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Motor pattern control for increasing crushing force in the striped burrfish (*Chilomycterus schoepfi*)

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Abstract

The relationship between muscular force modulation and the underlying nervous system control signals has been difficult to quantify for in vivo animal systems. Our goal was to understand how animals alter muscle activation patterns to increase bite forces and to evaluate how accurate these patterns are in predicting crushing forces. We examined the relationship between commonly used measures of cranial muscle activity and force production during feeding events of the striped burrfish (*Chilomycterus schoepfi*), a mollusc crushing specialist. We quantified the force required to crush a common gastropod prey item (*Littorina irrorata*) of burrfish using a materials testing device. Burrfish were fed these calibrated prey items while we recorded electromyograms (EMGs) from the main jaw closing muscles (adductor mandibulae A1β, A2α, and A2β). We quantified EMG activity by measuring the burst duration, rectified integrated area, and then calculated the intensity of activity from these two variables. Least squares regressions relating force to crush (F_{crush}) and all EMG variables were calculated for each fish. Multiple regression analyses were used to determine how much of the variation in F_{crush} could be explained by muscle activation patterns. We found that 20 cm burrfish are capable of generating extremely high crushing forces (380 N peak force) primarily by increasing the duration of muscle activity. EMG variables explained 71% of the total variation in force production. After accounting for the inherent variation in F_{crush} of snails, EMGs do a very good job of predicting bite forces for these fish.

Keywords: Bite force; Electromyography; Motor pattern; Fish; Calibrated prey

Introduction

Understanding how muscles function to generate force or enact movement has been one of the central goals of functional morphology and biomechanics in recent years (Alfaro and Herrel, 2001; Gillis and Biewener, 2000; Wainwright and Turingan, 1996). Unfortunately, the attempt to determine how nervous system control signals translate into functionally important behaviors like locomotion or feeding has often been challenging (Lauder, 1983; Wainwright, 1987; Wainwright and Friel, 2000; Westneat, 2003). The relationship between force production and neuromuscular activation patterns can be difficult to predict a priori because the linkages between structural elements that translate muscular tension into kinetic movement can be complicated and elusive. Indeed, because muscles that could function similarly across a joint can actually function in surprising and unexpected ways (e.g. Ahn

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and Full, 2002), to understand how muscles modulate force production, it is helpful to find simple systems in which the functional elements are understood and the measures of performance are appropriate and relevant.

The relationship between muscle function and the requisite neural control signals has been examined in several animal feeding studies (Alfaro et al., 2001; Bramble and Wake, 1985; Grubich, 2000; Herrel et al., 1997; Hylander et al., 2000; Lauder, 1985; Smith, 1994). Despite the diversity of studies that investigate correlations between muscle activity patterns and morphological specializations in feeding structures (Grubich, 2003; Lauder, 1983), or the correlations between morphology and maximal force production (Hernandez and Motta, 1997; Wainwright, 1987), the link between muscle activity patterns and force production is not well understood for fish feeding. If a general relationship were to be found, our ability to infer muscle function from easily recorded measures of activity would be greatly enhanced.

Durophagy, the ability to process hard shelled prey items, presents unique morphological and muscular challenges for fishes. Though skeletal morphology is generally considered the major determinant in the feeding abilities of fishes (e.g. pharyngeal plates, fusion of lateral jaws, etc.), the associated musculature involved in mastication has been shown to be a strong predictor of dietary specialization (Grubich, 2003; Meyer, 1990; Ralston and Wainwright, 1997; Turingan and Wainwright, 1993; Wainwright, 1996). Durophagy has been an important ecological transition for many fish species, even leading to evolutionary novel characters (Lauder, 1983).

The purpose of this study was to determine how muscle recruitment was modulated to alter force output. We investigated the relationship between the force needed to crush snails during feeding and the associated patterns of muscular activity for the durophagous striped burrfish (*Chilomycterus schoepfi*). Because the force required to crush snails was known, this system allowed us to examine the predictive power of commonly used muscle activation pattern variables in determining force production. Previous studies have sought to link motor patterns with suction feeding in fish (Lauder et al., 1986), buccal pressure during inflation of burrfish (Wainwright and Turingan, 1996), bite force in humans (Proeschel and Morneburg, 2002; Weijnen et al., 2000), yet there has been little work done linking bite force with patterns of muscle activation for non-human systems (but see McBrayer and White, 2002).

C. schoepfi were chosen for this study for two reasons. First, they are durophagous trophic specialists whose diet is more than 90% composed of sessile, hard prey items like gastropod mollusks (Ralston and Wainwright, 1997). Second, their simple musculoskeletal anatomy involved in prey processing is well documented and amenable to electrode implantation (Friel and Wainwright, 1997, 1999; Winterbottom, 1974). Burrfish (Fig. 1) have a crushing plate that is composed of the laterally fused left and right premaxillae and maxillae of the upper jaw and the fused dentary and articular bones of the lower jaw (Turingan, 1994; Winterbottom, 1974). The adductor mandibulae muscles (AM) are used to adduct the jaws and exert biting forces (Friel and Wainwright, 1997; Lauder, 1985). Adductor subdivision A1β attaches to the upper jaw and acts to rotate the upper jaw around a fulcrum on the ethmoid making a first class lever system. Adductor mandibulae A2 is divided into A2α and A2β subunits. Both muscles attach to the dentary, acting to adduct the mandible around the joint between the quadrate and articular forming a third class lever. The jaw is opened through the action of the levator operculi (LO) via a linkage connecting the opercular series to the articular bone. For a more detailed treatment of jaw morphology, including lever arms and muscle fiber orientations for the closely related porcupinefish, see Turingan (1994).

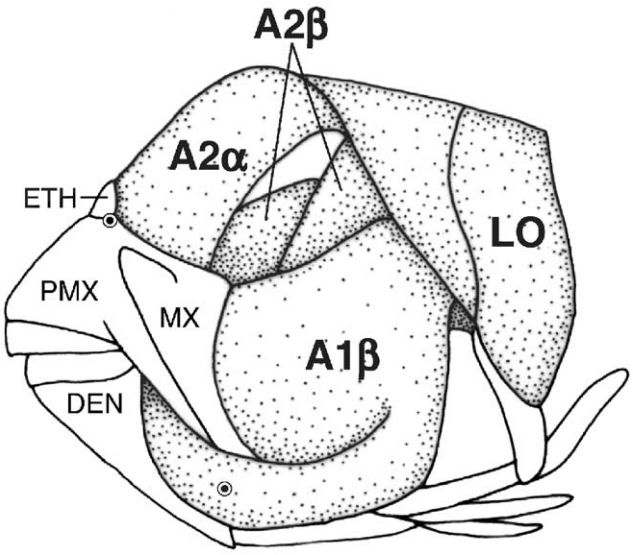

Fig. 1. Lateral view of the striped burrfish (*C. schoepfi*) adductor mandibulae complex and key skeletal elements of the oral jaw involved in prey crushing. Muscles are labeled in bold type while bones of the oral jaw are not. The upper jaw is made up of the fused premaxilla (PMX) and maxilla (MX). The dentary (DEN) is the toothed bone of the lower jaw. The upper jaw rotates around the ethmoid (ETH) and is closed through contraction of the A1β muscle whose action is transmitted to the upper jaw. Adductor mandibulae section A2 is subdivided into two functional units, A2α and A2β, that insert on the dentary rostral to the fulcrum (⊙) and act to close the lower jaw. The LO opens the jaw through a linkage between the opercular apparatus (not shown) and the dentary.

Materials and methods

Force calibration

The force required to crush a common marine gastropod prey item for burrfish was determined by mechanical testing. Salt-marsh periwinkles (*Littorina irrorata*) were collected from salt marshes in the northwest Gulf of Mexico near the Florida State University Marine Station. Animals were transported back to the lab and crushing experiments were conducted on live animals within two days of collection.

We measured the length of 294 snails (the maximum length along the long axis of the shell) ranging from 5 to 24 mm and then recorded the force required to crush the shell in a custom built crushing device fitted with a Cadet model Accuforce transducer (Ametek). Based on observations of snail orientation while burrfish fed in aquaria, snails were positioned operculum-down so that forces were exerted approximately normal to the long axis of the shell. Although previous work indicates that there is no appreciable difference in measured crushing forces when using flat metal plates or more tooth-like surfaces (Wainwright, 1987), in an attempt to keep our measurements of performance as biologically accurate as possible, we used a large plate that was slightly knurled to mimic the ridged surface of the burrfish crushing plate. Because the crushing plates of the materials testing device and the crushing surface of burrfish are both large, broad flat surfaces, the contact area between the shell and our crushing device was approximately the same as in burrfish. Shell strength (F_{crush}) was recorded as the compressive force necessary to fracture the periwinkle shell when slowly loaded as per Lowell et al. (1994). A least squares linear regression was fit to the $\log_{10}$-transformed data and this relationship was used subsequently to estimate the F_{crush} from shell length.

Animals

Striped burrfish (*C. schoepfi*) were hand-caught in seagrass beds near the Florida State University Marine Station in the northwest Gulf of Mexico. Five individuals, numbered 1–5 (17.5 ± 4 cm SL), were placed in insulated coolers and immediately taken back to the laboratory at Florida State University, Tallahassee, FL. Animals were housed in 100 l aquaria at room temperature (24 ± 2 °C) and maintained on a diet of salt-marsh periwinkles (*L. irrorata*) prior to experiments. Feeding experiments were conducted once the fish had become adjusted to the laboratory conditions but within 20 days of collection.

Electromyographic experiments

We recorded electromyograms (EMGs) from the left side of four bilaterally paired muscles while burrfish crushed and processed periwinkles. Simultaneous recordings were made from all three adductor mandibulae muscles, A1β, A2α, A2β and the LO. As the primary mouth opener in pufferfishes, the LO is commonly used as a reference for the timing of other muscles (Friel and Wainwright, 1999).

EMGs were recorded with fine-wire bipolar electrodes made from paired 1.5 m pieces of 0.051 mm diameter poly-insulated stainless steel wire (California Fine-Wire). The electrode ends were glued together to fix the distance between recording tips. Electrode tips were made by removing 0.5 mm of the insulation with a razor under a dissecting microscope to reveal the bare wire, then threading the leads through a hypodermic needle and bending the electrode tips back to form anchoring hooks. Fish were anesthetized with MS-222 (tricaine methanesulfonate, about $0.3\ g\ l^{-1}$), and electrodes were inserted percutaneously into the bellies of muscles using landmarks from prior dissections on preserved fish for reference. Electrode leads were color coded by muscle, tied to a loop of suture that was run through the skin on the dorsum of the fish for strain relief, and glued together to form a common cable. After surgery, fish were returned to their tank and allowed to recover until the effects of anesthesia had worn off (1–3 h).

Periwinkles were selected at random, their length measured with vernier calipers, then offered to the burrfish one at a time for feeding. Crushing of the periwinkle shell was signaled by a clearly audible "crunch" and shell fragments were subsequently expelled from the fish's mouth. When an animal became satiated and no longer responded to snails presented in the tank, it was euthanized with an overdose of MS-222 and the fish was dissected to verify electrode position.

Electrode leads were connected to Grass P-511 preamplifiers. The signal was amplified 10 000 times and filtered with a signal bandpass of 100–3000 Hz using a 60 Hz notch filter. EMGs from all four muscles, together with a simultaneous voice track for noting events, were recorded on a 14-channel TEAC XR-5000 FM analog recorder. Feeding sequences were later played back to produce hard copies used for visual inspection of the recorded events on a Graphtech thermal-array recorder.

At least 25 crushing events per individual were analyzed. We included only those trials in which an individual caused complete failure of the shell. The analog EMG data were digitized using a Keithley 500A system with an effective sampling rate of 8 kHz. A custom-designed software program was used to measure the onset of adductor mandibulae muscles relative to the jaw opening levator operculi. Burst duration and

rectified integrated area were measured for each of the four muscles (A1β, A2α, A2β, LO). We calculated the intensity of activity of each muscle burst by dividing the integrated area by the duration of activity. For a detailed explanation of these procedures, see Friel and Wainwright (1999).

Statistical analysis

The size of each snail was transformed into an expected crushing resistance using the regression equation (Eq. (1)) and the effect of snail size on the activity patterns of the three adductor muscles was investigated. All data were $\log_{10}$-transformed to provide a linear relationship and meet the assumptions of parametric regression. Least squares regressions relating force and each of the EMG variables were calculated for each fish.

To determine how much of the variation among snails in F_{crush} could be explained by muscle activation patterns, we used a series of General Linear Model (GLM) multiple regression analyses. Five models were constructed: an overall model that included as factors all EMG variables for all muscles, individuals, and all interactions, and an additional model for each of the EMG variable types (relative onset, duration, intensity, area) that included the adductor mandibulae muscles, individuals, and all interactions. We analyzed each model in a stepwise fashion. First, the entire model was run including the individual effect, all EMG variables and all interactions. Then those terms that did not substantially contribute to the model (arbitrarily determined as $p \geqslant 0.35$) were removed and the model was rerun. All statistical procedures were conducted with Systat v. 5 for Macintosh.

Results

Force calibration

During loading experiments, the snail shells crushed completely. Snails spanned a five-fold size range (4.8–23.2 mm), which corresponded to a fifty-fold range in measured crushing force (10.5–588.8 N). Snail length predicted three-quarters of the variation in force needed to crush snails ($r^2 = 0.75$, $p \geqslant 0.001$, Fig. 2). From the slope of the regression equation (GLM), we calculated the force to crush a snail (F_{crush}) as:

$$F_{\text{crush}} = 0.068 s^{2.92}, \quad (1)$$

where s is the length of the snail from the tip of the spire to the distalmost margin of the operculum.

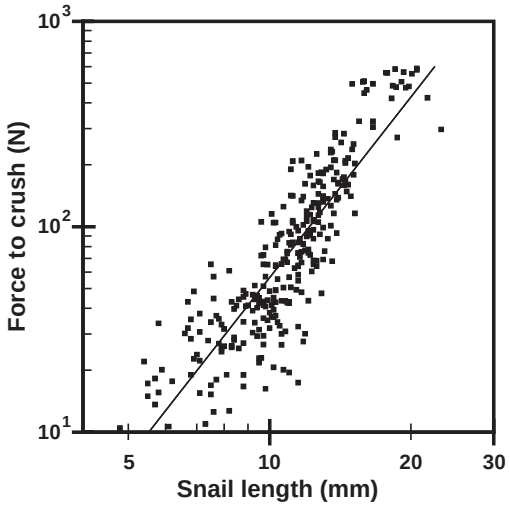

Fig. 2. Breaking force as a function of snail length for fresh salt-marsh periwinkle snails (*L. irrorata*). Snail length was determined as the maximum span of the shell along the longitudinal axis. A total of 294 snails with lengths ranging from 4.8 to 23.2 mm were cracked. Breaking force of the shell ranged between 10.5 and 588.8 N. Data were $\log_{10}$-transformed and a linear regression model was fit to the points. The force at fracture for a snail shell scales with the snail length 2.92, $r^2 = 0.75$.

Feeding trials

Burrfish picked up *L. irrorata* shells and, after orienting the snail between the crushing plates of the oral jaw, crushed the shell – usually on the first attempt. Shell fragments were expelled after separating the flesh from the shell. Individuals ate between 25 and 43 snails during feeding trials before becoming satiated (Table 1). All individuals easily processed smaller snails, but as snails approached the maximal size crushed in this study (19.2 mm), some fish were unable to fracture the snail. Unsuccessful attempts were usually followed by reorientation of the snail and further attempts to crush the snail were made. Although the skin around the jaws partially obscured the snail from view while being crushed, it was usually possible to visually confirm snail orientation in the mouth. Snails were always crushed in the crushing plates immediately posterior to the beak-like portion of the jaws for all individual burrfish.

C. schoepfi generally activated the three muscles of the adductor mandibulae complex simultaneously to produce isometric forces that were transmitted through the crushing plate to the periwinkle shells. One individual consistently activated A2α earlier than the other adductor mandibulae muscles (Animal #2, Fig. 3). All muscle activity variables increased with force output, though there was no single EMG variable that was

Table 1. Least squares regression slopes of EMG variables on force. Force was inferred from snail size

EMG variable	Individual (# of crushing events)									
	1 (25)		2 (29)		3 (43)		4 (27)		5 (28)	
	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2
Duration										
A1β	1.24***	0.67	0.95***	0.56	0.96***	0.43	1.52**	0.34	0.85**	0.25
A2α	1.48***	0.63	0.78***	0.40	0.90***	0.28	0.83**	0.25	0.82**	0.24
A2β	1.78***	0.63	0.91***	0.24	0.87***	0.30	0.52	0.13	0.84**	0.23
Area										
A1β	1.76***	0.58	2.02***	0.59	1.12***	0.23	0.62*	0.17	1.48*	0.18
A2α	2.14***	0.81	1.35***	0.57	0.76*	0.11	0.88*	0.16	0.82	0.07
A2β	1.35***	0.48	1.33***	0.43	1.16***	0.32	0.46	0.05	1.48*	0.19
Intensity										
A1β	0.52*	0.21	1.07***	0.34	0.16	0.01	0.90**	0.33	0.62	0.06
A2α	0.66***	0.41	0.57***	0.41	−0.14	0.01	0.05	0.00	−0.01	0.00
A2β	1.75	0.03	0.42*	0.16	0.26	0.05	−0.06	0.00	0.64	0.06

*$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$.

significant for all muscles across all individuals (Fig. 4, Table 1). Duration of muscle activation, and the related rectified integrated area, showed a strong positive correlation with oral jaw force output for all three muscles (Fig. 4, Table 1). Intensity of muscle activity was more variable, with only six out of 15 EMG variables showing a significant relationship between intensity and force output (Table 1). Adductor mandibulae A1β area showed a significant positive correlation to increased force production with regression slopes ranging from 0.62 to 2.02 (mean = 1.4). Area increased significantly for each lower jaw closing muscle in all but one instance (A2α for individual 5, A2β for individual 4).

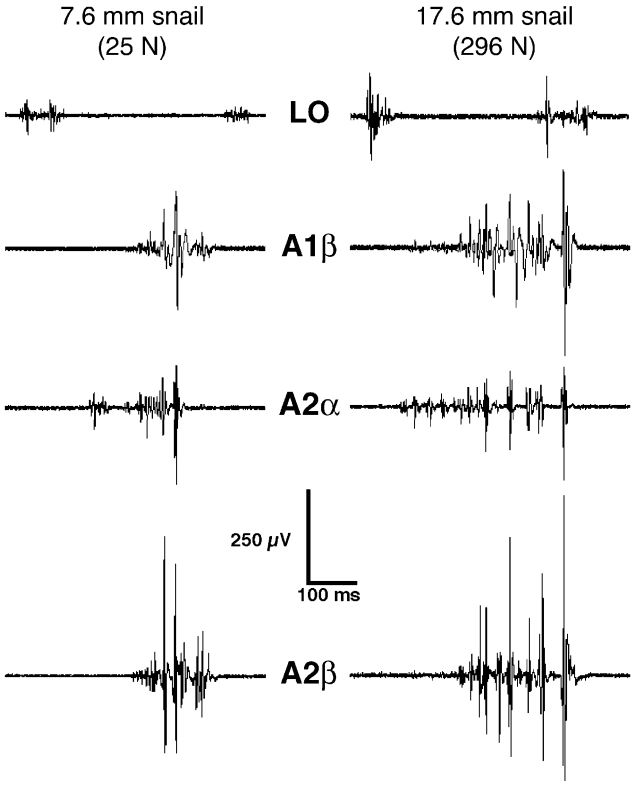

Fig. 3. Representative EMGs from a burrfish while feeding on snails of different sizes. Recordings were made from the same electrodes a few minutes apart. Muscle activity patterns are shown for the jaw opening LO and for the three jaw-adducting muscles A1β, A2α and A2β.

As shown in Table 2, there was significant variation between individuals in this study for all measured EMG variables except relative onset. This finding is consistent with previous studies that have sought to examine inter-individual effects on muscle patterns (Friel and Wainwright, 1999; Ralston and Wainwright, 1997; Reilly and Lauder, 1988; Sanderson, 1988; Wainwright and Lauder, 1986; Wainwright and Turingan, 1993).

Over 71% of the variation in inferred force production among feeding sequences was explained by EMG variables in the overall regression model (Table 2). Rectified integrated area alone accounted for more than 67% of the variation in muscle force output, while duration and intensity alone explained 60% and 50% of muscle force variation. Duration of A1β activation contributed most to the variation in the overall model ($F = 62.27$, Table 2), orders of magnitude more than any other variable. Adductor mandibulae section A1β was the best overall predictor of muscle force production (Table 2).

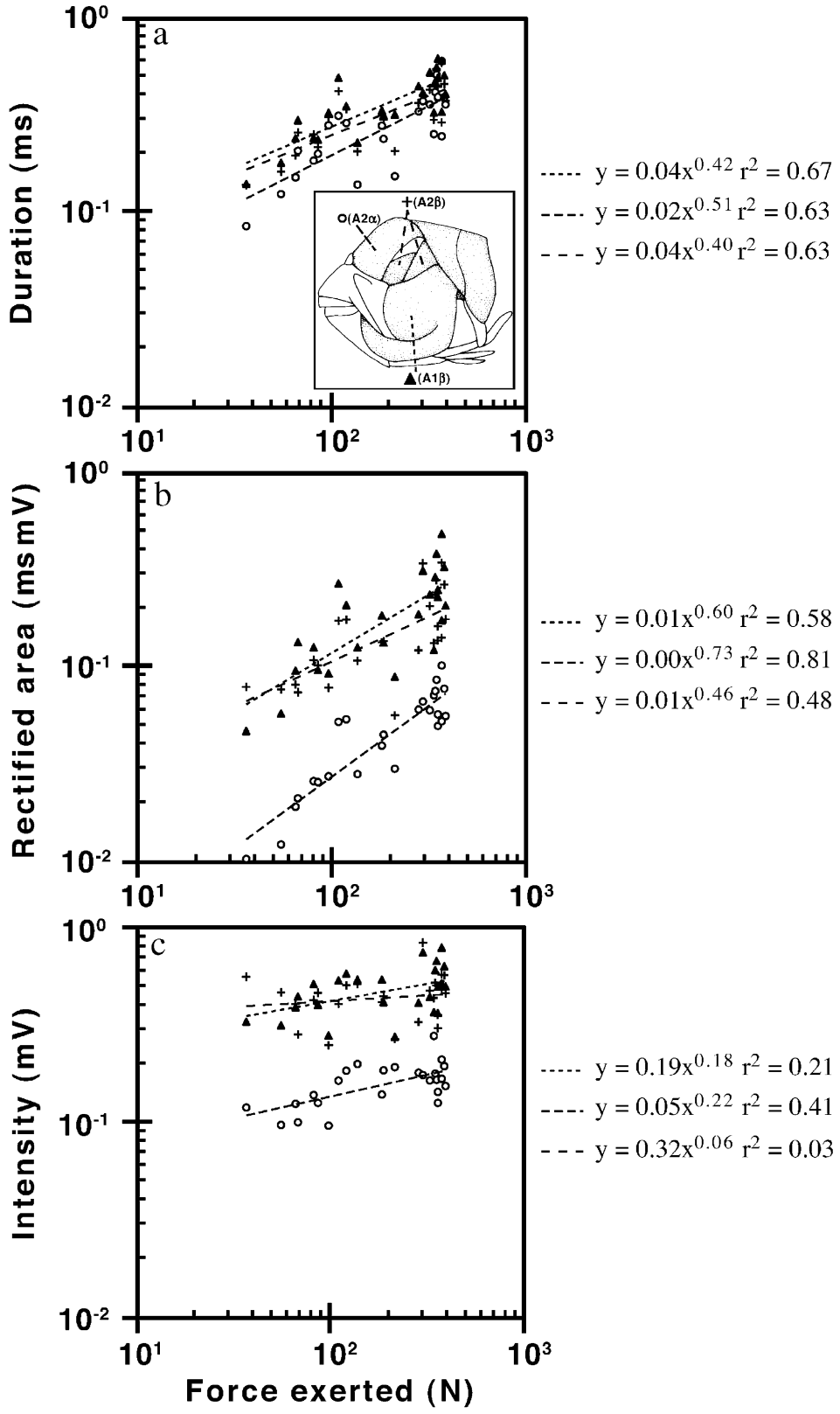

Fig. 4. Representative scatterplots of EMG activity variables in response to different force requirements for muscles involved in jaw adduction from one individual (#1) (see inset in (a) for symbol legend). Both duration (a) and rectified area (b) increase significantly with increased loading for all muscles. A1β and A2α increased intensity of activity (c), while A2β did not.

Table 2. Reduced model results from multiple regression analyses run to investigate how much variation in force production can be explained by EMG variables

Overall model ($r^2 = 0.71$)		Relative onset ($r^2 = 0.39$)		Duration ($r^2 = 0.60$)		Intensity ($r^2 = 0.50$)		Area ($r^2 = 0.67$)	
Categorical variable	F (df)	Categorical variable	F (df)	Categorical variable	F (df)	Categorical variable	F (df)	Categorical variable	F (df)
IND	9.92 (4,133)***	IND	2.135 (4,144)	IND	4.06 (4,141)**	IND	9.69 (4,137)***	IND	8.12 (4,132)***
A1β_RON	5.49 (1,133)*	A1β	2.14 (1,144)***	A1β	35.28 (1,141)***	A1β	30.24 (1,137)***	A1β	5.49 (1,132)**
A1β_DUR	62.27 (1,133)***	A2β	20.58 (1,144)***	A2β	3.61 (1,141)	A2α	0.71 (1,137)	A2α	1.04 (1,132)
A1β_INTN	10.41 (1,133)**	INDxA2β	2.86 (4,144)*	INDxA2β	2.33 (4,141)	INDxA1β	1.681 (4,137)	A2β	4.14 (1,132)*
A2α_INTN	3.98 (1,133)*					INDxA2α	9.30 (4,137)***	INDxA1β	1.73 (4,132)
A2β_RON	8.07 (1,133)**							INDxA2α	9.16 (4,132)***
A2β_INTN	2.32 (1,133)							INDxA2β	
INDxA1β_INTN	1.57 (4,133)								
INDxA2α_INTN	9.65 (4,133)***								

*$P \leqslant 0.05$, **$P \leqslant 0.01$, ***$P \leqslant 0.001$. Categorical variable abbreviations: IND, Individual; RON, Relative Onset; DUR, Duration; INTN, Intensity; x denotes an interaction between terms.

Discussion

Greater crushing forces were generated by a large increase in muscle activation patterns (EMGs), most notably duration and the related rectified integrated area of the signal. To measure the crushing forces of these fish, we developed an accurate method to evaluate force production by calibrating the strength of snail shell prey (*L. irrorata*). The patterns of muscle activity for the muscles of the adductor mandibulae complex predict over 71% of the variation in crushing force (F_{crush}). Rectified integrated area of the EMG signal, commonly used as a proxy for the total energy of muscular activation (Basmajian and De Luca, 1985; Loeb and Gans, 1986), is the best overall predictor of F_{crush}, alone explaining most of the variation in the total force output. Activity of the single upper jaw closing muscle, A1β, was the factor most highly correlated with increased force output of the jaws.

Can EMGs predict force output?

Commonly used measures of muscle activity are predictive of the overall force generated by burrfish when crushing shells, explaining over 71% of the variation in measured crushing force (Table 2). With r^2 values from 0.71 to 0.85, our results are comparable to patterns of EMG activity with isometric clenching forces for humans, an analogous functional behavior (Proeschel and Morneburg, 2002; Wang et al., 2000). Associations between common EMG activity patterns and the functional manifestations of muscular contraction, i.e. force generation, have been the focus of much research (Biewener et al., 1998; Jayne and Lauder, 1993; Johnston et al., 1995; Josephson, 1999; Wainwright and Turingan, 1996), and yet no universally agreed-upon inference guidelines have emerged. This lack of clear resolution is often attributed to the complex and integrated nature of force transmission involved in the behaviors studied. Unlike the complex mechanical linkages between jaw closing musculature and the bony elements involved in the feeding of most teleosts (Westneat, 2003), the oral jaws of *C. schoepfi* are simple first and third class mechanical levers. Because of this simple arrangement, muscle tension is translated directly into crushing force in a predictable manner, and therefore, EMG activity can characterize muscular function during feeding for these animals and may provide a useful study system for future research.

The strength of correlations between EMG activity and force production as investigated here may actually be underestimated because our method of determining crushing force was subject to some variation. We note that the r^2 value relating snail size with F_{crush} corresponds closely to the relationship of EMGs to F_{crush}, 0.75 and 0.71, respectively. Thus, when accounting for the error involved in estimating the force required to crush snails, there is only an additional 4% of the total variance left unexplained. In studies with humans that related EMG variables to direct measures of force output, up to 95% of the variation in force has been accounted for (Lawrence and Deluca, 1983; Moritani and Devries, 1978).

Force output by the oral jaws is best characterized by a significant increase in the total area of the EMG envelope. Integrated rectified area of the EMG signal is a measure of the real sum of mechanical activity of a muscle, and as such, is a surrogate for the total mechanical energy produced (Loeb and Gans, 1986). Since rectified integrated area is largely influenced by the duration of activation, our findings may be confounded by this dependence, especially because our results show that intensity of the EMG signal varies little with increased force production. If intensity does not increase with force output, or increases marginally as is the case here, then to cause an increase in area, the duration of muscular activation must increase since area is the product of intensity and duration. Thus, burst duration probably contributed the most to force production.

Intensity was the least predictive variable when assessing changes in force output, explaining only 50% of the variation in force output (Table 2). This is an unexpected result. Previous attempts at correlating muscle activity patterns and feeding performance have found that intensity is the best predictor of muscle output (Basmajian and De Luca, 1985; Herrel et al., 1997; Wainwright and Turingan, 1996). One explanation for the surprisingly poor predictive power of intensity in burrfish is that the inter- and intra-specific variation in EMG measurements in response to greater force production is higher than for other measures. Only six out of the 15 measured least squares regression slopes differed significantly from 0, and those that were significant showed a weaker correlation between F_{crush} and intensity of the EMG signal than for duration or area, with markedly lower r^2 values (Table 1). This high variability may simply be a measurement error with no functional consequence for feeding, or, alternatively, a telling indicator of how burrfish process prey. Based on the results of recent studies, it is likely that the variation in EMG parameters probably reflects a functional change in mechanical output (Jayne et al., 1990; Lauder et al., 1986; Wainwright and Friel, 2000).

EMG intensity is a measure of the average amplitude of electrical signal during a burst cycle, and as such, is a surrogate for the number of motor units activated at a given time (Basmajian and De Luca, 1985). If the intensity of EMG signals does not change over a wide range of force demands, then all of the motor units in the region of the EMG electrodes may be activated regardless of force output; in essence, the electrical

activation of the muscles has plateaued. Gans et al. (1985) found that the amplitude of electrical activity did not change for lizards between crushing of hard prey items and subsequent chewing, two very different functional behaviors. This suggests that it is not the number of motor units recruited that allow burrfish to crush hard prey items, but some other physiological phenomenon.

All three muscles of the adductor mandibulae complex seem to be activated in a pulsatile manner, with repeated clusters of depolarization spikes within each burst (Fig. 3). Repeated stimulation of muscles that are not allowed to relax can dramatically increase force generation relative to the twitch values alone (Loeb and Gans, 1986). Gans and De Vree (1986) found that when applying a constant amplitude external electrical stimulation to the adductor mandibulae of a shingle-back lizard, by summing the stimulation pulses (7–10 Hz pulsatile frequencies), force output could be increased by 250–350% over twitch values alone. During feeding trials of the same lizards, Gans et al. (1985) also found that the magnitude of EMG spikes did not differ between hard and soft prey items. They concluded that crushing hard prey items was not accomplished by increasing the intensity of muscle activity, but by synchronized, or unfused tetanus. If the duration of activation increases while the burrfish adductor mandibulae complex is held at a fixed length by a snail in the jaws, then the forces produced by the muscles can ramp up in a stair-step manner (Treppe) (Gans et al., 1985).

By gradually loading snails like this, burrfish may be using the minimum number of pulses, and thus duration, of electrical activity for a stepwise increase in force production towards the tetanus plateau. This slow loading may aid in the gradual development of high forces. Since the duration of activity was relatively long (100–300 ms), it is possible that muscle spindles in the adductor mandibulae complex could provide neural feedback to the animal during feeding. If a snail were to slip between the crushing plates, for example, burrfish could alter activation and consequent distribution of forces among the jaw closing musculature. This may help explain the variation in muscle activity we see between the α and β subunits of the A2 muscle. Both muscles insert on the dentary of the lower jaw, with A2α inserting rostrally relative to the A2β attachment. By altering the relative activation of these two muscles, burrfish may modulate the compressive force vectors of the lower jaw in response to perceived slipping of the prey. Though it is possible that the pulsatile nature of force application could lower the overall force needed to crush through the shell by stress softening (repeated loading), the effect of repetitive loading by predators on shell material at this timescale is thought to be minimal (Currey and Brear, 1984; Labarbera and Merz, 1992).

Of all the jaw closing muscles that constitute the adductor mandibulae complex, activation patterns of A1β explain the most variation in bite force. This result could be explained if A1β produced more force than its lower jaw closing counterparts, but since the total muscle mass of A2α and A2β is approximately twice that of A1β (Friel and Wainwright, 1999; Turingan, 1994), this is unlikely. Instead, we believe that because A1β is the only adductor that attaches to the upper jaw, the overall force output of the upper jaw lever system would be directly linked to the action of a single muscle. As such, A1β would be a more accurate predictor of force output than when multiple muscles function across a single joint. Since A2α and A2β insert on the lower jaw at different locations but both function to adduct the dentary, multiple combinations of A2 recruitment could produce similar forces, possibly explaining the lower predictive power of these two muscles considered alone. Though not the purpose of the present study, accurate measurements of lever arm moments and the physiological cross-sectional area of these muscles coupled with these results could provide a powerful means to investigate the relative contribution each muscle makes towards the total crushing force.

Crushing force measurements

In an attempt to obtain realistic measures of performance and minimize the influence of behavioral variation due to acclimation to testing equipment, we employed a biologically realistic, non-invasive method of force transduction that uses a calibrated prey item to measure the crushing force of *C. schoepfi*. Though crushing strength of hard prey items has been successfully used to evaluate trophic level feeding performance (Hernandez and Motta, 1997; Wainwright, 1987), this is the first study to use crushing performance to measure bite force in fishes. Prey items differing in physical characteristics (e.g. chewy, tough, elusive) have been used to evaluate EMG variability across prey type or across species in the past (Friel and Wainwright, 1998; Gans et al., 1990; Wainwright and Friel, 2000). Other methods of calculating bite force have been used with varying levels of success. One such technique that has been used for terrestrial feeding studies is a tuning fork-bite bar whereby an animal clamps down on instrumented metal plates (Erickson et al., 2004; McBrayer and White, 2002). This technique is difficult to implement for fish feeding studies because the loading of the device must be normal to the axis of measurement, and it is necessary to know the exact position of force application, both of which can be difficult in aquatic feeding studies. Electrical stimulation of adductor musculature has been used to study bite force in lizards (Gans and De Vree, 1986), and more recently by Huber

and Motta (2004) for sharks. This technique, according to the authors, often underestimates the total force produced during normal feeding because multiple muscles are involved in prey processing but not all were stimulated.

This work provides a method to quantify feeding performance while simultaneously investigating how animals modulate muscle activity patterns to increase force production, and provides an ecologically accurate measure of biomechanical performance because animals are eating calibrated prey.

High force output

The crushing force (380 N) produced by the burrfish oral jaw is one of the highest reported values for any bony fish to date. To produce these large forces, burrfish use the highly modified prey-processing structures of the oral jaw. In contrast to most teleosts, all tetraodontiform fishes, including burrfish, use their oral jaws for both prey capture and processing (Friel and Wainwright, 1998; Turingan and Wainwright, 1993). The jaws of these fish are robust with no noticeable streptostyly, and the musculature involved with jaw adduction fills the head. Turingan (1994) discusses numerous morphological modifications of the head bones and musculature, most notably the tightly fused bones constituting the oral jaw, which facilitate a durophagous lifestyle.

Given the role that diet plays in shaping musculoskeletal elements involved in feeding for durophagous trophic specialists (Lauder, 1983; Meyer, 1990; Ralston and Wainwright, 1997), it is no surprise that *C. schoepfi* can produce high forces. We were, however, impressed by the magnitude of force produced – the equivalent of a 38 kg load – especially because these fish are relatively small. Because crushing force increases with body size – often scaling allometrically (Erickson et al., 2003; Hernandez and Motta, 1997), we compared the absolute magnitude of our force data to the largest reported values for similar-sized bony fishes. Using jaw muscle cross-sectional area (CSA) to infer maximal force production abilities, Clifton and Motta (1998) estimated that a similar sized hogfish (*Lachnolaimus maximus*: Labridae) could generate upwards of 290 N of force with its pharyngeal jaws. When compared to crushing force estimates of another oral-jaw crushing specialist, the sheepshead (*Archosargus probatocephalus*: Sparidae), burrfish far surpass the 100 N of potential force (based on CSA) and 50 N of measured force (based on calibrated prey techniques) that a 20 cm individual could produce (Hernandez and Motta, 1997). Burrfish generate the largest crushing forces of any reported fish of similar size, making them a formidable durophagous predator.

General conclusions

Striped burrfish (*C. schoepfi*) are capable of generating extremely high forces with their oral jaws – upwards of 380 N. These compressive forces were sufficient to crush the shells of marine gastropods up to 19.2 mm in length. These forces exceed any measured values for any bony fish. Burrfish are able to modulate force production in different ways. Neuromotor systems are dynamic and plastic and can often produce the same force with different patterns. For all trials, the motor patterns of the upper jaw moving muscle (A1β) did not vary between individuals (Table 2), meaning that individuals used similar strategies to increase force production of the muscle. In contrast, for the two lower jaw muscles (A2α and A2β) there were significant individual interaction terms for burst area, and A2α had significant intensity interaction terms. Because more than one muscle inserts on the lower jaw, it appears that individuals recruit the muscles in different ways, perhaps allowing fine-scale control while crushing and possibly explaining the variation between individuals.

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