismal form and function that may take place over a relatively short time (e.g. Etkin & Gilbert, 1968; Dodd & Dodd, 1976; Gilbert & Frieden, 1981; Fox, 1984; Duellman & Trueb, 1986). In contrast to comparative analyses across taxa in which patterns of transformation in structure and function must be inferred (Lauder, 1981), major changes in morphology and physiology may be observed directly in metamorphosing individuals. In addition, the speed with which many metamorphic changes take place (in contrast to a more normal relatively continuous process of ontogenetic change) greatly facilitates experimental manipulation of development during this period to gain insight into causal relationships during ontogeny. A combination of experimental (manipulative) techniques and descriptive approaches facilitates understanding of rapid and substantial morphological changes.

Within vertebrates, one of the best known examples of metamorphosis occurs in amphibians (Noble, 1931; Duellman & Trueb, 1986) in which an aquatic larval stage may transform into a terrestrial adult form. This environmental transition necessitates many alterations in morphology and physiology as changes occur to permit function in a terrestrial environment. One of the systems that undergoes a particularly dramatic change is the skull and feeding mechanism. Previous studies have documented the transformation in tiger salamanders (*Ambystoma tigrinum*) from a larval suction feeding mechanism of prey capture (in which rapid buccal expansion is used to draw water and prey into the mouth; Lauder & Shaffer, 1985) to the post-metamorphic terrestrial feeding system in which prey capture involves tongue projection from the mouth (Bramble & Wake, 1985; Lauder, 1985; Lauder & Shaffer, 1986; Shaffer & Lauder, 1988; Reilly & Lauder, 1989). Many morphological changes in the head take place at metamorphosis to permit this change in feeding function. These include the appearance of the tongue, changes in the hyobranchial apparatus, changes in skull shape, and closing of the gill slits and loss of external gills (Bonebrake & Brandon, 1971; Reilly, 1986, 1987; Reilly & Lauder, 1988). These previous studies of metamorphosis in salamanders have focused on either changes in feeding function or changes in skull bone shape and position. One major unknown area of cranial metamorphosis in salamanders is muscle structure. No study has yet addressed possible changes that may occur in cranial muscle morphology during metamorphosis despite the fact that such changes might have a major effect on feeding function and the ability of salamanders to feed on land.

The goals of this paper are two-fold. First, we present an analysis of cranial muscle metamorphosis in the tiger salamander (*Ambystoma tigrinum*) to document changes in whole muscle structure and estimated force-generating capabilities. Secondly, we review previous analyses of cranial metamorphosis in salamanders and attempt to integrate these new data on muscles with previous data on the metamorphosis of cranial form, feeding performance and feeding function.

Materials and methods

Specimens

To investigate changes in cranial muscle architecture, mass and estimated force-generating capability across metamorphosis, samples of *Ambystoma tigrinum* were chosen from collections made just before and just after metamorphosis. It is important to emphasize that the *a priori* objective was to study the effects of metamorphosis *per se* on cranial muscles, and not larval or adult growth trajectories. Thus, samples of similarly-sized tiger salamanders were chosen on either side of metamorphosis. Specimens collected in Colorado Springs, Colorado, were obtained from the Museum of Natural History, University of Kansas, Lawrence KS (USA). Twelve larvae were used (specimen numbers: KU89119-89122, 89124, 89128, 89135, 89140-89141, 89144-89145, 89149) as were 8 individuals that had just metamorphosed (KU89091, 89096, 89102, 89107-89111). All specimens were collected from the same locality and were of similar size (the issue of size is discussed in detail below).

Measurements

Morphological changes at metamorphosis were characterized by measuring muscle fibre angle, fibre length and whole muscle mass for 9 cranial muscles. From these variables the peak tetanic tension that each muscle could generate was estimated from the physiological cross-sectional area (see below). Details of the morphology of these muscles in larval and transformed *Ambystoma tigrinum* have been described by Lauder & Shaffer (1985, 1988); here we describe only how these muscles were removed for measurement. In order to reduce variation in the data, one investigator (SMR) performed all the dissections; the other (GVL) weighed and measured the muscle fibres.

The specimens on which the muscle measurements were made were fixed with the jaw in a closed position; fibre lengths thus reflect the adducted position of the mandible. The epaxial muscles contribute to mouth opening during feeding by elevating the neurocranium and upper jaw (Lauder & Shaffer, 1985). The epaxial muscles were dissected out of larvae and transformed individuals by first removing the shoulder girdle and gill levators (in larvae) and then making a cut along the fourth myomere posterior to the skull. The muscle was then removed by peeling fibres from the underlying vertebrae and from the otic and parietal regions of the skull.

The adductor mandibulae internus and externus muscles are the major adductors of the lower jaw and function to close the mouth during prey capture (Lauder & Shaffer, 1985). The origins of these muscles were first cut from the skull, squamosal and quadrate and then the insertion was removed from the dentary and pre-articular.

The rectus cervicis muscle in larvae is the key muscle that generates negative buccal pressure by moving the hyobranchial apparatus posteroventrally during mouth opening. In terrestrial feeding, this muscle acts to retract the tongue and hyobranchial apparatus into the mouth (Reilly & Lauder, 1989). The rectus cervicis was cut along the first myomere posterior to the urohyal and was then pulled anteriorly and removed at its insertion on the hyobranchial apparatus. Care was taken to remove any adhering blood vessels or heart tissue.

The subarcualis rectus I muscle of larvae functions in gill arch abduction. In transformed tiger salamanders, this muscle functions, along with others, to project the tongue from the mouth by pulling ceratobranchial I anterodorsally (Reilly & Lauder, 1989). The subarcualis rectus I was cut anteriorly where the muscle fibres meet the tendon to the hyoid arch, and was peeled posteriorly from the ceratobranchial.

The interhyoideus and intermandibularis muscles form the musculature of the buccal floor and act to elevate the hyobranchial apparatus (Lauder & Shaffer, 1985, 1988; Reilly & Lauder, 1989). Both muscles were removed together because of their small size and intimate fibre association and were treated as one functional complex. Removal of these muscles was accomplished by first making an incision along the ventral midline of the buccal floor. Then a cut was made along the mandible to remove lateral and anterior attachments of these muscles.

The geniohyoideus is a longitudinal muscle that acts to protract the hyobranchial apparatus and to elevate the buccal floor (Lauder & Shaffer, 1985, 1988). The geniohyoideus was first removed from the urohyal, then detached from the basibranchial fascia, and finally severed at its attachment to the mandibular symphysis.

The depressor mandibulae muscle contributes to mouth opening by moving the tip of the mandible ventrally around its articulation with the quadrate. This muscle was first removed from its attachment to the

quadrate, then its insertion on the jaw was cut, and finally depressor mandibulae fibres were peeled from their attachments to the squamosal bone and epaxial muscles.

The branchiohyoideus muscle is typically lost at metamorphosis (Smith, 1920; Lauder & Shaffer, 1988; Reilly & Lauder, 1989) and a remnant muscle was present in 3 of the transformed individuals (which presumably had not quite completed full metamorphosis of the hyobranchial region). Statistical analyses (see below) were thus restricted to the 8 muscles common to both larval and transformed animals. The branchiohyoideus was teased from its origin on the surface of ceratobranchial I and then cut from its insertion on the hyoid arch.

After muscles from both sides of each preserved specimen were dissected out they were placed in a covered petri dish containing 70% EtOH. The total bilateral wet mass (g) for each muscle was measured on an analytical balance. Because the muscles were small, masses for each of the muscle pairs from each individual were measured once after gentle blotting and the muscle was then returned to the dish; this procedure was then repeated 3 times. Each muscle mass thus represents the mean value for both sides of the head weighed 3 times.

Muscle architecture was studied by digesting away intramuscular connective tissue with 25% nitric acid following the technique of Williams & Goldspink (1971; also see Gorniak, Rosenberg & Gans, 1982; Lauder, 1983; Powell *et al.*, 1984). After about a week of digestion (the exact length varied with muscle mass) the muscles were washed in distilled water and stored in a mixture of 50% glycerol and 50% distilled water. Dishes containing the muscle fibres were placed under a dissecting microscope (Zeiss IVB) and individual fibres gently separated with forceps. The angle of muscle fibres within the muscle to the centre line of the whole muscle (drawn from the midpoint of the muscle origin to the midpoint of the insertion) was measured with a camera lucida and digitizing tablet. Then, the muscle fibres were completely teased apart, laid out on the bottom of the dish, and the lengths of 25 representative fibres were measured. For both the angle and fibre length measurements, muscle fibres from all regions of the muscle were sampled.

The physiological cross-section of a muscle provides a measure of force-generating capability of the muscle, taking into account muscle architecture and fibre length (Gans & Bock, 1965; Alexander, 1968; Powell *et al.*, 1984). Maximum tetanic tension (MTT, in Newtons (N)) has been shown to be extremely highly correlated with physiological cross-sectional area by Powell *et al.* (1984). These authors compared MTT values estimated from muscle architecture to physiologically measured MTT values in 26 different guinea pig muscles in the hind limb. They found a highly significant correlation of 0·99 between the estimated and measured MTT values, indicating that MTT can be accurately predicted from morphological measurements alone. Maximum tetanic tension may be estimated by multiplying the physiological cross-sectional area (PCSA, in cm^2) by the specific tension (ST, in Ncm^{-2}): MTT = PCSA × ST. Specific tensions reported for vertebrate muscle have varied widely, but a value of 20 Ncm^{-2} represents a reasonable average value (Alexander, 1968; Altringham & Johnston, 1982; Powell *et al.*, 1984; Wainwright, 1987).

The PCSA was calculated by the following equation (Powell *et al.*, 1984): PCSA = ((muscle mass, in g) × (cos α, in degrees))/((fibre length, in cm) × (muscle density, in g/cm^3)). A muscle density of 1·05 g/cm^3 was used as in previous research (Lowndes, 1955; Alexander, 1968; Lauder, 1983). The average angle of individual muscle fibres to the line of whole muscle action is given by α. Our analysis assumes that the specific tension of the salamander jaw muscles does not change across metamorphosis.

Statistical approaches and analyses

Because the analysis of muscle architecture and mass involves dissecting out the muscles and is a destructive procedure, a longitudinal study of single individuals was impossible and we adopted a cross-sectional sampling approach. A useful way of visualizing the nature of metamorphic change in a cross-sectional sampling program is shown in Fig. 1 which depicts a hypothetical plot of the mass of one cranial muscle against time. Three individuals (A, B, and C) grow larger with time and muscle mass increases from time *t*0 to time *t*1. Metamorphosis occurs at time *t*1 (slightly different for each of the 3 individuals) and in this

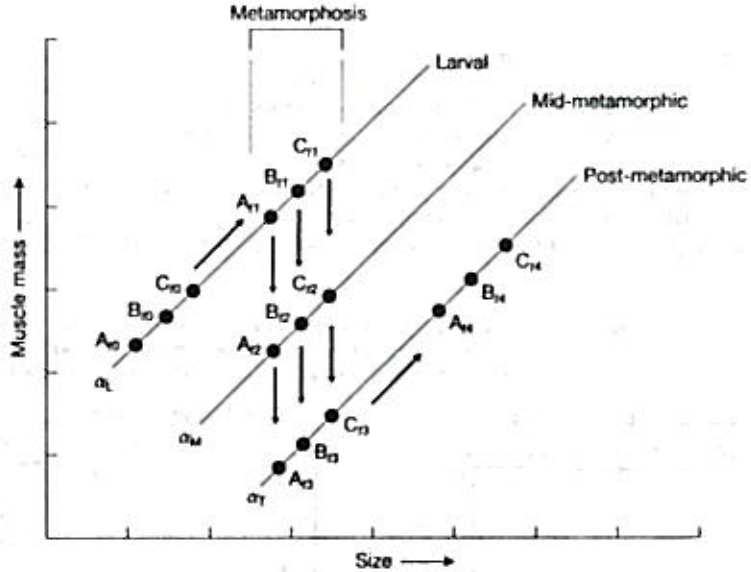

FIG. 1. Schematic diagram to illustrate one possible ontogenetic trajectory of muscle mass in an animal that metamorphoses. Three individuals, A, B and C, are shown and their positions along allometric trajectories are indicated by black dots. The time scale on the *x*-axis is arbitrary. As larvae grow (e.g. from time *t*0 to time *t*1) their muscles enlarge along a trajectory α_L. At metamorphosis, if muscle mass is lost as in this example, a regression line through mid-metamorphic individuals would reveal a change in intercept and (possibly) a change in slope (α_M). After metamorphosis (at *t*3), adult growth occurs with a slope α_T. Note that in a cross-sectional study, the intercepts of the regression lines for the three groups would decrease in the example shown. If samples were chosen just on either side of metamorphosis, and the size range of animals were small, then one would only observe a decrease in mean muscle mass between larval and post-metamorphic individuals.

hypothetical example the mass of the muscle decreases as metamorphosis occurs. After metamorphosis, at time *t*3, growth resumes and the mass of the cranial muscle increases again with time. If many individuals at each stage (larval, mid-metamorphic and post-metamorphic) were available for analysis, it would be observed that 3 regression lines with similar allometric coefficients (Fig. 1: α_L, α_M, α_T) could be drawn through the 3 ontogenetic stages. However, samples taken just before and just after metamorphosis would show little difference in size (Fig. 1, *t*1 to *t*3) although the mean values for muscle mass would differ (projections on to the *y*-axis). It is important to note that it is common in morphometric studies to plot variables against size (either of one variable, such as head or body size, or a general size vector; Humphries *et al.*, 1981; Bookstein *et al.*, 1985; Strauss, 1985) and in this case the independent variable (size) is being used as a surrogate for time. Here, the 2 samples differ in time (the transformed sample occurred about a month later in time than the larval sample) but do not differ in external head width or head length.

In this study our preliminary measurements showed that the 2 samples of tiger salamanders showed little variation in overall head size. However, we were concerned that even though the size range of the salamanders was small, significant relationships still could exist between muscle mass and head size. Because the transformed individuals were slightly older than the larval animals, they could show a change in muscle mass

due only to growth (or shrinkage) during and immediately after metamorphosis. Thus, 3 types of analyses were undertaken. Multivariate analysis was restricted to the estimated muscle tensions, a total of 8 variables, in order that the number of variables should not exceed the number of specimens in each group.

First, descriptive statistics were calculated for each variable and the mean tension compared for each muscle using a 2-sample *t*-test. The mean external head width for larval individuals was 20·2 mm (±1·1 S.E.) while the mean for transformed animals was 20·4 (±1·6 S.E.). The *t*-test analysis assumes that there is no significant relationship between the estimated muscle tensions and head size.

Secondly, analyses of covariance (ANCOVA) were conducted to compare intercepts of parallel regression lines for larval and transformed individuals. The estimated maximum tetanic tension for each muscle was regressed against a measure of head size (width of the head measured at the posterior margin of the mandible). Even though the overall means were similar between the 2 samples, it is still possible that size could confound the results of *t*-test comparisons (Packard & Boardman, 1987). We followed Crespi & Bookstein (1989) and used the ANCOVA results as a general guide to univariate relationships between the muscle tension variables and head width (the independent variable and univariate measure of size). The ANCOVA results should be interpreted with caution, however, because (1) many of the within-group regressions are not significant owing to the small size range of the specimens, and (2) the intercepts produced by the ANCOVA analysis (Table II) are well outside the natural size range. It may be misleading to compare intercepts at an animal size of zero centimetres.

For both the *t*-test and the ANCOVA analyses, because multiple comparisons were being conducted, we adopted a conservative 0·01 level of significance.

Thirdly, because size *per se* is a multivariate attribute of organisms (and multiple univariate regressions against one size variable do not take into account correlations among variables), approaches have been developed by Humphries *et al.* (1981), Bookstein *et al.* (1985) and Strauss (e.g. 1985) to remove size from among-group comparisons via modified principal component analysis. We therefore conducted a principal component analysis on 8 muscle tensions (all but the branchiohyoideus which is lost at metamorphosis) using log (10) -transformed data and the covariance matrix. To examine the effects of size on muscle masses, correlations of principal components 2 and 3 with size were removed by the 'shear' technique of Humphries *et al.* (1981; also see Strauss, 1984, 1985; Strauss & Fuiman, 1985) which removes within-group principal component 1 effects by regression.

Results

Summary statistics for larval and transformed individual muscle masses, fibre angles and estimated maximum tetanic tensions (MTT) are presented in Table I. A sample plot of the variables measured on the rectus cervicis muscle is presented in Fig. 2. Both larval and transformed individuals spanned a range of head widths from 17 mm to 22 mm.

Only the depressor mandibulae muscle showed any change in mean fibre angle during metamorphosis (an increase from 4·2° to 14·1°, $P<0·005$; Table I), and within-muscle variation in fibre angle is high for all muscles (although the mean fibre angles were generally low, indicating that the muscles were essentially parallel-fibred). Three muscles exhibited a change in muscle fibre length. The depressor mandibulae and interhyoideus/intermandibularis both had shorter fibres after metamorphosis, showing a decrease of 20% (6·9 mm to 5·5 mm; $P<0·001$) and 26% (10·0 mm to 7·4 mm; $P<0·001$), respectively. The subarcualis rectus I muscle increased in fibre length by 82% (3·9 mm to 7·1 mm; $P<0·01$) but showed no change in fibre angle.

Both univariate analyses indicate that only the rectus cervicis and depressor mandibulae MTTs change significantly at metamorphosis (Tables I and II). The *t*-tests and the ANCOVAs (when intercepts are compared within the range of actual animal size) indicate that the estimated MTTs in these muscles decrease at metamorphosis. The MTTs in the epaxial muscles and the adductor

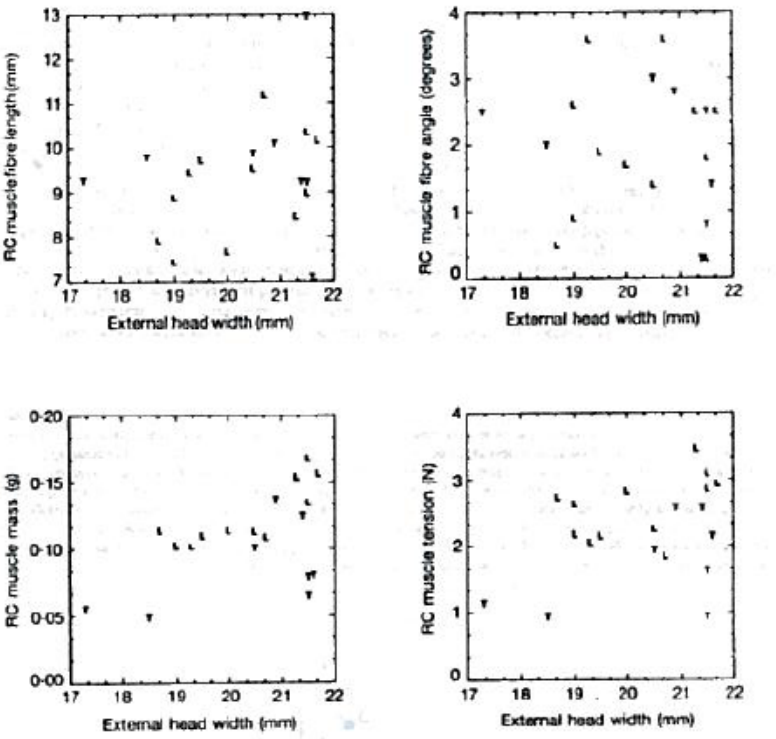

FIG. 2. Sample plots of four variables (fibre length, fibre angle, mass and estimated peak tetanic tension) for one muscle, the rectus cervicis (RC). Each variable is plotted against external head width. The position of each larval specimen is indicated by an L and the position of post-metamorphic (transformed) individuals is indicated by a T.

mandibulae internus and externus decrease after metamorphosis but the difference is not significant (Table I). Mean values for the subarcualis rectus I, interhyoideus/intermandibularis and geniohyoideus all increased during metamorphosis but not significantly (Table I).

A principal component analysis of MTTs from all eight muscles that are retained across metamorphosis (Figs 3, 4, 5) shows that there appears to be a multivariate size effect: all variables are highly and positively correlated with PC1 (Fig. 4), and larger individuals tend to have higher MTTs. However, there was no inter-group size effect (i.e. larval and transformed individuals do not separate along PC1). Sheared PC analysis (Bookstein *et al.*, 1985) which removed possible

TABLE I

*Mean estimated muscle tensions (in Newtons), t-tests on larval and transformed mean muscle tensions, muscle masses (g), and fibre angles (degrees), ± 1 S.D., for larval (n = 12) and transformed (n = 8) **Ambystoma tigrinum**. We were able to estimate muscle tension and fibre angles of the branchiohyoideus muscle for only one individual in our transformed population, so no standard deviations are given for these variables*

Muscle	Muscle tension			Muscle mass		Fibre angle	
	Larvae	Transformed	*t*-tests	Larvae	Transformed	Larvae	Transformed
Epaxial	25·9±6·2	24·5±8·8	n.s.	0·50±0·11	0·46±0·15	3·4±2·2	4·1±3·1
Adductor mandibulae internus	3·1±0·9	2·7±0·7	n.s.	0·10±0·03	0·09±0·03	20·1±6·0	15·9±11·5
Adductor mandibulae externus	3·2±0·9	2·8±0·6	n.s.	0·09±0·03	0·07±0·02	6·2±3·6	11·8±5·1
Rectus cervicis	2·6±0·5	1·7±0·7	0·01	0·12±0·02	0·09±0·03	1·9±1·1	1·9±1·0
Subarcualis rectus I	0·6±0·2	0·7±0·3	n.s.	0·012±0·003	0·01±0·01	2·5±1·2	4·2±3·0
Interhyoideus/intermandibularis	2·0±0·7	2·5±1·2	n.s.	0·10±0·04	0·10±0·05	2·7±1·6	4·9±4·9
Geniohyoideus	0·5±0·1	0·6±0·2	n.s.	0·03±0·01	0·01±0·01	2·2±1·4	2·6±1·3
Depressor mandibulae	2·7±0·8	1·7±0·3	0·001	0·10±0·03	0·05±0·02	4·2±3·6	14·1±7·2
Branchiohyoideus	2·0±0·5	1·8±—	—	0·14±0·03	0·04±0·03	3·3±3·0	0·8±—

n.s., not significant

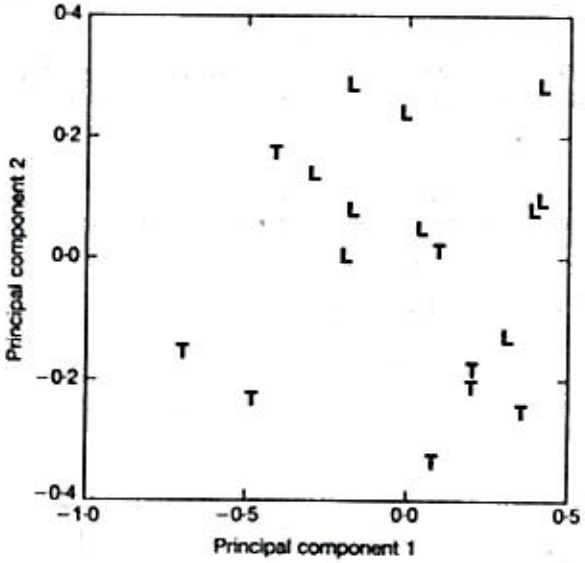

FIG. 3. Plot of principal component 1 against principal component 2 calculated for the estimated peak tetanic tensions in eight cranial muscles. PC1 accounts for 59% of the variance while PC2 accounts for 20·1% of the variance. Note that transformed individuals (T) tend to have negative scores on PC2 while larval individuals (L) tend to have high scores on PC2. Variables loading high and positive on PC2 include the estimated maximal tetanic tension (MTT) in the depressor mandibulae, adductor mandibulae externus and adductor mandibulae internus. Variables loading high and negatively on PC2 include estimated MTT in the subarcualis rectus I and the interhyoideus/intermandibularis.

TABLE II

*Parameters for log(10)-transformed linear regressions of muscle tensions (in Newtons) on external head width (in cm) for larval (n = 12) and transformed (n = 8) **Ambystoma tigrinum**. Analysis of covariance indicates significance of tests for transmetamorphic variation in larvae vs. transformed salamanders. The branchiohyoideus muscle is lost at metamorphosis and thus no data are available on transformed individuals. The 0·01 level was used to evaluate ANCOVA test significance*

Muscle	Larvae			Transformed			ANCOVA test *P* value		
	Slope	Intercept	*P* value	Slope	Intercept	*P* value	Slope	Intercept	R^2
Epaxial	2·52	−1·89	0·048	3·40	−3·08	0·04	n.s.	n.s.	0·47
Adductor mandibulae internus	3·38	−3·93	0·023	2·67	−3·09	0·04	n.s.	n.s.	0·50
Adductor mandibulae externus	3·45	−4·04	0·005	2·61	−2·99	0·01	n.s.	n.s.	0·63
Rectus cervicis	1·57	−1·65	n.s.	2·72	−3·35	n.s.	n.s.	0·001	0·55
Subarcualis rectus I	3·61	−4·96	n.s.	3·40	−4·60	n.s.	n.s.	n.s.	0·38
Interhyoideus/intermandibularis	4·04	−5·01	0·02	1·07	−1·05	n.s.	n.s.	n.s.	0·14
Geniohyoideus	2·14	−3·07	n.s.	3·49	−4·82	0·03	n.s.	n.s.	0·46
Depressor mandibulae	3·75	−4·48	0·004	2·07	−2·49	0·01	n.s.	0·001	0·76
Branchiohyoideus	13·3	−13·3	0·03	—	—	—	—	—	—

n.s., not significant

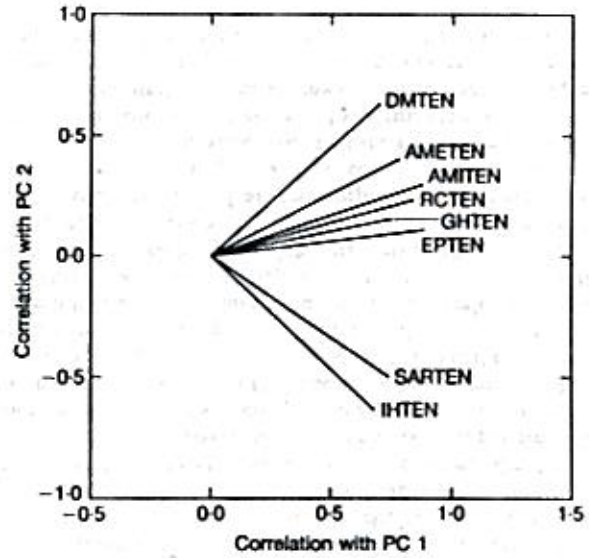

FIG. 4. Correlations of estimated maximal tetanic tension with principal components 1 and 2 presented in a vector diagram. Note that all variables correlate positively with PC1. The array of correlations with PC2 reflects the direction of transformation at metamorphosis. Variables with high positive correlations with PC2 (such as MTT of the depressor mandibulae) undergo reductions in mean value at metamorphosis. Variables with high negative correlations with PC2 undergo an increase in mean value at metamorphosis. TEN, estimated maximal tetanic tension in: AME, adductor mandibulae externus; AMI, adductor mandibulae internus; DM, depressor mandibulae; EP, epaxials; GH, geniohyoideus; IH, interhyoideus; RC, rectus cervicis; SAR, subarcualis rectus.

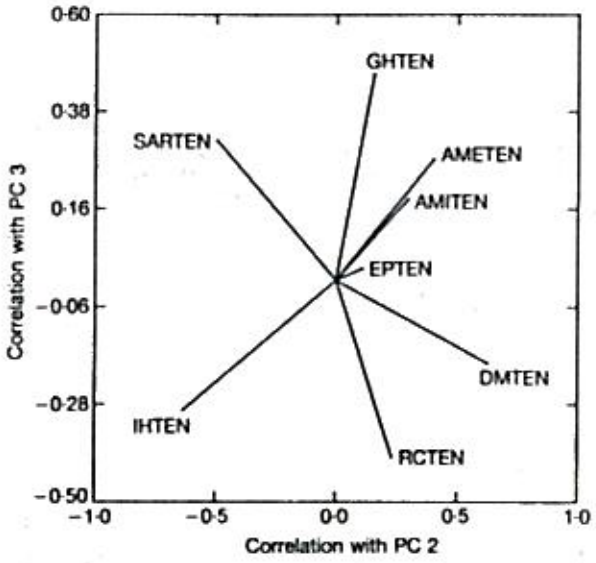

FIG. 5. Correlations of estimated maximal tetanic tension with principal components 2 and 3 presented in a vector diagram. The distribution of muscles reflects changes in muscle tension at metamorphosis and not differences in size of larval and transformed specimens. Abbreviations as in Fig. 4.

effects of size from PCs 2 and 3 altered the position of the larval and transformed individuals very little; thus, Figs 3, 4, and 5 show the results of the unsheared data. The presence of high and positive correlations of all variables with PC1 is due to similar within-group size correlations of muscle tensions with head width in both larvae and transformed individuals. The distribution of muscles in Fig. 5 thus reflects changes in muscle tension at metamorphosis and not differences in size of larvae and transformed specimens in our sample.

The subarcualis rectus I and interhyoideus/intermandibularis muscle tensions are negatively correlated with PC2 (Fig. 4) and both these muscles show slight increases in estimated tension at metamorphosis. The two muscles with significant decreases in tension at metamorphosis (the rectus cervicis and depressor mandibulae) are positively correlated with PC2 and negatively correlated with PC3 (Fig. 5).

Discussion

Previous research on the functional design of the musculoskeletal system in metamorphosing amphibians has focused on analyses of the skeleton and has only qualitatively considered changes in myology (e.g. De Jongh, 1968; Wassersug & Hoff, 1979; Trueb, 1985; Reilly, 1986, 1987; Lauder & Shaffer, 1988). Yet changes in muscle morphology and function are critical to a complete understanding of changes in function during development.

Here, we first consider the specifics of muscle metamorphosis in *Ambystoma tigrinum*, and then

provide an overview of the functional design of the head of the tiger salamander during development to place previous research and the present results into a single framework.

Muscle metamorphosis

During metamorphosis in tiger salamanders, two of the head muscles undergo a substantial decrease in estimated force-generating capability: the depressor mandibulae and the rectus cervicis muscles estimated peak tetanic tensions decrease by about 36% over a time period of several weeks. Because of our choice of pre- and post-metamorphic samples of individuals that just span metamorphosis, this decrease in estimated MTT is not confounded by larval or post-metamorphic growth. Rather, this decrease reflects a substantial change in head muscle design that takes place during metamorphosis itself. In addition, one muscle, the branchiohyoideus, is absent in transformed individuals as it is completely resorbed during metamorphosis.

While no muscles other than the rectus cervicis and depressor mandibulae exhibit a significant change in MTT when considered individually, several multivariate patterns of transformation at metamorphosis emerge when patterns of covariation among muscles are accounted for. Larval individuals tend to have higher scores on PC2 due to higher estimated MTTs in the depressor mandibulae and adductor mandibulae muscles, and lower MTTs in the interhyoideus/intermandibularis and subarcualis rectus I muscles (Figs 3, 4). Larval individuals also tend to have more positive scores on both PC1 and PC2 while transformed individuals have negative scores for the most part on PC2. There is a zone of overlap which is not surprising given the close temporal proximity of the two samples to each other and the relatively small sizes of our samples (Fig. 3). In general, PC2 scores reflect the direction of change in muscle tension at metamorphosis (Figs 4, 5): muscles that exhibit a reduction in MTT at metamorphosis are correlated positively with PC2, those that change little at metamorphosis have correlations that are near zero (Fig. 4: geniohyoideus and epaxial muscles), while those that increase at metamorphosis are negatively correlated with PC2 (subarcualis rectus I and interhyoideus/intermandibularis).

The process of metamorphosis thus appears to involve an increase in force-generating capability in the buccal floor muscles, a decrease in mouth-closing muscles and a decrease in the muscles involved in opening the mouth (depressor mandibulae and rectus cervicis). In addition, the subarcualis rectus I muscle (involved in movements of the hyobranchial apparatus) increases slightly in mass and MTT at metamorphosis and contributes to the negative scores of the transformed individuals on PC2 (Fig. 3).

Overall, the change in mass accounted for most of the change in estimated MTT of each cranial muscle at metamorphosis. Within-muscle variation in fibre angle and length tended to be high and muscle mass contributed the most to changes in MTT. For example, the rectus cervicis muscle (plotted in Fig. 2 to show the scatter of points for each variable measured from this muscle) did not change mean fibre angle (1·9°) or length (larval mean = 9·1 mm, transformed mean = 9·6 mm; not significant at $P = 0{\cdot}43$) while mass and MTT decreased significantly (Table I).

The subarcualis rectus I exhibited a large increase in fibre length of 82% while also increasing in mass from 0·012 g to 0·03 g. This muscle is the major muscle involved in projecting the tongue out of the mouth during terrestrial feeding (Reilly & Lauder, 1989), and it is thus not surprising that such dramatic changes should occur over the time course of metamorphosis. In larvae, the subarcualis rectus I originates on the hyoid arch and inserts proximally on ceratobranchial I near its articulation with the hypobranchial (Lauder & Shaffer, 1988). The branchiohyoideus muscle also originates from the hyoid but inserts on the distal end of ceratobranchial I. At

metamorphosis, the larval branchiohyoideus disintegrates and the distal attachment of the subarcualis rectus I migrates along ceratobranchial I toward the distal tip (Smith, 1920; Lauder & Shaffer, 1988; Reilly & Lauder, 1989).

Metamorphosis of the feeding mechanism

A critical problem in any analysis of the transformation of behaviour is the correlated change in many aspects of animal design. This problem is especially evident in animals that metamorphose as many changes in form and function take place over a short time span. In an effort to understand the modifications in feeding behaviour that occur during salamander metamorphosis, previous research has focused on one or more aspects of the ontogenetic change from a larval aquatic feeding system to a post-metamorphic terrestrial feeding system.

A key goal of these studies has been to document and analyse the ontogeny of functional design in the feeding mechanism of salamanders at several levels of organization. Thus, investigations of the feeding system of tiger salamanders during ontogeny have involved analysing several components of the head, including (but not limited to) muscle activity patterns, morphology, and kinematics of feeding behaviour.

The results of these studies are summarized in Fig. 6 and are reviewed here to provide an overview of recent results on the ontogeny of functional design in tiger salamanders. At metamorphosis there is a transformation in the hydrodynamic design of the feeding system in tiger salamanders from a unidirectional flow system in larvae to a bidirectional pattern (Fig. 6: panel 1; Lauder & Shaffer, 1986). In larvae, waters enters the mouth anteriorly (with the prey) and exits posteriorly from the gill slits. After metamorphosis the gill openings have closed and water brought into the mouth during suction feeding must also exit via the mouth. Terrestrial feeding is accomplished by projecting the tongue toward the prey and does not involve suction (Reilly & Lauder, 1989).

Many changes also occur in the osteology of the skull and hyobranchial apparatus at

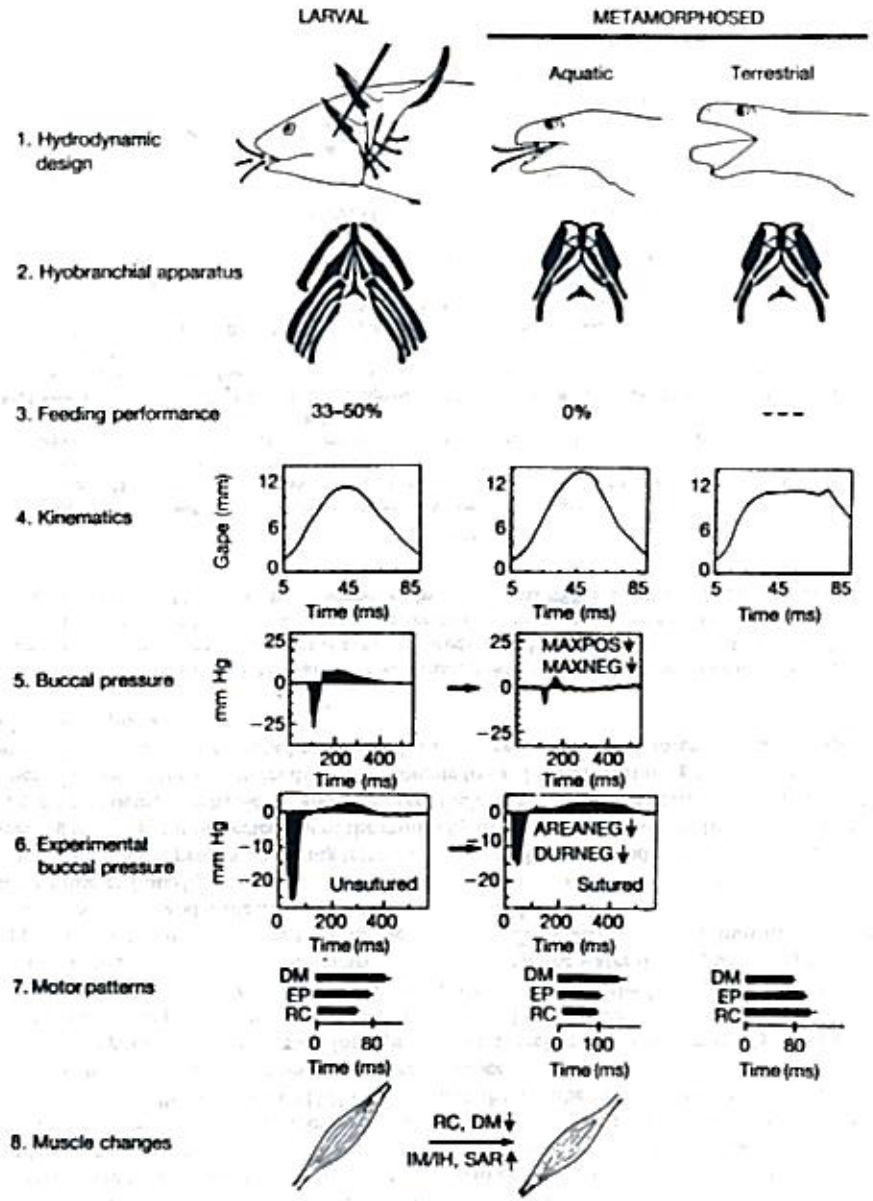

FIG. 6. Summary figure illustrating current data on the metamorphosis of functional design in the feeding mechanism of tiger salamanders (*Ambystoma tigrinum*). An important aspect of the analysis of the ontogeny of function in salamanders is the comparison of three 'stages' of ontogeny: larval, metamorphosed individuals feeding in the water, and metamorphosed animals feeding on land (Lauder & Shaffer, 1988).

Eight aspects of the metamorphosis of function are compared. Panel 1 shows changes in the hydrodynamic design of the feeding mechanism. Panel 2 shows the structure of the hyobranchial apparatus before and after metamorphosis. Panel 3 shows aquatic feeding performance (not measured for terrestrial feedings). Panel 4 presents the pattern of jaw bone movement during feeding (only the gape profile from the study of Shaffer & Lauder (1988) is shown). Panel 5 illustrates the pattern of pressure change in the mouth cavity during aquatic feeding (two of the pressure variables measured in the study of Lauder & Shaffer (1986) are shown; both the maximum negative (MAXNEG) and positive (MAXPOS) pressure variables decreased at metamorphosis). Panel 6 shows the pattern of buccal pressure change when the gill openings in an axolotl are experimentally sutured closed (Lauder & Reilly, 1988). The unsutured pressure pattern emulating the larval feeding system is shown on the left, and the sutured pattern imitating the post-metamorphic pattern is shown on the right. Two of the pressure variables are shown: duration of negative pressure (DURNEG) and the area under the negative portion of the pressure trace (AREANEG). Panel 7 illustrates the pattern of muscle activity during feeding (three representative muscles are shown from the study of Lauder & Shaffer, 1988). The black bars indicate the onset, cessation and standard error of muscle activity in each of three muscles: the depressor mandibulae (DM), epaxial (EP) and rectus cervicis (RC). Panel 8 summarizes the metamorphosis of muscle mass and estimated force-generating capabilities from this study. Estimated MTT decreases with metamorphosis in the rectus cervicis (RC) and depressor mandibulae (DM) while estimated MTT increases in the interhyoideus and intermandibularis (IH/IM) and the subarcualis rectus I (SAR).

metamorphosis (Fig. 6: panel 2; Lauder & Shaffer, 1988). For example, ceratobranchials II, III, and IV are lost at metamorphosis and extensive remodelling takes place in ceratobranchial I and the hyoid arch.

Analyses of the aquatic feeding ability of salamanders (Fig. 6: panel 3; Lauder & Shaffer, 1986; Reilly & Lauder, 1988) have shown that feeding performance decreases drastically after metamorphosis. Larval tiger salamanders capture small fish in a third to a half of all feeding attempts, while post-metamorphic tiger salamanders are able to capture only relatively slow-moving prey. Why does this drop in feeding performance occur?

One possible explanation could be that the pattern of jaw movement changes at metamorphosis so that in post-metamorphic individuals feeding kinematics are less effective in capturing prey. However, analysis of the pattern of jaw movements across metamorphosis has shown that this is not the case (Fig. 6: panel 4; Shaffer & Lauder, 1988): the kinematic pattern of feeding is extremely similar during aquatic feeding in both larval and post-metamorphic individuals. However, terrestrial feeding kinematics were found to differ significantly from aquatic feeding movements.

Accompanying the changes in osteology and hydrodynamic design at metamorphosis are changes in the pattern of buccal pressure used to capture prey (Fig. 6: panel 5; Lauder & Shaffer, 1986): the negative buccal pressure changes that drive the suction feeding mechanism are much greater in larvae than in metamorphosed individuals. In addition, the area under both the negative and positive portions of the pressure trace decreases at metamorphosis (Fig. 6: panel 5).

In order to understand just how much of the change in pressure profiles at metamorphosis was due to alterations in hydrodynamic design alone, Lauder & Reilly (1988) experimentally modified the feeding system in axolotls. These authors showed that converting the feeding system from unidirectional to bidirectional (by suturing closed the gill slits in axolotls and comparing the pressure profiles in sutured and unsutured individuals) feeding performance could be drastically reduced and the pressure patterns in the mouth altered significantly (Fig. 6: panel 6). Several of the changes that actually occur at metamorphosis were reproduced well by the experimental manipulation, but many (such as the decrease in maximum negative pressure and the decrease in the positive portions of the pressure trace) were not well imitated.

The changes in aquatic feeding performance at metamorphosis could also be due to alterations in the activity pattern in the jaw muscles. If post-metamorphic individuals use a different timing and sequence of jaw muscle activity than larvae, then this could explain differences in feeding performance. However, the pattern of muscle activity used during aquatic feeding has been shown not to change with metamorphosis (Fig. 6: panel 7; Lauder & Shaffer, 1988): only individuals feeding on land use a novel pattern of activity.

The results obtained in this paper on changes in muscle mass and estimated force-generating capability add a final piece of information to the study of ontogenetic changes in function (Fig. 6: panel 8). Changes in aquatic feeding performance observed by Lauder & Shaffer (1986) are due to *both* changes in hydrodynamic design *and* decreased force-generating capabilities of the cranial muscles. Changes in buccal pressure profiles across metamorphosis that occurred in the study of Lauder & Shaffer (1986) but not in the study of Lauder & Reilly (1988) should reflect changes in morphology of the feeding mechanism at metamorphosis that are not accounted for by alterations in hydrodynamic design alone. Thus, both the maximum negative pressure generated in the mouth cavity and the duration and amplitude of the positive after-pressure decreased across natural metamorphosis (Fig. 6) but not when the larval feeding system was experimentally converted to a bidirectional mechanism. These changes are best interpreted now as being due to reductions in mass of the major muscles generating negative buccal pressures, the rectus cervicis and depressor

mandibulae, and to reductions in force-generating capability of the depressor mandibulae and mouth-closing muscles. The decrease in positive afterpressure at metamorphosis (Fig. 6: panel 5) may also reflect a greatly reduced volume of water drawn into the mouth during prey capture.

These results do not rule out the possibility that changes in hyobranchial osteology and alterations in buccal volume at metamorphosis might also influence the pattern of pressure change and feeding performance across metamorphosis. However, the major changes in feeding behaviour appear to be the result of the metamorphosis of muscle mass and hydrodynamic design. In addition, Reilly & Lauder (1988) found that the number of ceratobranchials retained at metamorphosis had no effect on feeding performance.

The changes in estimated muscle force production correlate well with previously demonstrated changes in feeding behaviour across metamorphosis (Fig. 6: panels 1 and 3; Lauder & Shaffer, 1988; Reilly & Lauder, 1989). Specifically, after metamorphosis, the feeding mechanism on land involves projection of the tongue toward the prey, while in the water the tongue and hyobranchial apparatus do not project at the prey during the strike. Correlated with the acquisition of tongue-based feeding on land is the increase in mass of the geniohyoideus, subarcualis rectus I, and buccal floor muscles (interhyoideus and intermandibularis posterior). These muscles are all intimately involved in tongue projection during terrestrial feeding (Reilly & Lauder, 1989). During terrestrial feeding the tongue is projected out of the mouth by the combined actions of the subarcualis rectus I, geniohyoideus, interhyoideus and intermandibularis posterior: the vectorial summation of the lines of action of these muscles directs the tongue toward the prey. It is significant that these four muscles are the only ones in the head to exhibit an increase in estimated force-generating capability at metamorphosis.

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