

FUNCTIONAL DESIGN OF THE FEEDING MECHANISM IN SALAMANDERS: CAUSAL BASES OF ONTOGENETIC CHANGES IN FUNCTION

BY GEORGE V. LAUDER AND STEPHEN M. REILLY

School of Biological Sciences, University of California, Irvine, CA 92717, USA

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SUMMARY

Many correlated changes in the structure and function of the skull occur during metamorphosis in salamanders. To separate correlated from causal changes in form and function, we experimentally converted the unidirectional flow feeding system in axolotls (*Ambystoma mexicanum*) to a bidirectional system by suturing closed the gill slits. This mimics one of the major structural changes in the feeding mechanism that occur at metamorphosis. Eleven variables were measured from the intra-oral pressure traces recorded during feeding. The major changes in buccal pressure produced by suturing the gill slits were in the duration and area of the negative pressure traces. In addition, the ratio of positive to negative pressure areas increased by four times following closure of the gill slits. In contrast to pressure changes during metamorphosis in tiger salamanders, increases in the positive portion of the pressure traces occur in axolotls. These data corroborate the hypothesis that the decreased feeding performance after metamorphosis is a direct consequence of the change from a unidirectional to a bidirectional feeding mechanism, and demonstrate the causes of ontogenetic changes in function.

INTRODUCTION

A fundamental problem in studies of animal form and function is separating correlated changes from causal relationships. This is a special problem in analyses of ontogeny as many changes in morphology and physiology occur over a relatively short time span; it is difficult to identify specific morphological causes of functional changes during ontogeny. A good example within vertebrates is the process of cranial metamorphosis in urodeles. Morphological changes in the structure of the skull take place over a 2- to 3-week period, and many concomitant physiological and functional transformations also occur (Duellman & Trueb, 1986).

Some of the key morphological changes in the skull occurring during the metamorphosis of urodeles from an aquatic larva to an adult are: a reduction of buccal volume, a reduction in cranial muscle mass, a loss or alteration of many components of the hyobranchial skeleton, and the transformation from a unidirectional to a bidirectional hydrodynamic flow design (Bonebrake & Brandon, 1971;

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Latimer & Roofe, 1964; Lauder & Shaffer, 1986; Reilly, 1986, 1987; Smith, 1920). This last change involves closing the posterior gill flaps at metamorphosis, so that when an adult salamander feeds in the water, no fluid exits from the head posteriorly. In contrast, larval individuals possess open gill slits posteriorly so that water entering the mouth during prey capture travels unidirectionally through the mouth cavity and exits posteriorly through these gill slits (essentially the same feeding system as that of ray-finned fishes).

Lauder & Shaffer (1986) studied correlated changes in both form and function throughout metamorphosis in the tiger salamander *Ambystoma tigrinum*, and showed that there is a major decrease in aquatic feeding performance after metamorphosis. This has also been shown for *Notophthalmus viridescens* (Reilly & Lauder, 1988). Metamorphosed salamanders feeding in the water are significantly less able to capture elusive prey than are larvae. This decrease in feeding ability was correlated with alterations in the buccal pressure profile. Metamorphosed tiger salamanders generated significantly less pressure than larvae and this resulted in a decrease in the volume of water drawn into the buccal cavity per unit time (Lauder & Shaffer, 1986). However, Lauder & Shaffer (1986) were not able to say which of the many metamorphic morphological changes in the musculoskeletal system of the head were *causally* related to the changes in buccal pressure profiles. Yet, determining such causal relationships is essential to understanding form–function relationships and the bases of physiological transformations during ontogeny.

The purpose of this paper is to describe the results of experiments designed to dissect the network of correlated morphological and functional alterations in the skull of salamanders at metamorphosis. Our approach was to manipulate experimentally the major ontogenetic transformation in skull morphology, the transition from a unidirectional to a bidirectional feeding system, and assess the effect of changing this parameter *alone* on buccal pressure profiles and feeding performance. We experimentally converted axolotls (*Ambystoma mexicanum*) from a unidirectional feeding system to a bidirectional feeding system (thus mimicking the metamorphic change in feeding design) by suturing the gular membrane and gill slits. By comparing buccal pressure profiles and feeding performance before and after the closing of the gill slits, we were able to estimate the extent to which the alteration in hydrodynamic flow design *alone* is responsible for ontogenetic changes in pressure patterns and feeding performance.

MATERIALS AND METHODS

Experimental animals

Six axolotls (*Ambystoma mexicanum*) of similar size (mean snout–vent length = 11.0 cm, S.D. = 0.9) were isolated individually in 40-l glass aquaria at 20°C. All individuals were fed a maintenance diet of live earthworms (*Lumbricus*). Axolotls were chosen for these experiments because of their large size and their tolerance of experimental manipulation demonstrated by previous research (Lauder & Shaffer,

1985). During the experimental trials in which buccal pressures were measured, axolotls were fed earthworm pieces (about 1 cm long) held at the end of forceps. This feeding protocol minimized the variance in pressure due to differences in prey orientation and provided a uniform feeding stimulus as in previous research (Lauder & Shaffer, 1985). During the feeding performance trials, an elusive prey (live guppies, *Poecilia*, about 2 cm long) was used.

Experimental techniques

Buccal pressure recordings were made using similar procedures to those of Lauder & Shaffer (1986). Briefly, salamanders were anaesthetized using buffered tricaine methanesulphonate, and a polyethylene cannula (0.86 mm i.d., 1.52 mm o.d.) was passed through a hole in the skull just posterior to the vomer. The cannula was flanged so that it was held flush with the roof of the buccal cavity, and a slightly larger diameter cannula acted as a sleeve to hold the buccal cannula onto the skull. A Millar PC-350 catheter-tip pressure transducer (frequency response 0–10 000 Hz) was then passed through the cannula until the pressure-sensitive tip was within 1 cm of the buccal cavity. The axis of the pressure transducer was perpendicular to flow into the mouth cavity. Pressure signals were amplified 200 times with a UFI 2122 DC amplifier using a bandpass of 0–1000 Hz. The analog pressure trace was recorded on a Bell & Howell 4020A multichannel FM tape recorder for later analysis.

The feeding mechanism was converted from unidirectional to bidirectional by suturing the gular membrane and gill slits in each individual. This was a two-step procedure. First, a loop of suture was passed around the distal end of all four ceratobranchials and tied off. The ceratobranchials were not tied tightly together, but were held by the suture to prevent extreme gill bar abduction during feeding and to minimize the possibility of ripping out the other sutures. This loop of suture also had the effect of adducting the gill bars and causing the gill rakers on adjacent ceratobranchials to interlock loosely, forming a further barrier to water flow (Lauder, 1983, 1985a). Then, the gular membrane was sutured to the skin of the posterior body wall. A sufficient number of sutures was used to seal off the gill slits completely so that water could not flow out. The seal was tested in several individuals using methylene-blue-dyed water introduced into the buccal cavity during feedings: no dye was seen to emerge posteriorly from the gill slits.

Both control and experimental pressure measurements were made from each of the six *Ambystoma mexicanum*. At least 1 month was allowed between the two sets of measurements on each individual. In every case, the holes in the skin and skull, resulting from the sutures and insertion of the pressure cannulae, healed completely before the start of the second set of experiments. The experimental treatment consisted of suturing the gill slits closed as described above. For the control recordings, each individual was sutured in an identical manner as in the experimental treatment, but then the sutures were immediately cut and removed. Experimental and control treatments were alternated among the six animals, so that there would

be no bias during the 2 months of research. 198 feedings were obtained from the six *Ambystoma mexicanum*.

Feeding performance trials were conducted to determine if the surgical treatment had any measurable effect on behavioural efficiency in prey capture. The same six individuals used for pressure measurements were again subjected to control and sutured treatments. For each treatment, each individual was placed in an aquarium for 30 min with 30 live guppies (*Poecilia*) as prey. Each feeding attempt was observed in order to determine if the prey was caught or escaped. The proportion of prey captured per strike was then calculated over the entire 30-min trial (Table 4).

Data analysis and experimental design

Each pressure trace was converted to digital form using an IBM AT microcomputer and a 12-bit analog-to-digital converter sampling at 2050 Hz. These digitized pressure traces were then sent to a Tektronix 4107 graphics terminal where eight variables were measured following the procedures outlined in Lauder & Shaffer (1986). Fig. 1 illustrates how the variables were measured on a schematic pressure trace. These variables were (the abbreviations used here are those used subsequently in the paper): MAXNEG, the maximum negative pressure with respect to ambient in mmHg (1 mmHg = 133.3 Pa); MAXPOS, the maximum positive pressure in

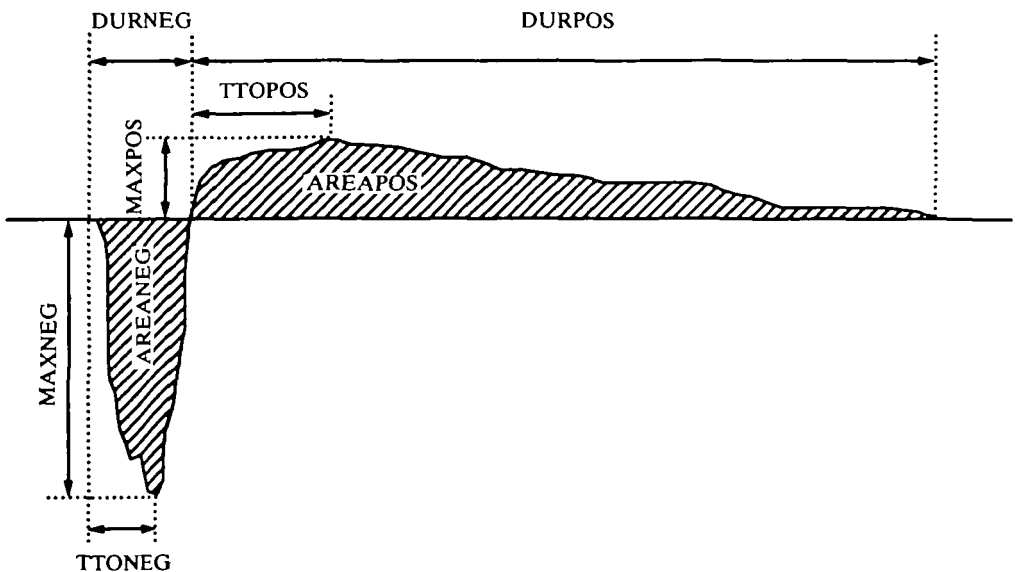


Fig. 1. Measurements taken from the buccal pressure traces recorded from normal (control) and experimental (gill slits sutured closed) *Ambystoma mexicanum*. Eight variables were measured from each pressure trace as shown and these are described in detail in the text. In addition, three ratio variables were derived from these measurements: MIPMN, the ratio of maximum positive to maximum negative pressure; DPDN, the ratio of negative to positive duration of the pressure waveform components; APAN, the ratio of the area under the positive portion of the pressure waveform to the area under the negative portion.

mmHg; DURNEG, the duration of the negative portion of the pressure trace in ms; DURPOS, the duration of the positive portion of the pressure trace in ms; TTOPOS, the time from the start of the positive portion of the pressure trace to the peak in ms; TTONEG, the time from the start of pressure decrease to the maximum negative value in ms; AREANEG, the area under the negative portion of the pressure trace in mmHg \times ms; AREAPOS, the area under the positive portion of the pressure trace in mmHg \times ms. In addition, three other dimensionless variables were derived from these eight: MPMN, the ratio of the maximum positive pressure to the maximum negative pressure; DPDN, the ratio of the duration of the positive portion of the waveform to the duration of the negative portion; APAN, the ratio of the area under the positive portion of the pressure trace to the area under the negative portion. Eleven variables were thus statistically analysed.

As in previous research (Lauder & Shaffer, 1986), it was realized that several of these variables provide partially redundant information (for example, high values of AREANEG would be expected to be related to large values of DURNEG and MAXNEG). Rather than make *a priori* decisions about which variables to measure, we chose to measure all 11 and subsequently use statistical tools (correlation matrices, principal components analysis) to quantify the extent of redundancy in the data set.

We used a two-way analysis of variance (ANOVA) experimental design (Sokal & Rohlf, 1981) with experimental treatment and individuals as the two main effects. In this design, treatment (sutured *vs* unsutured gill slits) was a fixed effect, while individual was a random effect. This experimental design was chosen because among-individual variance in pressure profiles may be large (Lauder & Shaffer, 1986) and we wished to compare treatment and control recordings from the same individual. The 0.01 level of statistical significance was used in Table 3 to lessen the chance of finding significant results by chance alone since 11 variables were tested (Sokal & Rohlf, 1981). Descriptive statistics were calculated for each cell of the ANOVA and are presented in Tables 1 and 2 as an aid to interpreting the results of the ANOVA (Table 3). Feeding performance data (Table 4) were analysed with a one-way paired ANOVA.

Two further analyses were conducted to assess multivariate patterns in the data. A principal components analysis was performed on the correlation matrix derived from a data set consisting of 183 feedings by five individuals. This analysis provided an indication of the multivariate pattern of dispersion between control and experimental treatments, and served to reduce the dimensionality of the data set from the original 11 variables to two, principal components one and two (Chatfield & Collins, 1980; Harris, 1975). The resulting factor scores were then analysed using a multivariate analysis of variance (MANOVA) to test for significant differences among the centroids of the polygons enclosing experimental and control treatments (Bray & Maxwell, 1985). A discriminant function analysis was also performed to ascertain the extent to which the 11 pressure variables could be used to predict whether the individual pressure trace resulted from a control or an experimental treatment.

RESULTS

Sample pressure traces for sutured and unsutured individuals are shown in Fig. 2, and descriptive statistics for each individual are shown in Tables 1 and 2. Suturing the gill slits closed results in statistically significant changes in three of the 11 variables (Table 3). (1) DURNEG, the duration of the negative portion of the pressure trace, is substantially shorter in individuals with sutured gill slits. (2) AREANEG, the area under the negative pressure component, is less than half as large in sutured individuals as it is in unsutured control recordings from the same individuals. This is due both to the shorter duration of the negative pressure (Fig. 2; Tables 1, 2: DURNEG) and to the reduction (not statistically significant) in peak negative pressure (Fig. 2; Tables 1, 2: MAXNEG). (3) APAN, the ratio of the area under the positive pressure pulse to the area under the negative phase is four times larger in individuals with gill slits sutured, reflecting both the increased durations and magnitudes of the positive pressure pulses and the decreased durations and magnitudes of the negative pressure pulses in sutured individuals.

Almost no treatment variation was detected in TTONEG (Table 3), indicating that the time taken to reach the maximum negative pressure is not affected by

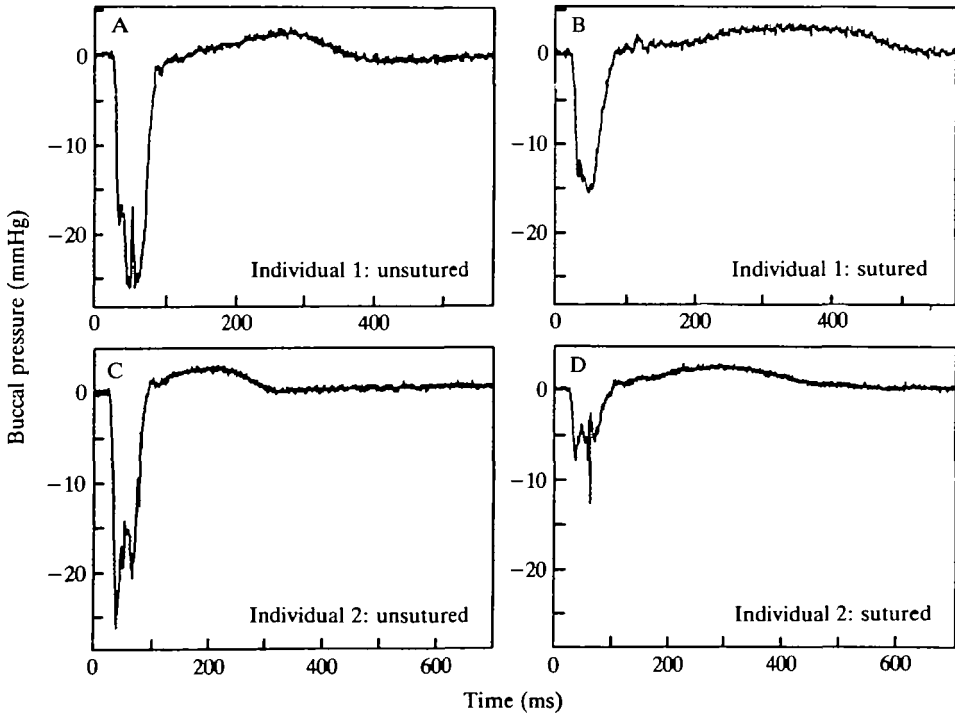


Fig. 2. Representative original pressure traces from two individual *Ambystoma mexicanum* showing the effect of suturing the gill slits closed. Note the decrease in negative pressure in sutured individuals. Statistical comparisons of the pressure traces from all sutured and control individuals are provided in Table 3.

Table 1. Summary statistics [mean (standard deviation)] for the 11 variables measured from buccal pressure recordings from six adult *Ambystoma mexicanum* with unsutured gill slits feeding in water

Variable	Individual						Overall mean
	1	2	3	4	5	6	
MAXNEG*	25.6 (7.2)	34.0 (7.1)	37.3 (17.4)	21.3 (5.1)	29.8 (7.7)	13.1 (2.2)	27.0 (11.5)
MAXPOS*	2.7 (0.7)	3.3 (1.1)	2.3 (0.8)	2.2 (0.6)	2.6 (0.5)	1.8 (0.4)	2.5 (0.9)
DURNEG†	115.8 (21.9)	77.0 (12.1)	155.9 (38.4)	87.8 (17.3)	103.6 (15.8)	133.1 (32.1)	111.6 (34.7)
DURPOST†	490.6 (317.4)	266.3 (100.6)	194.6 (142.1)	123.1 (36.1)	403.8 (201.9)	550.1 (486.8)	409.8 (323.0)
TTOPOST†	148.9 (45.9)	89.3 (52.0)	92.2 (69.3)	19.0 (8.7)	135.2 (60.3)	126.6 (36.4)	121.3 (55.2)
TTONNEG†	20.1 (6.6)	23.0 (7.2)	13.5 (3.5)	1510 (260)	23.6 (4.9)	18.3 (10.3)	20.0 (7.7)
AREANEG‡	1978 (406)	2445 (450)	1979 (628)	1510 (260)	2035 (360)	1093 (185)	1875 (584)
AREAPOST‡	1356 (1095)	767 (369)	331 (376)	0.10 (0.03)	945 (706)	538 (335)	846 (769)
MIPMIN§	0.11 (0.04)	0.10 (0.03)	0.08 (0.05)	0.10 (0.03)	0.09 (0.02)	0.14 (0.04)	0.11 (0.04)
DPDN§	4.4 (3.4)	3.4 (1.4)	1.2 (0.8)	0.10 (0.03)	4.1 (2.7)	4.7 (4.8)	3.9 (3.3)
APAN§	0.84 (0.72)	0.31 (0.14)	0.19 (0.24)	0.10 (0.03)	0.47 (0.32)	0.50 (0.28)	0.51 (0.48)

* In mmHg; † in ms; ‡ in mmHg × ms; § dimensionless.

Table 2. Summary statistics [mean (standard deviation)] for the 11 variables measured from buccal pressure recordings from six adult *Ambystoma mexicanum* with sutured gill slits feeding in water

Variable	Individual						Overall mean
	1	2	3	4	5	6	
MAXNEG*	21.5 (7.2)	19.3 (13.6)	10.8 (5.7)	12.9 (4.6)	18.3 (10.3)	19.0 (6.2)	17.3 (9.1)
MAXPOS*	5.1 (1.5)	4.2 (1.2)	3.1 (0.6)	4.8 (2.3)	2.9 (0.7)	2.5 (0.9)	3.8 (1.7)
DURNEG†	65.3 (12.2)	60.0 (12.4)	69.9 (10.9)	55.0 (12.2)	46.6 (7.1)	55.4 (15.8)	57.4 (13.8)
DURPOST	727.6 (449.5)	1237 (341)	457.3 (194.2)	1290 (494)	409.8 (81.5)	503.1 (216.1)	649.9 (401.7)
TTOPOST	245.8 (132.4)	144.5 (76.5)	198.6 (36.4)	159.5 (33.4)	150.8 (24.4)	91.5 (42.9)	157.0 (79.1)
TTONEG†	23.1 (7.9)	23.2 (9.6)	15.6 (2.3)	12.5 (3.9)	17.8 (7.6)	17.3 (5.0)	18.2 (7.5)
AREANEG‡	1275 (351)	989 (389)	512 (135)	619 (471)	809 (450)	837 (201)	837 (374)
AREAPOS‡	4595 (4020)	2468 (483)	953 (694)	3392 (471)	1017 (295)	953 (441)	1905 (2204)
MIPN§	0.26 (0.11)	0.27 (0.09)	0.33 (0.13)	0.37 (0.08)	0.19 (0.09)	0.15 (0.06)	0.25 (0.12)
DPDN§	11.6 (7.8)	20.8 (7.5)	6.7 (3.1)	22.8 (7.2)	9.0 (2.4)	10.0 (5.2)	11.6 (7.1)
APAN§	2.85 (2.48)	3.14 (1.42)	2.03 (1.77)	5.54 (1.36)	1.76 (1.58)	1.19 (0.6)	2.07 (1.7)

* In mmHg; † in ms; ‡ in mmHg × ms; § dimensionless.

suturing the gill slits. Similarly, AREAPOS, TTOPOS, DURPOS and MAXNEG all show little treatment effect.

More than half of the variables show a significant individual effect (Table 3), indicating that there is significant heterogeneity among individual *Ambystoma mexicanum* for these variables. As Tables 1 and 2 show, individual means for any given variable (within a treatment) may be quite different. That this variation is not consistent across treatments is shown by the significance of 10 of the 11 interaction terms (Table 3). Individual axolotls thus responded differently to suturing of their gill slits. A significant interaction term could result from individual variable means changing in inconsistent directions from control to treatment. For example, if one compares the value of the MAXNEG variable in Table 1 with the corresponding value for sutured individuals in Table 2; five of the six individuals show a decrease in mean MAXNEG under the treatment while one individual (number 6) shows an increase. In contrast, all individual means change in the same direction for the AREANEG variable, but the interaction term is significant due to differences in the magnitude of the change among individuals.

Correlations among variables generally were low, although several variables such as AREANEG and MAXNEG, and AREAPOS and DURPOS had correlations above 0.70. Of the three significant variables, the highest correlation among all possible pairs of variables was 0.48, and the correlation between AREANEG and DURNEG, two variables suspected *a priori* to be possibly highly intercorrelated, was 0.35. Principal component one had high (and positive) loadings by all variables associated with the positive portion of the pressure trace, and explained 46.5% of the variance in the data set. Variables describing the negative portion of the pressure

Table 3. *F values from a two-way analysis of variance on each of 11 variables measured from the buccal pressure recordings*

Variables	Factor		
	Treatment (1, 5)	Individuals (5, 198)	Treatment × Individuals (5, 198)
MAXNEG	5.292	19.748**	12.084**
MAXPOS†	11.441	5.165**	6.601**
DURNEG	23.301*	38.920**	13.540**
DURPOS	2.533	3.944*	13.330**
TTOPOS	4.436	3.656**	6.801**
TTONEG	0.651	5.472**	2.721
AREANEG	24.517*	32.441**	14.025**
AREAPOS†	3.962	1.751	8.318**
MPMN	15.263	2.065	18.695**
DPDN†	12.444	1.463	9.541**
APAN†	21.456*	1.017	4.436**

The treatment effect tests for the consequences of suturing the gill slits.

* $P < 0.01$; ** $P < 0.001$.

† Degrees of freedom: treatment (1,4); individuals (4,144); interaction (4,144).

Table 4. *Analysis of variance of feeding performance for control (unsutured) and experimental (sutured) Ambystoma mexicanum*

Effect	Degrees of freedom	Mean square	F-ratio	P-value
Treatment	1	0.777	61.76	0.0001
Error	10	0.013		

Mean performance (percent captures per attempts, \pm one standard deviation) for control individuals was 56.4% \pm 14.5, and was 5.5% \pm 6.4 for sutured individuals.

trace had negative loadings on the first principal component. Principal component two, accounting for 17.5% of the variance, had highest positive loadings by MAXNEG, AREANEG, MAXPOS and AREAPOS. The MANOVA on the factor scores showed that the mean principal component one scores for control and sutured individuals are significantly different ($F = 16.5$; d.f. 1,4; $P = 0.015$). Scores on principal component two were not significantly different. The omnibus MANOVA test was not significant ($F = 6.9$; d.f. 2,3; $P = 0.075$).

A discriminant function analysis using the 11 variables to predict which treatment each pressure trace belonged to correctly assigned 149 out of 150 feedings to the appropriate treatment, for an accuracy of over 99%.

Feeding performance trials (Table 4) showed that the sutured treatment reduced prey capture efficiency from 56 to 5.5%.

DISCUSSION

The distinction between unidirectional and bidirectional hydrodynamic design is the most basic and fundamental concept in aquatic vertebrate feeding systems. Yet, no research to date has quantitatively addressed the significance of this difference. These results clearly show that modifying the openings of the gill slits alone has a major effect on aquatic feeding performance and its physiological basis in *Ambystoma mexicanum*. Thus, transforming the feeding mechanism from a unidirectional to a bidirectional flow design (and altering no other parameters) has severe negative effects on the ability of salamanders feeding in the water to capture prey.

The three variables affected significantly by the closing of the gill slits are all related to the generation of negative buccal pressures. While the time taken to reach maximum negative pressure did not change when the gill slits were closed, the duration and area of the negative portion of the pressure trace decreased dramatically. Because the area under the negative portion of the pressure trace measures the momentum change of the water drawn into the mouth during prey capture (pressure area is the integral of force with respect to time, Lauder & Shaffer, 1986), the decrease in AREANEG following suture treatment directly reflects a decrease in energy imparted to the water by the head muscles. It is thus not surprising to find that feeding performance decreases significantly (Table 4).

What is the biomechanical basis for the changes produced by suturing the gill slits? Buccal cavity expansion during aquatic suction feeding in ambystomatid salamanders is the result of elevation of the skull by the epaxial muscles, slight lateral expansion of the suspensorium, lateral expansion of the hyobranchial apparatus, and ventral movement of the hyobranchial apparatus (Lauder & Shaffer, 1985). By preventing lateral expansion of the posterior portion of the hyobranchial apparatus (and thus the buccal cavity) by suturing the gill slits, the absolute volume change occurring during feeding is reduced. Because the magnitude of negative buccal pressure is a function of both the rate and the amount of the volume change (Lauder, 1980), the pressure generated during feeding will decrease.

The shifts in buccal pressure profiles (averaged over all feedings) that occur following closure of the gill slits are shown schematically in Fig. 3. From these curves it can be seen that suturing the gill slits produces a dramatic reduction in the negative pressure pulse, as well as increasing both the magnitude and the duration of the positive pressure component. While these two specific changes in the positive

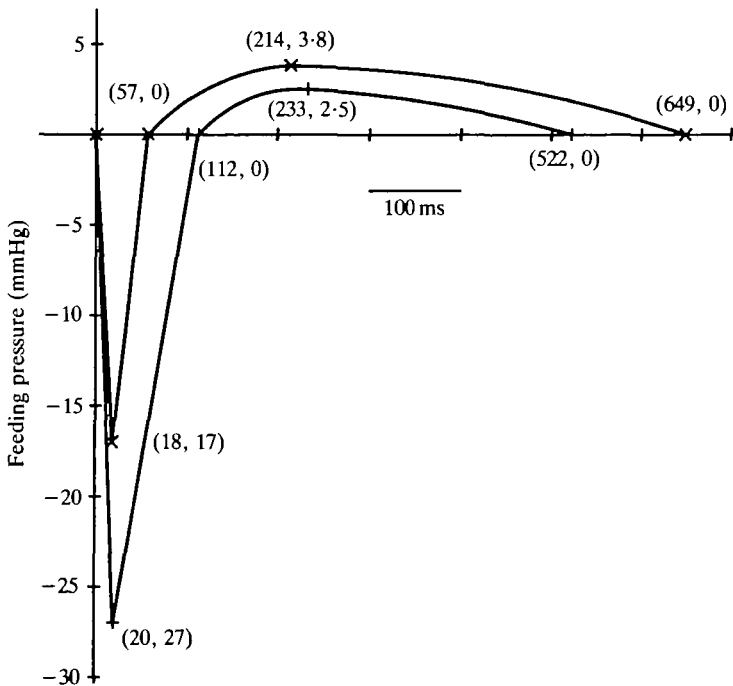


Fig. 3. Average pressure profiles reconstructed from the mean values for all sutured (x) and unsutured (+) individuals for each treatment. Pressure curves for the two treatments have been aligned so that the negative pressure decreases begin at the origin (0 ms, 0 mmHg). Note that a major effect of converting the feeding mechanism from a unidirectional to a bidirectional system is to decrease the area under the negative portion of the pressure trace and to increase the area under the positive portion of the trace. Coordinates indicate the position of digitized mean control and sutured values on the pressure curves.

pressure are not statistically significant (Table 3), the change in relative magnitude of the positive and negative areas is highly significant (Table 3), reflecting an alteration in the relative energy content of these two components of the pressure trace. This alteration is not entirely due to the significant change in the AREANEG variable (as variation in AREANEG only explains 23% of the variance in APAN), but also reflects the increased area of the positive pressure (Fig. 3).

The extent of overall differences in the two traces shown in Fig. 3 can be appreciated through the results of the discriminant function analysis, which correctly classified 149 out of 150 feedings by treatment type. The significant differences between the factor scores for control and experimental feedings on principal component one (and the loading patterns) indicate that sutured individuals are significantly different in a multivariate sense primarily in features of the positive portion of the pressure trace. The omnibus MANOVA just fails to be significant, mainly because the degrees of freedom for the effect of suturing the gill slits, 2 and 3, are so few. The high F -value (6.9) suggests that if more individuals were studied, the overall difference for both principal components together would be significant.

The increase in area of the positive pressure component reflects the inability of water drawn into the mouth in the early stages of prey capture to exit posteriorly. High-speed aquatic feeding (prey capture in *Ambystoma mexicanum* takes about 75 ms) is characterized by unsteady water flow with high inertia. Fluid inertia is responsible for the relatively high positive pressure seen in the bidirectional feeding system of *Ambystoma mexicanum*.

Previous predictions of differences between the function of unidirectional and bidirectional aquatic feeding systems (Lauder, 1985b) have proved to be simplistic. While the general prediction that, all other things being equal, bidirectional feeding systems should have an increased positive component of the pressure waveform has been corroborated, the extent of the alterations in the negative pressure component was not anticipated. Lauder (1985b, p.228) predicted that little difference would be found in the pressure profiles during the expansive and early compressive phases of feeding. These phases correspond roughly to the negative portion of the pressure waveform illustrated in Fig. 3. The data presented here unequivocally indicate that the duration and area of the negative pressure are significantly affected by converting the feeding system to a bidirectional design (Table 3; Fig. 3).

There is some possibility that in suturing the gill slits we prevented expansion of the posterior part of the buccal cavity and thus caused the reduction in negative pressure area and duration by factors other than the change to a bidirectional feeding design. While we are not able to rule out this possibility, the suturing of the gill slits did not affect hyoid motion [the major effector of buccal volume change (Lauder & Shaffer, 1985)] and the negative pressures generated were not significantly different (Table 3: MAXNEG). Lateral expansion of the buccal cavity was still possible as we did not suture the ceratobranchials tightly together.

What do these results tell us about the process of ontogenetic transformation of form and function in salamanders? During metamorphosis, many morphological changes take place in the structure of the skull, only one of which is the shift from a

unidirectional to a bidirectional feeding system. Lauder & Shaffer (1986) measured changes in pressure profiles accompanying metamorphosis in *Ambystoma tigrinum* (using the same 11 variables studied here), and those alterations in pressure reflect the total of structural changes in feeding design. The differences found in this study between unidirectional and bidirectional feeding systems in axolotls are due only to this particular structural change.

In comparing the results of Lauder & Shaffer (1986) with those of this paper, it is important to distinguish three possible patterns in the pressure variables. (1) Changes in the 11 pressure variables that occur both in this study and in that of Lauder & Shaffer (1986) indicate those attributes of feeding transformation at metamorphosis that are due to the shift from unidirectional to bidirectional flow. (2) Variables that were found to change by Lauder & Shaffer (1986) but did not change in this study reflect the effect of other morphological changes in the feeding mechanism at metamorphosis that are affecting the pressure profiles. (3) Variables that changed here but not in Lauder & Shaffer (1986) perhaps represent alterations in the pressure profile due to a unidirectional-to-bidirectional shift that is compensated for by other structural changes in the skull and their functional consequences, thus resulting in no net change in pressure during metamorphosis. In making these comparisons, we will use the 0.01 level of significance to determine those variables with significant changes in mean value.

Congruent changes between these two studies occur in the DURNEG and AREANEG variables. In addition, neither study found any change in the MAXPOS, TTOPOS and TTONEG variables. This clearly indicates that the negative portion of the pressure waveform is heavily influenced by the change from a unidirectional to a bidirectional flow design at metamorphosis. Furthermore, the transformation of flow regimes carries no change in timing of peak pressures.

The variables that were found to change by Lauder & Shaffer (1986) but did not change in this study were MAXNEG, DURPOS, AREAPOS, MPMN and DPDN. Other structural changes in the head at metamorphosis (such as the decrease in muscle mass, changes in muscle lever arms and changes in buccal volume due to the development of a fleshy mobile tongue) are thus affecting the pressure profiles significantly. A decrease in cranial muscle mass at metamorphosis may explain some of these changes, as MAXNEG, DURPOS and AREAPOS all *decreased* after metamorphosis (Lauder & Shaffer, 1986). An additional structural modification in the feeding mechanism that occurs at metamorphosis in *Ambystoma tigrinum* but is not mimicked here by experimentally modifying the axolotl feeding system is the change in mouth shape and the structure of the lips. In axolotls and larval *Ambystoma tigrinum*, the mouth is bordered by fleshy lips that occlude the margin of the gape when the mouth is opened during suction feeding. These lateral structures have been shown to play an important role in directing flow into the mouth during feeding in fishes (Lauder, 1979). The fact that these labial structures are still present in the axolotls with sutured gill slits, but not in metamorphosed *Ambystoma tigrinum*, may explain some of the variables that did not change in this study, but that did in *Ambystoma tigrinum* (Lauder & Shaffer, 1986).

One variable, APAN, changed in this study but not in Lauder & Shaffer (1986). This indicates that while the change in hydrodynamic design of the head does alter the relative contributions of the negative and positive portions of the pressure curve to the amount of energy imparted to the water by the head muscles, the other structural changes occurring at metamorphosis compensate for these changes. Because Lauder & Shaffer (1986) found that AREANEG did shift during metamorphosis, the lack of a change in APAN must be due to compensating alterations in AREAPOS. The decrease in mean AREAPOS with metamorphosis thus cancels out changes in AREANEG so that the ratio of the areas remains unaltered.

Despite the differences in some pressure variables between the two studies, performance changes are concordant: both metamorphosed and bidirectional individuals are much poorer at catching elusive prey (Table 4), reflecting the reduction in AREANEG.

This study has clarified the causal basis of previously documented ontogenetic changes in the functional design of urodeles. By experimentally manipulating the major ontogenetic design change, we have shown which of the many changes in structure are causally related to functional changes during ontogeny. Furthermore, we have identified the specific functional changes that underlie feeding performance shifts. However, changes in functional design may occur at many levels of biological organization, and we are only beginning to appreciate the potential extent of the transformation in the head of salamanders during metamorphosis. For example, physiological properties of the head muscles could change, as could muscle fibre lengths and fibre types. Alterations in structure and function at all levels of biological design must be studied in the future to achieve a more general understanding of the ontogeny of functional design in vertebrates.

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