

## Metamorphosis of Cranial Design in Tiger Salamanders (*Ambystoma tigrinum*): A Morphometric Analysis of Ontogenetic Change

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**ABSTRACT** While ontogenetic analyses of skull development have contributed to our understanding of phylogenetic patterns in vertebrates, there are few studies of taxa that undergo a relatively discrete and rapid change in morphology during development (metamorphosis). Morphological changes occurring in the head at metamorphosis in tiger salamanders (*Ambystoma tigrinum*) were quantified by a morphometric analysis of cranial osteology and myology to document patterns of change during metamorphosis. We employed a cross-sectional analysis using a sample of larvae just prior to metamorphosis and a sample of transformed individuals just after metamorphosis, as well as larvae undergoing metamorphosis. There were no differences in external size of the head among the larval and transformed samples. The hyobranchial apparatus showed many dramatic changes at metamorphosis, including shortening of ceratobranchial 1 and the basibranchial. The subarcualis rectus muscle increased greatly in length at metamorphosis, as did hypobranchial length and internasal distance. A truss analysis of dorsal skull shape showed that at metamorphosis the snout becomes wider, the maxillary and squamosal triangles rotate posteromedially, and the neurocranium shortens (while maintaining its width), resulting in an overall decrease in skull length at metamorphosis.

These morphometric differences are interpreted in light of recent data on the functional morphology of feeding in salamanders. Morphological reorganization of the hyobranchial apparatus and shape changes in the skull are related to the acquisition of a novel terrestrial feeding mode (tongue projection) at metamorphosis. Metamorphic changes (both internal and external) that can be used to judge metamorphic condition are discussed.

Analyses of ontogeny have contributed greatly to our understanding of form and function in the vertebrate skull. The analysis of vertebrate skull development has provided numerous characters for phylogenetic analysis (e.g., de Beer, '37; Goodrich, '30), has greatly increased our understanding of the developmental pathways producing the skull (Hall, '84a,b; Alberch and Alberch, '81; Hanken and Hall, '84), and has allowed scaling relationships for components of head design to be quantified (Albrecht, '78; Cochard, '85; Shea, '83, '84; Strauss, '84; Strauss and Fuiman, '85). However, despite many studies of cranial development in vertebrates, little is known about taxa that undergo a relatively discrete and rapid morphological change during ontogeny. Most ontogenetic analyses of head design focus on organisms that exhibit reasonably smooth, continuous modification of form during ontogeny: organisms that metamorphose are not often studied. Species

that metamorphose are often of special interest because the rapidity with which changes occur in both form and function provides an experimental opportunity to examine major changes in organismal design.

Within the vertebrates, many amphibian species exhibit abrupt postembryonic changes (metamorphosis) that accompany the transition from an aquatic to a terrestrial lifestyle. The physiological, biochemical, and behavioral transformations that occur during metamorphosis have received considerable study, and are summarized by Dodd and Dodd ('76), Duellman and Trueb ('86), Etkin and Gilbert ('68), Fox ('84), and Gilbert and Frieden ('81). Salamanders that metamorphose are especially useful as an experimental system for they exhibit a less radical metamorphosis than anurans, thus allowing homologous bones, muscles, and landmarks to be identified across metamorphosis. Yet morpholog-

ical changes are great enough in salamanders to challenge our attempts to understand the ontogeny of functional design.

Of importance for this paper is the body of background literature on the functional morphology of larval and adult salamanders (e.g., Drüner, '02, '04; Larsen and Beneski, '88; Lauder and Reilly, '88; Lauder and Shaffer, '85, '86, '88; Lombard and Wake, '76, '77; Reilly, '86, '87; Reilly and Lauder, '88a,b, '89; Roth, '76; Shaffer and Lauder, '85, '88). While none of these papers quantifies morphological changes per se at metamorphosis, they provide a basis for functional interpretations of morphometric changes in the skull.

The aims of this paper are twofold. First, we document patterns of morphometric change in head morphology across metamorphosis in the tiger salamander, *Ambystoma tigrinum*. We include in our analysis features of both the skull and the hyobranchial apparatus. Our specific aim is to examine morphological changes that occur at metamorphosis itself, not during larval or adult growth. Thus, we compare salamanders just before and just after metamorphosis. Secondly, we provide a functional interpretation of several of the key morphometric changes. This leads to a consideration of how well previous measures of the extent of metamorphosis reflect the important functional transformations that occur during the transition from the aquatic larval stage to the terrestrial adult.

#### ALLOMETRY AND METAMORPHOSIS

Bivariate and multivariate analyses of mensural characters have proven to be very useful in comparisons of relative growth and allometry in vertebrates (Bookstein et al., '85; Gould, '66, '71, '77; Shaffer, '84; Shea, '85; Sweet, '80). Kluge and Strauss ('85) have reviewed many of the key issues that relate to allometric studies of ontogeny. Here we consider how rapid morphological changes at metamorphosis may influence observed patterns of change in characters.

The generalized ontogenetic development of a character may exhibit a continuous (Fig. 1A), biphasic (Fig. 1B), or nonlinear growth trajectory. Changes in growth trajectories are usually associated with a biological perturbation during ontogeny, such as metamorphosis, and several possible patterns of morphological change may be observed. In addition, the graphical depiction of the metamorphic changes at ontogeny will depend on whether sampling of individuals is longitudinal or cross-sectional. In a longitudinal sampling across metamorphosis (where single individuals are measured at many sizes as they grow), allometric relationships may remain un-

changed; in other words, individual characters may be "ontogenetically scaled" (Gould, '75) through metamorphosis (Fig. 1C). Cross-sectional sampling of this pattern (in which different individuals are measured at a range of sizes) would reveal that larval, metamorphosing, and transformed samples have identical allometric coefficients and intercepts (Fig. 1F). Because of the variable size at which metamorphosis occurs, individuals in the middle of anatomical metamorphosis will span a size range, and this size range will overlap that of larvae on the low end and that of adults at larger sizes (Fig. 1F).

Longitudinal sampling during ontogeny might also reveal a vertical shift or "transposition" of the growth regressions. For example, as shown in Figure 1D, the length of a bone in the skull might decrease sharply at metamorphosis, and then resume growth with the same allometric coefficient. A cross-sectional analysis of different individuals would reveal (Fig. 1G) that larval, metamorphosing, and adult samples have the same coefficients but different intercepts. It is important to realize that the metamorphosing sample intercept moves down from the larval position to the adult position during metamorphosis.

Finally, longitudinal sampling might reveal that the regression coefficient after metamorphosis is not parallel to that during larval growth (Fig. 1E), indicating a different growth trajectory for the character after metamorphosis. In a cross-sectional analysis, this pattern will be shown by different slopes and intercepts for the three samples (Fig. 1H). In this case the slope of the metamorphosing sample will rotate from the larval position to the adult position during metamorphosis. The precise slope of the metamorphosing population will depend on how long metamorphosis was occurring before the sample of individuals was gathered for analysis.

In this paper the transformations that occur in the hyobranchial and cranial morphology of tiger salamanders, *Ambystoma tigrinum*, are compared in a cross-sectional analysis of metamorphosis. To focus on the metamorphic event itself, late larval, metamorphosing, and recently metamorphosed samples were analyzed to determine if transpositions in characters occurred across metamorphosis.

#### MATERIALS AND METHODS

##### *Specimens examined*

Metamorphic changes in the head of *Ambystoma tigrinum* (Ambystomatidae) were examined by measuring osteological features in samples preserved just before and after metamorphosis from the same (metamorphosing) popula-

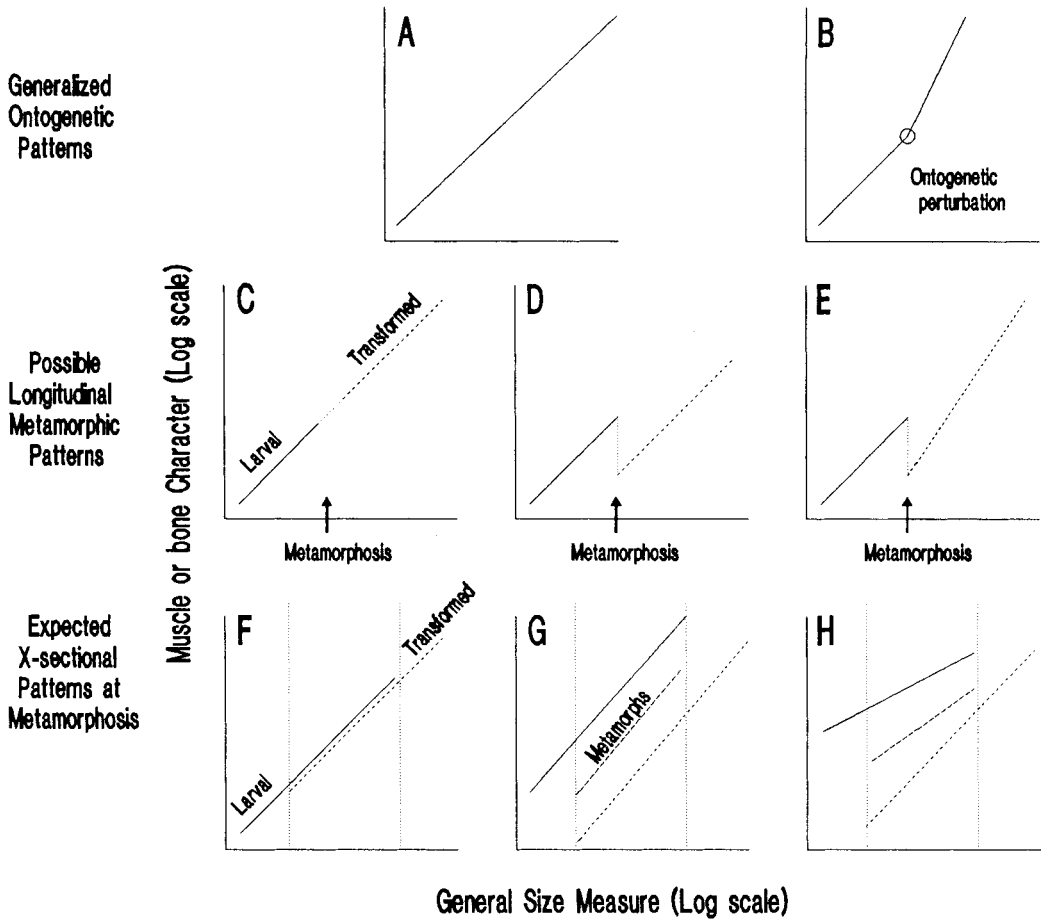


Fig. 1. Patterns of scaling in generalized, longitudinal, and cross-sectional views of the ontogeny of metamorphosing organisms. Axis labels apply to all graphs. Generalized growth trajectories can be **A**) linear, **B**) biphasic, or nonlinear (not depicted). Biphasic growth patterns could result from an ontogenetic perturbation. Longitudinal or cross-sectional sampling of characters across metamorphosis can reveal whether

a character is ontogenetically scaled (**C,F**), or ontogenetically transposed at metamorphosis with either the same trajectory (**D,G**) or a different trajectory (**E,H**) for the character after transformation. Ontogenetic samples are illustrated as larval (solid line), metamorphosing (long dashed line), and transformed (short dashed line).

tion (the samples are termed "larval" and "transformed," respectively). Cross-sectional analysis was chosen because the measurement of the characters of interest (especially muscles and bones of the hyobranchial apparatus) was too invasive to be possible in a longitudinal sampling of metamorphosing individuals. Specimens were collected in Colorado Springs, El Paso Co., Colorado, and were deposited in the Museum of Natural History, University of Kansas, Lawrence, Kansas (KU numbers given below). The samples included 12 larvae (KU89119–89122, 89124, 89128, 89135, 89140–89141, 89144–89145, 89149) at an ontogenetic stage just before metamorphosis with full bushy gills and complete open buc-

cal folds and gill slits, and 8 specimens preserved after transformation (KU89091, 89096, 89102, 89107–89111) with the gills completely resorbed. All specimens were preserved within 1 month of the same date.

Samples of larval and transformed individuals were selected for analysis on the basis of 1) their morphological proximity to metamorphosis as judged by external features (gill length, the extent of branchial openings, and tail fin condition), and 2) because the two samples did not differ significantly in external head size (head width, snout-mandible length, and snout-axilla length). This provided an a priori indication that they represented cross-sectional samples of the

same size taken at the endpoints of the metamorphic process.

An additional 28 individuals (collected in McGuires Pond, Jackson Co., IL: Deposited in Southern Illinois University Herpetology Collection) that were in the middle of metamorphosis (termed "metamorphs") were studied to determine the correlation of internal metamorphic changes relative to the external changes in morphology (gill condition, tail fin height) that are most commonly used to determine metamorphic stage. These specimens allowed us to analyze in detail, and with a larger sample size, the changes occurring during the process of metamorphosis per se.

### Measurements

The hyobranchial apparatus is often ignored in morphometric analyses of the vertebrate head, but this functional complex is critical to feeding behavior; where possible, functional considerations played an important role in choosing morphometric characters. For example, ceratobranchial 1 and the subarcualis rectus 1 muscle are important components of tongue-projection feeding behavior of salamanders (Lombard and Wake, '76; Reilly and Lauder, '89) and we included measurements of both in the analysis.

The following linear measurements were taken with calipers (variables marked with an asterisk were not measured on the sample of 28 metamorphs). From intact specimens we measured external head width (HW) measured just behind the mandibles; snout-mandible length (SML) from the tip of the snout to the posterior aspect of the mandible; snout-axilla length (SAL) from the snout to the posterior portion of the axilla when the arms are held perpendicular to the body; and interorbital distance\* (IOD) measured externally as the distance between the medial margins of the eyes. Nine cranial muscles were removed from these specimens for a separate analysis of muscle metamorphosis (Lauder and Reilly, '90) and the following measurements were taken from the dissected specimens: basibranchial length (BB) from anterior to posterior tips of the basibranchial; hypobranchial 1 length (HB) from the basibranchial-hypobranchial junction to the hypobranchial-ceratobranchial junction; ceratobranchial 1 length (CB) from the hypobranchial-ceratobranchial junction to the end of the straightened ceratobranchial; subarcualis rectus 1 muscle length (SARLENG) from anterior end of the muscle belly to the most posterior insertion of ceratobranchial 1; subarcualis rectus 1 muscle insertion point (SARINS) from the hypobranchial-ceratobranchial 1 junction to the most distal fibers of the subarcualis rectus 1

inserting on the ceratobranchial; internasal distance\* (IND) measured as the transverse distance between the outermost edges of the nasal bones (which reflects the separation of the narial openings); intersquamosal distance\* (ISD) measured between the lateralmost points of the squamosal bones; skull length\* (SKL) from the anterior margin of the premaxilla to the rear of the skull (exoccipitals) on the midline; skull width\* (SKW) of the braincase measured across the parietal bones along a line between jaw articulations. Several of these measurements are purposefully redundant so that correlation between external head measurements and similar measurements made directly on skull bones could be assessed.

Metamorphic changes in the overall shape of the skull were quantified using a truss analysis (Strauss and Bookstein, '82). Homologous landmark points chosen to archive the dorsal cranial shape (Fig. 2) were transferred to paper using a camera lucida attached to a Zeiss SV-8 dissecting microscope. Landmarks were clearly visible in both larval and transformed samples of salamanders. Because of the dorsoventral flattening of the head in salamanders, all of the landmark locations are very close to being in a single horizontal plane. Landmark configurations on paper were digitized (using Houston Instruments Hipad digitizing tablet) into a computer file as relative Cartesian coordinates. Lengths between landmarks, computed as Euclidean distances, were selected to form geometric trusses describing cranial shape as outlined in Figure 2B. Bilateral distance pairs were averaged within each individual truss to produce a total of 17 distances.

### Statistical analyses

Metamorphic variation in mensural variables was explored by computing principal components from the covariance matrix of logarithmically transformed data. Because our samples contained individuals that did not vary significantly in head size, the first principal component was not expected to be (and proved not to be) a measure of "general size" as is common in comparisons of size variant samples (e.g., Strauss, '85; Bookstein et al., '85). After principal components were computed, the corresponding correlations of mensural characters with principal components were portrayed as vectors following the methods of Strauss ('85) and Strauss and Fuiman ('85).

The two samples (larval and transformed) were compared using t-tests (on log-transformed data) using a conservative *P* value of 0.01 due to the multiple comparisons being performed. How-

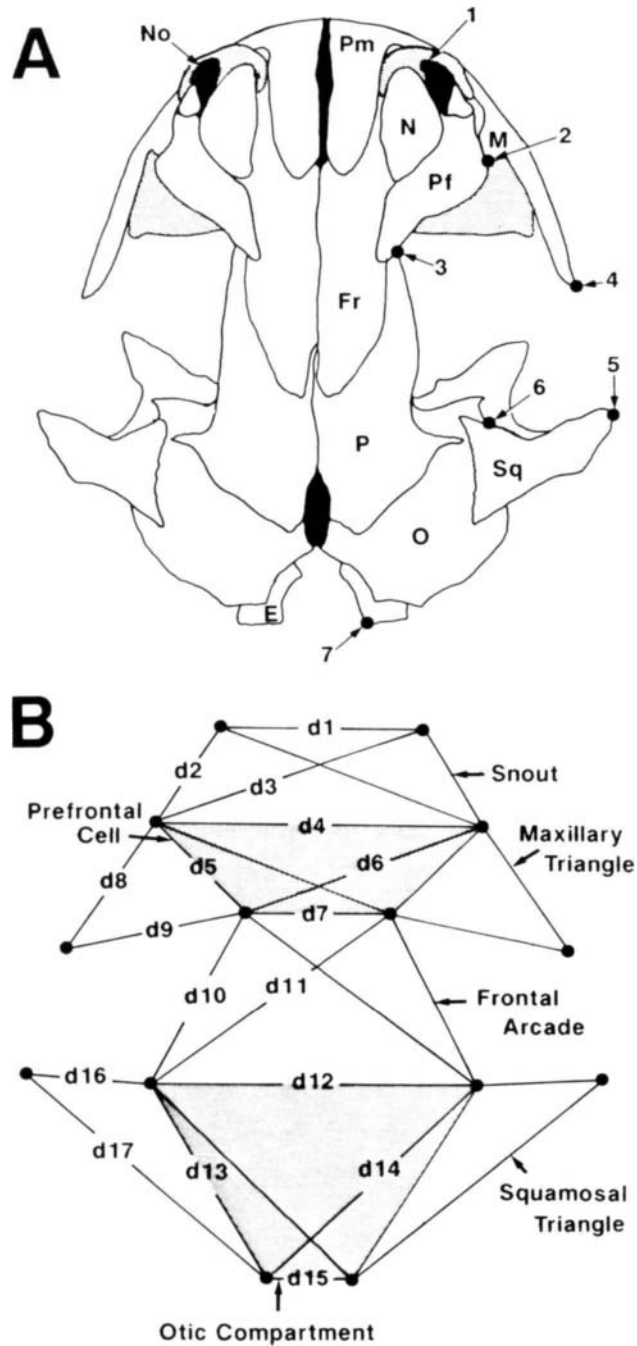


Fig. 2. Morphological landmarks (A) and truss distances among them (B) used to quantify dorsal head shape in larval and transformed *Ambystoma tigrinum*. Labelled truss cells in B indicate regional domains discussed in text. Distances d1 to d17 are discussed in the text. Landmarks were the anteromedial-most point of the external narial opening (1); the posterior end of the suture between the prefrontal and maxilla bones (2); the lateral point of intersection of the parietal

and prefrontal bones (3); the distal tip of the maxilla (4); the lateral tip of the squamosal bone (5); the anteromedial point of intersection of the squamosal and prootic bones (6); and the dorsomedial point on the exoccipital bone (7). E, exoccipital; Fr, frontal; M, maxilla; N, nasal; No, narial opening; O, otic capsule; P, parietal; Pf, prefrontal; Pm, premaxilla; Sq, squamosal.

ever, multiple t-tests may provide a misleading picture of mean changes between larval and transformed samples if individual variables are correlated with size (however measured) within samples (Packard and Boardman, '87). Even though the larval and transformed samples were sorted to be statistically equal in head length and there was no general size vector (Strauss, '85; Bookstein et al., '85), some variables did show significant correlations with HW. Therefore, analysis of covariance (ANCOVA) was used to compare intercepts of parallel regressions and identify transpositions. Where there was no correlation with either general size or with HW, t-tests provide a valid comparison between larval and transformed samples to identify transpositions.

Principal components analysis of truss distances (covariance matrix, logarithmically transformed data) was used to characterize cranial shape variation at metamorphosis. As with the mensural data, correlations of individual distances with the principal components were computed for interpretation of metamorphic shape transformations (e.g., Fig. 6C). Mean individual distances for each truss were used to reconstruct average cranial configurations for the larval and transformed samples.

## RESULTS

### Univariate analyses

Mean morphological measurements of pre- and postmetamorphic *Ambystoma tigrinum* are presented in Table 1. On the basis of t-tests, most variables reflective of skull size were not

significantly different in the two samples. However, internasal distance was larger and skull length was smaller in the transformed sample. Morphological aspects of the hyobranchial apparatus vary significantly in the two samples (Table 1). The transformed hypobranchial is larger while basi- and ceratobranchials are smaller. The transformed subarcualis rectus 1 muscle is longer and inserts more distally on ceratobranchial 1.

Analyses of covariance in larval and transformed samples are presented in Table 2 and representative plots are illustrated in Figure 3. Intercepts for most of the variables describing the skull were not significantly different for the larval and transformed samples (e.g., Fig. 3A). Skull length, internasal distance, and all aspects of the hyobranchial apparatus have significantly different intercepts (e.g., Fig. 3B-D).

ANCOVA tests comparing all three samples are presented in Table 3. For every variable the larval, metamorph, and transformed samples had slopes that were not significantly different from each other, although the sample sizes of larval and transformed individuals are small. Snout-mandible length had equal slopes and intercepts for all three samples. In the remaining variables, at least one of the samples has an intercept that is significantly different. Figure 4 illustrates this effect for hypobranchial length, and this pattern corresponds to the general relationship shown in Figure 1G.

### Multivariate analyses

Metamorphic differences in measurements among the larval and transformed samples are summarized by principal component analysis of the 13 head measurements (Fig. 5). Loadings on the first principal component were both positive and negative, indicating that it is not a generalized size component. The first principal component accounted for 77% of the variation and separated individuals on the basis of ontogenetic stage (Fig. 5A). Components 2 or 3 did not separate larval and transformed individuals (Fig. 5B). Correlations of the measurements with principal component 1 (Fig. 5C) show that high positive loadings are associated with increased internasal distance, subarcualis rectus 1 length, and subarcualis rectus 1 insertion point. High negative loadings on PC 1 are associated with smaller skull length, ceratobranchial length, and basi-branchial length. PC 2 appears to contain a minor effect of size as all correlations are positive except for the SARLENG variable; the mean of all vectors in Figure 5C is nearly parallel to PC2, suggesting that larger specimens may exhibit higher scores.

TABLE 1. Mean morphological measurements (mm  $\pm$  1 S.D.) for larval (N = 12) and transformed (N = 8) *Ambystoma tigrinum* and probabilities (P) for differences between them computed on logarithmically transformed data using t-tests.

Variable	Larvae	Transformed	t-test (P)
External head width	20.2 $\pm$ 1.1	20.4 $\pm$ 1.6	ns <sup>1</sup>
Snout-axilla length	34.6 $\pm$ 2.3	37.0 $\pm$ 3.5	ns
Intersquamosal distance	19.9 $\pm$ 1.1	19.6 $\pm$ 1.7	ns
Skull width	6.1 $\pm$ 0.5	6.5 $\pm$ 0.8	ns
Interorbital distance	11.3 $\pm$ 0.8	11.7 $\pm$ 1.1	ns
Snout-mandible length	17.6 $\pm$ 1.4	16.9 $\pm$ 1.1	ns
Skull length	18.6 $\pm$ 1.1	17.0 $\pm$ 1.0	0.005
Hypobranchial length	6.5 $\pm$ 0.6	7.4 $\pm$ 0.7	0.008
Internasal distance	9.8 $\pm$ 0.7	11.6 $\pm$ 1.1	0.001
Basibranchial length	4.1 $\pm$ 0.1	3.4 $\pm$ 0.3	0.000
Ceratobranchial length	17.3 $\pm$ 1.2	8.7 $\pm$ 2.8	0.000
Subarcualis rectus 1 length	6.6 $\pm$ 0.4	11.7 $\pm$ 3.7	0.002
Subarcualis rectus insertion	2.5 $\pm$ 0.3	6.0 $\pm$ 2.4	0.008

ns = P  $\geq$  0.01.

TABLE 2. Regression coefficients (slopes and intercepts) for log-transformed linear regressions of head measurements on external head width 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).

Variable	Larvae		Transformed		ANCOVA tests for —		
	Slope $\pm$ SE	Intercept	Slope $\pm$ SE	Intercept	Slope	Intercept	R <sup>2</sup>
Snout-axilla length	0.64 $\pm$ 0.32	0.71	0.78 $\pm$ 0.33	0.54	ns <sup>1</sup>	ns	0.48
Intersquamosal distance	0.88 $\pm$ 0.13*	0.15	0.90 $\pm$ 0.21*	0.12	ns	ns	0.77
Skull width	0.80 $\pm$ 0.42	-0.26	0.88 $\pm$ 0.46	-0.35	ns	ns	0.38
Interorbital distance	0.90 $\pm$ 0.25*	-0.12	0.88 $\pm$ 0.30*	-0.08	ns	ns	0.60
Snout-mandible length	0.41 $\pm$ 0.44	0.71	0.50 $\pm$ 0.25	0.57	ns	ns	0.24
Skull length	0.57 $\pm$ 0.29	0.52	0.59 $\pm$ 0.18*	0.45	ns	0.000	0.65
Hypobranchial length	1.17 $\pm$ 0.36*	-0.72	0.31 $\pm$ 0.42	0.47	ns	0.005	0.50
Internasal distance	1.13 $\pm$ 0.17*	-0.48	0.91 $\pm$ 0.30*	-0.12	ns	0.000	0.85
Basibranchial length	0.12 $\pm$ 0.16	0.45	-0.12 $\pm$ 0.43	0.69	ns	0.000	0.73
Ceratobranchial length	0.59 $\pm$ 0.34	0.47	2.61 $\pm$ 1.10	-2.50	ns	0.000	0.85
Subarcualis rectua 1 length	0.84 $\pm$ 0.26*	-0.28	-1.07 $\pm$ 1.50	2.45	ns	0.000	0.63
Subarcualis rect. insertion	0.29 $\pm$ 0.71	0.02	0.38 $\pm$ 2.30	0.18	ns	0.001	0.51

<sup>1</sup>ns =  $P \geq 0.01$ .

\* = Significant regression ( $P < 0.05$ ).

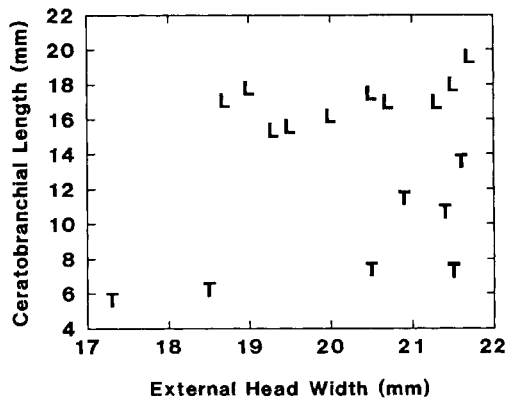
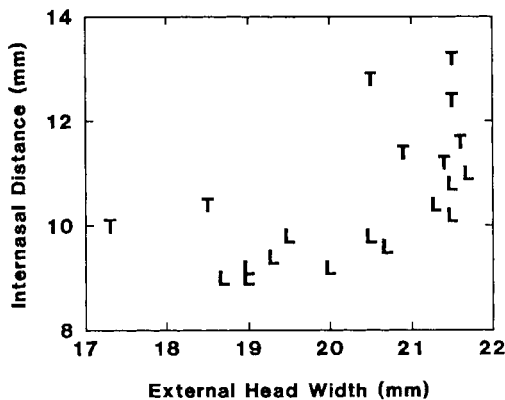
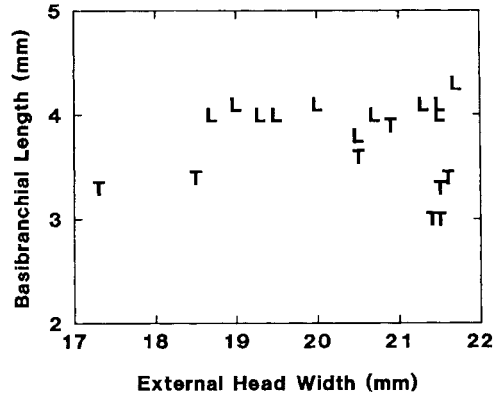
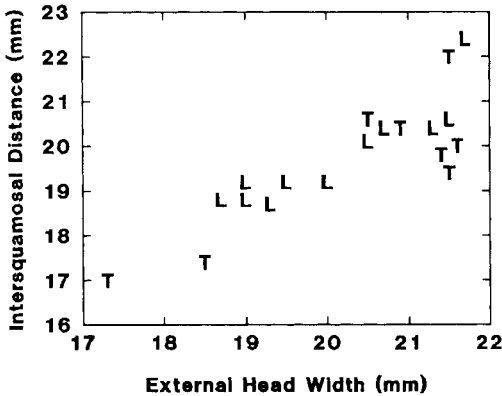


Fig. 3. Representative graphs of mensural characters (log-log plot) for larval (L) and transformed (T) samples of *Ambystoma tigrinum*. Transmetamorphic patterns were either ontogenetically scaled with equal intercepts (upper left panel), or ontogenetically transposed with different intercepts (other 3 panels).

TABLE 3. Regression coefficients (slopes) and intercepts for log-transformed linear regressions of head measurements on external head width for mid-metamorphosis *Ambystoma tigrinum* ( $N = 28$ ) (Analysis of covariance indicates significance of tests for transmetamorphic variation in larvae, metamorphosing, and transformed salamanders).

Variable	Metamorphs		ANCOVA tests for —		
	Slope	Intercept	Slope	Intercept	R <sup>2</sup>
Snout-axilla length	0.78 ± 0.06*	0.50	ns <sup>1</sup>	0.001	0.93
Snout-mandible length	0.97 ± 0.08*	0.01	ns	ns	0.84
Hypobranchial length	0.89 ± 0.10*	-0.33	ns	0.003	0.84
Basibranchial length	0.56 ± 0.16*	-0.18	ns	0.000	0.71
Ceratobranchial length	0.93 ± 0.13*	-0.35	ns	0.000	0.94
Subarcualis rectus 1 length	0.63 ± 0.14*	0.22	ns	0.000	0.62
Subarcualis rect. insertion	0.68 ± 0.21*	-0.17	ns	0.000	0.58

<sup>1</sup>ns =  $P \geq 0.01$ .

\* = significant regression ( $P < 0.01$ ).

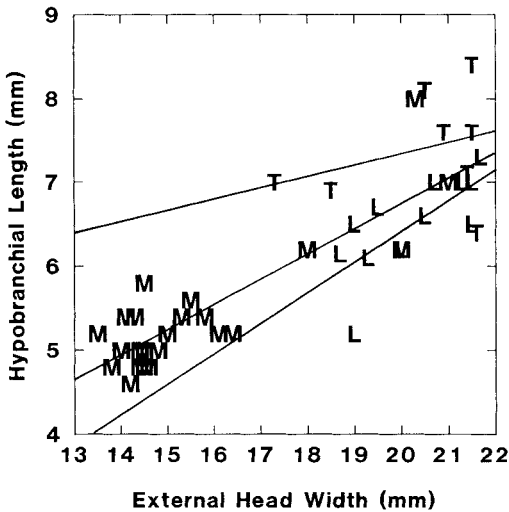


Fig. 4. Log-log plot of metamorphic character transposition in *Ambystoma tigrinum*. The allometric regressions for larval (L), metamorph (M), and transformed (T) samples are not significantly different, but their intercepts are positively shifted. For allometric parameters and tests for significance see Tables 2 and 3.

Multivariate differences in dorsal cranial shape are summarized by a principal component analysis of the 17 truss distances (Fig. 6). Truss distances loaded both positively and negatively on the first component showing that the truss measurements exhibit no general size component. Principal component 1 clearly separates the larval and transformed samples, while components 2 and 3 do not. Variables with large negative correlations with PC 1 were associated with the squamosal triangle and frontal arcade regions of the skull (Figs. 2B, 6C). Variables with high positive correlations primarily reflect changes in the snout, maxillary, and prefrontal regions of the skull (Figs. 2B, 6C). The mean

vector for all variables in Figure 6C has a positive correlation with both PC1 and PC2, suggesting that minor size effects are contained in both these components.

Cranial shape differences can be seen easily in the reconstructed larval and transformed skull trusses (Fig. 7). The significant shortening of the skull (Tables 1, 2; Fig. 7) can be attributed almost entirely to the shortening of the frontal arcade with some shortening of the prefrontal cell. Reduction in the size of the frontal arcade is reflected in the high negative loadings of d10 and d11 (Figs. 2, 6C, 7). The maxillary triangles rotate inward with the posterior shortening of the prefrontal cell. The otic compartment and the squamosal triangles are greatly remodeled, even though there is no significant difference in intersquamosal distance in the two samples. This is accomplished by a shortening of the lateral struts (d16, d17) of the squamosal triangles (Figs. 6C, 7), and a posterior enlargement of the otic compartment by the elongation of d13, d14, d15 (Figs. 6C, 7). This results in a shortening of the skull without a change in the snout-mandible length.

## DISCUSSION

The process of metamorphosis in salamanders is a complex one with changes in nearly every aspect of morphology taking place in less than one month (Duellman and Trueb, '86; Latimer and Rooffe, '64). While cranial morphology of *Ambystoma tigrinum* is changed by remodelling of skull bones, cranial muscles are altered by muscle fiber resorption and growth. In addition, major functional changes are taking place. The head changes from a functional design associated with aquatic prey capture to a feeding system involved in capturing prey on land (Bramble and Wake, '85; Lauder, '85; Lauder and Reilly, '90; Özeti and Wake, '69). Functional changes



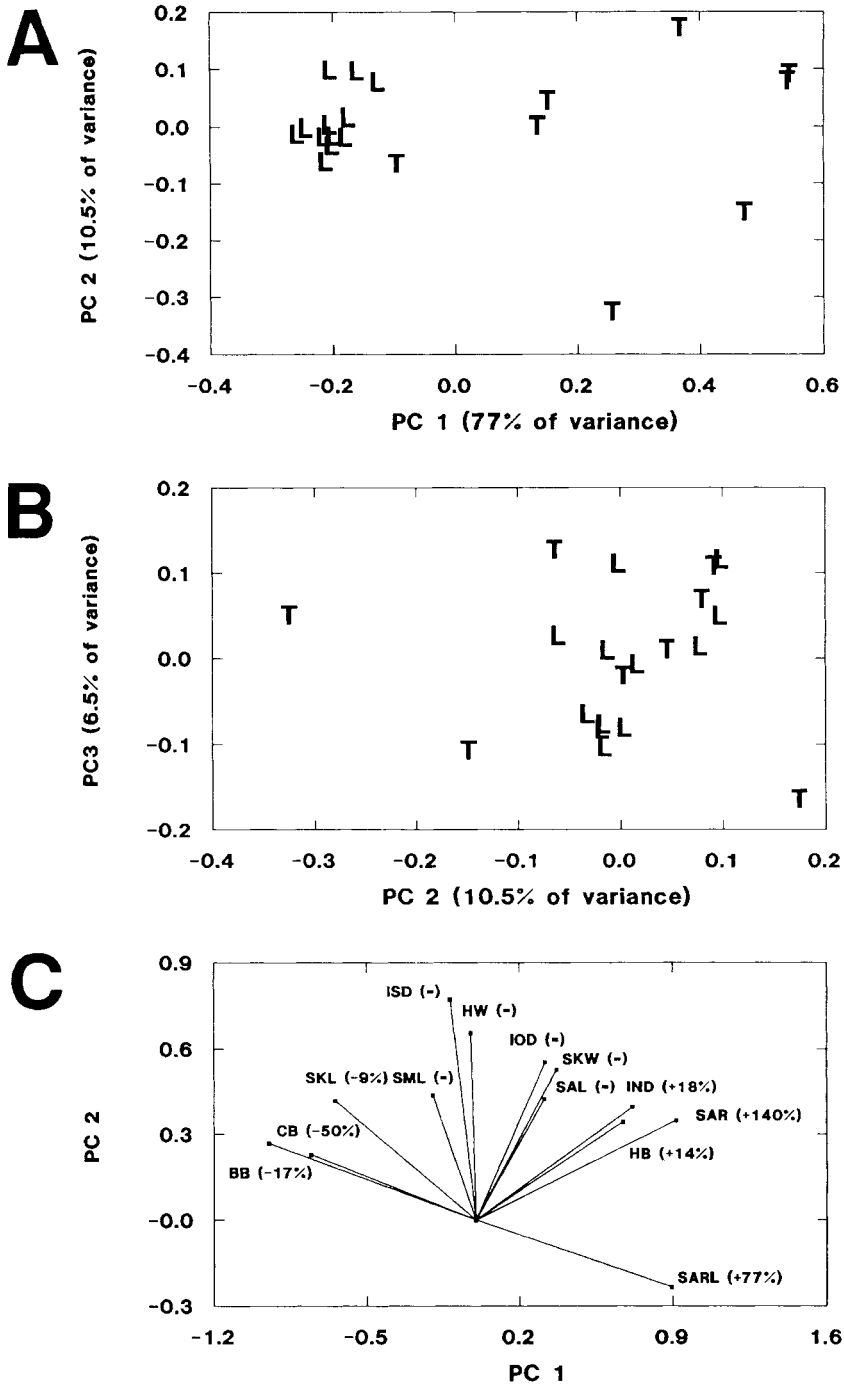


Fig. 5. Principal components analysis of 13 characters measured from the head, skull, and hyobranchial apparatus in *Ambystoma tigrinum*. **A,B:** Scatterplots of larval (L) and transformed (T) specimens on the first three principal components. The amount of variance explained by each component is given in parentheses. **C:** Corresponding correlations of mensural characters with principal components 1 and 2. Per-

centage change of each variable that differs significantly across metamorphosis (Table 1) is given in parentheses along with the sign of the change; a plus indicates that the variable increased at metamorphosis. Variables that do not change at metamorphosis are followed by an equal sign. Note that all variables do not have high and positive loadings on PC 1.

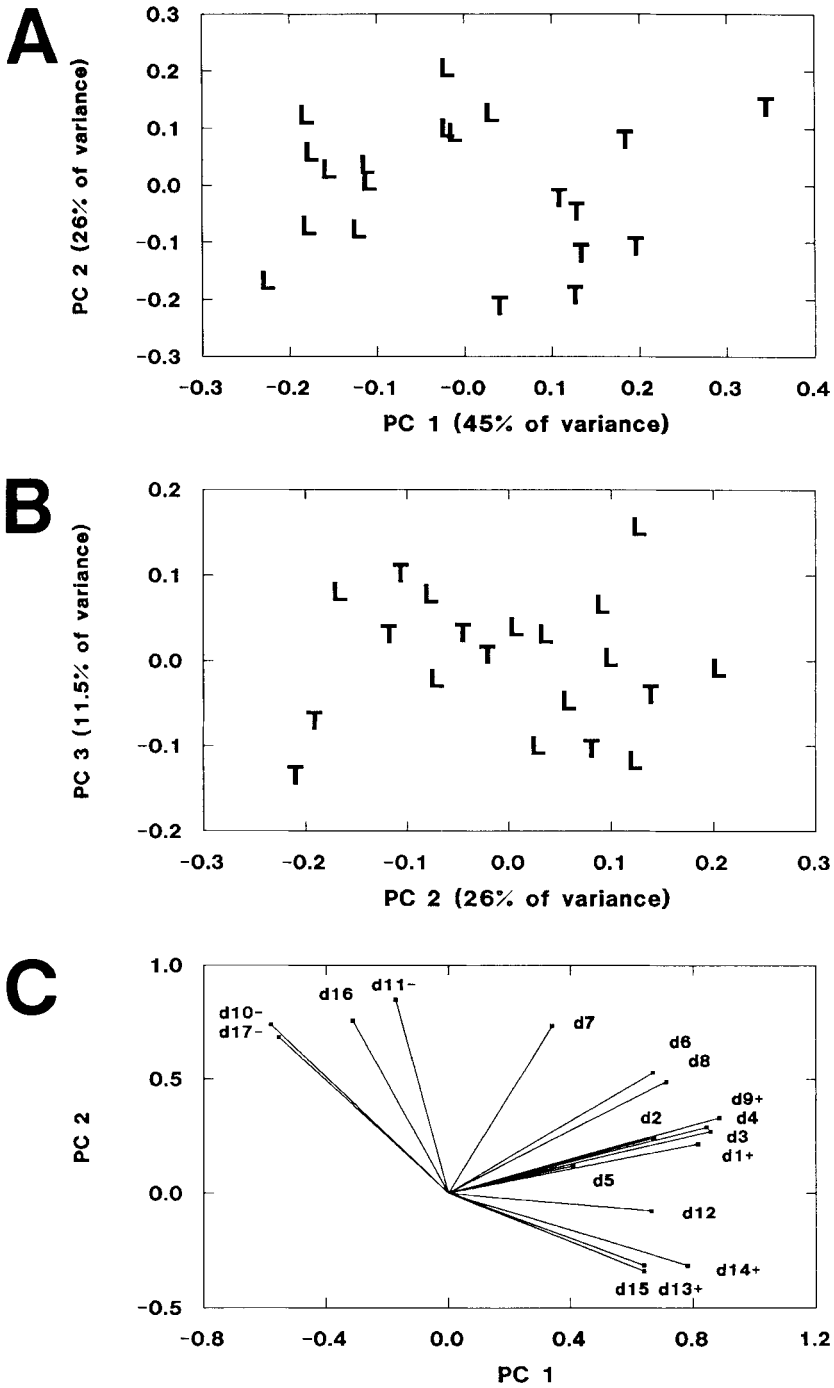


Fig. 6. Principal components analysis of 17 truss distances. **A,B:** Scatterplots of larval (L) and transformed (T) *Ambystoma tigrinum* on the first three principal components. **C:** Corresponding correlations of truss distances with

principal components. The direction of change (+ or -) is indicated for each truss distance that changes significantly at metamorphosis.

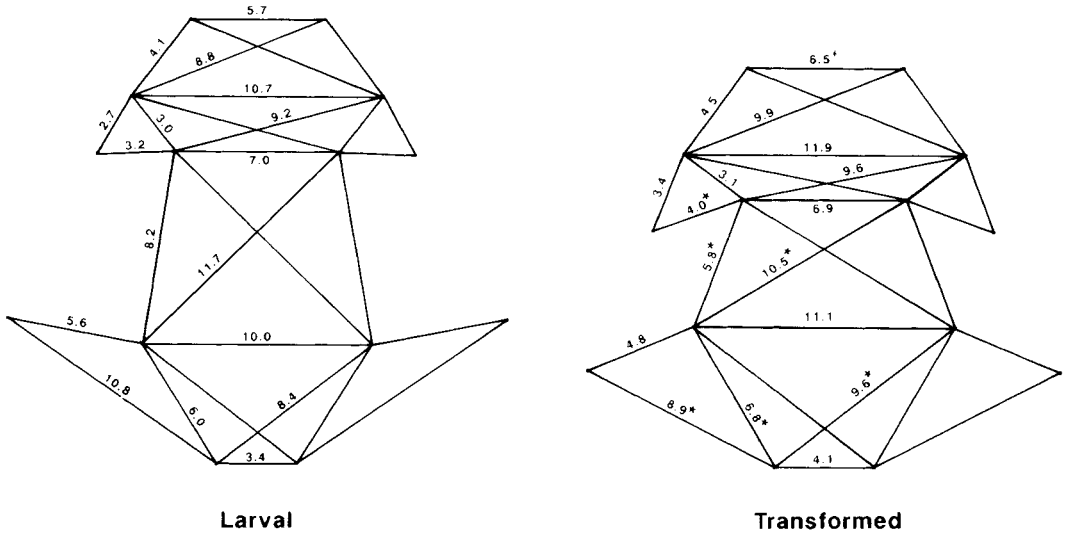


Fig. 7. Larval and transformed truss configurations for dorsal cranial shape reconstructed using mean truss distance values (mm). Asterisks indicate distances that differ significantly in the two samples (t-test,  $P \geq 0.01$ ). See Figure 2 for a key to the truss strut numbers.

associated with the aquatic-to-terrestrial transformation in *Ambystoma tigrinum* include changes in pressure patterns within the mouth cavity during prey capture (Lauder and Shaffer, '86), changes in muscle activity patterns used during feeding (Lauder and Shaffer, '88), and extensive changes in kinematic patterns of cranial components such as the hyobranchial skeleton (Reilly and Lauder, '89). Prey capture on land involves tongue projection from the mouth, while prey capture in the water is the result of suction feeding (Lauder, '85; Reilly and Lauder, '89).

#### Cranial metamorphosis

While most aspects of general head size were not significantly different between larval and transformed tiger salamanders, internasal distance and head length did differ. Skull length decreases dramatically during metamorphosis, becoming 9% shorter. Metamorphic increase in the width of the snout (Fig. 2) and separation of the narial openings is indicated by the increase in internasal distance as the nasal bones expand laterally to become 18% further apart on their lateral edges than in larvae. Changes in the shape of the snout can be seen in the reconstructed average transformed skull truss (Fig. 7).

The prefrontal cell (Fig. 2) becomes wider and shorter, producing the effect of rotating the maxillary triangles inward, thus rounding the lateral cheek area somewhat at the point where the

prefrontal bone meets the maxilla. Increase in length of the maxillary bones is known to occur at metamorphosis (Reilly, '87) and is reflected by the increase in the length and lateral position of the jugal process (d8 and d9, Figs. 2, 7).

At metamorphosis the frontal arcade exhibits greater change in shape than any other area of the skull. Its longitudinal and diagonal struts (d10, d11) decrease significantly (Fig. 7), indicating a large relative reduction in the distance between the prefrontals and squamosals (landmarks 3 and 6, Fig. 2). The intersection point of the prefrontal and frontal bones (landmark 3) is relatively stable. Some of the decrease in the length of the frontal arcade can be attributed to the anterior movement of landmark 6 due to the longitudinal expansion of the squamosal bone relative to the otic capsule at metamorphosis. Because skull width (across the parietals) and the distance across the frontals (d7) do not differ across metamorphosis the frontal arcade remains the same width as it shortens.

The otic compartment becomes wider and significantly longer at metamorphosis. The anterior expansion of the squamosal bone which contributes to the shortening of d10 in the frontal arcade also produces a significant increase in d13 shared by the otic compartment and the squamosal triangle. There is some lateral movement of landmark 6 as indicated by the increase in the distance between the squamosals (d12) and the significant increase in d11 and d14. Therefore,

the squamosal expands anteriorly and the otic capsules expand somewhat laterally, for a net anterolateral movement of about 0.8 mm (Fig. 7) in landmark 6. Widening of the posterior of the otic compartment is seen as the distance between the exoccipital bones (d15) increases.

Thus, it can be concluded from considerations of the changes in the four central cranial shape cells that the statistically significant decrease in skull length at metamorphosis (Tables 2, 3; Fig. 7) can be attributed to the decrease in length of the frontal arcade.

Movements of the anteromedial corners of the squamosals (landmark 6) also affect the squamosal triangles (Fig. 7). While the squamosal bone does expand anteriorly along the otic capsule, the distance from the exoccipital to the lateral tip of the squamosal (d17) becomes significantly shorter. This indicates there is considerable posterior rotation of the tips of the squamosal bones at metamorphosis. Because the intersquamosal distance does not change at metamorphosis (Tables 1, 2), there is a significant posterior shift in the point where the lower jaw articulates with the cranium. Wilder ('25) describes a similar posterior movement of the suspensorium at metamorphosis in *Eurycea bislineata*. Furthermore, because the snout-mandible length does not change, some movement of the jaw articulation would have to occur in order for the lower jaw to remain in alignment with the upper jaw as the frontal arcade shrinks and the entire maxillary arch (formed by premaxillae and maxillae) moves posteriorly. Because snout-axilla length does not change at metamorphosis and skull length decreases, the distance from the axilla to the skull must become longer.

#### *Hyobranchial metamorphosis*

Many changes occur in the hyobranchial apparatus at metamorphosis. Skeletal elements and muscles are lost, new structures appear, and many components change shape, size, and position (Fig. 8). Most obvious are the losses. The posterior three ceratobranchials (CB 2-4) and their associated muscles (subarcualis rectus 4 and subarcualis obliqui) are lost (Lauder and Shaffer, '88; Reilly, '87). The ceratobranchials are rapidly resorbed and disappear well before the gill muscles which often persist as a few muscle fibers in late metamorphosis with no traces of external gills or tail fin (three of the transformed specimens had traces of these muscles). The branchiohyoideus muscle, which extends from the ceratohyal to the distal end of ceratobranchial 1, is gradually lost at metamorphosis and remains as a few fibers until about the time the external gills completely disappear. The car-

tilaginous rod connecting the urohyal to the basibranchial is lost.

The new structures that appear at metamorphosis are associated with the development of the tongue (Yamasaki, '56). The thin second radials appear and project dorsolaterally from the basibranchial up into the tongue mass, their tips connected by the otoglossal cartilage (Fig. 8). Several new intrinsic tongue muscles (basiradialis, interradialis, and hyoglossus) develop along with these tongue skeletal structures.

Many changes take place in the structure of the hyobranchial apparatus (Fig. 8) as the remaining hyobranchial elements ossify to varying degrees. The hyoid arch (ceratohyal) changes shape from a curved rod to a spatulate platform and its attachments to the skull change at metamorphosis. At its distal end the hypohyals are replaced by the first radials creating a longer and less rigid connection of the ceratohyal to the basibranchial. Posteriorly, the ligament attaching the ceratohyal to the skull (HQL) moves from the prearticular to the dorsal aspect of the quadrate securing the ceratohyal to the rear of the skull (Fig. 8) instead of the lower jaw.

Our data show that elements of the first branchial arch exhibit a combination of negative and positive transposition during metamorphosis. The median basibranchial and distal ceratobranchial become shorter, while the bone connecting them, the hypobranchial, becomes longer (Fig. 8). The ceratobranchial shortens about 50% by the resorption of its distal end.

As ceratobranchial 1 shortens there is a great increase in the length of the subarcualis rectus 1 muscle. The change in this muscle is one of the most dramatic events of morphological metamorphosis (Fig. 8) and was first described in metamorphosing *Eurycea bislineata* (Smith, '20). Contrary to *Eurycea bislineata* there is no new growth or remodelling of the ceratobranchial with accompanying development of the subarcualis rectus 1. In *Ambystoma tigrinum* the insertion of the subarcualis rectus 1 moves from the proximal to the distal end of the shortening ceratobranchial (Fig. 8). The subarcualis rectus 1 fibers insert along the distal half of the anterolateral surface of the ceratobranchial, with some fibers wrapping ventrally to insert posteriorly on the ceratobranchial. The subarcualis rectus 1 muscle moves, enlarges, and laterally expands to exactly replace the larval branchiohyoideus which is lost during metamorphosis.

#### *Functional morphology of metamorphosis*

Transformation of the cranium involves changes in the shape of the snout, maxillary arch and the position of the jaw articulation relative

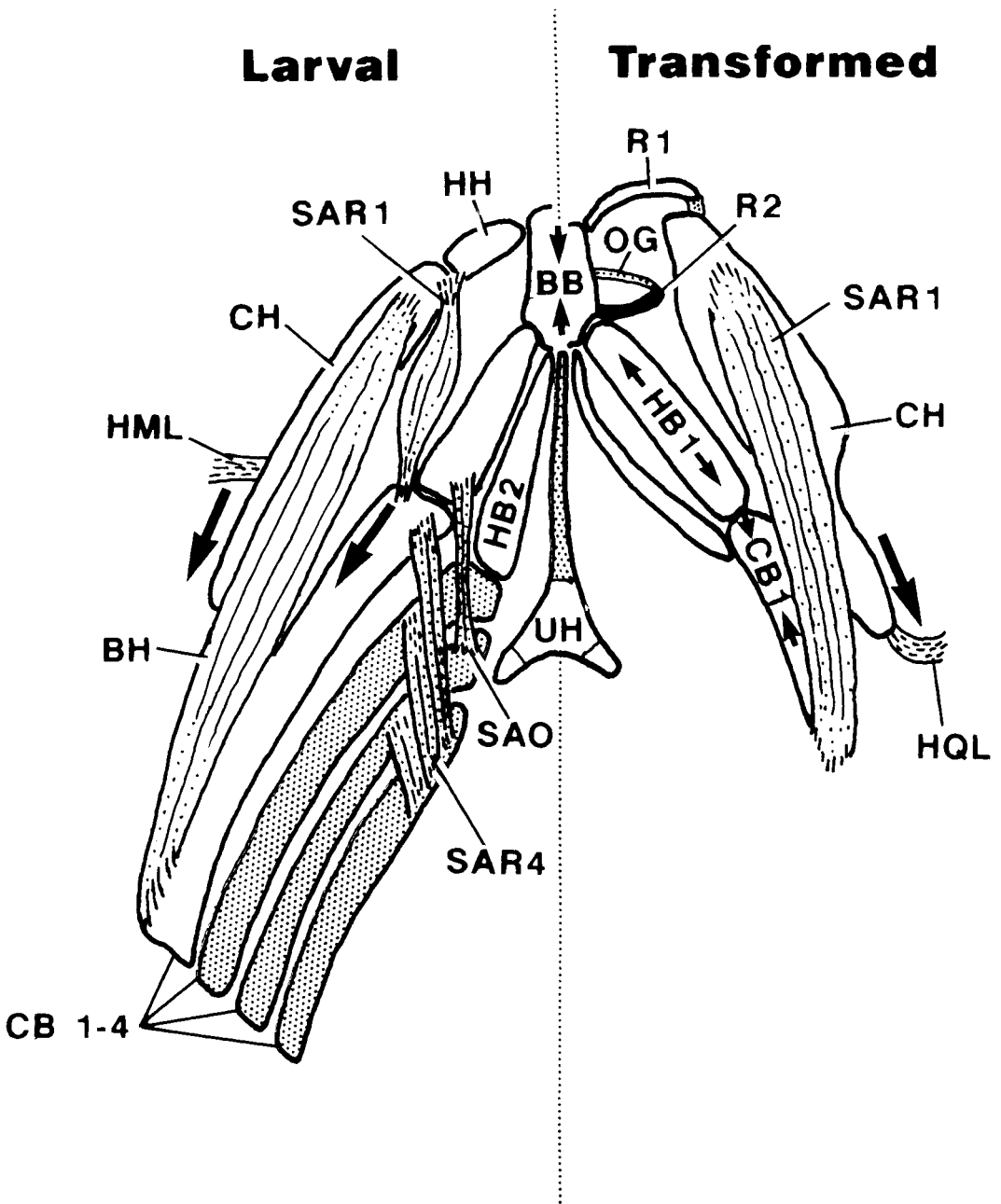


Fig. 8. Composite hyobranchial apparatus illustrating the morphological transformations that occur in muscles, bones and ligaments during metamorphosis in *Ambystoma tigrinum*. Metamorphic changes can be seen by comparing the larval (left side) to transformed (right side) morphology. Elements that change (arrows), are lost (shaded), and appear (solid) at metamorphosis are indicated. Single arrows show

structural movements and arrow pairs indicate size transpositions during metamorphosis. BB, basibranchial; BH, branchiohyoidens; CB, ceratobranchial; CH, ceratohyal; HB, hypobranchial; HH, hypohyal; HML, hyomandibular ligament; HQL, hyoquadrate ligament; OG, otoglossal cartilage; R, radial; SAR, subarcualis obliqui; SAR, subarcualis rectus; UH, urohyal.

to the otic compartment. Larval salamanders have a relatively long skull with the lower jaw articulating anterior to the otic capsules. The snout is narrow with short, weak maxillary bones projecting posterolaterally to curve inside the dentaries. Dentary teeth do not extend as far as the coronoid process of the prearticular. This results in a shorter edentate span of the mandible contacting the maxillary arch such that the effective bite is limited to about two thirds of the mandible. Large, well-developed labial lobes on the upper and lower jaws interdigitate to form a lateral cheek (Matthes, '34) at about the level of the maxillae. These flaps have two functions. First, they restrict water flow to the region anterior to the mouth and increase the volume flow rate of water entering the buccal cavity (Lauder and Shaffer, '86; Özeti and Wake, '69) in comparison to a mouth configuration with notched openings on the side (Lauder, '79). Secondly, structures lateral to the gape which occlude the margins increase the velocity of incoming water during suction feeding by decreasing the effective cross-sectional area of the mouth.

At metamorphosis the snout becomes significantly shorter and wider (Fig. 7). The skull shortens as the frontal arcade shrinks, pulling the snout posteriorly. Accordingly, the mandibles move posteriorly as the jaw articulation point (tips of the squamosal bones) shifts caudally relative to the otic capsules. The maxillary triangles rotate and the maxillae elongate to widen and extend the maxillary arch posteriorly, and dentary teeth extend past the coronoid process of the prearticular, creating a longer edentate apposition of the jaws. Now the gape can be opened wide enough for unobstructed tongue projection and subsequent capture and manipulation of large active terrestrial prey with the fully edentate jaws.

Elements of the hyobranchial apparatus function in suction feeding before and after metamorphosis. Aquatic feeding salamanders capture prey with a suction impulse resulting from the generation of negative buccal pressure by the retraction and ventral rotation of the hyobranchial apparatus on its articulation with the skull (Lauder and Shaffer, '86). Larvae employ a unidirectional hydrodynamic design in which captured water continues to flow posteriorly to exit through the gill slits while transformed salamanders which have lost gill openings must reverse the flow of captured water by expelling it back out the mouth. Both the amount of negative pressure generated and prey capture performance decrease significantly at metamorphosis (Lauder and Shaffer, '86; Reilly and Lauder,

'88a). While the drop in aquatic feeding performance results in part from the change in hydrodynamic design (Lauder and Reilly, '88), morphological changes in the hyobranchial apparatus associated with the development of tongue projection also contribute to the drop in performance.

Many metamorphic changes in the hyobranchial apparatus decrease its efficiency to function as a rotating skeletal basket to expand and contract the buccal cavity during suction feeding. The size of the branchial basket is decreased by the loss of the posterior three ceratobranchials, the shortening and thinning of the first branchial arch, and the shortening of the median basibranchial which loses its cartilaginous connection to the urohyal. Rigidity of the hyoid arch is greatly decreased by the change in the shape and length of the ceratohyal and the loss of its robust hypohyal connection to the basibranchial. Thus, changes in the extent and rigidity of the hyobranchial apparatus reduce its ability to expand the buccal cavity when it is retracted by the rectus cervicis muscle early during the strike (Lauder and Shaffer, '85). Buccal volume change per unit time is reduced after metamorphosis thus reducing suction pressures (Lauder and Shaffer, '86).

Another major change in the suction feeding mechanism at metamorphosis occurs in the point of rotation of the hyobranchial apparatus. In larval salamanders the rectus cervicis muscle attaches to the urohyal and basibranchial and functions to retract the hyobranchial apparatus rotating it ventrally on the mandibulohyoid ligament (Lauder and Shaffer, '85). This ligament transmits the posteroventral rotation of the hyobranchial apparatus to the lower jaw, causing depression and mouth opening. After metamorphosis the ceratohyal has a ligamentous connection to the posterior aspect of the quadrate (Fig. 8:HQL) and just posterior to this the distal end of ceratobranchial 1 lies in a fascial sheath limiting posterior movement. The rotation point for the hyobranchial apparatus is distal to the jaw articulation. Thus, after metamorphosis hyobranchial retraction is no longer linked biomechanically to lower jaw depression. In addition, the loose connection of the ceratohyal to the basibranchial and its increased flexibility allows the branchial skeleton to be protracted dorsally between the ceratohyals during tongue projection and manipulation of prey against the palate. In summary, the hyobranchial apparatus changes function at metamorphosis primarily from a complex system of interconnected gill arches that act to increase mouth volume during suction feed-

ing, to a less complex system that acts as the framework for terrestrial tongue projection.

The major myological change in the hyobranchial apparatus involves the subarcualis rectus 1, which changes in function from a weak gill abductor to one of the protractors of the hyobranchial apparatus during tongue projection (Reilly and Lauder, '89). At metamorphosis this muscle expands its origin to cover the anterior third of the ceratohyal. Its insertion moves from a small area on the proximal end of ceratobranchial 1 to wrap and insert widely around the distal end of the element. When this muscle contracts it pulls the ceratohyal posteriorly to the limit of the hyoquadrate ligament, then force is transferred to the first branchial arch which is buckled ventrally or dorsally depending on the position of the hyobranchial apparatus relative to the lower jaw (Reilly and Lauder, '89).

Changes in the size of individual branchial elements may also be interpreted in terms of their functions in the feeding mechanism. The larval basibranchial is the central anchor point for the hyoid and branchial arches (Fig. 8). It is the effective insertion point of the muscles that retract the hyobranchial apparatus during buccal expansion. At metamorphosis the basibranchial becomes smaller and loosely articulated with the hyoid arch. It still acts as the anchor point for hyobranchial retraction (to move prey or water), while its anterior end is free to bend ventrally relative to the hypobranchials when the hyobranchial apparatus is protracted during tongue projection (Reilly and Lauder, '89). It also becomes the foundation for several intrinsic tongue muscles and the radials used to flip the tongue pad.

#### *External vs. internal metamorphosis*

In most studies of salamander metamorphosis, the condition of the median fin, external gills, and gill slits is used to determine the metamorphic condition of specimens (e.g., Semlitsch and Wilbur, '89). Wilder ('25) commented that these characters "constitute the obvious and universally recognized evidences that metamorphosis is complete, and are, moreover, regarded by the majority of the investigators who have concerned themselves with the experimental study of urodele metamorphosis, as the only criterion of transformation." This statement holds true today except for a few studies which use the transformation of cranial and hyobranchial characters important in the feeding mechanism to judge the metamorphic condition of salamanders (Brandon and Bremer, '66; Duellman and Trueb, '86; Reilly, '86, '87; Reilly and Lauder, '88a).

Conspicuous changes in external morphology of the gills and tail fin comprise only a small part of the dynamic process of metamorphosis. If we examine the correlation of tail fin heights, gill length, and important internal changes in the hyobranchial apparatus (subarcualis rectus length, angle and insertion point) in "metamorphs" we find not only that internal and external changes are uncoupled (Table 4), but that gill length is not correlated with tail fin height. Thus, judging the metamorphic condition of salamanders solely on the basis of external features does not necessarily mean that the animals are fully larval or fully transformed internally.

For example, salamanders that have gill slits, external gills and a tail fin could be judged to have internal head morphology that is fully larval, when in fact they do. This is the case for any larval salamander which has not begun to metamorphose. This is also the case for neotenic salamanders in the family Ambystomatidae, which delay metamorphosis until after sexual maturity (Reilly, '87). On the other hand, salamanders with external gills and a tail fin could be judged to have fully larval internal head morphology when in fact they do not. This is the case in metamorphosing salamanders where internal changes precede the loss of external characters. This has been shown to be the case in neotenic salamanders in the family Salamandridae which have external gills and tail fins but are metamorphosed in all other respects except the retention of remnants of ceratobranchials 2-4 (Reilly, '86, '87). Similarly, in paedomorphic salamander families, reproducing animals are either mostly larval in head morphology or possess metamorphosed skulls and retain larval aspects of the posterior of the hyoid apparatus. Thus, presence of external larval characters might not coincide with internal change during metamorphosis and among different neotenic strategies.

Table 4. Correlations of tail fin and external gill morphology with aspects of internal hyobranchial structure that change at metamorphosis in midmetamorphic *Ambystoma tigrinum* ( $N = 28$ ). (Note that external morphology [1-3] is not correlated with metamorphic changes in the hyobranchial apparatus [4-7].)

	1	2	3	4	5	6
(2)	0.17	1.00				
(3)	0.34	0.29	1.00			
(4)	0.34	0.06	0.25	1.00		
(5)	0.23	0.03	-0.20	0.59	1.00	
(6)	0.05	-0.04	-0.18	0.62*	0.77*	1.00
(7)	0.08	-0.02	0.10	0.26	0.37	0.62*

<sup>1</sup>First gill length. <sup>2</sup>Tail fin height at pelvis. <sup>3</sup>Maximum tail fin height. <sup>4</sup>Ceratobranchial length. <sup>5</sup>Subarcualis rectus 1 length. <sup>6</sup>Subarcualis rectus 1 insertion point. <sup>7</sup>Subarcualis rectus 1 angle.

\* = Significant ( $P < 0.05$ ) Bonferroni adjusted correlation.

Salamander metamorphosis involves a complex series of parallel transformations that are not necessarily synchronous in different regions of the head and body. In judging the metamorphic condition of salamanders, it is important to check more than external characters, especially in the Salamandridae and paedomorphic families. Diagnostic changes that can be used (Fig. 8) involve structures that change position, appear or disappear. Based on changes in the three samples used in this study, the timing of loss and appearance of several features can be used to determine metamorphic condition. Ceratobranchials 2-4 are rapidly lost early in the metamorphic process. None of the metamorphs had these elements or any trace of them. A similar rapid loss of ceratobranchials 2-4 is seen in mid-metamorphic *Ambystoma talpoideum* and *Notophthalmus viridescens* (Reilly, '87). In fact, other than in neotenic salamandrids, we have never observed ceratobranchials 2-4 that were partially resorbed; they were either present or absent. Thus, the loss of these elements is a good indicator that internal morphological metamorphosis is underway. The muscles inserting onto the ceratobranchials (subarcualis rectus 4, subarcualis obliqui and branchiohyoideus) take much longer to disappear, and were present in all the metamorphs, and even lingered in two of the transformed animals. Thus, absence of these muscles is a good indicator of complete metamorphosis.

Less evident are the metamorphic transpositions that occur in various aspects of head morphology. While not necessarily diagnostic in nature, it is interesting that some morphometric characters may be affected by metamorphosis (Fig. 1B,D,G), while others are not (Fig. 1A,C,F). In *Ambystoma tigrinum*, most morphometric aspects of the skull do not change through metamorphosis, except skull length, which decreases significantly (Tables 1, 2). In addition, adjacent interconnected hyobranchial structures exhibit negative or positive transpositions during metamorphosis reflecting changes relating to the functional shift to terrestrial feeding (Fig. 8).

These results demonstrate that identification of the intricacies of metamorphic change and comparisons of metamorphic trajectories of morphological structures are necessary to understand metamorphic transitions in function and different developmental pathways exhibited by neotenic and paedomorphic salamanders.

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