

The ontogeny of functional design: metamorphosis of feeding behaviour in the tiger salamander (*Ambystoma tigrinum*)

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(Accepted 21 January 1988)

(With 2 plates and 6 figures in the text)

The ontogeny of feeding behaviour was studied quantitatively in the tiger salamander, *Ambystoma tigrinum*, to elucidate the relative importance of morphological and environmental changes on form and function. High-speed films of prey capture provided data for a frame-by-frame analysis of seven kinematic parameters of feeding behaviour. By comparing underwater feeding of larval and metamorphosed individuals, the effect of morphological changes occurring at metamorphosis on the feeding kinematic pattern was determined. By comparing metamorphosed animals feeding in the water and on land, changes in feeding kinematics associated with the environmental transition (and thus the differing physical properties of water and air) were determined. Both univariate and multivariate analyses failed to demonstrate any differences between larval and metamorphosed aquatic feedings for seven kinematic variables. However, when individuals feed on land, a radical shift in hyoid kinematics was observed. In addition, multivariate analysis showed that terrestrial feedings differed from aquatic feedings in having longer duration head movements. The lack of a kinematic difference between larval and metamorphosed individuals feeding in the water indicates that the morphological changes occurring at metamorphosis do not impose any obligatory kinematic consequences. Rather, metamorphosed *Ambystoma tigrinum* acquire the ability to modulate their kinematic pattern depending on the environment.

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Introduction

As animal form changes during phylogeny, there is often a corresponding change in function. The precision with which form maps to function is still a largely unresolved issue, and clarifying this question has been one of the primary goals of comparative functional studies over the last twenty years (e.g. Gans, 1969, 1974; Lauder, 1981, 1983; Liem & Wake, 1985). Recently, several studies have focused on functional and anatomical shifts during ontogeny to gain additional insights into the form-function relationship (e.g. Lakars & Herring, 1980; Gottfried, 1986; Herring & Wineski, 1986). While such studies lack the strict phylogenetic interpretations available in comparative analyses, they are also free of the problems inherent in evaluating the evolution of form among widely divergent taxa, and in determining the number of phylogenetically independent comparisons available within a clade (Felsenstein, 1985).

The transition from aquatic, free-living larvae to post-metamorphic terrestrial adults in amphibians represents one of the most complex ontogenetic transformations known among the vertebrates. This shift affects most aspects of the organism, involving fundamental changes in diet, locomotor mode and morphology, as well as the behavioural and performance shifts associated with the transition from aquatic to terrestrial life histories (Wilder, 1925; Noble, 1931; Duellman & Trueb, 1986). Unravelling the mechanisms by which these morphological, functional and physiological changes are accommodated within a single ontogeny remains both a major challenge to developmental and evolutionary biology, and a rich area to examine the form-function relationship during ontogeny.

The changes associated with amphibian metamorphosis are important in another respect: they constitute the best model available for the phylogenetic transitions involved in the invasion of land by primitive amphibians. The Late Devonian species that invaded terrestrial habitats are distantly related to modern amphibians, and are only similar to them in some extremely basic morphological and ecological respects (Romer, 1966; Bolt, 1969; Olson, 1971). However, since many modern amphibians undergo the transition from aquatic to terrestrial life histories ontogenetically, they may provide general clues as to the functional mechanisms involved in the phylogenetic invasion of land (Bramble & Wake, 1985). At the very least, amphibian metamorphosis provides us with the best *extant* system in which to examine morphological and functional patterns associated with a shift from aquatic to terrestrial life.

In this paper, we document the metamorphic changes that occur in the feeding behaviour of the tiger salamander, *Ambystoma tigrinum*. We chose this generalized salamander for several reasons. First, while it undergoes a major metamorphosis, movement patterns of different bones and muscles can still be homologized between larval and transformed individuals. This is not the case for anuran amphibians because of the extreme morphological reorganization that occurs at metamorphosis (Gilbert & Frieden, 1981; Wassersug & Hoff, 1982). Secondly, there already exist functional and morphological data on both larval (Shaffer & Lauder, 1985*a, b*; Lauder & Shaffer, 1985) and terrestrial (Larsen & Guthrie, 1975) feeding in this species, and these data serve as useful background information for this study. Thirdly, recent members of the *Ambystoma tigrinum* clade have evolved alternative metamorphic conditions several times (Shaffer, 1984*a, b*), suggesting that they have the genetic and physiological prerequisites for the loss and acquisition of metamorphosis. By quantifying the ways in which terrestriality *and* metamorphosis are achieved in this group, we may discern general processes that are important components of the evolution of terrestriality.

Our primary goal is to quantify the metamorphosis of feeding behaviour by examining the

kinematic changes that occur during the transition from a larval to a metamorphosed *morphology* and from an aquatic to terrestrial *habitat*. Generally, these two aspects of metamorphosis are by necessity confounded, since larval feeding performance must be assessed in an aquatic environment, while metamorphosed individuals are studied on land. However, because metamorphosed tiger salamanders will feed in the water (and do so in nature, Stebbins, 1951; Reese, 1969; Miller & Larsen, 1986, pers. obs.), we can take advantage of an experimental design that allows a separate assessment of the changes in feeding kinematics associated with shifts in morphology and environment. We can assess the effect of the morphological changes at metamorphosis by comparing prey capture of larval and metamorphosed aquatic individuals feeding in the water (the environment is held constant). Alternatively, by comparing metamorphosed aquatic and metamorphosed terrestrial individuals, we can assess the shifts in feeding behaviour associated purely with a change in environment (morphology is held constant). Our experimental design thus allows us to test rigorously the statement that 'the biophysical constraints associated with feeding in air and water influence the basic structure and performance of the tetrapod feeding mechanism' (Bramble & Wake, 1985: 230).

Materials and methods

Experimental animals

Kinematic data were obtained from 13 individual *Ambystoma tigrinum mavortium* collected near Limon, Lincoln Co., Colorado, USA. All individuals were collected as larvae, tested, and held singly in the lab. in 40 litre aquaria until metamorphosis occurred naturally. After metamorphosis, individuals were kept in shallow water (about 4 cm deep) in the same aquaria or were transferred into aquaria with moist paper towels. Salamanders were maintained on a diet of earthworms (*Lumbricus*) dusted with vitamins and bone meal; this diet was also used in all feeding trials. *Ambystoma tigrinum* is a generalized carnivore, and soft-bodied invertebrates form a major component of their natural diet, both on land and in water (Reese, 1969; Dodson & Dodson, 1971; Rose & Armentrout, 1976; Collins & Holomuzki, 1984; Miller & Larsen, 1986). Individuals were tested within 3 months of transformation to minimize size differences between larval and metamorphosed individuals. However, size independent shape changes do occur at metamorphosis, particularly in the head region (Latimer & Rooffe, 1964). No attempt was made to correct for these effects statistically, since they reflect part of the morphological component of metamorphosis. Mean morphological measurements (in cm) of the animals at the time of these experiments were: snout-vent length, larvae—9.9, metamorphosed—9.7; head width, larvae—2.7, metamorphosed—2.0; head length, larvae—3.4, metamorphosed—2.8.

Experimental design and statistical analysis

The primary goal of these analyses was to partition the total variance in the kinematic data into components attributable to variation within and among individuals and to variance among 'metamorphic stages'. In this paper, we refer to 3 stages. We use this term in a special sense, because strictly speaking we are not comparing only steps in a linear developmental sequence. The 3 stages are: (1) larvae, which feed in the water, (2) metamorphosed animals feeding in the water (referred to here as the metamorphosed-aquatic stage, and (3) metamorphosed animals feeding on land (referred to here as the metamorphosed-terrestrial stage). Thus, since stages include both comparisons of different morphologies in the same environment (larval vs. metamorphosed aquatic) and different environments for the same morphology (metamorphosed aquatic vs. metamorphosed terrestrial), among-stage variance can be decomposed into morphological and environmental components. As with any analysis of variance, this partitioning only targets the events associated with changes (or lack of changes) in feeding kinematics; further experimental manipulations are generally necessary to establish causal relationships.

This design has certain problems associated with the lack of independence among errors in the 3 stages, making a posteriori hypothesis testing of stage means formally invalid (Steele & Torrie, 1980). A solution to

TABLE I

Experimental design for longitudinal and cross-sectional kinematic data on prey capture in *Ambystoma tigrinum*. An 'x' indicates that data were obtained for that individual. Longitudinal data were unavailable at mid-metamorphosis for all individuals

Metamorphic stage	Individual									
	Longitudinal				Cross-sectional					
Larval	x	x	x	x	x	x				
Mid-metamorphosis	—	—	—	—			x	x	x	
Metamorphosed aquatic	x	x	x	x				x	x	
Metamorphosed terrestrial	x	x	x	—					x	x

this problem would be to include the fourth category of larvae feeding on land, and treat 'stage' as 2 factorial effects: environment (water or land) and metamorphosis (larval or transformed). Unfortunately, this is biologically impossible, since larvae do not feed on land. Therefore, we treat stage as an ordered single category (ordered in the sense that we expect larvae and metamorphosed terrestrial stages to be most different), and are cautious in our interpretation of differences among means.

Two different experimental designs were analysed as separate analyses of variance; they are depicted schematically in Table I. In the *longitudinal* design (Cock, 1966), 4 individuals were filmed as larvae feeding in the water and as fully metamorphosed individuals both in water and on land (Table I). Since each individual was filmed under all conditions, this was treated as a 2-way factorial analysis of variance (ANOVA) (Sokal & Rohlf, 1981). We considered metamorphic stage to be a fixed effect and individual to be a random effect. Therefore, this is a mixed model ANOVA; in constructing F-ratios for significance testing, stage was tested over interaction, while individual and stage x individual interaction were tested over the error mean square (Sokal & Rohlf, 1981). We used the Statistical Analysis System (SAS) GLM (General Linear Model) procedure to generate Type IV sums of squares since this is a mildly unbalanced design (Freund & Littell, 1981; SAS, 1982).

The *cross-sectional* design (Cock, 1966) consisted of 9 individuals that were each measured for 1 of the 3 metamorphic stages (Table I). In these experiments, we were able to collect some data on mid-metamorphic individuals, defined as salamanders that have partially resorbed their external gills and tail fin, but still have open gill slits (Table I). This design provides additional information on variances among individuals and stages, but *cannot* provide insights on individual x stage interactions, since each individual was tested in only one stage. It was analysed as a 3-level nested ANOVA with among-stage, among-individual and within-individual levels (Sokal & Rohlf, 1981). We computed variance components for all 3 levels, even though stage is a fixed effect. We followed this rather unusual strategy since we examined *all* biologically possible levels of the stage main effect. Thus, rather than being only interested in the mean differences among our prescribed experimental treatments, as is usually the case for a fixed effect, we felt that quantifying the proportion of the total variance found among *all* stages was an important issue.

In both designs, at least 10 feeding trials were filmed on one day for each individual to document the inherent variability within and among individuals, which previous work has indicated may be large (Shaffer & Lauder, 1985a; Lauder & Shaffer, 1985).

We also performed a principal component analysis to provide a multivariate description of the longitudinal data set. The analysis factored the correlation matrix of 7 variables for 113 individual feeding trials. Three principal components were extracted, and factor scores and plots of all pairwise combinations of components were examined for multivariate patterns of dispersion.

Filming procedures

Our filming procedures followed those in Shaffer & Lauder (1985a). Animals were trained to feed under bright lights (1,200 watts) and filmed with a Photosonics 16-1PL camera using Kodak 4X Reversal film at 200

frames per second. In the longitudinal study, salamanders were allowed to undergo normal metamorphosis. Aquatic feedings were conducted in the same tanks in 15 cm of water. Terrestrial feedings were accomplished by gently moving a piece of earthworm held at the end of a long pair of forceps. All feeding sequences except those where the animal contacted the forceps or moved out of the horizontal plane while feeding were analysed.

Variables measured

Films were projected frame by frame on to a Houston Instruments Hipad digitizer (50 μm accuracy), and distances and angles were measured directly. Representative feeding sequences are shown in Plates I & II. From every frame on each feeding 3 measurements were made. (1) *Head angle* reflects the elevation of the head on the vertebral column; 3 points were used to define the angle, one above the eye, one at the midpoint of the angle of the head with the trunk, and one several centimetres posterior to the head along the body. (2) *Hyoid distance* is measured from the lower margin of the eye to the ventral-most extension of the throat (see Plates I & II). (3) *Gape distance* is the linear distance between the anterior tips of the upper and lower jaws.

For each feeding, these 3 measurements were plotted against frame number, representing time, and the maximum values of head angle, gape distance and hyoid distance were measured as the peaks of each curve. Times to reach maximum were calculated from the start of mouth opening (measured in frames, and converted to milliseconds), while the total gape cycle time was measured as the time from the start of mouth opening until the gape distance returned to baseline level. Thus, a total of 7 variables were extracted from each feeding (abbreviations used here are those used throughout the paper and in the tables): (1) *Headang*, the maximum angle (in degrees) of cranial elevation; (2) *Gapedist*, the maximum gape (in cm) reached during a feeding; (3) *Hyoidist*, the maximum ventral excursion (in cm) of the hyoid during a feeding; (4) *Tpkhead*, the time (in milliseconds) from the start of mouth opening until peak head angle is reached; (5) *Tpkgape*, the time (in milliseconds) from the start of mouth opening until peak gape is reached; (6) *Gapecy*, the total gape cycle time (in milliseconds) from the start of mouth opening until the mouth has closed again; and (7) *Tpkhyoid*, the time (in milliseconds) from the start of mouth opening until peak hyoid excursion is reached.

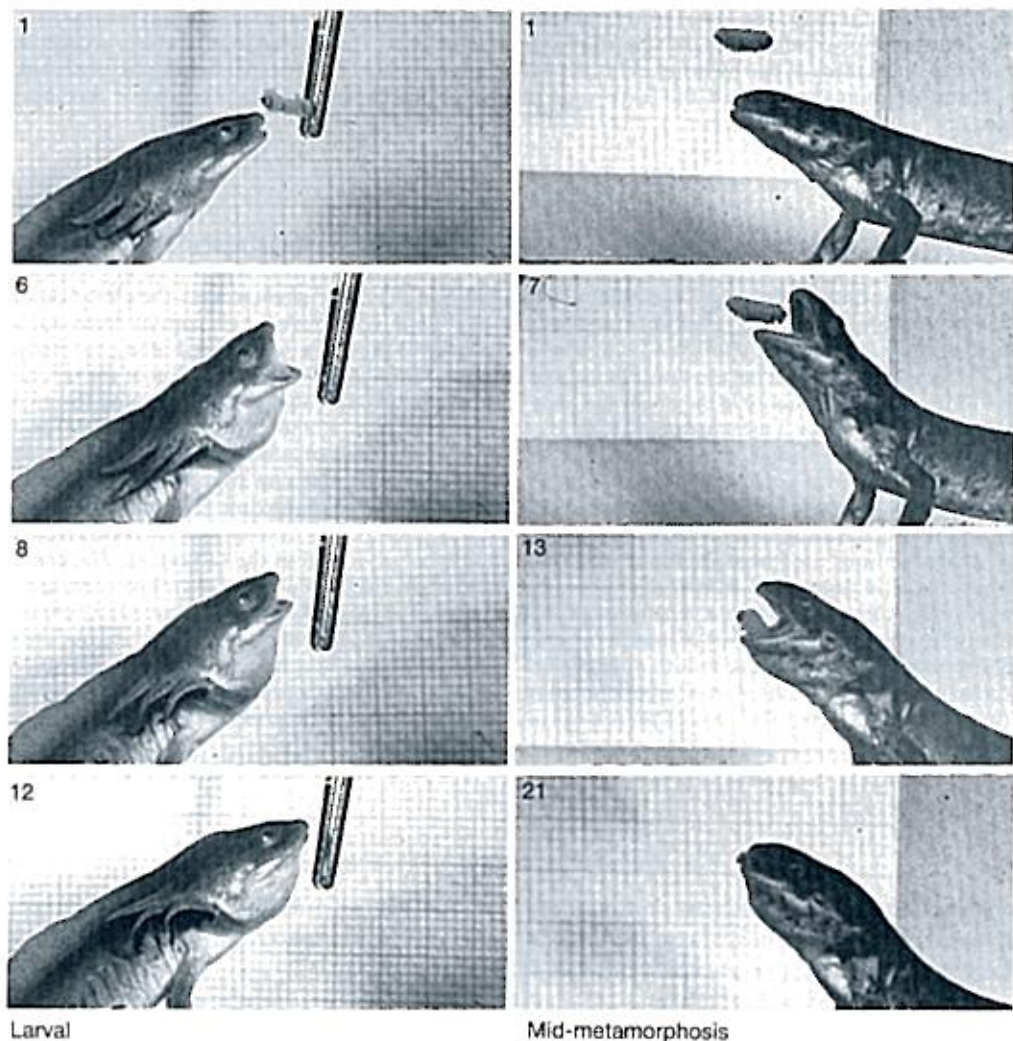
Results

Variance among metamorphic stages

Both the longitudinal and cross-sectional data sets provide information on differences among larval and transformed individuals feeding on land and in the water. In addition, the cross-sectional design includes an aquatic, mid-metamorphic stage.

The overwhelming conclusion from both analyses is that, for most variables, there is no effect of metamorphic stage on kinematic pattern (Table II, Fig. 1). For both the longitudinal (Table III) and cross-sectional (Table IV) data, there is no significant effect on head angle, time to peak head angle, time to peak hyoid depression, time to peak gape and gape cycle time. This is not simply a function of the low resolving power of these tests, since in all cases the associated F-ratios were very small (Tables III and IV). Rather, it reflects true similarity in kinematic values among stages. This similarity is further strengthened by examination of the mean values for each stage (Table II, Fig. 1). There is generally a high concordance of variable means from these analyses of different sets of individuals, further confirming their similarity.

Hyoid distance and gape distance showed the largest among-stage variances in both analyses. For gape distance, the significant effect in the longitudinal analysis is due to the high value of the metamorphosed aquatic stage ($\bar{x} = 1.4$ cm). Both the larval and metamorphosed terrestrial stages had identical means of 1.1 cm (Table II, Fig. 1). However, this pattern is not reflected in the cross-sectional analysis, where only the mid-metamorphic value differs from the other three (Table II, Fig. 1).

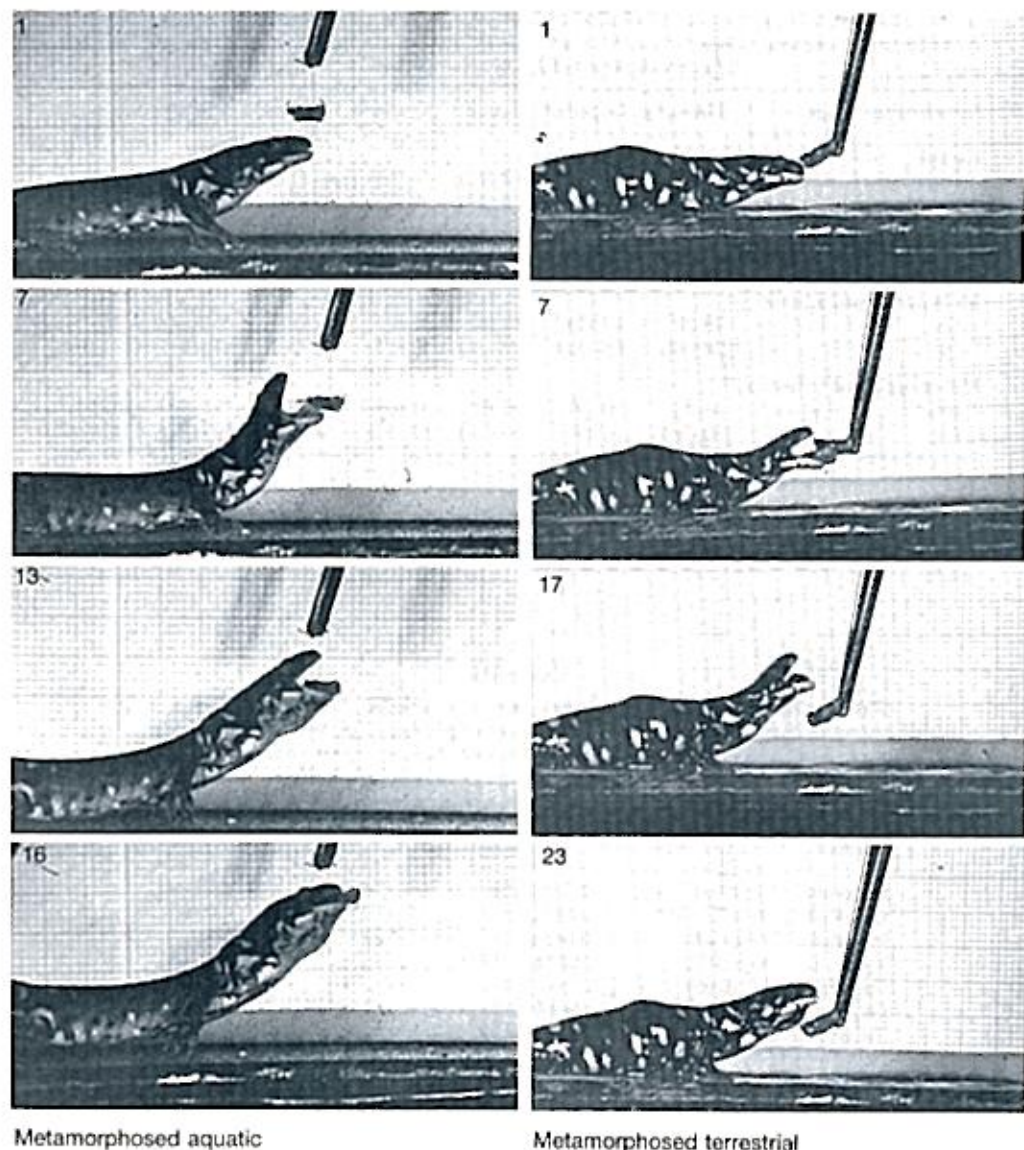


PLATES I and II. Four representative feeding sequences from the same individual tiger salamander at different ontogenetic stages. PLATE I. Film frames from larval and mid-metamorphic *Ambystoma tigrinum* feeding in the water. The time between sequential frames is 5 milliseconds. In both cases, a small piece of earthworm (*Lumbricus*) has been captured. Note the extent of hyoid depression in both feeding sequences, reaching its maximum at frame 8 in the larval sequence, and near frame 13 in the mid-metamorphic sequence.

Hyoid distance is the only variable showing unambiguous variation among stages. In both analyses, the larval and metamorphosed aquatic stages had mean values two to four times that of the metamorphosed terrestrial animals.

Variance among and within individuals

Although among-trial variation was a major component of variance (Tables III and IV), differences among individuals were generally large and significant. This effect is especially



Metamorphosed aquatic

Metamorphosed terrestrial

PLATE II. Film frames from metamorphosed *Ambystoma tigrinum* capturing a piece of earthworm. The sequence on the left was filmed underwater, and represents the metamorphosed aquatic 'stage'. Note that in frame 7 of the aquatic sequence, the tongue is contacting the earthworm and has been slightly projected above the lower jaw in lateral view. The sequence on the right represents terrestrial feeding. Note: (1) that the hyoid and throat region, visible below the lower jaw in frame 1, has moved *dorsally* by frame 7, and then moves *ventrally* and is visible again in frames 17 and 23; (2) that the tongue is projected out of the mouth toward the prey and contacts it in frame 7.

TABLE II

Means (sample size) for seven kinematic variables measured from high-speed films of prey capture by *Ambystoma tigrinum*. Statistics are presented separately for cross-sectional (cs) and longitudinal (l) data sets at each stage. Only cross-sectional data are available for the mid-metamorphic stage. Values for Headang are in degrees, values for Gapedist and Hyoidist are in centimetres, and values for Tpkhead, Tpkgape, Gapecy and Tpkhyoid are in milliseconds

Metamorphic stage	Headang	Gapedist	Hyoidist	Tpkhead	Tpkgape	Gapecy	Tpkhyoid
Larval							
(cs)	29 (13)	1.2 (13)	0.8 (13)	40 (13)	41 (13)	82 (12)	39 (13)
(l)	31 (32)	1.1 (32)	0.8 (32)	39 (32)	38 (32)	83 (29)	40 (32)
Mid-metamorphosis							
(cs)	26 (14)	0.8 (15)	0.6 (15)	30 (14)	39 (15)	99 (14)	39 (15)
Metamorphosed aquatic							
(cs)	19 (8)	1.1 (8)	0.7 (8)	31 (8)	32 (8)	61 (8)	35 (8)
(l)	28 (32)	1.4 (32)	0.7 (32)	33 (32)	36 (32)	75 (31)	42 (32)
Metamorphosed terrestrial							
(cs)	31 (9)	1.1 (9)	0.2 (9)	51 (9)	52 (9)	91 (9)	58 (9)
(l)	30 (38)	1.1 (39)	0.3 (38)	63 (38)	58 (39)	107 (39)	87 (38)

TABLE III

Two-way analysis of variance for seven kinematic variables (longitudinal data set) measured from *Ambystoma tigrinum* for three stages: larval, metamorphosed feeding in the water, and metamorphosed feeding on land. Table entries are mean squares (F value). Degrees of freedom are given below each effect

Variable	Metamorphic stage ($d.f. = 2$)	Individual ($d.f. = 3$)	Stage \times individual ($d.f. = 6$)	Error ($d.f. = 87-91$)
Headang	0.18 (0.001)	273.8 (3.2)	166.4 (2.0)	84.6
Gapedist	0.624 (11.5)**	0.084 (3.5)*	0.054 (2.2)	0.024
Hyoidist	0.082 (3.3)	0.062 (4.6)**	0.025 (1.9)	0.013
Tpkhead	263.5 (0.7)	1087.8 (5.3)**	358.1 (1.7)	205.5
Tpkgape	13.1 (0.05)	335.6 (1.1)	267.8 (0.9)	300.7
Gapecy	218.8 (0.12)	1364.9 (2.1)	1828.3 (2.8)*	645.7
Tpkhyoid	4.5 (0.01)	1934.6 (4.5)**	707.2 (1.6)	429.5

*Significant at $P=0.05$ level; ** $P=0.01$

pronounced for the cross-sectional data, where differences among individual means within stages accounted for between 16% and 47% of the total variation, and were always statistically significant. In the longitudinal design, the individual effect is less pronounced, at least in part because the individual by stage interaction is partitioned out of the individual component. However, the individual component was still large and significant in most cases, while the interaction term was not significant for six of seven variables.

TABLE IV

*Nested analysis of variance for seven kinematic variables (cross-sectional data set) measured from *Ambystoma tigrinum* for four stages: larval, mid-metamorphosis, metamorphosed aquatic, and metamorphosed terrestrial. Table entries are mean squares followed in parenthesis by the associated variance components (%). Degrees of freedom are given below each level*

Variables	Among stages (<i>d.f.</i> = 3)	Among individuals within stages (<i>d.f.</i> = 5)	Within individuals (<i>d.f.</i> = 34-36)
Headang	260.9 (0)	393.9 (40**)	94.1 (60)
Gapedist	0.335 (30)	0.109 (24*)	0.031 (46)
Hyoidist	0.815 (65*)	0.095 (16**)	0.019 (19)
Tpkhead	991.6 (28)	336.6 (24*)	99.6 (48)
Tpkgape	590.2 (0)	553.5 (45**)	112.9 (55)
Gapecy	2625.8 (5)	2044.2 (45**)	401.7 (50)
Tpkhyoid	1008.7 (2)	861.5 (47**)	157.7 (51)

*Significant at $P=0.05$ level; ** $P=0.01$

Multivariate analyses

The results of a principal component analysis for 113 feeding trials in the longitudinal design are presented graphically as a plot of the first two principal components (Fig. 2). Overlap between metamorphosed aquatic and larval feeding trials is high, while that between metamorphosed terrestrial and the two aquatic stages is relatively low. This pattern holds in addition to the differences among individuals in principal component scores (Fig. 2), although the unequal numbers of observations on different individuals precludes any formal statistical hypothesis testing.

An examination of the individual variable loadings suggests that the first component is strongly determined by the four timing variables and by hyoid distance (Table V). The high loadings of all four timing variables is not surprising, since they are strongly intercorrelated (correlation coefficients range from 0.68 to 0.78). However, the emergence of timing of the feeding events as a factor differentiating metamorphosed terrestrial feeding was not apparent in the univariate analyses, and is a unique feature of the multivariate analysis.

Movement patterns in different stages

While the quantitative analyses just described provide a powerful evaluation of several key variables, they do not describe all of the important differences between stages since they summarize only maximal excursions and timings. For a more complete view of stage effects, we averaged all the feeding trials for the three individuals with complete longitudinal data, and present the average curves for gape distance (Fig. 3), head lift (Fig. 4) and hyoid distance (Fig. 5). Since these curves represent only longitudinal data, each individual is equally represented in all stages, providing some control for variation among individuals.

In general, the curves for the aquatic larval and aquatic metamorphic feedings are very similar. For both hyoid depression (Fig. 5) and head angle (Fig. 4), total excursion and the timing of maximal excursion are virtually identical. The curves are offset throughout their lengths, presumably reflecting morphological changes in head dimensions occurring at metamorphosis.

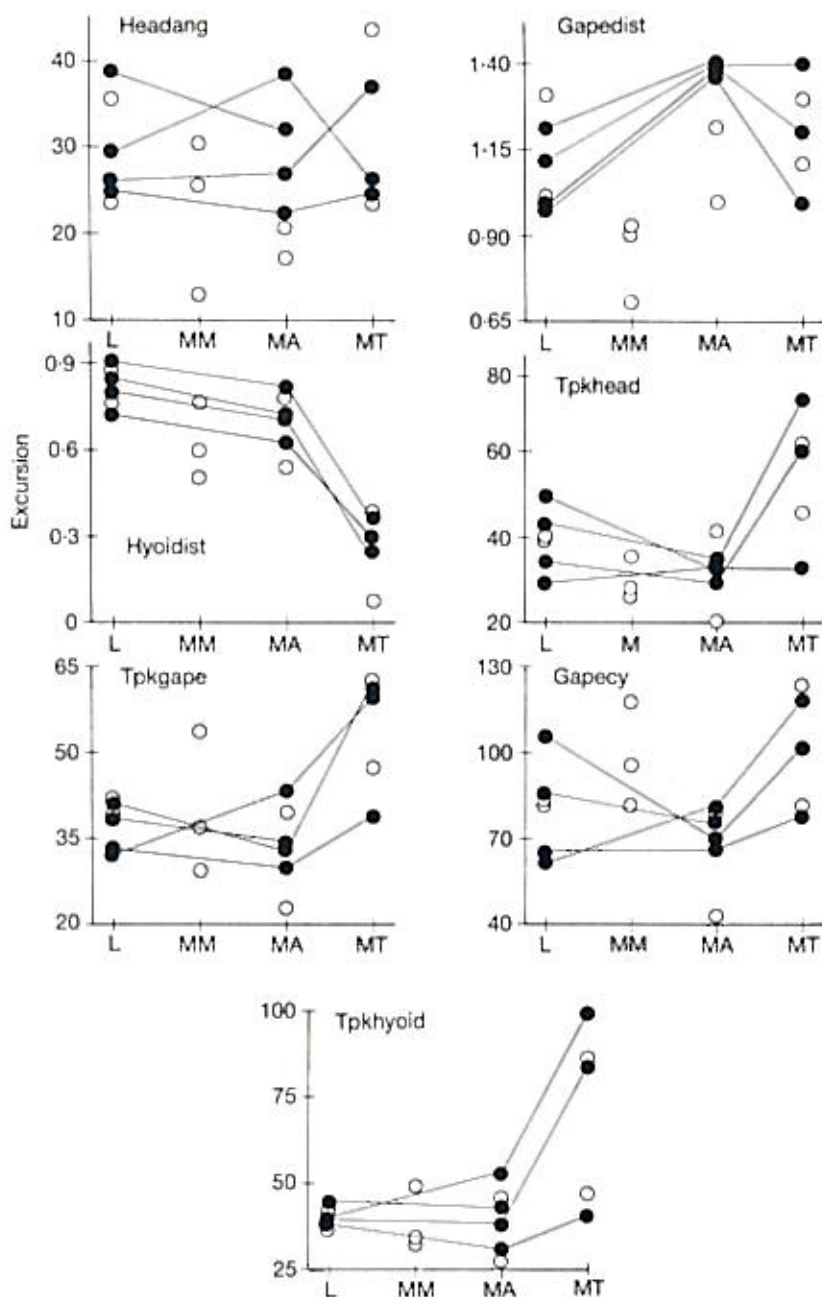


FIG. 1. Plots of individual means for longitudinal (solid black circles) and cross-sectional (open circles) data for four metamorphic stages. Longitudinal values for the same individual are connected with lines; mid-metamorphic values are only available for cross-sectional individuals. The y-axis is scaled in degrees for Headang, in centimetres for Gapedist and Hyoidist, and in milliseconds for Tpkhead, Tpkgape, Gapecy and Tpkhyoid. See text for a detailed description of these variables. L=larval stage, MM=mid-metamorphic stage, MA=metamorphosed aquatic stage, and MT=metamorphosed terrestrial stage.

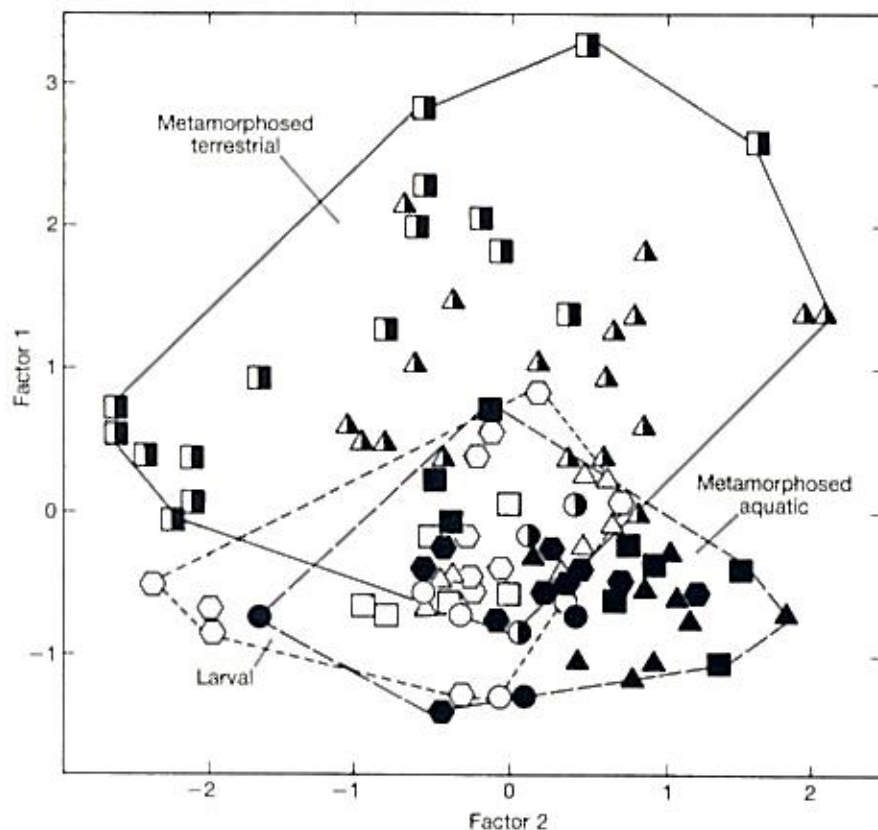


FIG. 2. Plot of principal component 1 (Factor 1) versus principal component 2 (Factor 2) for the seven kinematic variables from 113 feedings in 4 individuals. Metamorphosed aquatic feedings are solid black symbols, larval are open symbols, and metamorphosed terrestrial are half-shaded; the four individuals are represented by circles, squares, triangles and hexagons. Individual variation is always present. Additionally, aquatic feedings are more similar to each other than either is to terrestrial feedings.

TABLE V

Variable loadings and proportion of the total variance explained by the first three factors of a principal components analysis on three individuals with complete longitudinal data

Variable	Factor 1	Factor 2	Factor 3
Tpkhyoid	0.93	0.09	0.05
Tpkhead	0.89	-0.12	0.12
Gapecy	0.86	0.14	0.04
Tpkgape	0.83	0.25	0.21
Hyoidist	-0.60	0.22	0.22
Headang	0.00	-0.81	0.58
Gapedist	-0.32	0.52	0.72
Variance explained (%)	51	15	14

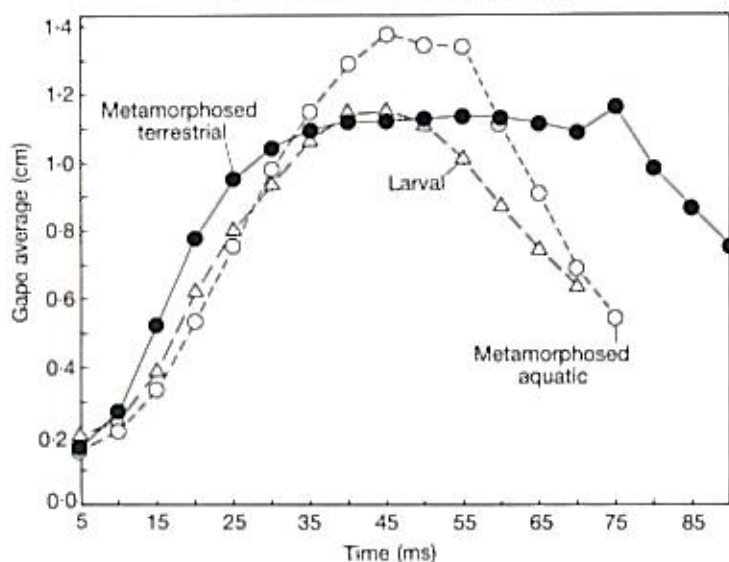


FIG. 3. Average kinematic pattern (from 3 individuals, 15 feedings) of gape distance (cm) versus time. Note the close similarity of the three gape profiles during the first 35 ms. Terrestrial feedings have a more extended period with the mouth open and a delayed closing relative to aquatic feedings of both larval and metamorphosed individuals. These average gape profiles do not return to the original starting distance because the jaws typically close on the prey.

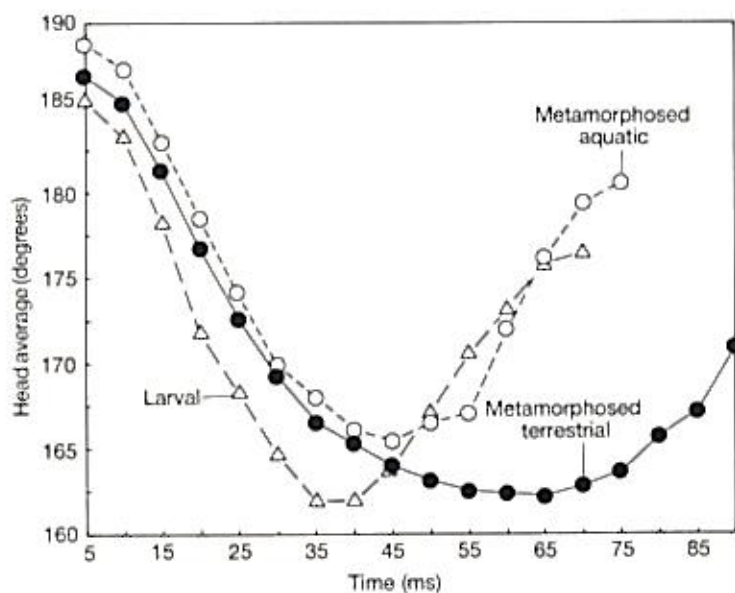


FIG. 4. Average kinematic pattern (from the same feedings as Fig. 3) for head angle (degrees) versus time. Again, the aquatic feedings are very similar, while terrestrial feedings are more prolonged. Note that a decrease in head angle indicates dorsal bending of the head on the vertebral column.

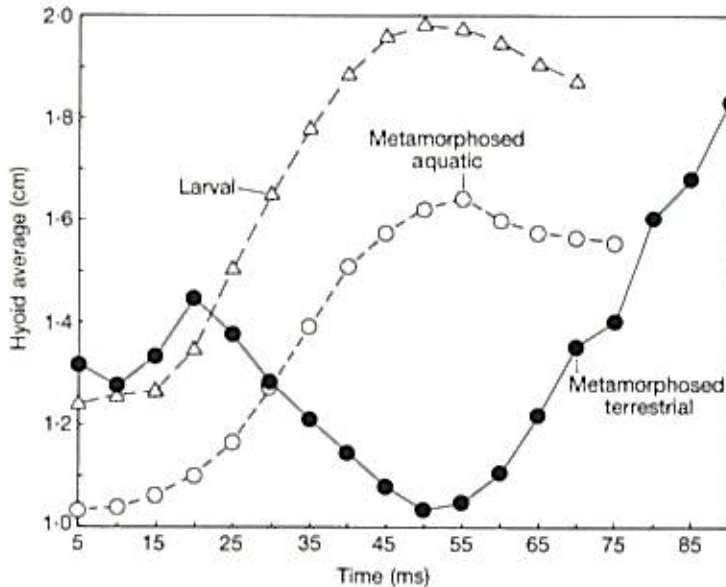


FIG. 5. Average kinematic pattern (from the same feedings as Fig. 3) for hyoid depression distance (cm) versus time. Note the close similarity in kinematic profile of the aquatic feedings, but the distinct hyoid profile used in terrestrial feedings. When feeding on land, *Ambystoma tigrinum* first elevates the hyoid apparatus, then depresses it ventrally. Peak elevation occurs at 50 ms, the time when the hyoid is maximally depressed in aquatic feedings.

As the ANOVAs suggest, when metamorphosed salamanders are fed on land, gape distance and head lift patterns remain similar to those in the water for the first half of the feeding cycle. However, the time to maximum excursion is considerably longer on land (Figs 3 and 4).

The hyoid shows a radically different movement pattern in the terrestrial stage, and some of these differences are not represented in the ANOVAs. The most striking difference is the incorporation of an entirely new movement pattern at the beginning of the strike (Fig. 5). Rather than the rapid and continuous increase in hyoid abduction seen in the water, the first hyoid movement is a short, rapid depression ending about 20 milliseconds after the initiation of feeding. The hyoid is then retracted far into the throat, such that maximal adduction coincides with maximal depression in the aquatic phases (Fig. 5: 50 ms time on the x-axis). During the time the hyoid is being slowly elevated in aquatic feedings, it is being depressed in the terrestrial feedings (Fig. 5: 50–85 ms). As in the other movement patterns, the recovery phase is longer for terrestrial individuals.

In Fig. 6, we show the coefficients of variation (CVs) for the data in Figs 3 and 5. All the CVs for the gape profiles reach a minimum 35 ms after the start of movement, and the pattern of variation among the gape profiles is remarkably consistent among stages. Relatively high variation occurs at the start and end of each gape profile. In contrast, the hyoid CVs are remarkably constant throughout the feeding cycle. Thus, in contrast to the average profiles, there is little difference among stages in the temporal profiles of kinematic CVs; differences in mean values cannot be attributed to different patterns of variability. On the contrary, the CVs are so low and consistent, especially for the hyoid, that a very precise modulation of movement pattern is suggested.

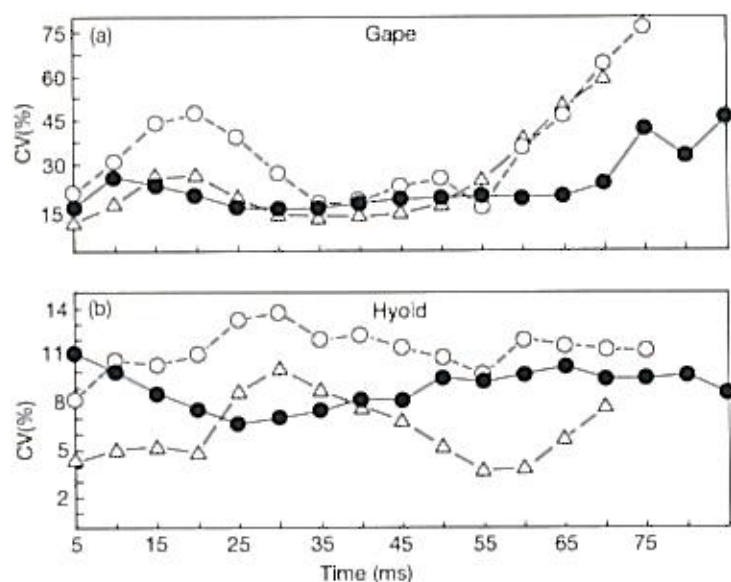


FIG. 6. Coefficients of variation (CV) in percent for the average gape profiles (a) and hyoid profiles (b) presented in Figs 3 and 5. Triangles indicate larval feedings, open circles indicate the metamorphosed aquatic stage, and solid circles the metamorphosed terrestrial stage.

Discussion

The ontogeny of functional design

The environmental and morphological shifts that occur across amphibian metamorphosis are among the most dramatic seen within vertebrates. The morphological changes associated with salamander metamorphosis include a major reorganization of the skull bones and musculature, and a complete restructuring of the hyoid cartilages (Wilder, 1925; Piatt, 1938; Regal, 1966; Ozeti & Wake, 1969; Bonebrake & Brandon, 1971; Reilly, 1986, 1987; Lauder & Shaffer, 1988). Furthermore, the environmental shift from water to air has obvious physical implications for organisms using suction feeding, and the inability to suction feed on land has been implicated as a major factor in the evolution of amphibious organisms (Ozeti & Wake, 1969; Bramble & Wake, 1985). Our experimental design allows a clear partitioning of variation in feeding performance, as reflected in kinematic patterns, into components attributable to morphological and to environmental shifts. In the discussion that follows, it is important to remember that a stage effect may represent a kinematic change associated with a shift in morphology or environment, or both; a posteriori examinations of differences among stage means are necessary to distinguish between these possibilities.

A strong, and unexpected, result of this study is that most kinematic variables we examined do not change from aquatic larval to metamorphosed aquatic feeding (Tables II, III, IV; Fig. 1). This holds for five of seven kinematic variables; only maximum hyoid depression and maximum gape distance showed univariate heterogeneity among stages. This result is surprising for two reasons. First, previous work on non-transforming ambystomatids (Shaffer & Lauder, 1985a, b)

demonstrated high levels of kinematic variation among three morphologically distinct species. While the extent to which this variation is genetically versus environmentally determined remains to be ascertained, these results suggest that considerable flexibility in kinematic patterns exists both among conspecific individuals and among taxa. However, the results reported here indicate that similar developmental flexibility among stages within a single ontogeny is constrained, and that this constraint is not broken at metamorphosis.

Secondly, previous work on buccal pressure recordings and feeding performance (in the sense of Arnold, 1983) from larval and metamorphosed aquatic tiger salamanders indicates that larval salamanders are more effective aquatic suction feeders than transformed individuals (Lauder & Shaffer, 1986). Larvae generate greater negative buccal pressures than transformed aquatic salamanders, and they are better able to capture elusive prey (Lauder & Shaffer, 1986). The kinematic data reported here suggest that such performance differences are achieved using very similar movement patterns during the feeding cycle, including similar timing of events. Additional electromyographic data on the same individuals also demonstrate similar muscle activity patterns among these aquatic stages (Lauder & Shaffer, 1988). Since kinematic and muscle activity patterns are the same in larvae and aquatic metamorphosed feedings, the reduced performance of transformed animals in the water *must* be a function of the actual anatomical changes that occur at metamorphosis. Exactly which changes are most important are not known, although the drastic reduction in relative head size of transformed animals and the shift from unidirectional to bidirectional flow patterns (Lauder & Shaffer, 1986; Lauder & Reilly, 1988), both probably contribute to this decrease in aquatic feeding performance.

The two variables which do show variation among stages are maximum hyoid depression and maximum gape distance (Tables III and IV, Fig. 1). For the longitudinal data, where gape distance differs the most among stages, larval and metamorphosed terrestrial individuals are identical, while metamorphosed aquatic salamanders have a larger gape. This unexpected result is not supported by the cross-sectional analysis (Table IV, Fig. 1), which shows all three as virtually identical. Our interpretation of these results is that gape distance is probably not significantly different among the three primary stages, although the longitudinal results indicate that some metamorphosed individuals feeding in the water may attempt to compensate for reduced buccal volume.

Hyoid distance, however, shows a clear pattern of differentiation in both analyses; metamorphosed terrestrial individuals have lower values than larval or aquatic metamorphosed ones. This suggests that metamorphosed salamanders are capable of altering the movement patterns of their hyoid depending on the environment. In the water, metamorphosed *Ambystoma tigrinum* are kinematically very similar to larvae (Fig. 1), even when all variables are viewed together in a multivariate context (Fig. 2). However, on land, individuals switch to lingual (tongue projection) feeding typical of other generalized urodeles (Regal, 1966; Ozeti & Wake, 1969; Larsen & Guthrie, 1975; Lombard & Wake, 1977; Bramble & Wake, 1985; also see Plates I & II). This suggests: (1) that the hyoid is the main functional unit involved in the ontogenetic shift in feeding; and (2) that the shift from aquatic suction to terrestrial tongue protrusion is not a consequence only of changes in morphology *per se*, but that significant environment input is also present. Obviously, some of the anatomical changes involved in metamorphosis (such as the acquisition of a fleshy tongue and associated musculature) must accompany the shift in feeding mode. However, these changes in muscle and bone architecture do not carry with them a *necessary* associated change in function, since metamorphosed individuals can modulate hyoid kinematics depending on the environment. Metamorphosis confers an increased *flexibility* in behavioural repertoire: a metamorphosed

salamander can utilize both feeding modes, depending on its environmental context, while a larva can only use suction feeding.

Finally, we note that the differences seen in the timing of the feeding cycle between metamorphosed individuals feeding in the water and on land is in the opposite direction to that which would be expected on the basis of the greater density of water alone. That is, completion of terrestrial feedings takes longer than aquatic sequences, while the time to maximum gape is roughly the same in both environments. This suggests that while the environmental context has an important influence on feeding kinematics, differences in prey capture are *not* purely a function of the biophysical differences involved with feeding in water and on land.

The evolution of terrestriality

A major conclusion of this study is that urodele metamorphosis is not accompanied by a complete reorganization of the feeding system; many aspects of larval feeding kinematics are retained after metamorphosis. Rather than acquire new kinematics, metamorphosed salamanders retain their larval movement patterns (when feeding in the water), and *add* new patterns when feeding on land to effect tongue protrusion. This observation is similar to the proposal of Bramble & Wake (1985: 232) for aquatic and terrestrial feeding in turtles, who note that, 'the same stereotyped sequence of kinematic (and probably motor) events can effect food transport in both air and water'.

By retaining the aquatic pattern and adding one or a few new behaviours when feeding terrestrially, maximum flexibility is allowed in the metamorphosed condition, since transformed animals are functional in both environments. Such flexibility, if present primitively in amphibians, may have been an important component of the invasion of land. Unfortunately, we lack the comparative data base for modern taxa to determine how primitive the ontogenetic addition of morphological and functional novelty may be. However, if this pattern is found in other urodeles, and in semi-terrestrial fishes, it may provide a general mechanism by which new habitats can be utilized without a loss of function in the ancestral environment.

We thank Steve Reilly, Peter Wainwright, Rich Lenski, Eric Findeis and Willy Bemis for enlightening comments on the manuscript and discussions on salamander feeding biology. A particularly careful statistical reviewer helped clarify the statistical presentation. Eric Findeis collected the digitized data from the many film frames and without him and the computer programming of Cathy Smither, this project would never have been completed. This research was supported by NSF BSR 85-19211 to HBS and NSF PCM 81-21649 and NSF DCB 86-02606 to GVL.

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