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PREY PROCESSING IN HAEMULID FISHES: PATTERNS OF VARIATION IN PHARYNGEAL JAW MUSCLE ACTIVITY

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Summary

This study examines patterns of variation in 15 electromyographic (EMG) variables measured from recordings of pharyngeal jaw muscle activity during prey processing in four species of the perciform fish family Haemulidae. Two questions were of primary interest. (1) Are motor patterns conserved across the four species? (2) Do the fishes alter (modulate) muscle activity patterns when feeding on different prey types? The experimental design used allowed the partitioning of variance in EMG variables among species, among individuals within species, among days within individuals, among feedings within days, and among prey types. Only one variable exhibited a significant species effect, indicating that the four species used virtually the same motor pattern during prey processing. In response to three prey types differing in hardness, all four species demonstrated an ability to modulate several EMG variables that characterized the intensity of electrical activity. However, variables characterizing the relative timing of muscle activities were not influenced by prey type. A significant variance component was found among recording days and, together with the possibility of variation among experimental preparations, this raises questions about the extent of previously reported inter-individual variation in EMGs. These results support a growing data base on aquatic feeding in lower vertebrates which finds that: (1) motor patterns tend to be highly conserved among closely related taxa; (2) the ability to modulate motor patterns in response to different prey types appears to be a general property of teleost fish feeding mechanisms; and (3) variation in experimental EMG data is ubiquitous and, when unaccounted for, confounds comparisons among treatment groups.

Introduction

In the past two decades of research on vertebrate functional morphology, electromyography has emerged as a powerful tool for studying muscle function. By documenting the time course and intensity of muscle electrical activity during natural behaviour (i.e. the motor pattern), electromyography has proved most useful in testing hypotheses about the function of specific muscles (Jacobson & Hollyday, 1981; Gans & Gorniak, 1982) and in comparisons of motor patterns,

Key words: Haemulidae, electromyography, fish feeding, pharyngeal jaws, motor patterns.

both among species performing the same behaviour (Shaffer & Lauder, 1985; Jenkins & Goslow, 1983) and among behaviours within species (Liem, 1980; Herring & Wineski, 1986; Wainwright & Lauder, 1986).

Recently, it has become clear that a successful comparison of motor patterns is dependent on an experimental design that accounts for sources of variation within the groups being contrasted (Shaffer & Lauder, 1985; Wainwright & Lauder, 1986; Sanderson, 1988). Variability in motor patterns is high (Shaffer & Lauder, 1985), and comparisons between species or experimental groups must take into account differences in the variables of interest among individuals within the groups, differences between experimental days from the same animal, and experimental measurement error. This has been shown to be particularly important in comparisons among species, because inter-individual variation can account for 30–90 % of the total variance in an EMG variable (e.g. Shaffer & Lauder, 1985). In spite of these recent discoveries of the extent of inter-individual variation, few studies have explicitly partitioned this source of variance in analyses of differences between experimental groups. Comparative data are needed to assess the generality of high inter-individual variation in the motor patterns underlying natural behaviour, and to improve our understanding of how animals adjust the neural basis of behaviour in response to sensory information.

The present study focuses on prey processing behaviour during feeding by fishes in the perciform family Haemulidae. Like many other fishes (Liem & Greenwood, 1981; Lauder, 1983*b*) haemulids manipulate, lacerate, chew and crush prey in their pharyngeal jaws before swallowing. Qualitative studies on pharyngeal jaw function in fishes have indicated that some species can alter the motor patterns controlling jaw movements when feeding on different prey (Lauder, 1983*a*,*b*; Sibbing *et al.* 1986). This phenomenon, termed modulation by Liem (1978), has also been observed to occur in the muscles controlling the strike in diverse fish taxa (Ballintijn *et al.* 1972; Elshoud-Oldenhave & Osse, 1976; Liem, 1980; Lauder, 1981; Wainwright & Lauder, 1986; Sanderson, 1988). However, no study to date has quantitatively assessed the ability of fish species to modulate pharyngeal jaw motor patterns in the light of the extensive inter-individual variation discussed above.

Two questions are of primary interest in this paper. First, are haemulids able to alter the motor pattern controlling prey processing when feeding on different prey types? Second, do different species use the same motor pattern when feeding on the same prey, despite some differences in morphology of the pharyngeal jaw apparatus? These questions are answered in the context of an analysis of variance experimental design that allows the partitioning of variance in EMG variables into differences among species, among individuals within species, among days within the same individual, among feedings within days, and among three prey types. The results show (1) that these fishes do modulate muscle activity when feeding on different prey types and (2) that different species use the same motor patterns. The specific changes that occur in response to each prey type permit insights into the functional significance of the motor patterns.

Materials and methods

Experimental animals

Four western Atlantic species of the cosmopolitan perciform fish family Haemulidae were compared in this study. Several formalin-preserved specimens of these and other haemulid species were examined for anatomical details of the pharyngeal jaw apparatus. Experimental data were collected from five *Anisotremus virginicus*, three *Haemulon plumieri*, three *Haemulon sciurus* and three *Haemulon aurolineatum*. Live specimens were purchased from commercial suppliers in Florida (United States) and Belize (Central America), who collected the fishes in coral reef habitats. All individuals were between 146 and 169 mm standard length. In the laboratory the fish were maintained in 100-l aquaria, held at constant temperature (19 °C) and fed a diet of the three experimental prey types. Like most haemulids, the adults of these species are nocturnally active predators on a broad range of benthic invertebrates, including polychaete worms, decapod crustaceans, amphipods, isopods, copepods, echinoderms, sipunculans, molluscs and others fishes (Randall, 1967).

Experimental techniques

The goals of these experiments were to compare patterns of muscle activity in different species and during feedings by the same fish on different prey types. For this reason three precautions were taken to standardize the experimental protocol with the aim of minimizing among-preparation variation induced by electrode shape, electrode placement and recording channel idiosyncrasies. First, because electromyographic (EMG) signals are affected by the morphology of the recording electrodes (Gans & Gorniak, 1980; Loeb & Gans, 1986), their construction was carefully standardized. EMG recordings were made through fine wire (0·051 mm diameter) steel alloy bipolar electrodes. The paired insulated electrode wires (75 cm long) were threaded through a hypodermic needle (26 gauge) and the first 15 cm were glued together with a thin coat of cyanoacrylate adhesive (Jayne, 1988). Under a dissecting microscope, a razor blade was then used to scrape away the insulation from each electrode tip, exposing 0·5 mm of steel wire. Finally, the electrode tips were bent back so that they were nearly parallel to each other and 1–2 mm apart. Gluing the wires together minimized the spreading of electrode tips that can occur during experiments due to normal animal movements. Post-experimental dissections confirmed that these electrodes maintained their original configuration better than standard, unglued tips.

Second, the method of electrode placement into each muscle was standardized for angle and location of hypodermic entry, and depth of electrode placement. Fishes were anaesthetized with tricaine methane sulphonate and colour-coded electrodes were implanted directly into the belly of the five branchial muscles, four of which were clearly visible through the thin epithelial membrane medial to the gill filaments. Electrode placement was verified by dissection following about one-third of the experiments. The electrodes were secured to a mid-dorsal suture behind the fish's head, and were glued together into a common cable. Electrical signals were amplified 10000 times (Grass P511J) with a bandpass of 100–3000 Hz and recorded on a Bell & Howell 4020A FM tape recorder at 19 cm s^{-1} tape speed. The 60 Hz notch filter was always used.

As a final precaution, each muscle was always recorded through the same tape channel, preamplifier and lead connectors. Since comparisons were always made among periods of activity in the same muscle, this prevented any differences among channels in recording properties from adding to the natural variation between individuals in EMG patterns.

Feeding sequences were later digitized at a sample rate of 2050 Hz with a Keithley DAS 12-bit analogue-to-digital converter and stored on an IBM AT microcomputer. A chart record (Gould 260) was made of each computer file for visual inspection.

Within 24 h of electrode implantation individuals were fed five each of the three prey types: 1 cm^3 pieces of frozen shrimp (*Penaeus*), 2–3 cm long pieces of live earthworm (*Lumbricus*) and 1–2 cm long live mole crabs (*Emerita*). These three prey were chosen to present a gradation of prey toughness and hardness for the fishes. The chitinous carapace of the mole crabs provided a hard barrier for the fish to break through and they were sometimes too hard for them to crush. In contrast, the worm pieces were pliant but their skin was tougher than the soft pieces of shelled shrimp and they were often chewed intermittently over several minutes before they were swallowed. Observations on the stomach contents of fish after experiments revealed that all prey items were considerably triturated before being swallowed.

To assess the extent of day-to-day variation in EMG patterns within the same individual, three *A. virginicus* and three *H. aurolineatum* were fed three worms per day for three consecutive days following electrode implantation.

All recordings were made from the left member of the same five paired branchial muscles (muscle names follow Winterbottom, 1974; Lauder, 1983*b*): the retractor dorsalis (RD), fourth levator externus (LE4), protractor pectoralis (PP), pharyngocleithralis externus (PCE) and pharyngocleithralis internus (PCI) (Fig. 1). These muscles were chosen because of their prominent role in the feeding mechanism and their easy access (only the retractor dorsalis could not be seen clearly through the mucous membrane that overlies the branchial muscles). The anatomical relationships of these muscles were the same in the four species and are the same as in other 'basal' perciform groups (see Lauder, 1983*a*,*b*), with the exception of the protractor pectoralis which exhibits a phylogenetically derived insertion on the posterior tip of the lower pharyngeal jaw, as opposed to the generalized insertion on the pectoral girdle (Greenwood & Lauder, 1981). Although a full functional analysis of the muscles will be published elsewhere, a brief description of each muscle and its action is presented here (see also Fig. 1). The retractor dorsalis passes from its origin on several anterior vertebrae to insert on the posterior face of the upper pharyngeal jaws; it retracts the upper pharyngeal jaws posteriorly. The fourth levator externus originates anteriorly from the otic

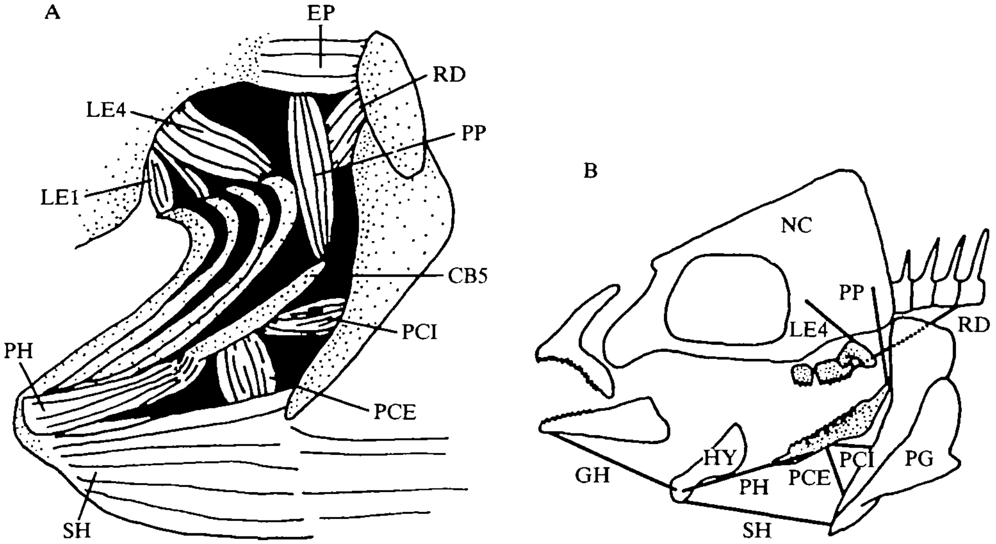

Fig. 1. (A) Diagram of the pharyngeal jaws of *Haemulon sciurus*, in lateral view. The operculum, gills and gill rakers, levator posterior muscle and the mucous membrane surrounding the pharyngeal muscles have been removed to highlight the five muscles studied in this paper: RD, retractor dorsalis; LE4, fourth levator externus; PP, protractor pectoralis; PCI, pharyngocleithralis internus; PCE, pharyngocleithralis externus. (B) A schematic diagram of the pharyngeal jaws in haemulid fishes. Black bars indicate the attachments and primary orientation of action of several prominent muscles, including the five that were the subject of the present study. Pharyngeal jaw bones are shown stippled, from upper left moving clockwise they are; pharyngobranchial three, pharyngobranchial four, epibranchial four and ceratobranchial five. See text for further discussion of muscle function. Other abbreviations: CB5, fifth ceratobranchial (lower pharyngeal jaw); EP, epaxial muscles; GH, geniohyoideus; HY, hyoid bar; LE1, first levator externus; NC, neurocranium; PG, pectoral girdle; PH, pharyngohyoideus; SH, sternohyoideus.

region of the skull and attaches to the uncinate process of the fourth epibranchial. Through the couplings between the epibranchial and both the upper and lower pharyngeal jaw, shortening of this muscle contributes to both upper jaw depression and lower jaw elevation. The protractor pectoralis runs from the skull to insert on the posterior limit of the fifth ceratobranchial (lower pharyngeal jaw) and strongly elevates this element dorsally, contributing to the pharyngeal jaw bite. The pharyngocleithralis externus, passing between the ventral margin of ceratobranchial five and the cleithrum, retracts the lower jaws ventrally. The pharyngocleithralis internus also passes between the fifth ceratobranchial and the pectoral girdle but in a more anterior-to-posterior orientation than the pharyngocleithralis externus (Fig. 1). This muscle retracts the lower jaws posteriorly.

The digital file of each feeding was played into a Tektronix 4107 colour graphics terminal, and a computer program was used first to rectify all EMGs and then to digitize variables. The behaviour being analysed in this study, termed pharyngeal

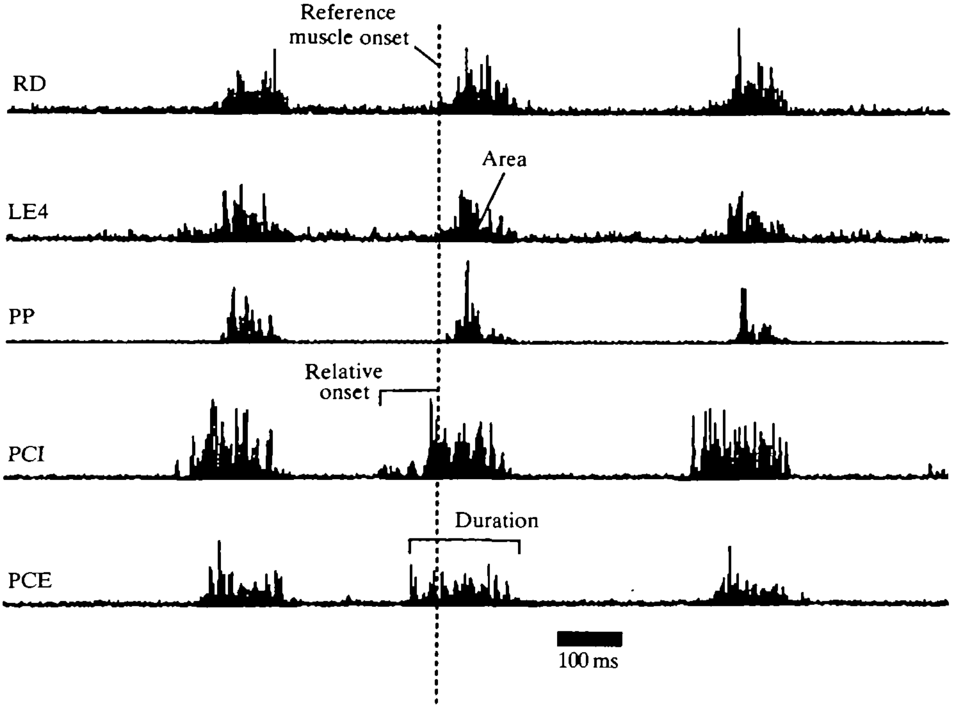

Fig. 2. Sample rectified electromyograms showing three muscle activity cycles from *Haemulon aurolineatum* chewing a piece of earthworm, to illustrate the EMG variables that were measured in this study. For each muscle the integrated area under the rectified EMG and the duration of activity were calculated, as was the onset time of each muscle relative to the onset of the retractor dorsalis (RD). See text for further discussion of variables and other muscle abbreviations.

transport by Lauder (1983*b*), is a repetitive process involving cyclical bursts of activity in the branchial muscles (Fig. 2). Fifteen variables were measured from each cycle of activity, for up to 10 cycles per feeding (mean = 7·1 cycles per feeding). If more than 10 cycles occurred in a feeding, only the first 10 were analysed. The variables summarized the pattern of activity in the five pharyngeal jaw muscles (Fig. 2). For each muscle the duration of activity (in ms) was measured (RDDUR, LE4DUR, PPDUR, PCEDUR, PCIDUR) and the area under the rectified curves (in mV·ms) was calculated (RDAREA, LE4AREA, PPAREA, PCEAREA, PCIAREA). Also, using the onset of activity of the retractor dorsalis muscle as a reference, the relative time of onset of activity for each of the other four muscles was measured (RD–LE4, RD–PP, RD–PCI, RD–PCE), as was the time to the onset of activity of the retractor dorsalis in the following cycle (RD–RD). The retractor dorsalis was selected as the reference muscle because it exhibited conservative activity (was active in all cycles) and has been used in previous studies (Lauder, 1983*a*,*b*), thus facilitating comparison.

Muscles occasionally exhibited two bursts of activity per cycle, but the analysis presented here concerns only the first activity burst in each muscle.

Experimental design

The overall experimental design was a two-way analysis of variance (ANOVA) with a nested level. Nested within species were varying numbers of individuals. Crossed with species and individuals was the prey-type factor. Five replicate feedings of each prey type for each fish constituted the contents of a single cell in this design. Slight variations of this experimental design have been used in earlier studies of muscular modulation in the sunfish family Centrarchidae (Wainwright & Lauder, 1986) and the marine Labridae (Sanderson, 1988). The cycles of muscle activity in each feeding clearly represent a time series event, and thus should not be considered as independent observations because this artificially increases the experimental degrees of freedom (Sokal & Rohlf, 1981). To avoid this problem, the mean of each variable was taken across the multiple cycles of activity in each feeding and was used as the basic observation in the main analysis. The species, prey type and species by prey-type interaction term were fixed effects in this model whereas all other terms were random effects. Guided by Scheffe (1959), appropriate F-ratios were constructed to test each effect. In all 203 feedings and 1417 cycles of muscle activity were analysed in this part of the study.

To assess the ability of each species to modulate pharyngeal muscle activity, the data were analysed separately for each species. Here, the designs were a two-way, mixed model, analysis of variance; individuals crossed with prey type. Because this is a mixed model, the F-ratio for the prey-type effect was constructed with the interaction term mean squares in the denominator (Sokal & Rohlf, 1981). Significant tests of the prey-type effect would indicate that the species was modulating the EMG variable in response to different prey.

A third analysis was performed to examine the extent of day-to-day variation within experimental animals feeding on worms. A four-level nested analysis of variance permitted the partitioning of EMG variance into that due to differences among species (*A. virginicus* and *H. aurolineatum*), among individuals within species, among days within individuals and among feedings within days. Because this is strictly a nested ANOVA, it was possible to examine explicitly the contribution of between-cycle variation without compromising the experimental design with increased degrees of freedom. This design involved the analysis of 54 feedings and 482 cycles of muscle activity.

Because many significance tests were performed in this study, a conservative probability of $P < 0.01$ was used to establish statistical significance. This lessened the chances of finding significant effects simply because so many tests were performed. All computations were performed on untransformed data with Systat (Wilkinson, 1986) on a microcomputer or with a mainframe SAS (version 5, 1985), using the general linear model procedure, with type four sums of squares (Freund & Littell, 1981).

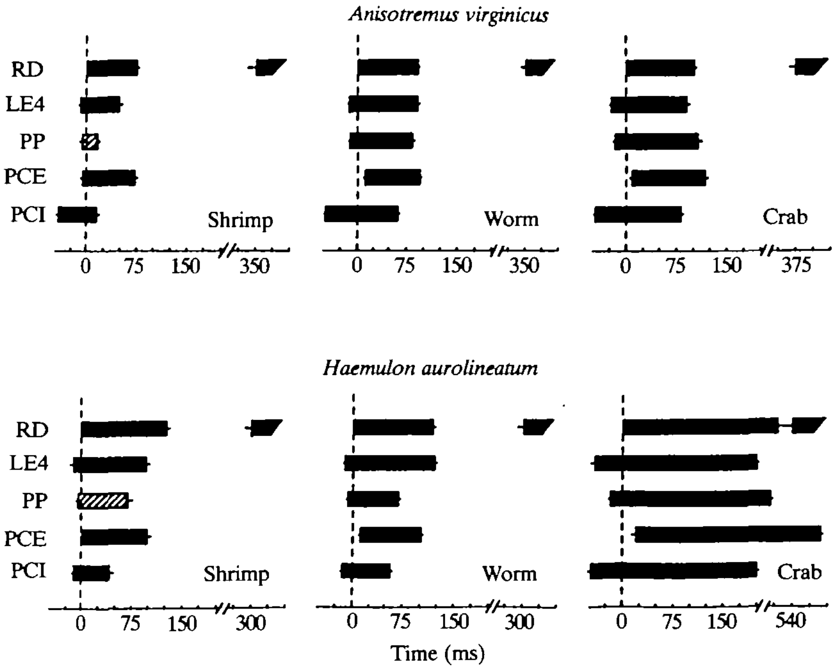

Fig. 3. Summary pattern of muscle activity in *Anisotremus virginicus* and *Haemulon aurolineatum* feeding on three experimental prey types. Bars indicate mean duration and relative onset times across all feedings on each prey type, analysed for each species. Error bars on right-hand side of bars are 1 s.e. for average duration, those to the left are 1 s.e. for relative onset times. Solid bars indicate muscle was active in over 75 % of all activity cycles, striped bars indicate activity in less than 50 % of all activity cycles analysed. The second activity bout in the retractor dorsalis (RD) shows mean and 1 s.e. for time between activity cycles. Because several levels of variation are confounded in obtaining the means plotted here, these data should not be used to make statistical comparisons, rather reference should be made to Tables 1, 2 and 4. Other muscle abbreviations: LE4, fourth levator externus; PP, protractor pectoralis; PCE, pharyngocleithralis externus; PCI, pharyngocleithralis internus.

Results

The patterns of muscle activity exhibited by the four species were very similar (Figs 3 & 4). In the overall analysis (Table 1) only one of the 15 EMG variables was significantly different among species: the activity area of the fourth levator externus muscle (LE4AREA). In contrast, all 15 variables showed significant differences among individuals within species. In this overall model, the prey-type effect was significant for eight of the 10 duration and area variables, but not for any relative onset time variables. The interaction terms showed a conservative pattern, with few variables having significant tests, indicating that the influence of different prey on the motor pattern was generally the same for all species and individuals within species.

When the data for each species were analysed separately in two-way ANOVAs

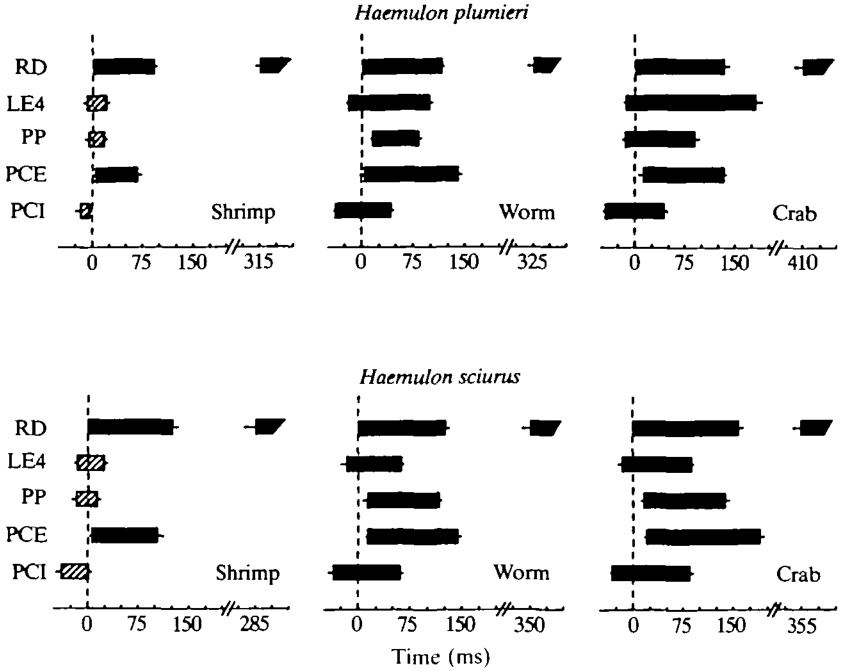

Fig. 4. Summary bar diagram of muscle activity patterns in five pharyngeal jaw muscles in *Haemulon plumieri* and *H. sciurus* chewing three experimental prey types. Conventions as in Fig. 3. As in Fig. 3, notice that hardness of prey is correlated with increased durations of activity. See Tables 1, 2 and 4 for statistical comparisons.

(Table 2), all four species showed about the same ability to modulate motor activity in response to different prey. Two variables, the duration and area variables for the protractor pectoralis muscle (PPDUR, PPAREA), had significant tests of the prey-type effect in all four species (compare prey types in Figs 3 & 4). Each fish species had a significant prey-type effect in five or six of the duration and area variables. All these variables, except RDDUR and PCEAREA were significant in at least one fish species. None of the five relative onset variables showed a significant prey-type effect in any species.

The means for the EMG timing variables in each species are shown graphically in Figs 3 and 4. Area variable means are presented in Table 3. In all species a strong effect of prey type can be seen for the 10 duration and area variable means. These variables consistently increased in magnitude across the three types of prey, with the longest durations and largest areas occurring when fishes fed on the mole crabs.

The nested ANOVAs permitted estimates of the percentage of total variance in each EMG variable attributable to each of the levels in the design (Table 4). Only a small fraction of variance in the motor variables could be attributed to differences among cycles of activity within each feeding and among feedings within each day. Day-to-day differences were significant in 13 of the 15 variables and

Table 1. *Results from two-way nested ANOVAs for 15 electromyographic variables measured from five muscles during pharyngeal transport in four species of fish, feeding on three prey types*

	Factor				
Variable	Species (3, 10)*	Individuals (10, 210)	Prey type (2, 18)	Species × prey type (6, 18)	Individuals × prey type (18, 206)
RDDUR	NS†	0·0001	NS	NS	0·0001
LE4DUR	NS	0·0001	0·0001	0·001	NS
PPDUR	NS	0·0001	0·0001	NS	NS
PCEDUR	NS	0·0001	0·0001	NS	NS
PCIDUR	NS	0·0001	0·0001	NS	0·01
RDAREA	NS	0·0001	0·001	NS	NS
LE4AREA	0·005	0·01	0·0001	NS	NS
PPAREA	NS	0·0008	0·007	NS	NS
PCEAREA	NS	0·0001	NS	NS	NS
PCIAREA	NS	0·0001	0·0001	0·005	NS
RD-RD	NS	0·0001	NS	NS	0·01
RD-LE4	NS	0·0001	NS	NS	0·0001
RD-PP	NS	0·0001	NS	NS	NS
RD-PCE	NS	0·0001	NS	NS	NS
RD-PCI	NS	0·0001	NS	NS	0·0001

Entries indicate the probabilities associated with tests of significance for each factor.
* Degrees of freedom.
† NS = $P > 0{\cdot}01$.

accounted for up to 31 % of the total variance. In contrast to the results of the overall model (Table 1), only three variables were significantly different among individuals within the two species. There was a wide range among variables in the percentage of variance that the among-individual factor accounted for; from as little as 1 % up to 86 %. Similarly, in tests of the species effect, several variables were conservative among taxa, and one was found to have over 90 % of its variance attributable to this level.

Discussion

Quantitative electromyographic data on aquatic feeding in lower vertebrates are available for other perciform fishes (Wainwright, 1986; Wainwright & Lauder, 1986; Sanderson, 1988), lungfishes (Bemis & Lauder, 1986) and ambystomatid salamanders (Shaffer & Lauder, 1985; Lauder & Shaffer, 1985). Three general conclusions can be drawn from this work, and below I discuss them as they relate to the findings of the present study. These are: (1) motor patterns tend to be highly conserved among closely related taxa; (2) the ability to modulate motor patterns in response to different prey appears to be ubiquitous among teleost fishes; and

Table 2. *Results of two-way ANOVAs testing the significance of the prey-type effect on EMG variables in each of four fish species*

Variable	*Anisotremus virginicus* (2,8)*	*Haemulon plumieri* (2,4)	*Haemulon sciurus* (2,4)	*Haemulon aurolineatum* (2,4)
RDDUR	NS†	NS	NS	NS
LE4DUR	NS	0·009	0·01	0·005
PPDUR	0·0004	0·01	0·0001	0·0001
PCEDUR	0·0001	NS	NS	NS
PCIDUR	0·01	0·007	NS	0·0004
RDAREA	NS	NS	0·01	NS
LE4AREA	0·01	0·001	0·003	NS
PPAREA	0·001	0·0001	0·002	0·002
PCEAREA	NS	NS	NS	NS
PCIAREA	0·001	NS	0·004	0·004
RD-RD	NS	NS	NS	NS
RD-LE4	NS	NS	NS	NS
RD-PP	NS	NS	NS	NS
RD-PCE	NS	NS	NS	NS
RD-PCI	NS	NS	NS	NS

Entries are P values associated with F-tests of significance.
Significant tests indicate an ability of the species to modulate that variable.
* Degrees of freedom in F-tests.
† NS = $P > 0{\cdot}01$.

(3) differences in motor pattern among individuals within species, among days within individuals and among feedings within days are significant sources of variation in EMG variables which, if unaccounted for, can confound comparisons among experimental groups.

Motor patterns are conservative

One of the central findings of this study is that the four haemulid species exhibited nearly identical patterns of muscle activity. Only one EMG variable was significantly different among species, the integrated area for the fourth levator externus EMG (Tables 1 & 4). This difference was due to a consistently high value for this variable in *H. aurolineatum* (Table 3). A strikingly similar pattern has been found in all previous quantitative assessments of differences between species in oral jaw EMGs recorded during prey striking behaviour in both fishes (Wainwright & Lauder, 1986; Sanderson, 1988) and ambystomatid salamanders (Shaffer & Lauder, 1985). In these studies, fewer than 10 % of the EMG variables exhibited a significant species effect.

A strong picture is emerging that motor patterns tend to be conserved across closely related taxa. This result is perhaps not surprising in the present study, since the four haemulid species in question have similar pharyngeal jaw morphology (P. C. Wainwright, unpublished observations). However, comparisons of morpho-

Table 3. *Mean (±one standard error) for the integrated area electromyographic variables from feeding on three prey types, compared between four fish species*

	Variable				
	RDAREA	LE4AREA	PPAREA	PCEAREA	PCIAREA
Anisotremus virginicus					
Shrimp	0·88 ± 0·075	0·91 ± 0·11	1·04 ± 0·27	2·75 ± 0·24	1·53 ± 0·13
Worm	1·54 ± 0·092	2·36 ± 0·13	6·92 ± 0·61	4·90 ± 0·21	5·44 ± 0·36
Crab	2·21 ± 0·20	3·88 ± 0·16	14·30 ± 1·34	9·99 ± 0·64	7·20 ± 0·36
Haemulon aurolineatum					
Shrimp	2·35 ± 0·12	3·54 ± 0·30	2·38 ± 0·31	4·17 ± 0·35	1·87 ± 0·20
Worm	3·18 ± 0·14	6·96 ± 0·24	1·90 ± 0·36	3·15 ± 0·29	1·96 ± 0·09
Crab	5·74 ± 0·88	12·13 ± 1·28	22·89 ± 3·08	15·84 ± 1·90	9·05 ± 1·12
Haemulon plumieri					
Shrimp	1·71 ± 0·19	0·43 ± 0·086	0·39 ± 0·079	3·43 ± 0·49	0·18 ± 0·043
Worm	4·49 ± 0·45	2·51 ± 0·23	2·46 ± 0·13	4·90 ± 0·45	1·79 ± 0·17
Crab	4·83 ± 0·29	5·42 ± 0·32	3·56 ± 0·39	2·67 ± 0·41	2·28 ± 0·17
Haemulon sciurus					
Shrimp	2·17 ± 0·22	0·65 ± 0·08	0·73 ± 0·12	1·92 ± 0·18	0·47 ± 0·055
Worm	4·04 ± 0·28	1·69 ± 0·12	2·25 ± 0·16	3·74 ± 0·21	1·77 ± 0·15
Crab	5·46 ± 0·30	2·59 ± 0·16	2·86 ± 0·14	7·47 ± 0·48	3·42 ± 0·22

See Table 2 for statistical comparisons. Note: multiply all entries by 0·4878 for proper scaling. Units are mV · ms; sample sizes vary between 27 and 161 cycles of motor activity.

logically more diverse taxa have given the same result (e.g. *Lepomis vs Micropterus*, Wainwright & Lauder, 1986; *Thalassoma vs Gomphosus*, Sanderson, 1988), demonstrating that motor patterns can be conserved even in the face of considerable structural evolution. This result is particularly significant in the light of the widely held view that behaviour tends to be more plastic than morphology in evolution (e.g. Morse, 1980; Krebs & McCleery, 1984). A key question in future research will be the extent to which motor patterns remain conservative when the morphological and phylogenetic breadth of the lineage under consideration is increased.

Modulation

Many electromyographic studies have examined the motor response of teleost fishes feeding on different prey (Ballintijn *et al.* 1972; Elshoud-Oldenhave & Osse, 1976; Liem, 1978, 1979, 1980; Elshoud-Oldenhave, 1979; Lauder, 1981, 1983*a*; Sibbing *et al.* 1986; Wainwright & Lauder, 1986; Sanderson, 1988). The consensus from this data base is that, although a few taxa have a highly stereotyped, narrow feeding repertoire, most teleosts modulate muscular activity; apparently to 'fine tune' the feeding response for the task of capturing or processing specific prey types. In marked contrast, aquatic ambystomatid salamanders do not appear to alter muscle activity when feeding on different prey (Reilly & Lauder, 1989).

Table 4. *Results of nested ANOVAs partitioning variation in EMG variables*

Variable	Among species (1,4)*	Among individuals within species (4,12)	Among days within individuals (12,36)	Among feedings within days (36,456)	Among cycles within feedings (error)
RDDUR	35·1	48·9	6·2	3·3†	6·4
LE4DUR	16·5	58·3	18·3†	2·8†	4·1
PPDUR	3·2	60·9	31·1†	1·5†	3·6
PCEDUR	0·0	66·7	28·7†	1·1†	3·6
PCIDUR	4·1	74·9	17·9†	0·7†	2·4
RDAREA	1·0	86·1†	11·0†	0·6†	1·2
LE4AREA	91·0†	0·9	7·3†	0·2†	0·4
PPAREA	76·0	9·3	13·5†	0·5†	0·9
PCEAREA	19·5	58·1	21·4†	0·2†	0·7
PCIAREA	0·2	68·1†	28·5†	0·9†	2·2
RD-RD	62·6	29·9	2·2	1·7†	3·7
RD-LE4	0·0	63·2	22·8†	3·0†	11·0
RD-PP	69·6	3·6	24·4†	0·3†	2·1
RD-PCE	11·1	77·1†	10·8†	0·2†	0·7
RD-PCI	49·1	35·1	14·3†	0·3†	1·2

Entries are percentages of total variance in each variable attributable to that level of the experimental design.

* Degrees of freedom.

† *P*<0·01.

Studies have focused on function in both the oral and the pharyngeal jaws. In both functional systems, different prey usually cause fish to employ the same two types of motor pattern changes: modulation of the intensity of muscle electrical activity and changes in the sequence of activity in the relevant muscles. Thus, strikes at elusive prey, compared with slow or immobile prey, are associated with greater activity and shorter relative onset times in oral jaw muscles (Elshoud-Oldenhave, 1979; Liem, 1979; Sanderson, 1988). Similarly, processing very hard prey elicits the same changes in pharyngeal muscles (see Lauder, 1983*a*).

Pharyngeal jaw modulation by the four haemulids only partly followed this trend. In all muscles, prey hardness was correlated with increased electrical activity during pharyngeal transport (see Figs 3 & 4). At least two of the muscles, the fourth levator externus and the protractor pectoralis, contribute directly to occlusal movements of the pharyngeal jaws. Electrical activity, as measured by integrated EMGs, has been shown to correlate positively with mechanical tension developed by skeletal muscles (Bigland & Lippold, 1954; Walmsley *et al.* 1978; Ringelberg, 1985). Thus, the observed increases in integrated areas and activity durations underlie more forceful and longer triturating action of the pharyngeal jaws during processing of harder prey types.

Harder, tougher prey elicited greater electrical activity in the five muscles, but no effect was seen on the relative onset time variables. This result is in contrast to the two previous studies of pharyngeal jaw modulation in teleosts. Work with the carp, *Cyprinus carpio* (Sibbing *et al.* 1986), and sunfishes, *Lepomis* spp. (Lauder, 1983*a*), showed that some prey elicited distintly different sequences of muscle activity. In these studies, particularly hard (molluscs; Lauder, 1983*a*) or soft prey (tubifex worm–soil mixture; Sibbing *et al.* 1986) caused fishes to change the basic motor pattern. Although such extreme prey consistencies were not fed to the haemulids in this study, the mole crabs were frequently rejected by fish after unsuccessful attempts to crush their carapace, indicating that this prey often matched the maximum crushing capability of individuals. Since the sequence of muscle use remained the same, it may be inferred that the jaw movement pattern did not vary with prey type. Hence, the overall effect of the prey types used in this study was that the fishes modulated the intensity of muscle contractions but did not adapt the basic pattern of jaw movements.

Inter-individual variability

Every study which has quantitatively partitioned electromyographic variation has found differences among individuals to be a major source of experimental variance (Shaffer & Lauder, 1985; Bemis & Lauder, 1986; Wainwright, 1986; Wainwright & Lauder, 1986; Sanderson, 1988). Inter-individual differences in EMG patterns are a compelling type of phenotypic variance in functionally important variables, that have not previously been recognized. A quantitative link between variation in EMG patterns measured at the strike and feeding performance has been demonstrated in *Lepomis* (Wainwright, 1986), suggesting that differences among individuals in muscle activity patterns could underlie differences in feeding ability. Although the ecological and evolutionary implications of this have yet to be explored, intraspecific variation in motor patterns may represent a fertile, although presently underutilized, resource for research (Shaffer & Lauder, 1985; Bennett, 1987).

A cautionary note is warranted, however, because the among-individuals component in experimental designs usually confounds at least two additional sources of variance. These are: differences among experimental days and differences among experimental preparations. Day-to-day differences accounted for as much as 31 % of the total variance in the haemulid EMG variables (Table 4). If this source of variance is not explicitly partitioned it becomes subsumed into the individual effect, artificially increasing the apparent differences among individuals. This was the case in the overall model (Table 1) where all variables showed a significant among-individuals component. Yet, when day-to-day variance was assessed, only three variables remained significantly different among individuals within species (Table 4).

One study (Sanderson, 1988) has examined the effect of different experimental preparations by repeating the entire implantation protocol on the same individuals. Sanderson found the among-preparation variance component to be significant in three of nine EMG variables and it accounted for as much as 67 % of the total variance in one case. In other work, multiple electrodes have been implanted into single muscles to assess the effect of different electrodes and their placement (Shaffer & Lauder, 1985; Bemis & Lauder, 1986). In neither study were differences among electrodes in the activity patterns recorded found to be statistically significant, although Bemis & Lauder (1986) did find this level of their experimental design accounted for the greatest fraction (31 %) of variation in the EMG variable they analysed.

Clearly, the extent of individual variation in motor patterns may be greatly exaggerated when these two factors are confounded with the among-individuals level of experimental designs. It is important to emphasize that when these sources of variance are not explicitly partitioned, they *will* be confounded with the individual factor and *will* artificially enhance apparent inter-individual differences. Both sources of variance will be present, even when data are collected on only one day or from only one implantation. Until a complete assessment of this issue is made, it will not be known if the ubiquitous and significant differences among individuals in EMG characters that have been reported truly represent intraspecific or phenotypic variation, or simply an artefact of the experimental design.

This is a particularly troublesome problem because no data are available describing the causes of day-to-day variance, so it cannot be controlled for without explicitly estimating its contribution. Similarly, among-preparation variance is neither entirely understood (although see Gans & Gorniak, 1980) nor easily controlled. Unfortunately, estimating these sources of variance greatly increases the complexity of experimental designs and the amount of data that must be gathered to obtain reasonably powerful statistical tests (see Sanderson, 1988).

Oral jaws vs *pharyngeal jaws*

One unexpected result in the nested analysis was the very small percentage of the total EMG variance attributable to differences among feedings within days and among cycles of activity within feedings (Table 3). These low variance components suggest that haemulid pharyngeal transport is composed of a repeated, highly stereotyped, basic pattern of muscle activity that varies little among cycles of activity within each feeding or among feedings within the same day. Previous investigations have focused on strike behaviour during prey capture and found large variance components among feedings within days (this level is usually the error variance). Estimates of the percentage of total variance due to feeding-to-feeding differences have ranged as high as 87 % and 99 % in salamanders (Shaffer & Lauder, 1985), 88 % in labrid fishes (Sanderson, 1988) and 27 % in *Lepidosiren* (Bemis & Lauder, 1986). In the haemulid data this source never accounted for more than 3·3 % of the total variance in any variable. Hence, the neural control of prey processing behaviour in the pharyngeal jaws of these fishes seems to differ substantially from that reported for prey striking behaviour.

Strike behaviour is associated with only a single burst of activity in each muscle, whereas pharyngeal transport is cyclical, involving repeated bursts from all muscles. This is similar to mammalian chewing, where internal oscillators are thought to produce stereotyped EMG and movement patterns that can be modulated by sensory feedback (Thexton, 1974, 1976). Comparable quantitative data are not yet available for mammalian chewing but the low feeding and cycle variances seen here suggest the presence of an oscillating pattern generator in the central nervous system of these fishes. The extent to which other lower vertebrate taxa exhibit similar differences in motor patterns between oral and pharyngeal jaw systems must await further study.

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