J. exp. Biol. 141, 343–358 (1989)
Printed in Great Britain

PHYSIOLOGICAL BASES OF FEEDING BEHAVIOUR IN SALAMANDERS: DO MOTOR PATTERNS VARY WITH PREY TYPE?

BY S. M. REILLY AND G. V. LAUDER

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

Accepted 27 June 1988

Summary

Muscle activity patterns (motor patterns) of the jaw musculature of all vertebrates studied to date (primarily fishes and amniotes) vary considerably when they feed on different types of prey. Previous data on buccal pressure patterns suggested that feeding in the aquatic salamander, *Ambystoma mexicanum* (Shaw), is highly stereotyped. This hypothesis was tested by quantifying the motor pattern used during feeding on two prey types: earthworms and guppies. Twenty-nine variables were measured from the activity pattern of six cranial muscles in the feeding mechanism of *Ambystoma mexicanum*. These variables included the area under the electromyogram of each muscle, relative muscle onset times, and the amplitudes and durations of muscle bursts. Univariate and multivariate statistical analyses demonstrate that the feeding motor pattern of *Ambystoma mexicanum* is stereotyped and does not change with prey type, in contrast to motor patterns of other vertebrates studied to date. Individual salamanders use significantly different motor patterns from one another during feeding, but do not alter their motor pattern during feeding on different prey.

Introduction

The physiological basis of variation in the behaviour of an individual animal is variation in the pattern of muscle activity used to produce the behaviour. If a mammal, for example, exhibits two types of locomotor behaviour, one predicts that the difference in behaviour is correlated with a difference in the muscle activity patterns used in the two locomotor situations. Thus, the study of variation in muscle activity patterns (also referred to here as motor patterns) is fundamental to understanding how and why animals alter their behaviour and to establishing the mechanistic bases of animal movement (Goslow, 1985; Hiiemae & Crompton, 1985; Lauder, 1985; Liem, 1978; Wainwright, 1986).

Recent research on vertebrate motor patterns during natural behaviours has demonstrated considerable variation when different stimuli are presented to an individual. For example, in the cranial muscles involved during the prey capture

Key words: motor pattern, muscle, salamanders, feeding behaviour.

and processing behaviour of both fishes and amniotes, different foods elicit different patterns of muscle activity (Crompton *et al.* 1977; DeVree & Gans, 1973, 1976; Elshoud-Oldenhave & Osse, 1976; Gorniak, 1977; Gorniak *et al.* 1982; Hiiemae & Crompton, 1985; Hiiemae *et al.* 1978, 1981; Liem, 1979, 1980; Sanderson, 1988; Wainwright, 1989; Wainwright & Lauder, 1986; Weijs & Dantuma, 1981). The motor pattern governing feeding behaviour thus varies and may be modulated by complex central feedback mechanisms (Hiiemae & Crompton, 1985; Thexton, 1976) to effect behaviour appropriate for the stimuli presented. All previous studies that have tested for an effect of food type on the feeding motor pattern of vertebrates have found a significant effect, indicating that however stereotyped the motor pattern for any single prey type among species, individuals within a species possess the ability to modulate the motor pattern in response to prey characteristics.

Recent research on the aquatic feeding system of salamanders has provided an indication, as yet unexplored, that the feeding motor pattern may be more stereotyped than in other vertebrates (Lauder & Shaffer, 1985). In the course of their study of the functional morphology of aquatic prey capture in salamanders, these authors provided pressure measurements within the buccal cavity of axolotls (*Ambystoma mexicanum*) and showed, for one individual, that the negative pressure generated during aquatic prey capture did not change with the number of prey eaten. There was thus no effect of satiation on the magnitude of negative pressure generated during prey capture (Lauder & Shaffer, 1985; fig. 17). This observation suggests that the motor pattern of axolotls might be relatively stereotyped.

The purpose of this work was to test directly the hypothesis that axolotls (*Ambystoma mexicanum*) possess a feeding motor pattern that does not vary with the type of prey eaten. We present the results of a statistical test of motor pattern variability and conclude by corroborating the initial hypothesis: the feeding motor pattern of *Ambystoma mexicanum* does not vary with prey type.

Materials and methods

Experimental animals

Six axolotls (*Ambystoma mexicanum*) were chosen for these experiments from a laboratory-maintained colony. The six individuals were of similar size (mean snout–vent length = 106·5 mm, S.D. = 5·1 mm) and each animal was housed individually in a 40-l aquarium at 20°C.

Two experimental prey were chosen with the *a priori* aim of presenting prey that differ considerably in escape ability to maximize the chance of detecting a difference between prey in cranial muscle motor pattern elicited. The two prey chosen were earthworm (*Lumbricus*) pieces, about 1 cm long, and live guppies (*Poecilia*), about 2 cm long. Previous research (Lauder & Shaffer, 1985; Lauder & Reilly, 1988) has shown that *Ambystoma mexicanum* readily eat both worms and guppies, and that these prey represent extremes of a prey mobility spectrum that

challenge the feeding performance of axolotls. Earthworm pieces were presented on the end of long forceps, each piece about 1 cm anterodorsal to the mouth as in previous research (Lauder & Shaffer, 1985, 1986). During feeding, the mouth opens, buccal pressure drops and the worm is drawn off the forceps by the flow of water into the mouth. Worm pieces were relatively immobile, had zero escape velocity, and were captured about 93 % of the time (Lauder & Shaffer, 1986). In contrast, live guppies were presented by introducing about 20 individuals into the experimental aquarium and allowing the axolotls to feed at will. Guppies have an extremely rapid Mauthner-cell-mediated escape response (Eaton, 1984), and *Ambystoma mexicanum* capture guppies only about 50 % of the time (Lauder & Reilly, 1988). This prey type was presented to elicit the most rapid feeding behaviour possible.

Feeding performances (calculated as the percentage of all strikes that resulted in the successful capture of prey) were measured for each individual on both prey types. These performance values were obtained while the electromyographic recordings were being conducted (see below) and reflect both the presence of electrodes in the cranial muscles and the high densities of prey in this experimental situation. They should thus be compared to previous values (Lauder & Reilly, 1988) with caution.

Not all strikes were successful, and we analysed only feedings that were successful in capturing prey. We interspersed worm feedings with guppy captures to prevent the predator from becoming habituated to a single prey type.

Experimental techniques

The motor pattern of the jaw musculature was quantified by measuring muscle electrical activity patterns produced by six cranial muscles during feeding in six axolotls. Electromyographic recordings were made from the six muscles simultaneously by implanting bipolar stainless-steel electrodes into each muscle as in previous research (e.g. Wainwright & Lauder, 1986; Lauder & Shaffer, 1988). All electrode implantations were made while the animals were under anaesthesia, induced by placing the salamanders in a solution of tricaine methane sulphonate ($1\,g\,l^{-1}$) for about 15 min. The bared metal tips of each electrode were about 0·7 mm long and the insulated portions were glued together proximal to the bared ends with a cyanoacrylate adhesive to prevent tip displacement within the muscle (Jayne, 1988). The six pairs of electrodes were then glued to each other and attached to the back of the animal with a loop of suture. Electrodes were sutured individually to the skin to prevent movement of the electrode and to minimize movement artefacts during feeding.

Electrodes were implanted percutaneously directly into the belly of each muscle. The six muscles studied are not surrounded by other muscles into which the electrodes might stray (Lauder & Shaffer, 1985; Fig. 1). In no case did an implantation into a muscle of interest involve penetrating another superficial muscle. Electrode position was verified visually; in some muscles (e.g. the geniohyoideus) the tips of the electrodes could be seen directly through the thin

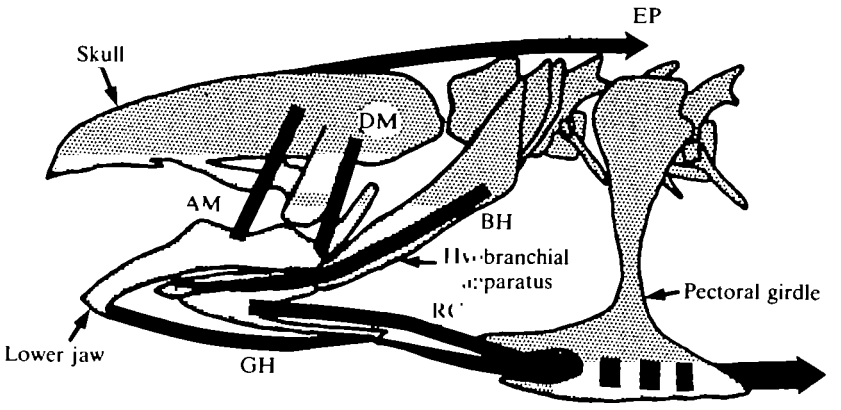

Fig. 1. Schematic diagram of the skull, hyobranchial apparatus and pectoral girdle in *Ambystoma mexicanum* to show the muscles from which recordings were made in this study. A more detailed treatment of cranial morphology and muscle function during feeding is provided in Lauder & Shaffer (1985). Stipple indicates bony or cartilaginous elements of the head. The six muscles used in this investigation of motor patterns are shown schematically by black lines extending from muscle origin to insertion. AM, adductor mandibulae externus muscle; BH, branchiohyoideus muscle; DM, depressor mandibulae muscle; EP, epaxial muscles; GH, geniohyoideus muscle; RC, rectus cervicis muscle.

translucent skin covering the muscle belly. In other muscles (e.g. the epaxialis) the electrode was simply implanted directly into the thick muscle mass. Although we did not test directly to ascertain that electrode implantation did not affect feeding behaviour, previous analyses (Lauder & Shaffer, 1985) have shown that feeding kinematics with electrodes are similar to those without electrodes. Also, we note that *both* prey types were tested on the same animal during the same implantation, and that feeding performance by these experimental animals exceeded performance measurements on animals without electrodes (compare the performance results shown in Fig. 3 with the data of Lauder & Shaffer, 1986).

The electromyographic signals were amplified 10000 times using Grass AC P511J preamplifiers with a bandpass of 100–3000 Hz, and stored on a Bell & Howell 4020A multichannel FM tape recorder for subsequent computer analysis.

The six muscles chosen for analysis are muscles involved in all major phases of the feeding mechanism (Fig. 1). Anatomical descriptions and biomechanical analyses of these muscles have been provided elsewhere (Lauder & Shaffer, 1985, 1988), and only a brief summary of muscle action will be provided here. The depressor mandibulae (DM) and rectus cervicis (RC) muscles are the major muscles mediating mouth opening (Fig. 1). In addition, the rectus cervicis moves the hyoid apparatus posteroventrally, greatly expanding the volume inside the mouth and contributing significantly to the decrease in intraoral pressure (Lauder & Shaffer, 1985). The anterior epaxial muscles (EP) elevate the skull on the vertebral column during feeding and contribute to the increase in gape. The

adductor mandibulae externus (AM) muscle functions mainly to close the mouth, adducting the mandible against the upper jaw (Fig. 1). The geniohyoideus (GH) acts to protract the hyoid apparatus, to elevate the floor of the buccal cavity and to depress the mandible. Finally, the branchiohyoideus (BH) is a gill arch muscle which elevates the hyoid apparatus and abducts the gill arches. Functionally, these muscles can be divided into two major groups: (1) those involved in the modulation of pressure change in the oral cavity (the branchiohyoideus, geniohyoideus and rectus cervicis) and (2) those that function to open and close the mouth (the epaxial muscles, depressor mandibulae and adductor mandibulae externus).

Data analysis and experimental design

Each feeding by each *Ambystoma mexicanum* was converted into a digital data file with a Keithley analog-to-digital converter and an IBM AT microcomputer. The sample rate for each of the six channels was 2050 Hz at 12-bit resolution. To ensure that each feeding was completely recorded on disc, data were collected off the tape recorder for about 2·5 s. Each feeding was thus represented on disc by a digital data file (data matrix) with six columns (one for each muscle) and 5000 rows (corresponding to the 2·44 s each feeding was sampled).

The digital data file for each feeding was then analysed using a Tektronix 4107 graphics terminal (for measuring integrated electromyographic activity in each muscle) and a computer program that measured the other variables (described below) directly from the file. Our aim was to characterize the motor pattern relatively completely in order to detect any change when *Ambystoma mexicanum* fed on different prey types (see Fig. 2). As in previous research (Lauder & Shaffer, 1985, 1988), the depressor mandibulae was used as a reference muscle against which to measure the onset time of activity in the other five muscles: it is the major mouth opener and has a high-amplitude and consistent activity pattern (Lauder & Shaffer, 1985).

Twenty-nine variables were measured from each feeding (Fig. 2; the abbreviations given here are those used in the text and tables). The maximum amplitude (MAX) of electrical activity (in V) was measured for each muscle, for six variables: AM/MAX, DM/MAX, RC/MAX, GH/MAX, BH/MAX and EP/MAX. The time to peak voltage (TMAX) within a burst from the start of activity in the depressor mandibulae (in ms) was measured for each muscle, for six variables: AM/TMAX, DM/TMAX, RC/TMAX, GH/TMAX, BH/TMAX and EP/TMAX. The duration (DUR) of electrical activity (in ms) in each of the six muscles was measured: AM/DUR, DM/DUR, RC/DUR, GH/DUR, BH/DUR and EP/DUR. The onset of activity (ON) (in ms) was measured for each muscle relative to the start of activity in the depressor mandibulae muscle, for five variables: AM/ON, RC/ON, GH/ON, BH/ON and EP/ON. Finally, the area (AREA) under each rectified muscle burst (in V × ms) was measured using the Tektronix graphics terminal (Fig. 2): AM/AREA, DM/AREA, RC/AREA, GH/AREA, BH/AREA and EP/AREA.

These measurements provide some redundancy in capturing the morphology of the motor pattern. Thus, depending on the shape of the burst of electrical activity, the area of the burst might be expected to correlate highly with the amplitude and duration of activity. However, we decided not to make *a priori* decisions about variable redundancy, and instead to let the statistical analyses provide quantitative data on the extent of intercorrelation among variables.

The primary experimental design used in this study was a two-way analysis of variance (ANOVA) (Sokal & Rohlf, 1981) with individuals and prey type as the two main effects. In this design, each of six individuals was tested on both worm and guppy prey types, and prey type is treated as a fixed effect and individuals as a random effect. Thus, the *F*-ratios for the prey-type effect were constructed by dividing the mean square for prey type by the interaction mean square, whereas *F*-ratios for individuals and the interaction terms were constructed using the error mean square as the denominator. The 0·01 level of significance was chosen because multiple univariate comparisons were being conducted (Sokal & Rohlf, 1981). The two-way ANOVA design has the advantages of controlling for individual differences in the response to prey types and of allowing differential responses to prey by individuals to be quantified.

Both prey types were presented within a 2-h period to each individual, and thus none of the variation between prey types within an individual can be attributed to differences among electrode implants or to differences among experimental days. However, our experimental design does not take into account differences among experimental days or implants when comparisons among individuals are considered, and variation due to these causes will inflate differences among individuals (see Wainwright, 1989, for a detailed discussion of this issue). Caution must therefore be exercised when interpreting among-individual variance, as these data provide an upper bound on the extent of such variance.

123 feedings were obtained from the six individuals giving an average of about 10 feedings per individual per prey type. Thus, about 20 recordings were obtained from each muscle, 10 on each prey type. The degrees of freedom for the ANOVA tests vary (see Table 2) because of missing values in the experimental design for some variables. For example, *Ambystoma mexicanum* no. 3 pulled out the epaxial muscle electrode during the experiment, and data are thus not available on this muscle for this individual.

Because of problems that arise in interpreting significance levels when many univariate analyses of variance are conducted (Lauder & Shaffer, 1988) and because of the difficulty in clearly summarizing the results of many statistical tests, two multivariate analyses were conducted on the data set. Because the large number of variables analysed (29) was so much greater than the average number of feedings in each cell of the ANOVA design (about 10), we first reduced the dimensionality of the data set (Chatfield & Collins, 1980; Lauder & Shaffer, 1988) to four factors through a principal components analysis (PCA) on five of the six area variables. The area variables were chosen *a priori* as capturing a high proportion of information about the motor pattern. The PCA factored the

correlation matrix of five area variables (presented as Table 3) representing feedings from the six individuals. PCA values calculated with the covariance matrix (and the logarithm of original variable values) produced closely similar loading patterns. The principal components analysis allows the examination of linear combinations of variables to determine if major axes of variation in the multivariate data set are attributable to the effect of prey type.

A multivariate analysis of variance (MANOVA) was performed on the factor scores from principal components 1 and 2 to test the overall hypothesis of no difference between feedings on the two prey types when many variables are considered simultaneously. This MANOVA tests the centroids of the two prey-type polygons for a significant difference between them (see Fig. 3). As in the univariate ANOVAs, the multivariate test statistics for the prey-type effect were constructed using the interaction mean square.

Results

Summary statistics for the six area variables are given in Table 1, and representative bursts of activity of three muscles during feeding on a piece of worm are shown in Fig. 2. The results of the univariate ANOVAs for the 29 measurements of the motor pattern are presented in Table 2, and the ANOVA results are consistent across variables. There is no significant prey-type effect for any variable and thus no indication from the univariate analyses that the motor pattern differs when *Ambystoma mexicanum* feeds on worms or live fish. The results also indicate that there is significant variation among individuals for these variables, with nearly every aspect of the motor pattern displaying significant variation among the six individuals studied. The interaction term (Table 2) is significant in only three of 29 variables, indicating that each individual responded in the same way to each prey type, despite the extensive variation among individuals. Thus, individuals were consistent in how they responded to the two prey, even though they differed from each other in their mean motor pattern.

The correlation matrix for the area variables (Table 3) shows that the area of electrical activity in the branchiohyoideus, rectus cervicis and geniohyoideus (the three muscles involved in generating negative pressures within the buccal cavity) are all intercorrelated, and that there are relatively low correlations between these area variables and the mouth opening and closing muscle areas. Three variables (GH, RC and BH) load positively and highly on principal component 1 (Table 4), whereas component 2 reflects the high loading of mouth opening and closing muscles (AM and DM). Together components 1 and 2 account for 79·3 % of the total variation, and components 3 and 4 together account for only 16·6 % of the variation.

We interpret component 1 as a suction component, with feedings scoring highly on this factor having greater negative pressure and higher velocity flows into the mouth, from the greater electrical energy present in the electromyograms of the suction-generating muscles. Feedings scoring highly on principal component 2 are

Table 1. *Summary statistics for the area variables digitized from electromyographic recordings from six muscles during feeding on two prey types by* Ambystoma mexicanum

		Individual					
Variable	Prey type	1	2	3	4	5	6
AM/AREA	Worms	10·4 ± 4·8 (15)	29·5 ± 17·4 (10)	9·9 ± 4·0 (9)	8·8 ± 5·0 (9)	14·3 ± 7·9 (12)	47·2 ± 13·2 (9)
	Guppies	20·7 ± 6·9 (13)	42·2 ± 29·0 (11)	14·4 ± 8·7 (8)	13·3 ± 5·5 (5)	26·3 ± 13·1 (10)	31·3 ± 18·2 (8)
DM/AREA	Worms	48·5 ± 13·3 (15)	75·0 ± 25·1 (11)	32·5 ± 11·9 (11)	13·5 ± 7·1 (10)	71·3 ± 21·6 (12)	121·2 ± 10·6 (9)
	Guppies	80·4 ± 10·7 (13)	100·6 ± 27·4 (11)	36·3 ± 9·8 (8)	15·9 ± 4·9 (5)	75·9 ± 27·0 (10)	141·1 ± 35·0 (7)
RC/AREA	Worms	21·6 ± 11·2 (15)	3·1 ± 2·8 (6)	15·0 ± 7·4 (11)	3·3 ± 2·2 (7)	4·3 ± 2·7 (10)	6·0 ± 4·5 (8)
	Guppies	36·3 ± 12·6 (13)	14·7 ± 16·3 (7)	23·2 ± 20·8 (8)	4·6 ± 1·7 (4)	9·2 ± 7·6 (9)	3·6 ± 4·5 (3)
GH/AREA	Worms	25·8 ± 17·6 (15)	6·4 ± 6·3 (7)	10·2 ± 11·0 (8)	7·5 ± 5·9 (7)	2·7 ± 2·1 (4)	5·0 ± 2·9 (8)
	Guppies	53·6 ± 26·4 (13)	14·5 ± 14·4 (10)	25·2 ± 12·8 (7)	5·7 ± 1·8 (4)	2·7 ± 0·8 (2)	3·1 ± 3·3 (2)
BH/AREA	Worms	56·8 ± 5·1 (8)	21·9 ± 10·6 (11)	27·5 ± 25·8 (11)	15·9 ± 8·2 (10)	36·2 ± 22·2 (11)	40·3 ± 16·0 (9)
	Guppies	87·1 ± 16·9 (13)	37·6 ± 18·3 (11)	40·5 ± 23·0 (8)	14·6 ± 4·6 (5)	31·0 ± 13·4 (10)	15·4 ± 8·2 (8)
EP/AREA	Worms	27·7 ± 14·1 (9)	20·0 ± 23·0 (11)		36·8 ± 23·3 (10)	13·9 ± 9·6 (11)	45·8 ± 13·0 (9)
	Guppies	38·0 ± 8·1 (13)	23·2 ± 11·8 (11)		44·9 ± 14·7 (4)	28·1 ± 19·4 (8)	35·9 ± 14·9 (8)

Units of the area variables are volts × milliseconds.
Values are means ± S.D. (*N*) and should be multiplied by 0·4878.
See text for an explanation of abbreviations.

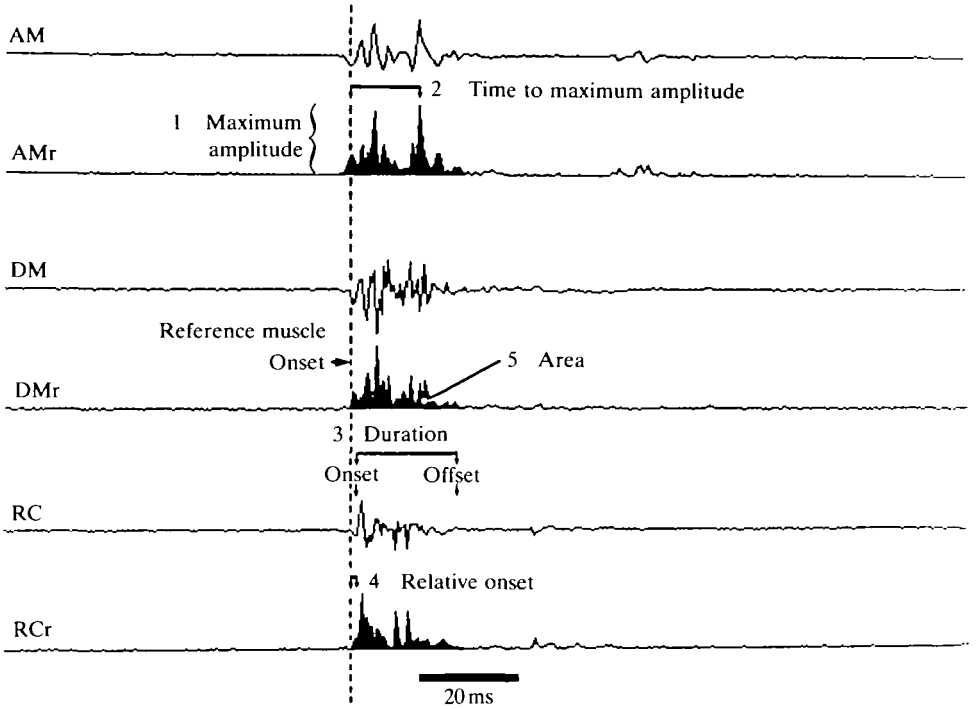

Fig. 2. Representative electromyograms for three muscles (the adductor mandibulae externus (AM), depressor mandibulae (DM), and rectus cervicis (RC)) to show the variables measured to characterize the motor pattern. The upper panel for each pair is the unrectified electromyographic trace (AM, DM, RC), and the lower panel shows the rectified trace (AMr, DMr, RCr). This figure shows muscle activity that was converted from analogue to digital form at 2050 Hz and 12-bit resolution, as displayed on a printout of the Tektronix graphics terminal screen (see text for further discussion). Resolution of the data as digitized is substantially greater than this printed output. The five types of variables measured from each of the six muscles (numbered 1 to 5) are shown and are explained in detail in the text.

interpreted as reflecting a greater speed of the strike from the increase in electrical activity present in the mouth opening and closing muscles.

A plot of the position of each feeding in principal component space is presented in Fig. 3. There is extensive overlap in the polygons enclosing the worm and guppy feedings. A MANOVA testing for a significant difference between the centroids of the worm and guppy polygons reveals no significant differences ($F = 1{\cdot}7$; df = 2, 2; $P = 0{\cdot}37$). Even with the limited degrees of freedom available for this test, the low F-statistic provides little indication that increased sample sizes would help to distinguish worm from guppy feedings.

Individual salamanders do, however, occupy different areas of multivariate space (Fig. 3), indicating that although there is no overall prey-type effect, individuals do have motor patterns that differ along the suction or strike-rapidity axes in multivariate space. Salamander no. 1 had the best feeding performance on

Table 2. *Univariate two-way ANOVA F-statistics (prey-type effect is fixed, individual effect is random) for 29 variables digitized from electromyographic recordings of six muscles in* Ambystoma mexicanum *feeding on two prey types*

Variable	Prey type (df = 1,5)	Individual (df = 5,72–111)	Individual× prey-type (df = 5,72–111)
AM/ON	2·742	29·814**	3·066
AM/MAX	0·002	46·423**	2·059
AM/TMAX	0·100	20·661**	3·115
AM/DUR	0·006	21·868**	3·229*
AM/AREA	1·172	15·100**	2·916
DM/MAX	1·775	107·065**	2·219
DM/TMAX	3·041	5·079**	0·353
DM/DUR	9·171	14·211**	0·829
DM/AREA	7·020	71·403**	2·364
RC/ON	4·655	12·366**	0·947
RC/MAX	1·933	10·743**	2·009
RC/TMAX	0·393	4·785**	3·157
RC/DUR	3·474	4·031*	0·928
RC/AREA	5·607	17·628**	1·380
GH/ON	0·051	7·004**	1·365
GH/MAX	0·711	7·204**	3·026
GH/TMAX	0·007	4·703**	1·000
GH/DUR[1]	0·034	4·966*	0·242
GH/AREA	1·646	15·401**	2·457
BH/ON	0·094	32·924**	2·861
BH/MAX	0·132	87·878**	2·250
BH/TMAX	0·962	1·784	2·253
BH/DUR	0·096	6·051**	3·650*
BH/AREA	0·333	24·347**	6·228**
EP/ON[1]	1·140	11·209**	0·458
EP/MAX[1]	1·337	5·776**	1·238
EP/TMAX[1]	0·134	1·562	0·851
EP/DUR[1]	1·982	7·785**	0·335
EP/AREA[1]	1·490	6·483**	1·502

* Significant at $P \leq 0{\cdot}01$; ** $P \leq 0{\cdot}001$.
[1] Degrees of freedom for each column: 1,4; 4,57–86; 4,57–86.
See text for an explanation of the abbreviations.

guppies, with a success rate of 93%, and all other individuals exhibited substantially lower performances that ranged from 67% to 83% (Fig. 3).

Discussion

Motor pattern variation

Our results demonstrate that *Ambystoma mexicanum* does not modulate its feeding motor pattern in response to different prey. Both the multivariate

Table 3. *Correlation matrix for five area variables measured from the electromyographic recordings of feeding by* Ambystoma mexicanum

	AM/AREA	DM/AREA	RC/AREA	GH/AREA	BH/AREA
AM/AREA	1·00				
DM/AREA	0·47	1·00			
RC/AREA	−0·05	0·07	1·00		
GH/AREA	−0·10	0·07	0·79	1·00	
BH/AREA	0·09	0·19	0·70	0·70	1·00

Entries in the table are Pearson product-moment correlations, calculated pairwise for each set of variables.

Each correlation is based on an average sample size of 65 feedings.

See text for an explanation of the abbreviations.

Table 4. *Factor loadings (for principal components 1–4, PC1–4) for the area variables measured from the electromyographic recordings of feeding in* Ambystoma mexicanum

	Factor loadings			
Variable	PC1	PC2	PC3	PC4
RC/AREA	0·91	−0·13	0·05	0·22
GH/AREA	0·91	−0·16	−0·03	0·21
BH/AREA	0·88	0·07	0·09	−0·45
AM/AREA	0·03	0·87	0·49	0·09
DM/AREA	0·21	0·83	−0·52	0·02
Proportion of total variance explained	49·7%	29·6%	10·5%	6·1%

See text for an explanation of the abbreviations.

(Table 4; Fig. 3) and univariate analyses (Table 2) illustrate a lack of change in motor pattern when feeding on the two prey types.

This result contrasts sharply with the results of other quantitative analyses of motor pattern variation in feeding systems (Wainwright & Lauder, 1986; Wainwright, 1988; Sanderson, 1988) on fishes, and with studies of feeding systems in fishes and amniotes (Crompton *et al.* 1977; DeVree & Gans, 1973, 1976; Elshoud-Oldenhave & Osse, 1976; Gorniak, 1977; Gorniak *et al.* 1982; Hiiemae & Crompton, 1985; Liem, 1979, 1980; Weijs & Dantuma, 1981). These studies have all found that modulation of the feeding motor pattern occurs when different prey are presented to the animal. For example, Wainwright & Lauder (1986) measured 11 variables from the motor pattern used during feeding by four genera of sunfishes (Centrarchidae) and found a statistically significant prey-type effect in nine of the 11 variables. Sanderson (1988) found prey-type effects in the feeding

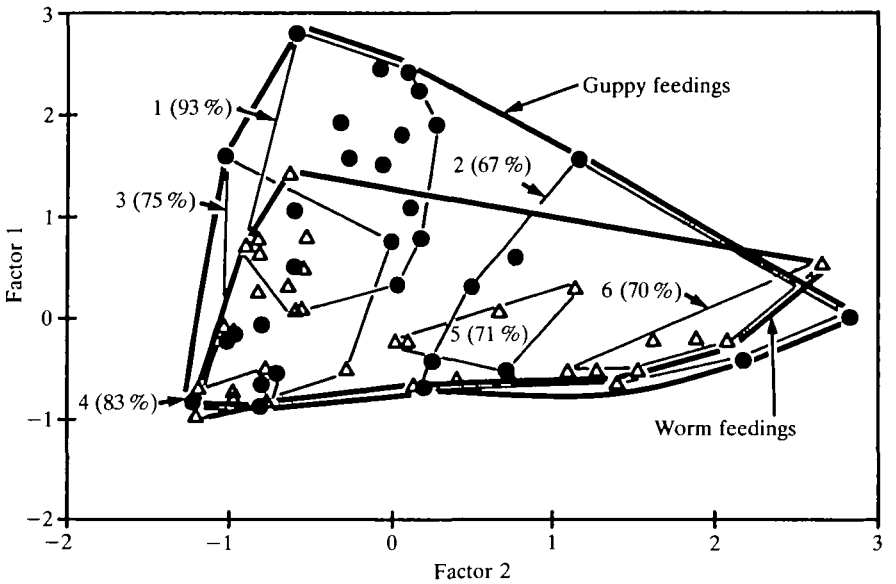

Fig. 3. Principal components analysis of 65 feedings and five variables (see text for details) to illustrate the multivariate relationship between the guppy and worm motor patterns. Each point represents one feeding: solid circles, guppies, open triangles, worms. Heavy solid lines enclose the worm and guppy feeding polygons. There is extensive overlap between the polygons, and the centroids of the worm and guppy polygons are not significantly different (MANOVA $F = 1{\cdot}7$; df = 2, 2; $P = 0{\cdot}37$) indicating that the motor pattern does not change with prey type in *Ambystoma mexicanum*. Thin solid lines enclose polygons outlining the feedings for each of the six individuals. The percentages next to the polygons for each individual reflect feeding performances (percentage of all guppy strikes that are successful) for that individual. Note that although worm and guppy feedings are not significantly different among individuals, individual animals occupy different areas of multivariate space. Loadings of the variables on components 1–4 are given in Table 4. Factor 1 is interpreted as a suction axis. Feedings with large positive loadings are predicted to involve the generation of greater negative buccal pressures than feedings with negative scores. Factor 2 is interpreted as a strike speed axis. Feedings with large positive loadings are predicted to involve more rapid jaw movements than those with negative loadings.

motor pattern of labrid fishes, and Liem (1978, 1979, 1980) has repeatedly noted extensive qualitative variation in motor patterns used by cichlid fishes to capture prey. The motor pattern in *Ambystoma mexicanum* thus appears to be stereotyped compared with the motor systems of the fishes and terrestrial vertebrates so far examined. Comparative analyses of the motor patterns used by amphibians feeding on more than one prey type are not available, as studies have typically focused on one prey species (Ewert, 1980; Gans & Gorniak, 1982).

Our result of motor pattern stereotypy must be considered, however, within the limits of the experimental design which focused on two prey types of similar size. Since we treated prey type as a fixed statistical effect, we are not able to generalize this result to all possible prey types or size classes of prey. Thus, it remains true

that the motor pattern of *Ambystoma mexicanum* might be shown to vary if prey of widely differing size classes were used as the treatment.

One interesting aspect of our results is the dispersion of individual salamanders in principal component space (Fig. 3) (bearing in mind the cautionary notes mentioned in Materials and methods concerning confounding of variation due to implants and days). Although individuals do not use a different motor pattern for the two prey types, each individual possessed a different motor pattern from other individuals. The occurrence of high levels of among-individual variation in motor patterns in salamanders has been noted previously (Shaffer & Lauder, 1985; Lauder & Shaffer, 1985, 1988), but the available data are not sufficient to allow an understanding of the functional significance of this variation.

The pattern of individual variation shown in Fig. 3 could be attributable to random variation, such that motor patterns vary randomly among individuals (within the limits that produce a functional feeding system). Under this view, minor variations in motor patterns have no functional significance and each individual possesses a fully functional motor pattern that differs in small, random ways from that of other individuals. If differences among individuals in feeding motor patterns are random, then individuals should not stay in the same relative position.

An alternative view is that the differences among the motor patterns of the six individuals shown in Fig. 3 are meaningful and reflect differences in feeding performance that have not been detected by this study. Under this view, differences in the motor patterns could be produced either by genetic differences among individuals in output from the central nervous system or, perhaps, by the effects of early experience with particular prey types.

The feeding performance data shown in Fig. 3 indicate that feedings that load highly on principal component 1 are associated with greater capture success, and this result is consistent with the interpretation that factor 1 represents a suction axis. This indicates that interindividual variation in motor pattern might not be random and might, in fact, reflect differences in feeding ability among individuals.

Currently there are few data in the literature that allow electromyographic variables to be related quantitatively to behavioural performance, and it is not possible here to document the quantitative relationship among variation in muscle activity patterns, buccal pressure changes and feeding performance. This makes interpreting the variation in feedings along the principal components more difficult. However, Lauder & Shaffer (1985) did conduct multiple regressions using patterns of muscle activity during feeding in *Ambystoma mexicanum* as independent variables, in an attempt to predict variation in mouth cavity pressures and jaw bone movement. They showed that muscle activity patterns are related both to the speed of the strike and to the magnitude of negative pressure generated in the oral cavity.

For example, variation in the duration of rectus cervicis muscle activity is significantly related to variation in the magnitude of negative buccal pressure (Lauder & Shaffer, 1985). This supports our interpretation of principal component

1 as a suction factor. Furthermore, variation in the duration of negative pressure generated in the buccal cavity is significantly related to variation in the duration of depressor mandibulae muscle activity (Lauder & Shaffer, 1985). This supports our interpretation of component 2 as an axis reflecting the speed of the strike.

The stereotyped prey capture behaviour of *Ambystoma mexicanum* indicates that the neural control for suction feeding appears to lack the complex feedback pathways and modulatory channels that have been postulated in mammals (Hiiemae *et al.* 1978; Hiiemae & Crompton, 1985). At least initial prey capture does not seem to be *modulated* by changes in (1) visual stimuli, (2) variation among prey types in input to the snout neuromasts or (3) differential olfactory excitation by prey. As yet, no research provides any indication of the neural pathways used in feeding or whether these differ between a stereotyped feeding species such as *Ambystoma mexicanum* and other species that show modulation of the feeding motor pattern. The neural basis of prey detection in aquatic salamanders has not been the subject of the elegant neuroethological research such as that conducted on several terrestrial amphibians (e.g. Ewert, 1980; Roth, 1982, 1986; Roth *et al.* 1983). Studies of this type would aid in determining the neural basis of behavioural stereotypy.

Testing the hypothesis of motor pattern stereotypy

How constrained is the pattern of motor output to the jaw muscles in aquatic salamanders? Three sets of tests might be conducted that would help define the extent of motor pattern stereotypy. First, a comparative, phylogenetic analysis could be conducted using different genera of aquatic salamanders to see if the lack of variability observed in this study is general to caudates or if it is unique to *Ambystoma mexicanum* or some subclade within urodeles. Second, a direct experimental test of the ability of axolotls to modulate the motor pattern could be conducted by experimentally modifying the musculoskeletal system. If the pattern of motor output from the nervous system to the jaw muscles remained unchanged following modification of the peripheral morphology, this would support the view that the feeding motor pattern is stereotyped. Third, the motor pattern could be studied quantitatively to see if there is an effect of satiation. Does muscle activity change with the number of prey eaten? If not, then the lack of a satiation effect can be taken as further evidence of stereotypy in the motor pattern of aquatic salamanders.

The extent and significance of motor pattern variation in vertebrates has not yet been examined in any systematic and quantitative manner. Differences among species in musculoskeletal morphology and neuroanatomy have been documented with increasing accuracy (e.g. Hanken & Hall, 1989; Northcutt & Davis, 1983), but relatively little quantitative information is available on both intraspecific and interspecific patterns of variation in motor output to peripheral musculature. Such data are essential if we are to understand the mechanistic basis of behavioural variation in vertebrates.

We thank Sue Bryant and Warren Fox for the gift of the *Ambystoma mexicanum* used in this paper. Peter Wainwright, Chris Sanford, Ron Brandon, Mark Westneat, Brad Shaffer and Bruce Jayne provided many helpful comments on the manuscript, as did two anonymous reviewers. We also thank Heidi Mumford for her invaluable assistance in the laboratory. This research was supported by NSF DCB 8602606 and NSF DCB 8721010.

References

CHATFIELD, C. & COLLINS, A. J. (1980). *Introduction to Multivariate Statistics.* London: Chapman & Hall.

CROMPTON, A. W., THEXTON, A. J., PARKER, P. & HIIEMAE, K. (1977). The activity of the jaw and hyoid musculature in the Virginia opossum, *Didelphis virginiana.* In *The Biology of Marsupials* (ed. B. Stonehouse & G. Gilmore), pp. 287–305. New York: MacMillan.

DEVREE, F. & GANS, C. (1973). Masticatory responses of pygmy goats (*Capra hircus*) to different foods. *Am. Zool.* **13**, 1342–1343.

DEVREE, F. & GANS, C. (1976). Mastication in pygmy goats *Capra hircus*. *Annls Soc. r. zool. Belg.* **105**, 255–306.

EATON, R. C. (1984). *Neural Mechanisms of Startle Behavior.* New York: Plenum Press.

ELSHOUD-OLDENHAVE, M. J. W. & OSSE, J. (1976). Functional morphology of the feeding system in the ruff – *Gymnocephalus cernua* (L. 1758) – (Teleostei, Percidae). *J. Morph.* **150**, 399–422.

EWERT, J.-P. (1980). *Neuroethology.* New York: Springer-Verlag.

GANS, C. & GORNIAK, G. C. (1982). Functional morphology of lingual protrusion in marine toads (*Bufo marinus*). *Am. J. Anat.* **163**, 195–222.

GORNIAK, G. C. (1977). Feeding in golden hamsters, *Mesocricetus auratus*. *J. Morph.* **154**, 427–458.

GORNIAK, G. C., ROSENBERG, H. I. & GANS, C. (1982). Mastication in the Tuatara, *Sphenodon punctatus* (Reptilia: Rhynchocephalia): structure and activity of the motor system. *J. Morph.* **171**, 321–353.

GOSLOW, G. E. (1985). Neural control of locomotion. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem & D. B. Wake), pp. 338–365. Cambridge: Harvard University Press.

HANKEN, J. & HALL, B. K. (1989). *The Vertebrate Skull*, vols. 1–3. Chicago: University of Chicago Press (in press).

HIIEMAE, K. & CROMPTON, A. W. (1985). Mastication, food transport, and swallowing. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem & D. B. Wake), pp. 262–290. Cambridge: Harvard University Press.

HIIEMAE, K., THEXTON, A. J. & CROMPTON, A. W. (1978). Intra-oral food transport: the fundamental mechanism of feeding. In *Muscle Adaptation in the Craniofacial Region* (ed. D. Carlson & J. MacNamara), pp. 181–208. Ann Arbor: University of Michigan Press.

HIIEMAE, K., THEXTON, A. J., MCGARRICK, J. & CROMPTON, A. W. (1981). The movement of the cat hyoid during feeding. *Archs oral Biol.* **26**, 65–81.

JAYNE, B. C. (1988). Muscular mechanisms of snake locomotion: An electromyographic study of lateral undulation of the Florida banded water snake *(Nerodia fasciata)* and the yellow rat snake (*Elaphe obsoleta*). *J. Morph.* **197**, 159–181.

LAUDER, G. V. (1985). Homology, analogy, and the evolution of behaviour. In *The Evolution of Behaviour* (ed. M. Nitecki & J. Kitchell), pp. 9–40. London: Oxford University Press.

LAUDER, G. V. & REILLY, S. M. (1988). Functional design of the feeding mechanism in salamanders: causal bases of ontogenetic changes in function. *J. exp. Biol.* **134**, 219–233.

LAUDER, G. V. & SHAFFER, H. B. (1985). Functional morphology of the feeding mechanism in aquatic ambystomatid salamanders. *J. Morph.* **185**, 297–326.

LAUDER, G. V. & SHAFFER, H. B. (1986). Functional design of the feeding mechanism in lower vertebrates: unidirectional and bidirectional flow systems in the tiger salamander. *J. Linn. Soc. (Zool.)* **88**, 277–290.

LAUDER, G. V. & SHAFFER, H. B. (1988). The ontogeny of functional design in the tiger salamander (*Ambystoma tigrinum*): are motor patterns conserved during major morphological transformations? *J. Morph.* **197**, 249–268.

LIEM, K. F. (1978). Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes. I. Piscivores. *J. Morph.* **158**, 323–360.

LIEM, K. F. (1979). Modulatory multiplicity in the feeding mechanism in cichlid fishes, as exemplified by the invertebrate pickers of Lake Tanganyika. *J. Zool., Lond.* **189**, 93–125.

LIEM, K. F. (1980). Adaptive significance of intra- and interspecific differences in the feeding repertoires of cichlid fishes. *Am. Zool.* **20**, 295–314.

NORTHCUTT, R. G. & DAVIS, R. (1983). *Fish Neurobiology.* Ann Arbor: University of Michigan Press.

ROTH, G. (1982). Responses of the optic tectum of the salamander *Hydromantes italicus* to moving prey stimuli. *Expl Brain Res.* **45**, 386–392.

ROTH, G. (1986). Neural mechanisms of prey recognition: an example in amphibians. In *Predator–Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates* (ed. M. E. Feder & G. V. Lauder), pp. 42–68. Chicago: University of Chicago Press.

ROTH, G., GRUNWALD, R., LINKE, G., RETTIG, G. & ROTTLUFF, B. (1983). Evolutionary patterns in the visual system of lungless salamanders (fam. Plethodontidae). *Archs Biol. Med. exp.* **16**, 329–341.

SANDERSON, S. L. (1988). Variation in neuromuscular activity during prey capture by trophic specialists and generalists (Pisces: Labridae). *Brain Behav. Evol.* (in press).

SHAFFER, H. B. & LAUDER, G. V. (1985). Aquatic prey capture in ambystomatid salamanders: patterns of variation in muscle activity. *J. Morph.* **183**, 273–284.

SOKAL, R. R. & ROHLF, F. J. (1981). *Biometry* (2nd edn). New York: Freeman Press.

THEXTON, A. J. (1976). To what extent is mastication programmed and independent of peripheral feed-back? In *Clinical and Physiological Aspects of Mastication* (ed. D. J. Anderson & B. Matthews), pp. 201–214. Bristol: John Wright.

WAINWRIGHT, P. C. (1986). Motor correlates of learning behavior: feeding on novel prey by pumpkinseed sunfish (*Lepomis gibbosus*). *J. exp. Biol.* **126**, 237–247.

WAINWRIGHT, P. C. (1989). Prey processing in haemulid fishes: patterns of variation in pharyngeal jaw muscle activity. *J. exp. Biol.* **141**, 359–375.

WAINWRIGHT, P. C. & LAUDER, G. V. (1986). Feeding biology of sunfishes: patterns of variation in prey capture. *J. Linn. Soc. (Zool.)* **88**, 217–228.

WEIJS, W. A. & DANTUMA, R. (1981). Functional anatomy of the masticatory apparatus in the rabbit (*Oryctolagus cuniculus* L.). *Neth. J. Zool.* **31**, 99–147.