

Motor Control Across Trophic Strategies: Muscle Activity of Biting and Suction Feeding Fishes¹

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SYNOPSIS. Many fishes use a powerful bite of the oral jaws to capture or tear their prey. This behavior has received less study from functional morphologists and physiologists than suction feeding, and presents an opportunity to examine motor control of fish feeding across alternative prey-capture strategies. We used electromyography to compare muscle activity patterns of the feeding bite in five teleost fishes representing at least three lineages in which biting has been independently acquired: two parrotfish (*Cetoscarus bicolor* and *Scarus iseri*), a wrasse (*Cheilinus chlorourus*), and two serrasalmines, a pacu (*Piaractus brachypomus*) and a piranha (*Pygocentrus nattereri*). Multivariate analysis indicated that muscle activity patterns differed significantly among species, although a four-way ANOVA designed to test for differences within a phylogenetic hierarchy revealed that the biting motor pattern was largely similar for both narrow and broad phylogenetic comparisons. A comparison of the motor patterns of biting and suction feeding species revealed that biters had significantly shorter durations of the epaxialis and sternohyoideus and significantly longer relative onset times of the epaxialis, adductor mandibulae, and sternohyoideus. Character mapping of timing variables suggested that short relative onset times are primitive for suction feeders and that this characteristic is generally retained in more advanced species. Despite these differences, all species overlap extensively in multivariate EMG space. Our results demonstrate that change in the feeding motor pattern has accompanied morphological and behavioral change in transitions from suction to biting, which suggests that the neuromotor system has not acted as a constraint on the evolution of the feeding system in fishes.

INTRODUCTION

Fishes capture a diversity of prey using a wide range of movements of the skull, suspensorium, hyoid, and the oral jaws (mandible, maxilla, and premaxilla) (*e.g.*, Lauder, 1985; Lauder and Shaffer, 1993). These behaviors have been described as suction feeding, biting, filter feeding, and ram feeding: non-exclusive categories that designate the central strategy for obtaining food (*e.g.*, Lauder and Shaffer, 1985). Historically, the study of fish feeding has largely focused on suction feeding. We now

know a great deal about the morphology (*e.g.*, Anker, 1974; Barel, 1983; Westneat, 1994), kinematics (*e.g.*, Lauder, 1980a, 1982, 1983; Westneat, 1990), linkage mechanics (*e.g.*, Alexander, 1966, 1967; Westneat, 1990, 1991), muscle activity patterns (Lauder, 1980b, 1985; Wainwright *et al.*, 1989; Lauder and Shaffer, 1993), and even the fluid mechanics (Muller and Osse, 1984; Muller, 1989; Ferry-Graham and Lauder, 2001) of this behavior. The suction feeding paradigm, summarized by Lauder (1985), and Lauder and Shaffer (1993), has shaped inquiry into the functional morphology of actinopterygian feeding, and strongly contributed to our current assessment of the major themes of organismal design. Surprisingly, the functional morphology of oral jaw biting, an important feeding strategy employed by representatives of every major fish lineage, remains mostly un-

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explored (but see Bellwood and Choat, 1990; Turingan and Wainwright, 1993; Wainwright and Turingan, 1993). In this paper, we examine the activity patterns of the cranial muscles during biting in five species of fish to address a number of important questions regarding diversity and evolution of motor control in fishes.

What defines biting prey capture in fish? For the purposes of this study, we define biters as those species that depend on forceful contact of the oral jaws with the prey for prey acquisition. Some biters may employ suction to help position prey while biting. In other species, particularly those that feed on algal mats, coral, or similarly large, immobile prey, suction may play no role in determining capture success. Thus, the defining feature of biting, prehension of the prey by the oral jaws, does not necessarily preclude suction generation, and considerable overlap may occur between these two broad strategies. All biters are expected to retain the ability to generate suction for intraoral transport of captured prey. Nevertheless, biters usually possess other anatomical, functional, dietary, and behavioral characters that distinguish them from species that rely primarily upon suction feeding (without jaw prehension) to capture prey. Biters usually take large prey, and biting itself may be viewed as a strategy for overcoming the restrictions that maximum gape size places on diet. As a consequence of this, biting behavior frequently involves piece removal in which portions of a large prey item are freed via action of the oral jaws. Examples of piece removal include scale eating in cichlids and serrasalmins, flesh removal in sharks, piranhas, and ceptosid catfishes, sea urchin predation by triggerfishes, and algal grazing in parrotfishes and chaetodontids. Bout feeding, which involves repeated instances of piece removal, also characterizes many biting species.

There are many functional consequences of bringing the oral jaws into contact with the prey during prey capture. Biters are predicted to maximize force production rather than velocity advantage in the oral jaws, a design optimization that potentially influences head shape (Barel, 1983; Westneat,

1994). Biters also tend to have robust oral jaws and a well-developed adductor mandibulae complex (Bellwood and Choat, 1990; Turingan and Wainwright, 1993). The extent to which diversification of motor control has accompanied morphological change in biting taxa is currently unknown as there have been no comparative EMG studies explicitly focused on biting species.

In this study, we present the first quantitative comparative examination of the motor patterns of biting in fishes. We examined three issues of motor control evolution in biters. First, we tested for similarity of biting motor patterns at three phylogenetic levels (among species within families, between two families, and between two orders) using multivariate and univariate analysis of EMG variables. Second, using multivariate and univariate techniques, we tested the hypothesis that motor control between biters and suction feeders is conserved. Lastly, using multivariate and univariate techniques, we explored the variability and degree of overlap in muscle activity patterns across biters and suction feeders. Muscle timing variables were mapped onto a phylogeny to explore patterns of evolution in the motor control of feeding in fishes.

METHODS

The original research reported herein was performed under guidelines established by the Field Museum Animal Care Committee and approved protocol 94-6. Experiments were performed on three individuals of the following species: *Cetoscarus bicolor* (SL 86 mm to 112 mm) *Scarus iseri* (SL 74 mm to 118 mm), *Cheilinus chlorourus* (SL 97 mm to 116 mm) *Piaractus brachypomus* (SL 85 mm to 122 mm), and *Pygocentrus nattereri* (SL 118 mm to 128 mm). Fishes were purchased from aquarium suppliers and maintained in 200-liter aquaria at a temperature of 25–28°C on a diet of commercially available pellets, live fish, aquatic plants, and algae cubes, depending on the species.

Electromyography

Procedures for electrode construction and EMG surgery are detailed elsewhere (Al-

faro and Westneat, 1999). We measured EMGs in up to six cranial muscles, depending on the species: the epaxialis, levator operculi, sternohyoideus, and up to three paralogous subdivisions of the adductor mandibulae (Friel and Wainwright, 1997). Electrodes were implanted into the muscles of the left side by sliding a syringe needle beneath scales, through skin, and into the target muscle. After recovery (1 hr minimum), the fish were offered prey. Due to dietary differences shown by the species in this study we were unable to discover a single prey item that all five species would readily accept. Therefore, we fed each species items that approximated their natural prey, elicited a range of feeding behaviors, and presented similar problems for capture and ingestion: commercially available algae cubes for the parrotfish, small live fiddler crabs (genus *Uca*) for *Cheilinus*, raw beef heart for *Pygocentrus*, and Amazon sword plant leaves (genus *Echinodorus*) for *Piaractus*. Prey items were held in place using an alligator clip affixed to a small stand except for algae cubes, which were smeared onto a small coral head.

Procedures for EMG data acquisition and signal processing are detailed elsewhere (Alfaro and Westneat, 1999). Feeding EMGs typically show a high degree of variation both among individuals of the same species and among strikes of the same individual (Shaffer and Lauder, 1985; Sanderson, 1988). Three individuals of each species and from seven to twenty-nine bites per individual were analyzed, for a total of 242 total bites. The muscle activity pattern of a bite was quantified by measuring 19 EMG variables in four groups: (1) duration (ms) of muscle activity; (2) onset time (msec) of other muscles relative to the onset of levator operculi activity; (3) mean intensity (mV) of each burst of activity; and (4) area under the rectified (absolute value) EMG trace in mV·msec (see Alfaro and Westneat, 1999). We also calculated total bite cycle time, defined as beginning when the earliest muscle in any bout fired (usually LOP) and as ending when the last muscle ceased firing (usually AM).

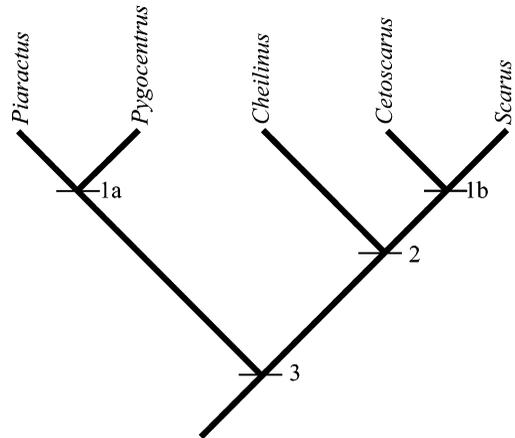


FIG. 1. Currently accepted phylogenetic relationship of the biters that were compared in this study (Lauder and Liem, 1983; Westneat and Alfaro, unpublished data). Numbers at nodes indicate phylogenetic levels at which ANOVAs were used to compare biting motor patterns.

Statistical analysis: biters

Residual analysis revealed that raw EMG data met the necessary assumptions for parametric statistics. To test the hypothesis that all biters used the same motor pattern as well as to test for among individual variation, a multivariate analyses of variance (MANOVA) was performed using species as a fixed effect and individuals nested within species as a random effect. A significant species effect was accepted as indicating that muscle activity patterns were distinct. To examine where motor pattern change occurred within the context of a phylogeny, we performed a four-way ANOVA of EMG variables using comparisons that corresponded to successively higher phylogenetic levels (Fig. 1). Our sampling design allowed us to ask whether motor pattern differences had evolved at relative low (roughly subfamilial level between *Scarus* and *Cetoscarus* and between *Piaractus* and *Pygocentrus*) medium (familial level between *Cheilinus* and *Scarus* + *Cetoscarus*) and at high (ordinal level between *Piaractus* + *Pygocentrus* and *Cheilinus* + *Scarus* + *Cetoscarus*) levels in the phylogeny. These tests were performed on the 16 EMG variables the species had in common (A1 was the only adductor subunit compared across species). We accepted a significant

species effect as indicating that elements of the feeding motor pattern were not shared between groups.

To determine the primary axes of variance in the EMG data, principal components analyses (PCA) were performed on the muscle duration and relative onset variables. Epaxialis muscle onset time was omitted from the dataset due to the infrequent firing of this muscle in some biters. The principal components of the covariance matrix of the raw data were calculated, and a scatter plot of the two major axes of variance (PC1 and PC2) was used as a way of plotting individuals in multivariate motor pattern space. All analyses were performed using JMP 3.1 (SAS, 1995).

Statistical comparisons between biting and suction feeding

To compare our results with studies of feeding in other species, we combined our data with previously published EMG data on *Balistes vetula* (Wainwright and Turinagan, 1993), a biter, and the suction feeders *Amia calva*, *Micropterus salmoides*, *Lepomis macrochirus*, *Notopterus chitala* (Wainwright *et al.*, 1989), and *Oxycheilinus unifasciatus* (Sanderson, 1988) made available to us by the authors. We compared raw data from epaxialis (EP), levator operculi (LOP), adductor mandibulae (AM), and sternohyoideus (SH) muscles and their onsets relative to LOP. We did not use data on mean peak amplitude and integrated areas from other studies as we judged that these variables would be more susceptible than muscle durations and relative onsets to differences in equipment and experimental protocol. The parrotfish data set contained a large number of bites in which the EP did not fire and because relative onset is undefined for a muscle that does not fire, these cells showed missing data. To avoid the exclusion of entire feeding events as a result of cells with missing data, we included epaxialis duration, but excluded the variable of epaxialis onset time for all multivariate analyses. The combined data set was subjected to a nested MANOVA with feeding mode (suction *versus* biting) as a fixed effect and species within mode and individuals within species within mode as random

effects to test the hypothesis that biters and suction feeders share the same motor pattern. In addition, nested ANOVAs with mode as a fixed effect and species within mode and individuals within species within mode as random effects were used to test for significant differences between feeding types, species, and individuals for each of the EMG variables measured. To reduce Type I error rates due to multiple tests we applied a sequential Bonferroni correction to p values from the univariate tests (Rice, 1989). To explore the pattern of evolution of timing variables, we mapped mean species onset times for EP, AM, and SH onto the currently accepted phylogeny for the species in this study using the squared changes option in MacClade 4.0 (Maddison and Maddison, 2000).

To further explore the pattern of variation in this data set, a principal components analysis was performed on the covariance matrix using raw durations and relative onset times for the eleven species. The PCA allowed us to visualize what portions of the multivariate EMG space were occupied by particular species and feeding types and to assess bite stereotypy.

RESULTS

Muscle firing order was generally the same in all five biting species, with the levator operculi firing first, followed by the epaxialis, sternohyoideus, and then the adductor mandibulae (Fig. 2). This is the same sequence of muscle activity that is found during suction feeding. However, the amount of concurrent activity varied widely among biters. This differs from suction feeders, which show a high degree of overlap of muscle activity (Wainwright *et al.*, 1989). Recruitment of the epaxialis muscle also varied among species, firing regularly in *Pygocentrus* and *Cheilinus*, less frequently in *Piaractus*, and infrequently in both parrotfish species.

Biting in parrotfishes

Muscle activity patterns (MAPs) in *Cetoscarus bicolor* and *Scarus iseri* reflect behavioral and functional differences in feeding strategy between excavating and scraping species (Bellwood and Choat, 1990; Al-

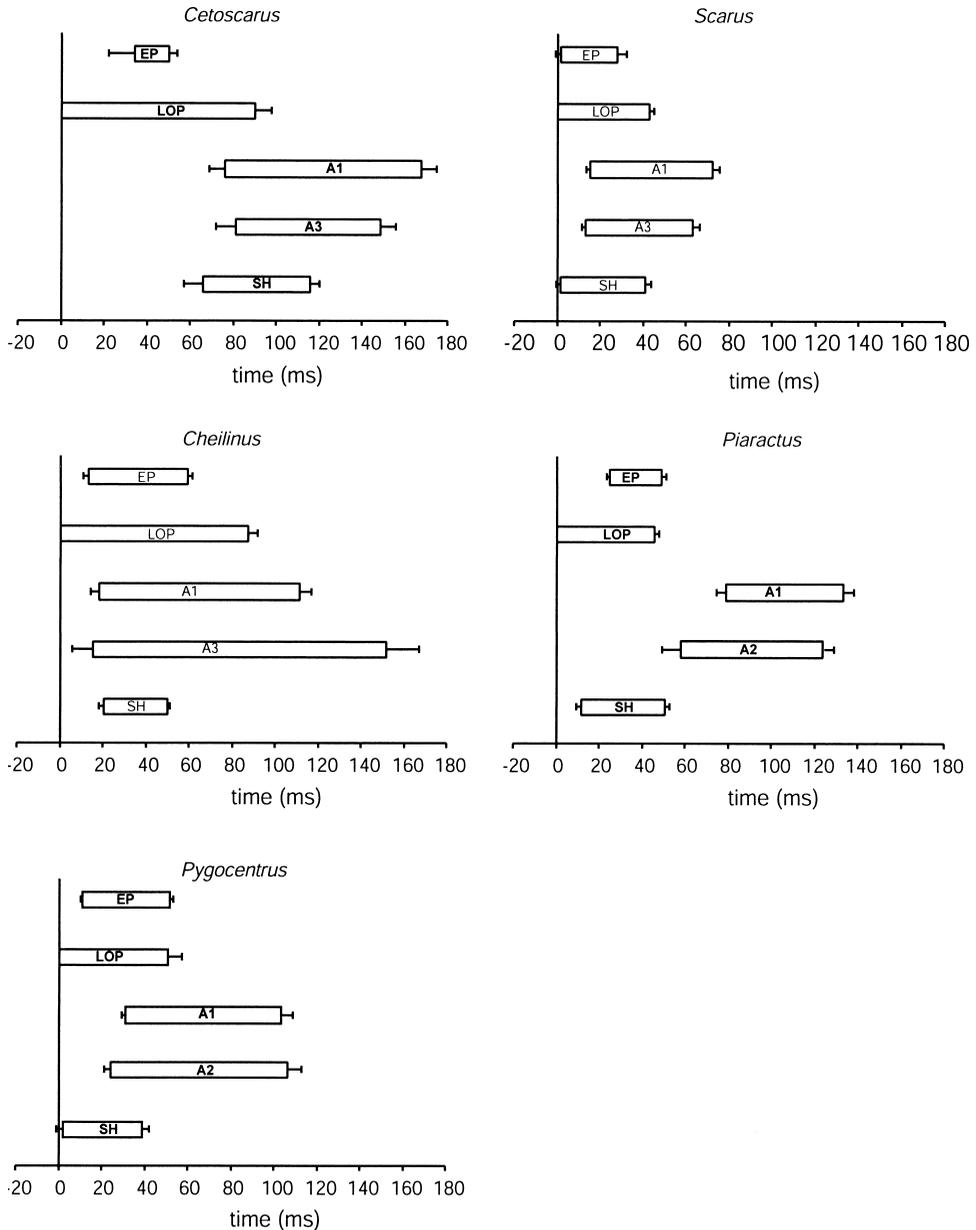


FIG. 2. Box plot summary of biting EMGs. Shown are the average patterns of activity for five cranial muscles in the five species examined in this study. Rectangles represent mean muscle duration with error bars to the right indicating one standard error of the mean. Mean onset times relative to the LOP are indicated by the distance of each bar to the line to the left with error bars to the left indicating one standard error of the mean. EP = epaxialis, LOP = levator operculi, A1–A3 = adductor mandibulae subunit, SH = sternohyoideus.

faro and Westneat, 1999). *Cetoscarus* generally takes a few, slow (≈ 175 msec) bites during feeding bouts. The MAP is characterized by a distinct offset of the adductor mandibulae relative to the epaxialis

and levator operculi muscles (Fig. 2, Table 1). In contrast, *S. iseri* takes a dozen or more rapid (≈ 80 msec) bites during a feeding bout. MAPs in *Scarus iseri* show short durations for all muscles relative to *Cetos-*

carus bicolor, and a high degree of overlap among jaw openers (epaxialis, levator operculi, and sternohyoideus) and jaw closers (adductor mandibulae subdivisions A1 and A3). Another notable characteristic of the MAP in parrotfishes is irregular recruitment of the epaxialis: although most fish species studied to date use the epaxialis muscle during the feeding strike, *Cetoscarus bicolor* and *Scarus iseri* frequently do not recruit this muscle while biting (Alfaro and Westneat, 1999). As a result of a moderate frequency of bites with no observable EP activity, EP duration for both parrotfishes appears short in Figure 2.

Biting in Cheilinus chlorourus

This species vigorously attacked crabs as they were introduced to the tank. Feeding bouts generally consisted of a single bite in which a portion of the prey, usually a leg, was removed. In some instances, *Cheilinus* sucked the crab into the oral jaws prior to piece removal although most bites were accomplished without visible suction. After piece removal, individuals would swim a short distance away and process the prey item with their pharyngeal jaws. Processing would sometimes last as long as a minute, after which time individuals would return to the crab and initiate further attacks. In some cases, individuals failed to remove a portion of the prey. In these instances, the fish performed a rapid shaking behavior wherein the prey was violently thrust against the substrate while held in the oral jaws. Individuals were usually successful in removing pieces of the prey after one or two such shakes.

Relatively long bite cycles (≈ 130 msec) characterized this species. The MAP showed long durations for all muscles except the sternohyoideus, and short relative onset times, so that there was high overlap of muscle activity (Fig. 2, Table 1). The epaxialis muscle was active during all recorded bites. We also noted a novel muscle activity pattern that accompanied shaking behavior, characterized by early and sustained high amplitude activity in the adductor mandibulae and late onset of activity in the jaw opening muscles (epaxialis, levator operculi, and sternohyoideus).

Biting in Piaractus brachipomus and Pygocentrus nattereri

Pacu (*Piaractus*) and piranhas (*Pygocentrus*) both fed in multi-bite bouts, although pacu bouts contained higher number of bites. However, there were substantial differences in the ability of these species to remove pieces of the prey. At the end of all pacu bouts, one-half or more of the original prey item remained. In contrast, piranhas quite easily consumed the quantity of prey offered within a few seconds, effectively ending the feeding bout. Pacu bite cycles were similar to *Cheilinus*, lasting approximately 130 msec. In contrast, piranha bite times (≈ 110 msec) were only slightly longer than *S. iseri* bites.

MAPs differed in a number of features between these species (Fig. 2, Table 1). Epaxialis duration was shorter in *Piaractus* than in *Pygocentrus*, and this muscle did not fire in approximately 10% of the bites in this species. Relative onset times for all muscles were longer in *Piaractus* than in *Pygocentrus*. This resulted in similarities between the box plots of *Piaractus* and *Cetoscarus* wherein both displayed distinct phases of jaw opener and jaw closer activity. In contrast, *Pygocentrus* jaw opening muscle activity overlapped with activity in both subdivisions of the adductor mandibulae.

Statistical analysis of biters

A MANOVA of EMG variables revealed a highly significant effect of species ($P < 0.001$, $F = 20.38$, $df = 60/544$) and individuals ($P < 0.001$, $F = 11.35$, $df = 120/1,000.8$). Four way ANOVA (Fig. 1, Table 2) detected significant differences in only four EMG variables at three levels of comparison: between *Scarus* and *Cetoscarus*, between *Scarus* + *Cetoscarus* and *Cheilinus* and between *Scarus* + *Cetoscarus* + *Cheilinus* and *Piaractus* + *Pygocentrus* (Table 2). We found no significant difference in motor pattern between *Piaractus* and *Pygocentrus*. Despite this lack of significance in the univariate tests, EMG variables showed a two to threefold difference among species means (Table 1). Principal components analysis (Fig. 3) illustrated the

TABLE 1. Mean and one standard error of mean muscle duration (ms), relative onset (ms), peak amplitude, and integrated area of cranial muscles of species examined in this study.*

Variable	<i>Cetoscarus</i> n = 3/35		<i>Scarus</i> n = 3/45		<i>Cheilinus</i> n = 3/61		<i>Piaractus</i> n = 3/45		<i>Pygocentrus</i> n = 3/46	
	m	SE	m	SE	m	SE	m	SE	m	SE
EP	16.10	3.83	26.68	4.05	46.39	2.19	24.39	2.01	40.56	1.95
LOP	90.10	7.76	42.81	2.36	87.28	4.39	45.61	2.28	50.54	6.49
AM	91.85	7.02	57.08	3.62	93.58	5.37	54.29	5.07	72.71	5.43
SH	50.03	4.52	39.71	2.85	29.66	1.54	39.00	2.53	37.11	3.11
EP-LOP	34.07	12.23	1.68	2.79	14.18	2.09	25.08	1.76	11.20	0.77
AM-LOP	76.62	7.78	15.76	1.52	19.04	3.96	78.94	4.37	31.83	1.35
SH-LOP	66.74	8.87	1.68	1.61	21.36	2.17	12.04	1.94	2.35	2.61
MP EP (mV)	0.019	0.003	0.027	0.003	0.029	0.002	0.032	0.005	0.089	0.006
MP LOP (mV)	0.007	0.001	0.022	0.001	0.041	0.002	0.025	0.001	0.055	0.009
MP AM (mV)	0.026	0.003	0.038	0.003	0.043	0.004	0.048	0.005	0.044	0.006
MP SH (mV)	0.017	0.002	0.052	0.008	0.043	0.004	0.021	0.003	0.046	0.005
IA EP (mV*ms)	1.66	0.47	3.82	0.48	6.93	0.55	5.43	1.08	18.93	2.07
IA LOP (mV*ms)	3.06	0.29	4.48	0.29	16.66	0.85	5.46	0.29	12.56	2.30
IA AM (mV*ms)	10.32	0.97	10.57	0.87	22.57	2.86	15.03	1.61	19.27	3.72
IA SH (mV*ms)	4.28	0.62	10.64	2.41	6.95	0.70	4.17	0.66	10.11	1.40

* Bold indicates biting species. n = number of individuals/number of recorded bites; EP = epaxialis duration; LOP = levator operculi duration; AM = adductor mandibulae duration; SH = sternohyoideus duration; EP-LOP = relative onset of epaxialis; AM-LOP = relative onset of adductor mandibulae; SH = relative onset of sternohyoideus, MP = mean peak; IA = integrated area; mV = millivolt; mV*ms = millivolt-milliseconds; m = mean; SE = standard error. Raw data for *Balistes*, *Amia*, *Lepomis*, *Micropterus*, and *Notopterus* provided by PC Wainwright. Raw data on *Oxycheilinus* provided by S. L. Sanderson.

variability of the muscle activity pattern (MAP) within and among biting species. The first two principal components accounted for 67% of the total variance (Table 3). LOP duration, relative onset of AM, and relative onset of SH loaded showed high, positive coefficients along PC1. AM duration showed a high, positive coefficient along PC2. All five species overlapped to varying degrees within MAP space (Fig. 3). *Cetoscarus* appeared to occupy the most distinct portion of the space and was characterized by relatively long durations of LOP and long relative onset times of AM and SH. *Piaractus* also occupied a relatively discrete portion of the multivariate space, characterized by short LOP duration and short relative onset times. Despite feeding on entirely different kinds of prey, *Pygocentrus* and *Scarus* strongly overlapped with each other. Short LOP durations and relatively short muscle onset times characterized these species.

Bite stereotypy, as assessed by polygon size in MAP space, showed substantial variation among species. The parrotfishes showed both the largest (*Cetoscarus*) and the smallest (*Scarus*) degree of bite stereo-

typy. In general, species with shorter bite cycle times, such as *Scarus* and *Pygocentrus* showed greater bite stereotypy than species with longer average bite cycle times, such as *Cetoscarus* and *Cheilinus*.

Statistical analysis of biters and suckers

MANOVA of EMG variables revealed a highly significant effect of feeding mode ($P < 0.0001$, $F = 69.11$, $df = 6, 384$) species ($P < 0.0001$, $F = 20.46$, $df = 54, 1,962$), and individual ($P < 0.0001$, $F = 5.72$, $df = 144, 2,252$). Qualitatively, biters showed shorter durations of muscle activity and longer relative onset times (Fig. 4). Univariate ANOVAs revealed that these differences were significant between modes for all variables except LOP and AM duration (Table 4). Phylogenetic mapping of onset variables suggested that short relative onset times were primitive for teleosts (Fig. 5). While the suction feeding taxa in our study appear to have largely retained this ancestral condition, there appear to have been multiple independent evolutions of increased onset times in biters.

Principal components analysis revealed that, despite significant differences in mul-

TABLE 1. *Extended.*

<i>Balistes</i> n = 1/10		<i>Amia</i> n = 3/33		<i>Lepomis</i> n = 4/37		Micropterus n = 7/64		<i>Notopterus</i> n = 3/21		<i>Oxycheilinus</i> n = 3/41	
m	SE	m	SE	m	SE	m	SE	m	SE	m	SE
34.68	7.70	104.32	6.06	52.92	3.31	55.88	2.28	49.27	3.49	40.12	1.92
69.50	11.24	116.02	7.98	57.77	3.54	61.80	4.23	83.93	6.56	49.88	3.45
83.05	16.62	108.18	5.20	79.19	5.20	75.06	4.92	88.36	9.75	49.88	4.00
15.38	10.93	98.51	4.65	50.65	2.88	62.06	2.70	65.99	5.41	35.85	1.79
11.25	4.78	1.49	1.29	4.56	1.34	5.81	1.16	9.52	3.24	5.98	2.98
51.56	10.37	7.64	2.50	27.69	3.38	28.81	2.78	12.51	4.02	24.76	3.25
7.27	4.70	6.47	2.64	6.88	1.31	4.76	1.36	14.62	3.81	8.29	3.11

tivariate and univariate analyses, suckers and biters exhibit substantial overlap in their pattern of muscle activity (Fig. 6). The first two principal components explained approximately 66% of the total variance (Table 5), and were most useful in heuristically visualizing similarities and differences among feeding mode and species. Duration variables, particularly for LOP and AM, showed high, positive coefficients along PC1 while relative onset variables loaded high on PC2. Biters with short bite cycles and short relative onset times, like *Scarus* and *Pygocentrus*, overlapped entire-

ly with *Micropterus*, *Lepomis*, and *Oxycheilinus*. *Cetoscarus* occupied the largest and most distinct portion of the MAP space among biters. Much of the variation among bites within this species, as well as in *Cheilinus*, and *Piaractus* was oriented along PC2. In contrast, the suckers *Micropterus*, and *Amia* and the biter *Pygocentrus* showed most variation along PC1. Strikes by the suckers *Notopterus* and *Lepomis*, and the biters *Oxycheilinus* and *Scarus* showed moderate variation along both PC1 and PC2.

DISCUSSION

The data presented here increase our understanding of vertebrate feeding by exploring muscle activity patterns of biting in several diverse groups of fishes. Our results show that, qualitatively, biters differ among

TABLE 2. *F ratios from ANOVAs of muscle activity pattern variables in five biting species.*¹

Variable	Comparison			
	1a	1b	2	3
EP (ms)	0	.9	2.3	0
LOP (ms)	4.0	0	4.3	5.3
AM (ms)	4.9	4.0	1.5	3.2
SH (ms)	.1	.5	.1	.4
EP-LOP (ms)	8.5*	.6	.5	.3
AM-LOP (ms)	7.8	3.8	1.6	1.6
SH-LOP (ms)	29.8**	0	.7	7.5
MP EP (mV)	.3	5.7	.1	10.6*
MP LOP (mV)	.3	3	1	1.7
MP AM (mV)	.6	0	1.6	.4
MP SH (mV)	4.3	.1	.2	.1
IA EP (mV*ms)	.1	6.8	.7	10.1*
IA LOP (mV*ms)	.3	4.8	16.8*	.2
IA AM (mV*ms)	0	1.2	4.6	0
IA SH (mV*ms)	1.9	.1	0	.1

¹ EP = epaxialis; LOP = levator operculi; AM = adductor mandibulae; SH = sternohyoideus; EP-LOP = relative onset of epaxialis; AM-LOP = relative onset of adductor mandibulae; SH = relative onset of sternohyoideus; MP = mean peak signal; IA = integrated area. * = $P < 0.05$; ** = $P < 0.001$. Comparison numbers refer to phylogeny in Figure 1.

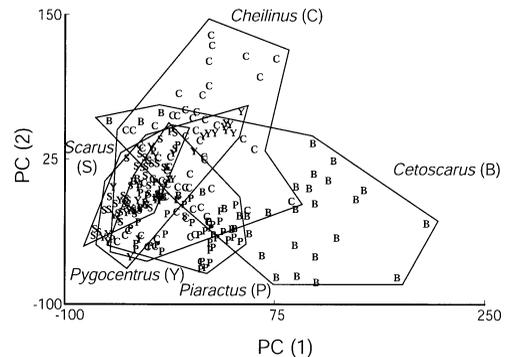


FIG. 3. Plot of bite scores along principal components (PC) one and two for five biting species. Letters represent individual bites, and polygons enclose all scores for each species. Biters: B = *C. bicolor*; S = *S. iseri*; C = *C. chlorurus*; P = *P. brachypomus*; Y = *P. natterii*; E = *B. vetula*.

TABLE 3. *Principal components analysis of cranial muscle timing variables in five biters.**

Variable	PC1	PC2	PC3	PC4	PC5	PC6
EP	-0.07	0.24	-0.08	0.71	0.41	-0.51
LOP	0.55	0.32	-0.74	0.02	-0.22	0.04
AM	0.38	0.66	0.62	0.03	-0.14	0.09
SH	0.02	0.04	-0.07	0.27	0.51	0.81
AM-LOP	0.53	-0.60	0.23	0.47	-0.27	0.05
SH-LOP	0.51	-0.19	0.08	-0.45	0.65	-0.26
Variation (%)	40	27	15	7	6	5
Cumulative (%)	40	67	82	90	95	100

* EP = epaxialis; LOP = levator operculi; AM = adductor mandibulae; SH = sternohyoideus; EP-LOP = relative onset of epaxialis; AM-LOP = relative onset of adductor mandibulae; SH = relative onset of sternohyoideus; variation = percent of total variance explained by each principal component; cumulative = percent of total variance explained by successive principal components.

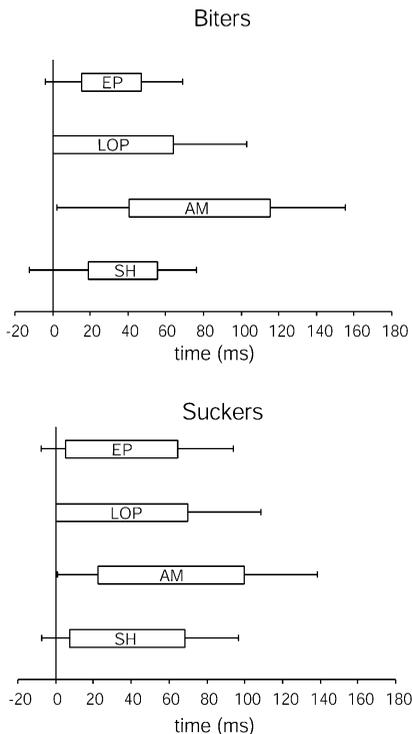


FIG. 4. Box plot summary of aquatic feeding EMGs. Shown are the average patterns of activity for four cranial muscles for six biting species and five suction feeding species. Rectangles represent mean muscle duration with error bars to the right indicating one standard deviation of the mean. Mean onset times relative to the LOP are indicated by the distance of each bar to the line to the left with error bars to the left indicating one standard deviation of the mean. EP = epaxialis, LOP = levator operculi, AM = adductor mandibulae subunit, SH = sternohyoideus.

species in many aspects of muscle timing, and that when the motor pattern is considered as a whole, there exist significant quantitative differences among biting species. However, there are relatively few detectable differences in specific EMG variables when the motor pattern is examined at various levels of the phylogeny, suggesting that some features of the biting MAP are conservative or convergent. Our higher-level analysis of fish feeding motor patterns reveals that biting is distinct from suction feeding in several key features, particularly in variables associated with the timing of muscle firing. Phylogenetic mapping suggests that biting lineages have independently departed from a primitive motor pattern that was characterized by short relative onset times. Despite these differences between suction feeders and biters, there is broad overlap in motorspace across behaviors and

TABLE 4. *F ratios from nested ANOVAs of cranial timing variables in biting and suction feeding teleosts.¹*

Variable	Factor		
	Mode	Species	Individual
EP (ms)	58.18**	3.39**	9.96**
LOP (ms)	6.92	4.27**	4.79**
AM (ms)	1.50	2.08	5.92**
SH (ms)	68.65**	4.98**	6.87**
EP-LOP (ms)	28.13**	1.53	3.16**
AM-LOP (ms)	28.51*	2.90*	6.20**
SH-LOP (ms)	12.70*	6.93**	2.68**

¹ EP = epaxialis; LOP = levator operculi; AM = adductor mandibulae; SH = sternohyoideus; EP-LOP = relative onset of epaxialis; AM-LOP = relative onset of adductor mandibulae; SH = relative onset of sternohyoideus; * = $P < 0.05$; ** = $P < 0.001$.

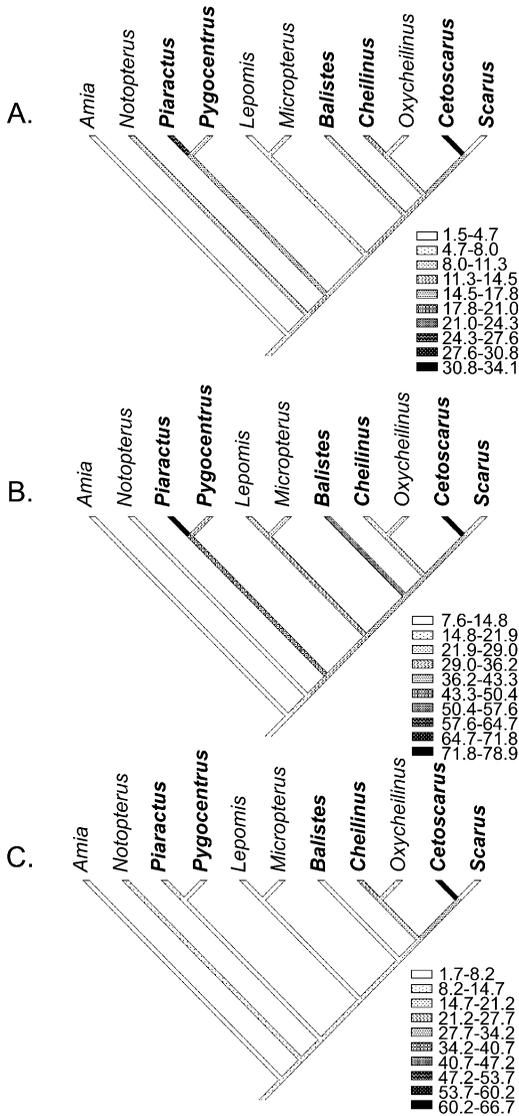


FIG. 5. Evolution of muscle timing variables in teleost fishes. Shown are the terminal states and minimized squared change ancestral reconstructions of EPL-LOP (A), AM-LOP (B), and SH-LOP (C) mapped onto the currently accepted phylogeny of teleosts is characterized by short relative onset times. While advanced suction feeders have generally retained relatively short onset times, long onset times have been acquired independently in biting lineages.

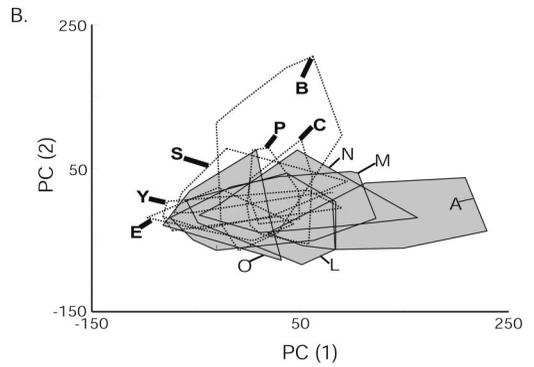


FIG. 6. Plot of bite scores along principal components (PC) one and two for six biting and five suction feeding species. Polygons enclose all scores for each species, and the shaded area denotes multivariate space occupied by suction feeders. Bold letters indicate biters, regular letters indicate suction feeders. Biters: B = *C. bicolor*; S = *S. iseri*; C = *C. chlorurus*; P = *P. brachypomus*; Y = *P. natterii*; E = *B. vetula*; Suckers: A = *A. calva*; L = *L. macrochirus*; M = *M. salmoides*; N = *N. chitala*; O = *O. unifasciatus*.

species. Below we consider the implications of these results for our understanding of motor pattern evolution.

Muscle activity pattern evolution in biters

Wainwright *et al.* (1989) found that suction feeding MAPs showed strong differentiation at high phylogenetic levels. Interestingly, although we detected a significant effect of species in multivariate analysis, we found few significant differences among the MAPs of biters at all phylogenetic levels in our study (Fig. 1, Table 2). The lack of significance in univariate tests of EMG variables might suggest that the MAP of biters is even more similar than that of suction feeders in some aspects. As serrasalmins, scarids, and *Cheilinus* are all thought to have independently acquired biting as their primary feeding mode, this motor pattern similarity could be interpreted as functional convergence. However, species do differ substantially in some aspects of their muscle activity pattern. Mean muscle activity, muscle timing, peak amplitude, and muscle firing intensity vary widely across species (Table 1), box plots of the average motor pattern vary dramatically (Fig. 2) and MANOVA reveals a significant species-level effect. It seems likely that this variation

TABLE 5. *Principal components analysis of cranial muscle timing variables in six biters and five suction feeders.**

Variable	PC1	PC2	PC3	PC4	PC5	PC6
EP	0.37	-0.31	0.23	0.46	-0.66	0.25
LOP	0.63	0.25	0.49	-0.52	-0.03	-0.18
AM	0.60	0.09	-0.79	0.07	0.02	-0.12
SH	0.31	-0.21	0.29	0.52	0.71	-0.07
AM-LOP	-0.10	0.73	0.11	0.48	-0.19	-0.43
SH-LOP	0.08	0.51	0.00	0.05	0.16	0.84
Variation (%)	40	26	16	9	5	4
Cumulative (%)	40	66	81	90	96	100

* EP = epaxialis; LOP = levator operculi; AM = adductor mandibulae; SH = sternohyoideus; EP-LOP = relative onset of epaxialis; AM-LOP = relative onset of adductor mandibulae; SH = relative onset of sternohyoideus; variation = percent of total variance explained by each principal component; cumulative = percent of total variance explained by successive principal components.

among species is biologically significant and that it is associated with the striking behavioral and functional diversity of the biting strategies employed by the species in this study: parrotfishes scrape and tear algae from rocks, *Cheilinus* dismembers and crushes benthic invertebrates, pacus remove bites from leaves and seeds, and piranhas use a powerful slicing bite to remove chunks of flesh from their prey. Thus we remain open to the possibility that our inability to reject the null hypothesis of motor pattern similarity at many levels of the phylogeny might be a consequence of small sample size and high variation at the level of individuals (Shaffer and Lauder, 1985; Sanderson, 1988).

Qualitatively, biters appear to differ from each other in the recruitment of the epaxialis muscle. Many biting species, including parrotfishes (Alfaro and Westneat, 1999), *Piaractus*, and *Balistes vetula* (Wainwright and Turingan, 1993) do not regularly recruit this muscle during feeding, and show relatively short duration of epaxialis activity during bites when it is recruited. This would suggest the testable hypothesis that species like *Scarus*, *Cetoscarus*, and *Piaractus*, which show low EP activity, achieve lower gape angles and/or have lower jaw opening velocities than species that show relatively greater EP activity like *Cheilinus* and *Pygocentrus*. The apparent functional convergence of this trait in diverse lineages also suggests that biting may sometimes place a reduced demand on neurocranial rotation (contra Barel, 1983). Experimental

studies relating EMGs to kinematics and feeding performance in biters are necessary to test these predictions and to determine the role of the epaxialis in biters.

Biters also differ from each other in the degree of overlap of muscle activity and in relative onset times. Although *Scarus iseri* possesses a muscle activity pattern that is qualitatively similar to many suction feeders (Wainwright *et al.*, 1989; Alfaro and Westneat, 1999), other biting species show considerable variation in muscle relative onset times, particularly in the adductor mandibulae and the sternohyoideus. Within suction feeding species, longer relative onset times of the adductor mandibulae are suggested to be related to the jaw protrusion mechanism of derived teleosts (Wainwright *et al.*, 1989). Jaw protrusion is not likely to be the explanation for increased relative onset times in biters, since the species with the longest relative onset times, *Cetoscarus*, *Piaractus*, and *Pygocentrus*, all have reduced or absent premaxillary protrusion during feeding (Alfaro and Westneat, 1995; Janovetz, 2001). Longer relative onset times of the adductor mandibulae in suction feeders are also associated with feeding on immobile or non-evasive prey (Liem, 1978; Wainwright, 1986; Wainwright and Lauder, 1986; Sanderson, 1988). Given this trend, one might predict that all biters would show pronounced AM offset times since they all were fed relatively non-evasive prey. However, some biting species (*Scarus*, *Cheilinus*) did show relatively short onset times of the AM. It seems clear that the functional

significance of muscle timing variation is poorly understood and warrants future study.

Muscle activity patterns in biters and suction feeders

Biters differ from suction feeders in two general ways: in biters, duration of the EP and SH are shorter, and relative onset times of EP, AM, and SH are longer. This effectively produces an average firing pattern in biters that shows reduced overlap of muscle activity compared to suction feeders (Fig. 4). Differences between the two groups in sternohyoideus activity may have especially important functional significance. In suction feeders, the sternohyoideus is critically important to the creation of suction because it lowers and pulls posteriorly the hyoid apparatus, which is primarily responsible for generating negative intra-oral pressures (Lauder, 1985; Lauder and Shaffer, 1993). The pattern of variation in timing and duration of this muscle suggests that it has diversified in function to a greater extent in biters than it has in suction feeders. Since biters are presumably less dependent on suction for prey capture, the apparent increased diversification of SH activity across biting species could be due to this muscle being freed from some of the functional demands associated with suction. Similarly, if short onset times are a broad requirement for high performance suction feeding, some of the differences in motor pattern between biters and suction feeders could be due to the biting motor pattern in general being more free to vary along timing axes than the suction feeding motor pattern. Again, this stems from the hypothesis that capture success in biters is less dependent on (or entirely independent of) producing a rapid suction vortex.

Behavioral observations and preliminary kinematics are at least consistent with the hypothesis that biters are not maximizing suction generation. *Scarus*, *Cetoscarus*, and *Pygocentrus* individuals were all observed to begin some bites with their mouths already open, which presumably would reduce their ability to develop high intra-oral negative pressure. Kinematic analysis reveals that *Scarus* and *Cetoscarus* individu-

als typically do not rotate the neurocranium during jaw opening (Alfaro and Westneat, 1995), which would also presumably reduce their ability to generate suction via an explosive jaw-opening phase. Phylogenetic mapping of timing variable reinforces the hypothesis that short onset times are correlated with suction feeding (Fig. 5). Short onset times appear to be primitive for teleosts and suction feeders have generally retained this character, with biters typically showing the most extreme cases of onset time increase.

Despite these differences, muscle activity patterns of all species show a great deal of overlap in multivariate space (Fig. 6). The functional consequences of both this overlap among species and of the relative degree of variation within species has yet to be explored. Future work relating muscle activity patterns to kinematic performance would be valuable in generating theoretical predictions of muscle activity patterns for specific behaviors.

Conservation, constraint, and the evolution of muscle activity patterns

Qualitative studies of cichlid feeding (Liem, 1978, 1979) found substantial variation in muscle activity pattern among species with different diets, and even within species during different feeding behaviors. These results demonstrated that muscle activity patterns were not evolutionarily conserved at low levels and suggested that the motor pattern was a relatively plastic component of organismal design. However, subsequent quantitative work on a range of teleosts found muscle activity patterns to be conserved at low phylogenetic levels (*e.g.*, Wainwright and Lauder, 1986; Sanderson, 1988; Westneat and Wainwright, 1989). From these quantitative studies grew the idea that motor patterns associated with suction feeding were a generally conserved feature of vertebrate design, leading to hypotheses of intrinsic and extrinsic constraint to explain the pattern (*e.g.*, Lauder and Shaffer, 1993; Smith, 1994). Testing conservatism hypotheses has proved problematic, although Smith (1994) outlined one approach. For rigorously defined motor patterns, the hypothesis of motor pattern

conservation is supported if the primitive motor patterns are retained when anatomy, kinematics, and/or behavior change (Smith, 1994).

Our study allows us to approach the question of motor pattern conservation within Smith's framework. The comparison of muscle activity patterns from biting taxa to those that use suction feeding as their primary mode of prey capture represents a study of the evolutionary response of a homologous feeding mechanism (the oral jaws and associated musculature) to changes in feeding behavior. In contrast to suction feeding, biting is a derived behavior, independently acquired in labrids, scarids and serrasalmins. Our results showing significant statistical differences between the muscle activity patterns of biters and suckers reject the hypothesis that motor patterns of aquatic prey capture are conserved at high phylogenetic levels. Rather, change in muscle firing pattern has repeatedly accompanied the shifts in morphology and behavior that are associated with novel feeding strategies (Figs. 4, 5). By rejecting the hypothesis of conservation at this level, one may also reject the hypothesis of intrinsic constraint of motor pattern evolution since it is clear that motor patterns are not actually constrained from changing. Additional work on motor control of feeding should focus on the diversity of aquatic feeding modes present in fish in order to more accurately characterize the range of control solutions to the problem of aquatic prey capture. Further inquiry into the evolution of motor patterns would greatly benefit from studies that incorporate detailed EMG data and thorough taxon sampling within the framework of a well-supported phylogeny.

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