COUPLED *VERSUS* UNCOUPLED FUNCTIONAL SYSTEMS: MOTOR PLASTICITY IN THE QUEEN TRIGGERFISH *BALISTES VETULA*

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Summary

Teleost fishes typically capture prey with the oral jaws and perform most types of prey-processing behavior with the pharyngeal jaw apparatus. In these fishes, the motor patterns associated with the different stages of feeding are quite distinct, and fish can modify muscle activity patterns when feeding on different prey. We examined motor pattern variation in the queen triggerfish, *Balistes vetula*, a versatile predator that both captures and processes prey with its oral jaws. During feeding on three prey that differed in hardness and elusiveness, three distinct patterns of behavior could be identified on the basis of patterns of muscle activity: prey capture, buccal manipulation and blowing. During prey capture by suction feeding, the retractor arcus palatini muscle (RAP) commenced activity before the levator operculi muscle (LOP). In both buccal manipulation and blowing, the RAP began activity well after the onset of activity in the LOP. Both prey capture and buccal manipulation motor patterns varied when fish fed on different prey. When capturing hard-shelled and non-elusive prey, *B. vetula* did not employ suction feeding but, instead, the fish directly bit parts of its prey. The motor pattern exhibited during direct biting to capture prey was different from that during suction feeding, but was indistinguishable from the pattern seen during the repeated cycles of buccal manipulation. Harder prey elicited significantly longer bursts of activity in the jaw adductor muscles than did soft prey. In spite of the involvement of the oral jaws in virtually all stages of feeding, *B*. *vetula* shows levels of variation between patterns of behavior and types of prey characteristic of previously studied teleost fishes. Thus, the coupling of capture and processing behavior patterns in the repertoire of the oral jaws does not appear to constrain the behavioral versatility of this species.

Introduction

A feeding event in a typical fish involves distinct and usually sequential behavior patterns: prey are captured, briefly manipulated, chewed, shredded or crushed, and then swallowed. Previous functional morphological studies of actinopterygian fishes have

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shown that the oral jaw apparatus is primarily used for prey capture and manipulation while the pharyngeal jaw apparatus is used for chewing, crushing and swallowing prey (e.g. Liem, 1979, 1986; Sibbing, 1982; Lauder, 1983a,b; Wainwright, 1988, 1989a; Drucker and Jensen, 1991). Two of the general features of the muscle activity patterns (motor patterns) used during these various types of feeding behavior that have been revealed by electromyographic research are: (1) that different behavior patterns are associated with distinct motor patterns (Lauder, 1983a; Sibbing et al. 1986; Wainwright, 1989a), and (2) that the motor pattern associated with each behavior varies depending on the size, consistency and escape ability of the prey (Elshoud-Oldenhave and Osse, 1976; Lauder, 1981, 1983a,b; Liem, 1978, 1979, 1980; Sanderson, 1988; Sibbing et al. 1986; Wainwright and Lauder, 1986; Wainwright, 1989a). It has been suggested that the functional decoupling of oral and pharyngeal jaw systems may promote the morphological specializations seen in each unit and the behavioral versatility that characterizes actinopterygian feeding mechanisms (Liem, 1973, 1984).

In this study, we examine motor versatility in a fish species that has secondarily coupled prey capture and processing behavior within the repertoire of the oral jaws. The queen triggerfish (*Balistes vetula*) is a native of Caribbean coral reefs where it feeds on a broad range of benthic prey, principally echinoid and ophiuroid echinoderms, chitons, decapod crabs, polychaetes, bivalve and gastropod molluscs, small fish and amphipods (Randall, 1967; Reinthal et al. 1984). Unlike most actinopterygians, balistids capture, manipulate and reduce prey with their oral jaws. The pharyngeal jaw apparatus is formed by thin bones, small teeth and small muscles and probably does not play a major role in the reduction of prey (Randall, 1967; Winterbottom, 1974; Turingan and Wainwright, 1993). Electromyographic recordings of muscle activity were obtained from seven cranial muscles during prey capture, buccal manipulation and a novel 'blowing' behavior. Our aim was to test specific hypotheses about the relationship between coupling and the control of muscle activity patterns. Other issues, such as the effects of coupling on feeding performance and buccal pressures, are left to future studies.

In the light of the multiple functions performed by balistid oral jaws, and in contrast to the decoupled condition found in most fish feeding systems, we ask the following questions. (1) Does *B*. *vetula* exhibit different muscle activity patterns during the different types of behavior executed by the oral jaws? (2) Does *B*. *vetula* alter the muscle activity patterns associated with each behavior in response to prey that differ in hardness and escape ability? (3) Do the oral jaws of *B*. *vetula* show increased levels of modulatory capabilities relative to more generalized percomorph taxa?

Materials and methods

Balistes vetula Linnaeus (Teleostei, Balistidae) is a member of the cosmopolitan marine teleost family Balistidae (order Tetraodontiformes). The species was selected for this study because it represents a generalized balistid morphology and exhibits the broad, principally durophagous, diet considered typical of the family (Randall, 1967). Five live specimens of *B. vetula*, ranging from 145 to 175mm standard length (SL), were collected by hand from coral reefs along the southwest coast of Puerto Rico and shipped by air to

Florida State University, Tallahassee. These individuals were maintained separately in 120l aquaria for at least 3 weeks prior to experimentation. Anatomical observations were initially made on two formalin-preserved specimens (152 and 163mm SL) to determine the precise position of the muscles studied electromyographically.

Electromyography

The techniques used during electromyography (EMG) were designed to minimize the effect of the experimental apparatus on the behavior of *B. vetula*. During recording sessions, the fish moved freely in the aquarium to pursue and manipulate prey.

Electromyographic recordings of muscle activity were made through bipolar electrodes implanted into the cranial muscles. Each electrode was constructed from two 120cm sections of 0.002 gauge (0.051mm diameter) insulated stainless-steel wire. The two wire pieces were laid side by side and glued together along the first 15cm of one end. The wires were threaded through a 26 gauge, 13mm hypodermic needle, insulation was scraped from the ends of each wire, exposing 0.5mm electrode tips, and the electrodes were bent back against the shaft of the needle. This configuration formed a hook which anchored the electrode in the belly of the muscle during electromyographic recordings.

Fish were anesthetized gradually in a solution of no more than $1\,gl^{-1}$ of MS-222, and electrodes were implanted percutaneously into the cranial muscles using the hypodermic needles. The electrode wires from up to seven muscles were bundled together, glued into a common cable, and tied to a loop of suture located just lateral to the first dorsal spine. Following electrode implantation, each fish was returned to its aquarium and allowed to recover from anesthesia. Recording sessions did not begin until 2–3h following recovery from anesthesia to ensure minimal residual effects of the anesthesia. After each experiment, the fish was killed by over-anesthesia, fixed in buffered formalin, and electrode position was confirmed by dissection.

During recording sessions, electrical signals were amplified 10000 times with Grass P511 preamplifiers and filtered with a bandpass between 100 and 3000Hz. The 60Hz notch filter was always used. Electromyographic data and a simultaneous voice description of fish behavior were recorded on high-grade VHS tapes with a TEAC XR-5000 tape recorder. Following the experiments, feeding sequences were played back at one-quarter recorded speed on a Western Graphtek mark-11 thermal array recorder running at $50\,mm\,s^{-1}$. This produced a resolution of $5\,ms\,mm^{-1}$ of chart paper.

Seven muscles were selected for this study (Fig. 1) based on their prominent role in the feeding mechanism of generalized percomorph fishes or because of their anticipated significance in *B. vetula*. The levator operculi (LOP) attaches the neurocranium to the opercle bone and functions to depress the mandible through ligamentous connections between the opercle and the proximal margin of the lower jaw. The A2α, A2β and A2γ sections of the adductor mandibulae muscle originate from the suspensorium and insert on the dentary. The retractor arcus palatini (RAP) is a novel derivation of the adductor arcus palatini found in balistoid tetraodontiforms and apparently convergently in fishes of the family Acanthuridae (including the Zanclinae; Winterbottom, 1974). This muscle originates from the neurocranium and inserts on the anterior region of the suspensorium. The attachments of the RAP suggest that the muscle is a strong abductor of the
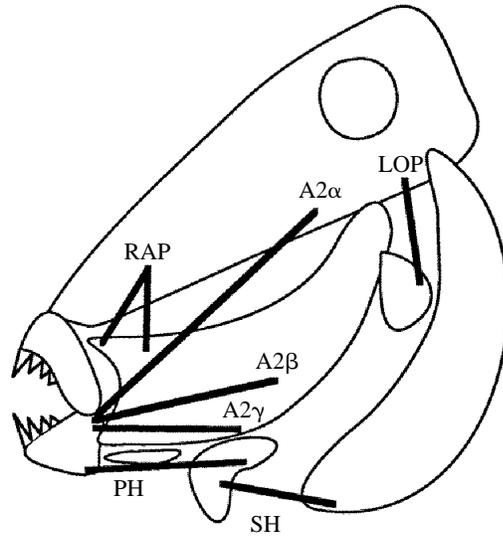
Fig. 1. Diagram of the cranial skeleton of *Balistes vetula* with the attachment relationships of the seven study muscles indicated by thick black lines. A2α, A2β and A2γ, sections 2α, 2β and 2γ of the adductor mandibulae; LOP, levator operculi; PH, protractor hyoideus; RAP, retractor arcus palatini; SH, sternohyoideus.

suspensorium. Other work with *B. vetula* (Turingan and Wainwright, 1993) indicates that the RAP functions in much the same fashion as has been found for the unspecialized levator arcus palatini of generalized percomorph taxa. The protractor hyoideus (PH) connects the ventral margin of the lower jaw to the ceratohyals. Depending on how firmly fixed the hyoid structures are, this muscle may protract the hyoid or depress the lower jaw. The sternohyoideus (SH) attaches the ventral regions of the pectoral girdle to the urohyal. This muscle is greatly reduced in size relative to the generalized percomorph condition but is still positioned to retract and depress the urohyal.

In order to determine the effects of prey type on capture and processing behavior, four experimental prey were chosen that represented extremes of the attributes that *B. vetula* encounters in its natural food. Pieces of squid mantle (*Loligo*, 20mm) were the least challenging and smallest prey. Live intact earthworms (*Lumbricus*, 80mm) were used as a surrogate for the large polychaete worms that make up a major part of the natural diet. Live fish (*Poecilia latipinna*, 40–50mm) were elusive and elicited strong suction feeding behavior. A heavily armored decapod crab (*Mithrax sculptus*, carapace width 20–25mm) was not elusive, but its strong exoskeleton had to be reduced through strong biting actions. The four experimental prey were fed to each fish in random order until the fish stopped feeding.

Feeding sequences were divided into three categories which were analyzed separately: prey capture, buccal manipulation and 'blowing'. The types of behavior were identified with the aid of the simultaneous voice record. Prey capture was defined as the cycle of muscle activity corresponding to initial capture of prey. Buccal manipulation was a cyclic behavior pattern in which prey was moved anteriorly and posteriorly in the mouth while

being bitten repeatedly. The behavior pattern that we term 'blowing' was used to manipulate prey and involved the formation of strong jets of water forcefully expelled from the mouth. Blowing behavior was usually preceded by several buccal manipulation cycles and was characterized by a single cycle of activity during which unwanted parts of the prey were ejected from the mouth.

Each feeding sequence began with a single prey capture cycle of motor activity, usually followed by 1–25 cycles of buccal manipulation. In about 35% of feeding sequences, buccal manipulation was interrupted by at least one blowing event. In total, 43 cycles of prey capture, 523 cycles of buccal manipulation and 40 cycles of blowing were analyzed.

The temporal pattern of muscle activity associated with each behavior pattern was quantified by measuring two types of variable from the chart recording of each feeding. For each cycle of activity, a digital caliper was used to measure the duration of activity of each muscle (LOP-DUR, A2α-DUR, A2β-DUR, A2γ-DUR, RAP-DUR, PH-DUR, SH-DUR) and the onset time of each muscle relative to the onset of activity in the levator operculi muscle (LOP-A2α, LOP-A2β, LOP-A2γ, LOP-RAP, LOP-PH, LOP-SH). The levator operculi was chosen as the reference muscle because it was the only muscle consistently active in all behavior cycles and because it has been used in previous studies of fish motor patterns (Wainwright, 1986; Wainwright and Lauder, 1986; Wainwright *et al.* 1989), facilitating comparisons with other taxa. Caliper measurements were made to the nearest 0.1mm on printed electromyograms that had a magnification of 5msmm^{-1}. This yielded a resolution of 0.5ms for the activity timing variables.

Experimental design

There were two primary objectives of the statistical comparisons: (1) to determine whether the muscle activity patterns used during prey capture, buccal manipulation and blowing behavior differed and, if so, in what manner, and (2) to determine whether the motor patterns used during the three types of behavior differed when *B. vetula* fed on different prey and, if so, in what manner. Because the answers to these two questions may be related (i.e. motor patterns of some types of behavior may be affected by prey type while others are not), each question was analyzed separately for the different categories of prey and behavior. Thus, the comparison of motor pattern variables from different *behavior patterns* was accomplished by analyzing the data for three of the prey types separately. For earthworm, fish and crab prey, two-way analyses of variance (ANOVA) were employed with individuals (random effect) and behavior pattern (fixed effect) as the two main effects in a mixed model. Squid were not included in this analysis because fish frequently did not exhibit buccal manipulation or blowing after capturing the small squid pieces. *F*-ratios constructed with the behavior mean squares in the numerator and the interaction term mean squares in the denominator (Zar, 1984) were used to test the significance of the behavior effect.

The effect of *prey type* on motor pattern was analyzed separately for each behavior pattern. Mixed-model two-way ANOVAs were used with individuals (random effect) and prey type (fixed effect) as the crossed main effects. Tests of the significance of the prey type effect were *F*-ratios constructed with the prey type mean squares in the numerator and the interaction term mean squares in the denominator. To improve the shape of

distributions for each variable within the cells of the experimental designs, all ANOVAs were run on $\log_{10}$-transformed values.

The data set was not completely balanced because only the levator operculi muscle was recorded from all five fish. The number of individuals from which data were successfully obtained varied among muscles: five individuals (LOP), four individuals (PH, SH, A2α), three individuals (A2β, A2γ) or two individuals (RAP). Owing to the mosaic nature of the data set, multivariate analyses could not be run to look at overall effects of feeding behavior and prey type on motor pattern or patterns of correlation among different muscles.

Finally, the frequency of use of each muscle was compared among the four types of behavior. For each behavior pattern, the proportion of cycles that each muscle was active was calculated for each prey type. A comparison was made across behavior patterns by combining all prey within each behavior pattern and, for each muscle, a *G*-test (Sokal and Rohlf, 1981*a*) was used to test the null hypothesis that the frequency of use of each muscle did not differ among behavior patterns.

Results

In our experiments, *B. vetula* exhibited a highly variable motor pattern (Figs 2–7). Considerable variation was found at virtually all levels, including between consecutive cycles of activity of a single feeding sequence (Fig. 2), among behavior patterns (e.g. Figs 4–7) and among prey types (Figs 2 and 3). Over 70% of our ANOVA results indicated significant differences among experimental individuals in the EMG variable under consideration. Our statistical results indicated significant effects of both behavior and prey type on the timing of muscle activity.

Behavioral effects

Comparisons of muscle activity variables for the three feeding behavior patterns are presented for a representative elusive prey (fish) and a non-elusive prey (crab) in Table 1. Because blowing behavior was rarely exhibited during feeding on pieces of squid or worm, results can only be shown for fish and crab. Qualitative contrasts between prey capture and buccal manipulation for squid and earthworm prey can be made visually by inspecting Figs 4 and 5.

Three of the thirteen EMG variables varied significantly among behavior patterns when *B. vetula* fed on fish prey, and two variables differed among behavior patterns when they fed on crab prey (Table 1). The only variable that varied significantly among behavior patterns during feeding on both fish and crab prey was the duration of activity of the levator operculi muscle. The LOP activity burst was much longer during blowing behavior than during either prey capture or buccal manipulation (compare Figs 4–6). The duration of LOP activity during prey capture and buccal manipulation ranged from 35 to 65ms, but during blowing behavior it averaged over 160ms while feeding on crabs and 121.7ms while feeding on fish.

The most striking difference in motor patterns among the different types of behavior was found in the relative onset of activity of the retractor arcus palatini muscle (Table 1;
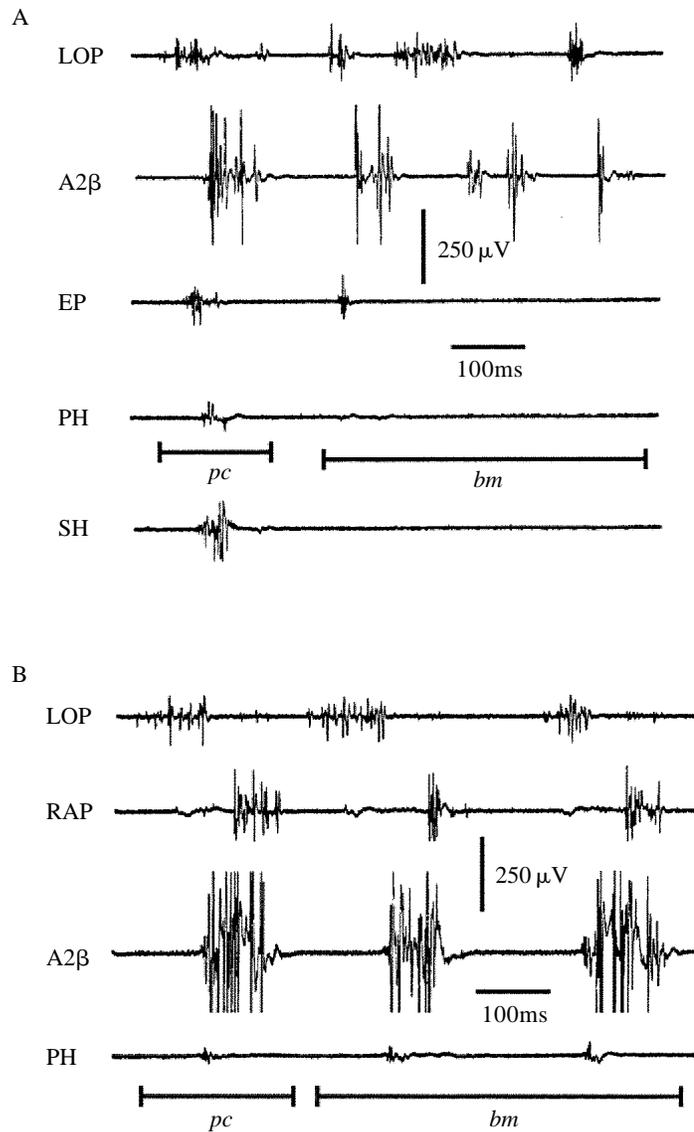
Fig. 2. Simultaneous electromyographic recordings from five cranial muscles in *Balistes vetula* during capture (*pc*) and buccal manipulation (*bm*) of (A) a fish prey and (B) a crab prey. A2β, adductor mandibulae; EP, epaxialis (not analyzed in this study); LOP, levator operculi; PH, protractor hyoideus; RAP, retractor arcus palatini; SH, sternohyoideus.

RAP-LOP). During capture of fish prey, the RAP commenced activity an average of 3.52ms prior to the onset of activity in the LOP. This was the only muscle that consistently showed activity before the LOP in any of the types of behavior. In marked contrast, during buccal manipulation and blowing of fish prey, the RAP commenced activity an average of 61.7ms after the onset of activity in the LOP. The ANOVA comparison for this variable yielded highly significant differences among the behavior
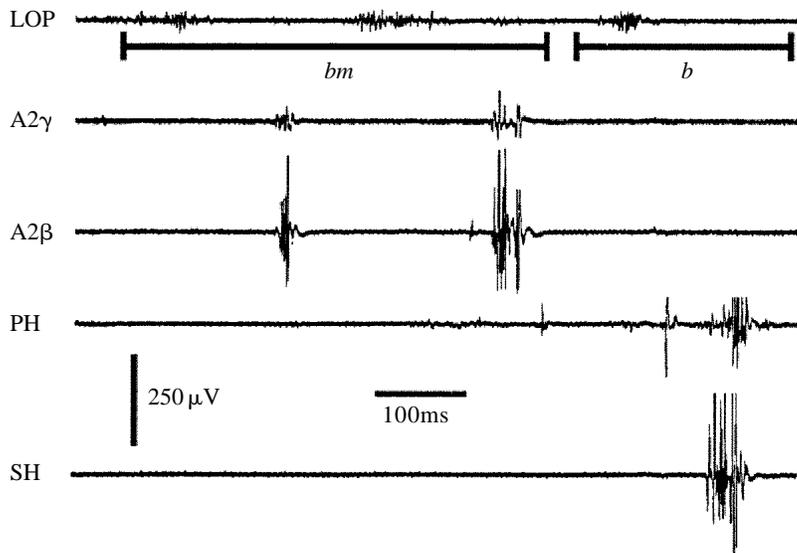
Fig. 3. Simultaneous electromyographic recordings from five cranial muscles in *Balistes vetula* during buccal manipulation (*bm*) and 'blowing' (*b*) behavior with a fish prey. Note that the PH and SH muscles are not active during buccal manipulation but are strongly active during blowing behavior. This is a key distinction between the types of behavior. A2β and A2γ, sections of the adductor mandibulae; LOP, levator operculi; PH, protractor hyoideus; SH, sternohyoideus.

patterns elicited during feeding on fish prey (*F*-ratio=323.1), underscoring the striking difference in use pattern seen in this muscle during various types of behavior. Interestingly, when feeding on crab prey, the onset of activity in the RAP did not differ among behavior patterns (Table 1; see below: *Prey-type effects*).

When crabs were the prey, section A2γ of the adductor mandibulae muscle had a significantly briefer activity duration during blowing (Table 1, Figs 4–6). Because insufficient recordings were made of blowing behavior from this muscle during fish feedings, this behavior was omitted from the fish analysis for this muscle. It was the brief activity of this muscle that drove the significant behavior effect in the analysis of crab feeding behaviors. Thus, the difference between fish and crab prey in terms of this variable may be misleading.

The relative onset time of activity in the A2α section of the adductor mandibulae muscle also differed among the types of behavior elicited during feeding on fish prey (Table 1, A2α-LOP). This muscle commenced activity significantly earlier during prey capture than during buccal manipulation and blowing of fish prey (Figs 4–6). In general, all muscles appeared to show earlier onset of activities relative to the LOP during prey capture of fish (e.g. Fig. 4; PH, SH) but, except for the A2α and RAP, no muscle was found to show significantly different onset times across all types of behavior exhibited while feeding on either fish or crab prey (Table 1). To understand why this variable did not differ among behavior patterns during crab feedings, it is necessary to see that, compared with fish captures, during capture of crabs, triggerfish exhibited a motor pattern

Table 1. *Results of two-way ANOVAs run on 13 EMG variables comparing muscle activity among different types of feeding behavior used by* Balistes vetula

Variable	Fish	Crab
LOP-DUR	27.2 (2, 8)*	17.3 (2, 8)*
A2α-DUR	5.0 (2, 6)	7.1 (2, 6)
A2β-DUR	3.1 (2, 4)	5.1 (2, 4)
A2γ-DUR	2.6 (1, 2)	25.3 (2, 4)*
RAP-DUR	1.7 (2, 1)	0.9 (2, 1)
PH-DUR	3.8 (2, 6)	1.1 (2, 6)
SH-DUR	0.7 (2, 6)	3.8 (2, 6)
A2α-LOP	19.6 (2, 6)*	1.7 (2, 6)
A2β-LOP	9.6 (2, 4)	2.0 (2, 4)
A2γ-LOP	2.6 (1, 2)	2.3 (1, 2)
RAP-LOP	323.1 (2, 1)*	0.9 (2, 1)
PH-LOP	0.3 (2, 6)	0.7 (2, 6)
SH-LOP	0.1 (2, 6)	2.5 (2, 6)

Tests were run separately for behavior patterns executed while feeding on fish and crab prey. The mixed-model ANOVAs are the Behavior factor crossed with the Individual factor.

Table entries are *F*-ratios for the Behavior factor formed by the mean squares for the Behavior factor in the numerator and the mean squares for the interaction term in the denominator.

*Significant at the column-wise Bonferroni correction of $P<0.05$.

Degrees of freedom for each test are given in parentheses.

characterized by longer relative onset times in most muscles (Fig. 4). Thus, in crab feedings there were no muscles that showed significant differences among types of behavior in relative onset times of muscle activity, while this was a strong trend during fish feedings.

Finally, there was a major difference between behavior patterns in the proportion of cycles in which each muscle was active. The results of *G*-tests showed that the frequency of use of all six muscles varied among behavior patterns. With two degrees of freedom, a significance level of $P<0.001$ is achieved by a *G*-value of 13.82 (Sokal and Rohlf, 1981*b*). *G*-values from the tests for all six muscles far exceeded this value: A2α=133.3, A2β=140.4, A2γ=87.4, RAP=174.3, PH=70.16, SH=46.5. During prey capture and buccal manipulation, the adductor mandibulae subdivisions and the RAP were virtually always active, while these muscles were generally active in fewer than 50% of blowing cycles (Figs 2B and 7). In contrast, the PH and SH were active in most blowing cycles but were employed in less than 50% of prey capture or buccal manipulation cycles. Thus, a key difference between these types of behavior was that prey capture and buccal manipulation were dominated by activity of the jaw muscles, while blowing behavior was accomplished predominantly by the hyoid musculature.

Prey-type effects

Prey type had a substantial impact on both the buccal manipulation and prey capture muscle activity patterns. The results of ANOVA tests of the prey-type effect are shown for each of the three behavior patterns in Table 2. Prey type altered both activity duration

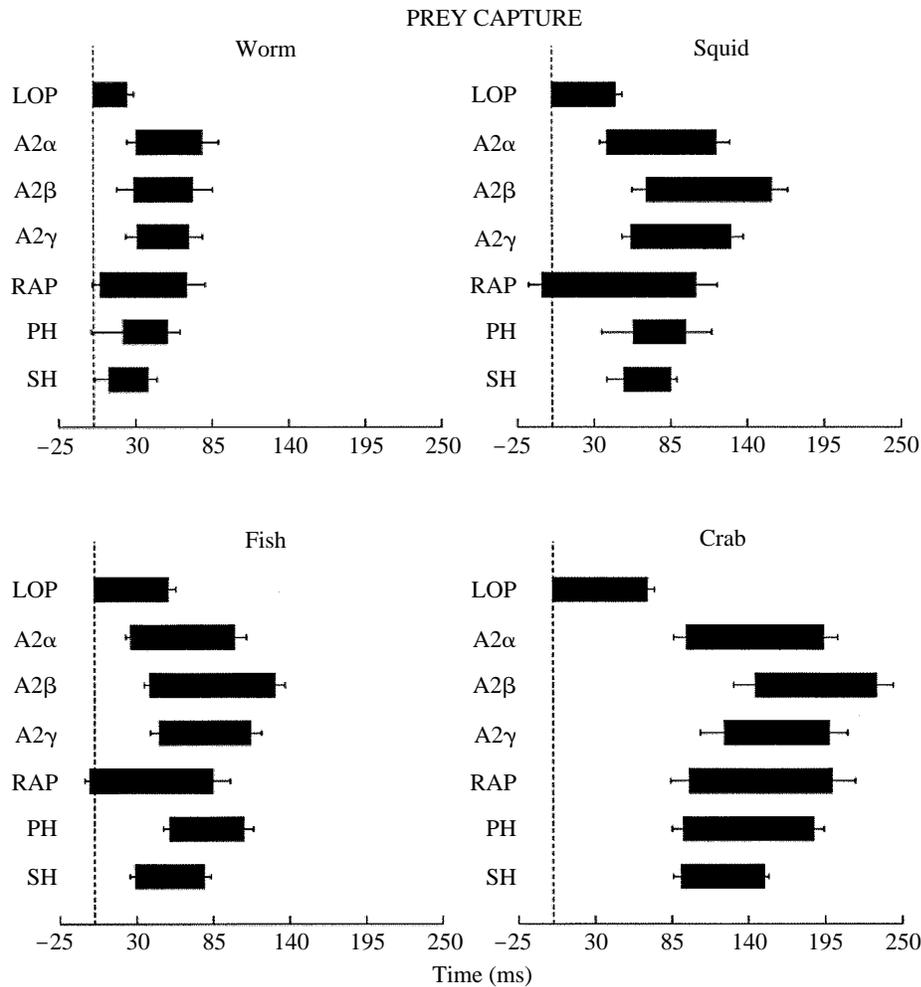P. C. WAINWRIGHT and R. G. TURINGAN

Fig. 4. Electromyographic bar diagrams illustrating the mean values of 13 EMG variables measured from recordings of seven muscles during prey capture behavior on four experimental prey. The width of each bar indicates the mean duration of activity for the muscle, the right-hand error bar indicates the standard error of the mean activity duration, the relative position of each bar indicates the time of onset of activity relative to activity in the LOP muscle, and the left-hand error bar indicates the standard error of onset time.

and relative timing variables of the prey capture behavior (Table 2; Fig. 4). In contrast, only activity duration variables differed among prey types during buccal manipulation (Table 2; Fig. 5). No variables differed significantly between prey types during blowing behavior (Table 2; Fig. 6).

Seven of the thirteen EMG variables were significantly altered when *B. vetula* captured different prey (Table 2). Interestingly, these significant effects were largely due to the influence of crab feeding values. When the crab data were omitted from the analyses, only two activity duration variables (A2β-DUR, A2γ-DUR) showed a significant effect of
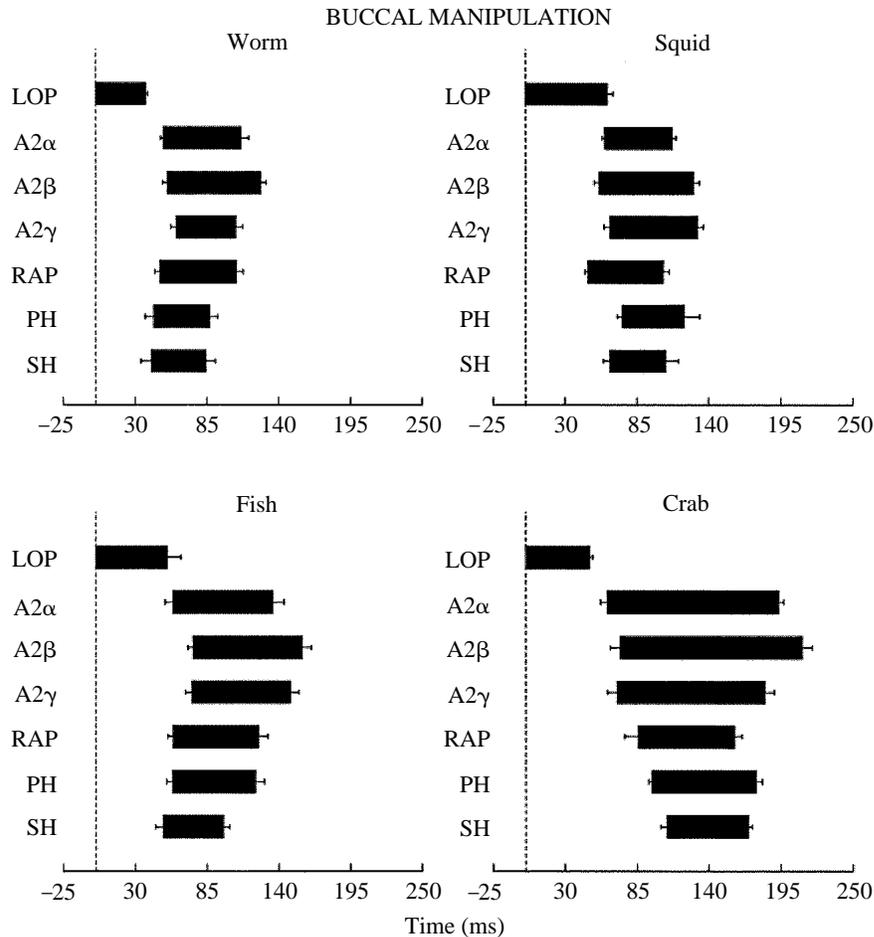
Fig. 5. Electromyographic bar diagrams illustrating the mean values of 13 EMG variables measured from recordings of buccal manipulation during feeding on four experimental prey. The width of each bar indicates the mean duration of activity for the muscle, the right-hand error bar indicates the standard error of the mean activity duration, the relative position of each bar indicates the time of onset of activity relative to activity in the LOP muscle, and the left-hand error bar indicates the standard error of onset time.

prey type. In both cases, this was due to the low values observed during capture of worms. During capture of crab prey, queen triggerfish employed a distinctly different temporal sequence of muscle activity. Five of the six relative onset variables were significantly longer during capture of crabs (Table 2), with the sixth variable (PH-LOP) also showing this trend. The most striking change occurred in the RAP muscle, which began activity an average of 2.24ms prior to the onset of activity in the LOP muscle during capture of worm, squid and fish, but did not commence activity until 92.06ms after the onset of activity in the LOP during crab capture (Figs 2 and 4). In general, capture of crabs evoked a motor pattern characterized by a longer delay between the onset of activity in the LOP and activity in the other muscles.
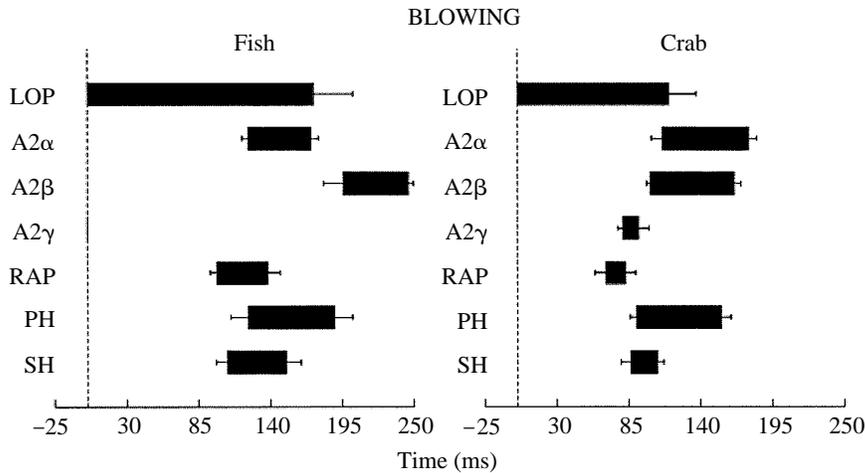
Fig. 6. Electromyographic bar diagrams illustrating the mean values of 13 EMG variables measured from recordings of 'blowing' behavior during manipulation of two experimental prey. The width of each bar indicates the mean duration of activity for the muscle, the right-hand error bar indicates the standard error of the mean activity duration, the relative position of each bar indicates the time of onset of activity relative to activity in the LOP muscle, and the left-hand error bar indicates the standard error of onset time.

The duration of activity in the three adductor mandibulae muscles varied significantly during buccal manipulation of the four prey types (Table 2; Fig. 5). The trend here was for the duration of activities to be longer with harder or tougher prey. Thus, the longest activity durations in the three jaw adductors were found during buccal manipulation of crabs and the briefest were found in worm and squid sequences (Fig. 5). In contrast to this trend, worm and fish prey involved the most cycles of buccal manipulation, activity frequently continuing for more than 10 cycles per prey (mean cycles per bout of activity during buccal manipulation: squid 0.9 ± 0.31, worm 9.2 ± 0.63, fish 8.9 ± 0.73, crab 3.1 ± 0.22). Squid rarely elicited more than two cycles of buccal manipulation because in most cases they were swallowed immediately after capture. Although crabs brought about the greatest degree of muscle activity and a tendency for the longest cycles of buccal manipulation activity (crab cycles averaged about twice the duration of a worm cycle), only about three cycles of buccal manipulation were used per strike during feeding on crabs.

Blowing behavior involved a highly variable motor pattern typified by a long burst of activity in the LOP muscle and irregular bursts of activity in all of the other muscles, beginning some time after the midpoint of LOP activity (Figs 3 and 6). No variables differed significantly between fish and crab prey during blowing behavior (Table 2). In spite of major differences in the means of some variables, this is true because of the high variability found in muscle activity during this behavior (Fig. 6).

Few differences were apparent in the relative frequency of use of muscles during feeding on different prey (Fig. 7). The sternohyoideus muscle was active in 54% of fish

capture sequences while it was only active in about 15% of worm, squid and crab captures (Fig. 7). In general, the pattern of frequency of use of muscles varied much more among behavior patterns than among prey types within each behavior pattern.
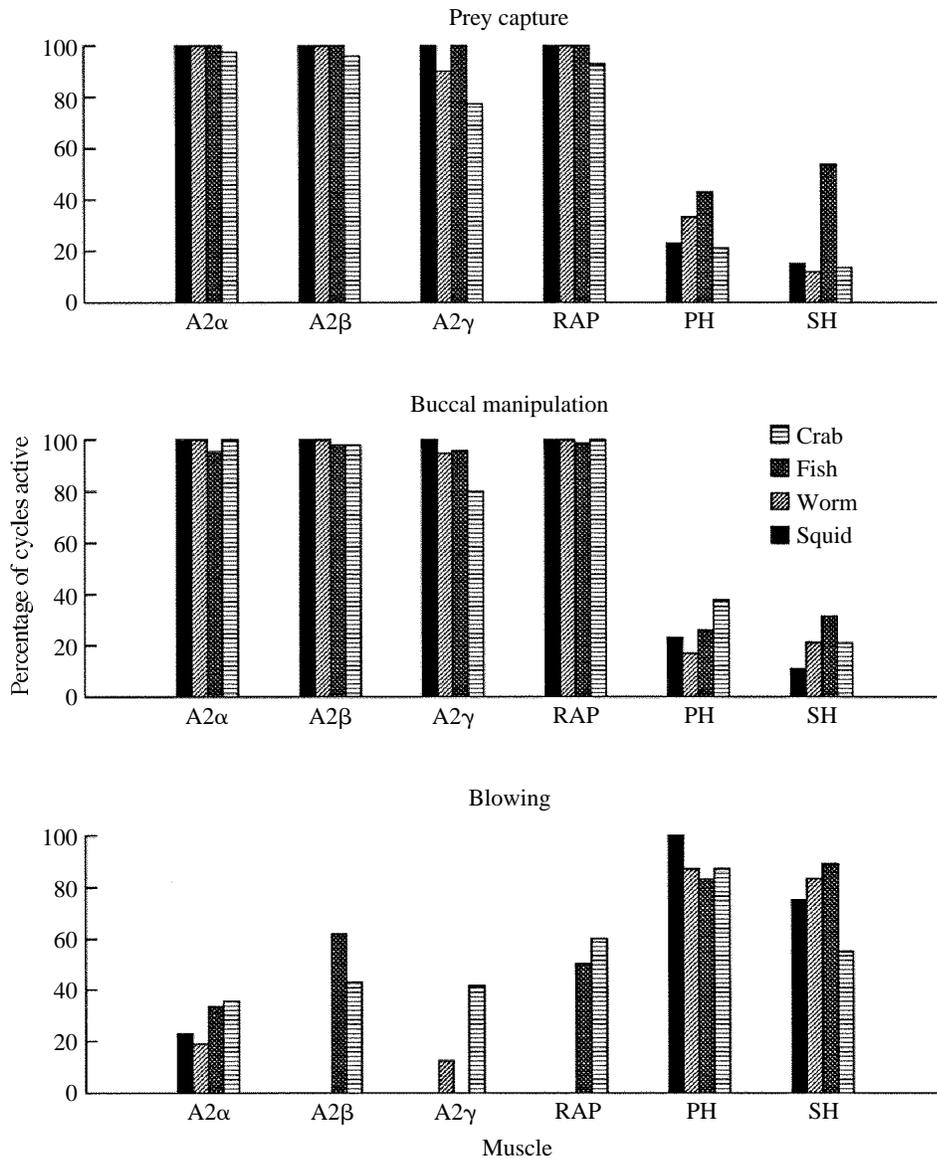

Fig. 7. Frequency histograms of the proportion of cycles of activity during which six cranial muscles are active. The height of each bar indicates the percentage of cycles of the behavior pattern for which activity was observed in that muscle, from a total pool of five individuals. Data are broken down by prey type within each of three types of behavior. Note the different pattern between 'blowing', and prey capture and buccal manipulation. Generally, muscles that were nearly always active in prey capture and buccal manipulation were only rarely active during blowing behavior.

Table 2. *Results of two-way ANOVAs on the effect of prey type on 13 motor pattern variables from three types of feeding behaviors exhibited by* Balistes vetula

Variable	Prey capture	Buccal manipulation	Blowing
LOP-DUR	3.8 (3, 12)	2.1 (3, 12)	2.9 (1, 4)
A2α-DUR	7.8 (3, 9)	14.9 (3, 9)*	0.2 (1, 3)
A2β-DUR	21.3 (3, 6)*	23.2 (3, 6)*	7.8 (1, 2)
A2γ-DUR	12.0 (3, 6)*	14.9 (3, 6)*	0.5 (1, 2)
RAP-DUR	0.2 (3, 3)	7.5 (3, 3)	0.1 (1, 2)
PH-DUR	0.1 (3, 9)	0.7 (3, 9)	1.3 (1, 3)
SH-DUR	0.3 (3, 9)	0.8 (3, 9)	1.4 (1, 3)
A2α-LOP	38.9 (3, 9)*	0.5 (3, 9)	5.0 (1, 3)
A2β-LOP	17.6 (3, 6)*	4.1 (3, 6)	0.9 (1, 2)
A2γ-LOP	22.3 (3, 6)*	0.2 (3, 6)	NA
RAP-LOP	63.3 (3, 3)*	7.3 (3, 3)	0.3 (1, 1)
PH-LOP	0.2 (3, 9)	1.5 (3, 9)	0.1 (1, 3)
SH-LOP	21.1 (3, 9)*	1.6 (3, 9)	2.1 (1, 3)

The mixed-model ANOVAs crossed the Prey Type factor with the Individual factor. Only the significance tests of the Prey Type factor are reported below.

Entries are the *F*-ratios for the Prey Type effect constructed with the mean squares for the Prey Type effect in the numerator and the mean squares for the interaction term in the denominator.

Degrees of freedom for each test are reported in parentheses.

*Significant at the column-wise Bonferroni correction of $P<0.05$.

NA, not applicable.

Discussion

The queen triggerfish shows an ability to alter the activity patterns of its cranial muscles in response to different prey and during the sequential activities that occur in a single feeding event. Three distinct behavior patterns were used during capture and processing of the four experimental prey; prey capture, buccal manipulation and blowing. The EMG data indicate that these behavior patterns may also be defined on the basis of unique features of the motor patterns. Prey type had a substantial impact on the motor patterns of prey capture and buccal manipulation, but not on blowing behavior. Below we discuss these results and their relationship to the findings of similar studies in other fish taxa, and we evaluate the evidence that the coupling of prey capture and processing behavior into the repertoire of the oral jaws has had an influence on the plasticity of the system.

Motor variation among behavior patterns

Balistes vetula displayed distinct prey capture, buccal manipulation and blowing motor patterns for use in capturing and handling prey. It should be noted that this species may also be capable of additional behavior patterns that were not elicited in our experiments. Feeding behavior that this species is known to execute but which was not investigated in the present study includes prying or wrenching prey away from their holdfast and manipulation of large prey (e.g. sea urchins) with strong jets of water, blown from the mouth. On the Caribbean coral reefs where it occurs, this species has a broad diet

including echinoids, chitons, decapod crabs and polychaete worms (Randall, 1967; Reinthal *et al*. 1984). Most of these prey are armored and strongly attached to the substratum, requiring extensive manipulation during either capture or processing. Thus, these features of the queen triggerfish's feeding repertoire appear to play a central role in its trophic ecology.

We initially distinguished the three behavior patterns on the basis of features that could be observed during feeding trials and noted on a voice recording. Prey capture was the first cycle of motor activity in each feeding sequence and corresponded with the strike at the prey. Buccal manipulation was associated with anterior–posterior movement of the prey in the buccal cavity, coupled with biting action. Blowing behavior was characterized by a long period of mouth opening in conjunction with forceful expulsion of water and pieces of the bitten prey. Cycles of muscular activity that could not be clearly identified on the basis of these criteria were not analyzed.

In addition to these *a priori* features, the three behavior patterns may now be distinguished on the basis of the characteristics of the motor pattern observed in this study. Before elaborating on this point, it is important to emphasize that when feeding on crabs, *B. vetula* exhibited a prey capture motor pattern qualitatively different from the motor pattern used during capture of the other three prey types (Fig. 4). Indeed, the queen triggerfish used a motor pattern indistinguishable from buccal manipulation when capturing crabs (see below).

The key feature distinguishing prey capture from buccal manipulation was the early onset of activity in the retractor arcus palatini muscle (RAP) during prey capture. During prey capture, the RAP became active an average of about 3ms prior to the onset of the LOP, while during buccal manipulation the RAP did not commence activity until at least 50ms after the onset of the LOP. In addition, there was a tendency for the LOP to be completely out of phase with the other seven muscles during buccal manipulation (Figs 2 and 5), while prey capture often involved strong overlap between activity in the LOP and activity in other muscles (Figs 2 and 4). However, only the relative onset time between the LOP and the A2α section of the adductor mandibulae varied significantly between prey capture and buccal manipulation (Table 1).

Blowing behavior was characterized by a long burst of activity in the LOP muscle (Fig. 6), typically two or three times longer than that found in prey capture or buccal manipulation. Furthermore, the duration of activity in the A2γ section of the adductor mandibulae was significantly shorter than that during capture or buccal manipulation (Table 1). One major feature of the blowing motor pattern was the consistency of activity in the protractor hyoideus and sternohyoideus muscles. Both muscles were active in over 85% of blowing cycles but they were active in fewer than 35% of capture and buccal manipulation cycles (Figs 2, 3 and 7).

The motor pattern observed during capture of squid pieces, earthworms and live fish shows many similarities to the suction feeding motor pattern that has been described in numerous acanthopterygian taxa (summarized in Lauder, 1985). Early onset of the levator operculi is followed by activity in the sternohyoideus, the epaxialis and eventually the adductor mandibulae (Liem, 1979; Lauder, 1981, 1985; Wainwright and Lauder, 1986; Sanford and Lauder, 1989; Fig. 4). However, a key difference between these earlier

studies and our findings with *B. vetula* involves the activity pattern of the sternohyoideus muscle. This muscle plays a central role in the feeding mechanism of most fishes as it is the principal depressor of the hyoid apparatus (Lauder, 1985), but in *B. vetula* it was rarely active during suction feeding (Fig. 7). It appears that the role of this muscle in suction feeding is reduced in the queen triggerfish. It is noteworthy that this clear trend in relative frequency of use of the sternohyoideus muscle is not apparent from examining the mean duration of activity and relative onset time of the muscle (Fig. 4) and that relative frequency of muscle use is often not considered in treatments of motor pattern modulation (e.g. Liem, 1980; Sanderson, 1988; Wainwright and Lauder, 1986; Wainwright, 1989*a*).

Modulation with respect to prey type

The strongest effects on motor pattern variation observed in our experiments were those attributable to prey type. The queen triggerfish is able to modulate muscle activity patterns during capture and buccal manipulation of different prey. The ability to alter motor patterns when feeding on different prey has been noted in numerous fish taxa, including members of the Cyprinidae (Sibbing *et al.* 1986), Cichlidae (Liem, 1979, 1980), Embiotocidae (Drucker and Jensen, 1991), Centrarchidae (Lauder, 1983*b*; Wainwright and Lauder, 1986), Haemulidae (Wainwright, 1989*a*) and Labridae (Sanderson, 1988). Although it has been suggested that some fish species do not alter their motor patterns in response to prey type (e.g. Lauder, 1983*b*; Wainwright and Lauder, 1986), it appears that the ability to modulate muscle activity during feeding behavior in response to differing prey is a general characteristic of teleost feeding mechanisms.

Prey type had a strong influence on both prey capture and buccal manipulation motor patterns in *B. vetula*. Most of the effects were similar to those previously reported in other fish taxa. The duration of activity in most muscles tended to be briefer during capture of non-elusive worm prey than during capture of elusive fish prey (Fig. 4). A similar trend has been reported in species of centrarchid sunfishes (Wainwright and Lauder, 1986) and labrids (Sanderson, 1988). One trend typical of previous studies but not observed in our experiments is the tendency for the capture of more elusive prey to elicit shorter relative onset times in muscles (Liem, 1980; Wainwright and Lauder, 1986; Sanderson, 1988). Our results generally showed the opposite trend. Non-elusive worm prey tended to have the shortest relative onset times (Fig. 4) and the elusive fish prey elicited intermediate values for the timing of muscle activity. Although it was evident from visual observations that *B. vetula* were more challenged by the fish prey (they often made several unsuccessful capture attempts before finally subduing fish) and the strikes appeared more 'energetic', this distinction was not apparent in the relative onset times of muscles.

One striking result of our analysis of the effects of prey type on motor pattern occurred during feeding on crab prey. When capturing crabs, *B. vetula* exhibited a motor pattern that differed from that seen during feeding on the other three prey types in five of the 13 electromyographic variables (Table 2). Most conspicuously, the relative onset time of the RAP was about 100ms greater during the capture of crab prey than during the capture of the other prey (Fig. 4). The crab capture motor pattern conformed with the buccal

manipulation muscle activity pattern and was statistically indistinguishable from it. During capture of the other three prey types, in particular fish prey, we noted the frequent occurrence of a loud 'popping' sound, apparently due to cavitation of water as a result of rapid changes in buccal pressure at the strike. This never occurred during capture of crabs. When capturing crabs, triggerfish bit off appendages or parts of the carapace rather than engulfing the entire prey. Therefore, it appears that the characteristic prey capture motor pattern seen during squid, earthworm and fish strikes is used during suction feeding, while some prey (i.e. crabs) are bitten initially, rather than being taken whole into the buccal cavity.

The motor pattern for buccal manipulation appeared to respond mostly to differences in the consistency of prey. Harder prey elicited longer bursts of muscle activity (Fig. 5). There are no previous studies of the effects of prey type on motor patterns of oral jaw muscles during buccal manipulation. Previous work on this type of behavior has focused on the pharyngeal jaw apparatus, which plays a major role in post-capture prey reduction in other fish taxa. Prey consistency does have a similar effect on pharyngeal jaw motor patterns: harder prey elicit greater muscle activity (Lauder, 1983*a*; Wainwright, 1989*a*).

Coupled versus *decoupled feeding systems*

The patterns of variation in muscle activity observed in *B. vetula* were markedly similar to those previously reported for other teleosts. Motor patterns differed among types of behavior and among feedings on different prey in much the same fashion as reported for other taxa. We see little support for the notion that the coupling of capture and processing behavior in the repertoire of the oral jaws of *B. vetula* constrains the motor flexibility of the system. In fact, this species shows a particularly plastic prey capture motor pattern, employing suction feeding to capture elusive prey and direct biting (Figs 2–6) to capture immobile, hard prey.

At least three major types of behavior have been recognized in the feeding repertoire of generalized teleost fishes: prey capture, buccal manipulation and pharyngeal transport (Lauder, 1983*a*; Wainwright, 1989*b*). In generalized teleosts, reduction of prey (chewing, crushing and shredding) is primarily accomplished by the pharyngeal jaw apparatus during pharyngeal transport (Lauder, 1983*a,b*; Wainwright, 1989*b*). Pharyngeal transport is also used during swallowing behavior. The key distinction between the generalized teleost and *B. vetula* is that, in the latter species, most of the prey-reducing functions are fulfilled by the oral jaws during buccal manipulation. While the reduced pharyngeal jaws may retain some pharyngeal transport functions in *B. vetula*, it is likely that this behavior is used mainly during swallowing. Thus, prey capture, prey manipulation and prey reduction are coupled as part of the oral jaw repertoire.

A few studies have made qualitative comparisons of the motor patterns used during different types of behavior in species with the generalized, decoupled feeding apparatus. These studies typically focused on either the oral jaws or the pharyngeal jaw apparatus. Data from *Esox lucius* and several species of the Centrarchidae indicate that the timing of muscular activity varies substantially between prey capture and pharyngeal transport behavior (Lauder, 1983*a*). Similarly, haemulid fishes show marked temporal variation in pharyngeal jaw muscle activity during prey capture and pharyngeal transport

(Wainwright, 1989*a*). It is important to realize, however, that because the studies cited above did not make quantitative comparisons among behavior patterns, the distinct motor patterns that are apparent in the bar diagrams may actually be so variable that the differences do not show statistical significance (see Wainwright, 1989*b*). In the current study, we found that only three of the 13 variables differed among the types of behavior, indicating that prey capture and buccal manipulation differ slightly less in the oral jaws of *B. vetula* than do prey capture and pharyngeal transport in previously studied taxa (Lauder, 1983*a,b*; Wainwright, 1989*b*). However, we see little evidence of a substantial difference in the patterns of modulation.

Two studies have examined quantitatively the effect of prey type on the oral jaw motor patterns of other teleost fishes. In three species of the Centrarchidae, an average of six out of 11 EMG variables varied with the type of prey (Wainwright and Lauder, 1986). Three species of the Labridae showed significant prey type effects in about 50% of the muscle activity timing variables (Sanderson, 1988). Of our 13 EMG variables, seven showed a significant prey type effect during prey capture behavior. Thus, our results compare closely with other quantitative studies that used a similar experimental design and suggest that the modulatory capacity of the queen triggerfish is not limited by the multiple roles of the oral jaws.

Although we find no evidence to support the hypothesis of constrained motor control in the queen triggerfish, future work may profitably investigate other aspects of the feeding mechanism in search of limitations related to the behavioral coupling. For example, the specializations of *B. vetula* for strong oral jaw biting may have some cost in suction feeding performance and in the ability to generate strong negative buccal pressures. Our laboratory observations suggest that *B. vetula* has about the same success rate in capturing elusive prey as species that possess a similar-sized mouth and are considered to be strong suction feeders (e.g. *Lepomis macrochirus*). Quantification of suction feeding performance and buccal pressure patterns in the queen triggerfish, and other balistoid taxa, would provide an additional test of the possible costs of coupling prey capture and prey processing behavior patterns.

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