

MUSCULAR BASIS OF BUCCAL PRESSURE: INFLATION BEHAVIOR IN THE STRIPED BURRFISH *CHILOMYCTERUS SCHOEPFI*

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Summary

We examined the relationship between commonly measured features of cranial muscle activity and the magnitude of sub- and superambient pressure measured inside the buccal cavity of the striped burrfish *Chilomycterus schoepfi* during inflation behavior. Buccal pressure was recorded simultaneously with electromyographic (EMG) records of activity from three expansive-phase muscles (levator operculi, levator pectoralis and hyohyoideus abductor) and three compressive-phase muscles (adductor mandibulae, protractor hyoideus and protractor pectoralis) in eight individuals. We quantified EMG activity in approximately 30 inflation cycles per fish by measuring the burst duration, rectified integrated area, intensity of activity (area divided by duration) and onset time relative to the onset of subambient pressure at the beginning of the cycle. Multiple regressions were calculated separately for data from each fish to investigate the relationships between pressure and

EMG variables. The percentage of variation in minimum buccal pressure or area under the subambient pressure curve explained by the multiple-regression models ranged among individuals from approximately 52 to 84%. The regression models accounted for more variation in peak pressure and the integrated area of superambient pressure; r^2 ranged from 76% to 97%. The strong relationship between EMG activity and superambient buccal pressure suggests that the latter is probably a direct function of the strength of compressive-muscle contraction. In contrast, the magnitude of subambient pressure is a complex function of the area of the oral opening and the rate of buccal expansion, factors that do not appear to be as directly indicated by the degree of muscle activity.

Key words: pufferfish, *Chilomycterus schoepfi*, inflation behavior, electromyography, buccal pressure.

Introduction

A common approach used to investigate vertebrate muscle function is to record electromyograms (EMGs) from the muscles in question during specific behaviors and to relate patterns of muscle activity to some mechanical manifestation of muscular contraction, such as movement or force (Johnson *et al.* 1994; Biewener and Dial, 1995; Jayne and Lauder, 1995). Such studies have been instrumental in developing our current understanding of the functional morphology of vertebrate musculoskeletal systems (e.g. Gans and Gorniak, 1982; Reilly and Lauder, 1990; Wainwright and Bennett, 1992; Jayne and Lauder, 1993). In attempts to gain the maximum amount of information from electromyograms, the activity patterns of muscles are often quantified by measurement of variables such as burst duration, integrated rectified area of the burst, spike amplitude and the relative timing of activity in different muscles. Considerable effort has been directed at determining the precise form of the relationship between EMG activity and tension of muscles (Bigland and Lippold, 1954; Millner-Brown and Stein, 1975; Lawrence and De Luca, 1983) and to

developing theoretical predictions (Bernshtein, 1967; Libkind, 1969). In controlled human studies on single muscles working across single joints, over 95% of the variation in tension is explained by measures of EMG amplitude (Moritani and DeVries, 1978; Lawrence and De Luca, 1983). Not surprisingly, when more complex musculoskeletal systems are considered, in which more than one muscle acts across more than one joint, the relationship between EMG activity and kinematic or mechanical output is typically less precise; EMGs of individual muscles explain 20–75% of variation in mechanical output variables (Lauder *et al.* 1986; Jayne *et al.* 1990).

Two major factors contribute to the reduced predictive performance of EMG activity variables in studies of whole-organism behaviors. First, muscular tension is usually not measured directly; instead, more integrated measures of performance are assessed, such as locomotor speed (Jayne *et al.* 1990; Jayne and Lauder, 1995) or suction pressure during prey capture by fishes (Lauder *et al.* 1986). Although muscular

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tension contributes to these quantities, it may not be the sole factor determining their magnitude. The second factor is that whole-animal behaviors typically involve the actions of several muscles simultaneously, often acting antagonistically or across a series of joints. Such complexity can obscure the roles of individual muscles, although understanding the functional role of isolated components is often possible only when the whole system is studied simultaneously (e.g. Wardle *et al.* 1995).

In this study, we investigate the relationship between cranial-muscle activation patterns and buccal pressure in the striped burrfish *Chilomycterus schoepfi*. The inflation behavior in this species, as in other pufferfish, involves a cyclical pattern of buccal expansion and compression as water is repeatedly drawn into the mouth and pumped into the stomach through the esophagus (Brainerd, 1994; Wainwright *et al.* 1995). Buccal expansion is characterized by a pulse of subambient pressure that is eliminated as water fills the buccal cavity. Expansion is followed immediately by buccal compression and a pulse of superambient buccal pressure corresponding to a period when water is forced posteriad through the esophagus and into the stomach (Brainerd, 1994). On the basis of our previous work, we selected and recorded from six muscles that we expected to be key effectors in these buccal-expansion and -compression actions. This study has two primary purposes. First, we test the power of commonly used electromyographic indicators of muscle activity in the six muscles to predict the magnitude of buccal pressure. Buccal pressure can be viewed as an integrated measure of the consequences of muscle contraction in this musculoskeletal system. Second, buccal expansion and compression are antagonistic actions of the same musculoskeletal system with different functional determinants, and we contrast the ability of EMG activity variables to account for sub- and superambient pressure.

Materials and methods

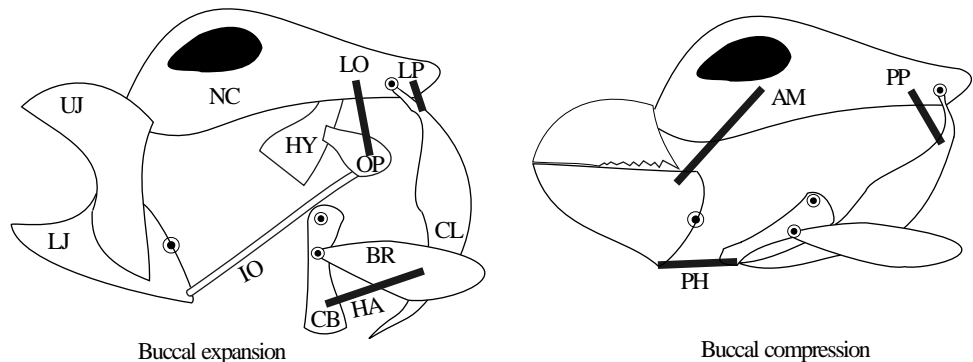
We studied the striped burrfish (*Chilomycterus schoepfi*) for several reasons. First, like other pufferfishes (Tetraodontidae), burrfish are able to inflate their bodies when disturbed, and this behavior involves substantial sub- and

superambient buccal pressure pulses as the fish draws water into the buccal cavity and pumps it into the stomach (Wainwright *et al.* 1995). Second, we have previously conducted research with this species elucidating the musculoskeletal mechanisms of buccal expansion and compression used during inflation behavior (Wainwright *et al.* 1995). For the present study, eight burrfish were collected in sea-grass beds near the Florida State University Marine Laboratory in the northeastern Gulf of Mexico. The standard lengths of the individuals, numbered 1–8, were 165, 170, 165, 160, 170, 165, 175, 145 mm, respectively. The fish were transported immediately to the laboratory at Florida State University, where they were maintained separately in 100 l aquaria at room temperature (approximately 21 °C).

During experiments, buccal pressure and cranial-muscle electromyograms were recorded simultaneously while the animal underwent several inflation bouts. Pressure was measured in the buccal cavity using a Millar SPR-407 microcatheter-tipped pressure transducer that was threaded through a plastic cannula that had been implanted under anesthesia (see below) in the neurocranium, on the dorsal midline between the anterior margins of the orbits. The cannula was flanged at the distal end, holding it in place on the superior wall of the buccal cavity.

To document patterns of muscle contraction during inflation behavior, we recorded EMGs from the left half of six bilaterally paired muscles during each experiment (Fig. 1). These muscles were selected because our previous research indicated that they are primarily responsible for the buccal expansion and compression movements that are repeated during inflation behavior (Wainwright *et al.* 1995). The expansion-phase muscles (Fig. 1) included the levator operculi (LO), the major mouth-opening muscle; the hyohyoideus abductor (HA), a large ventral muscle that causes depression of the hyoid apparatus and posterior rotation of the pectoral girdle; and the levator pectoralis (LP), a muscle that extends the cleithrum on the neurocranium, causing the latter to rotate posteriad. The compressive-phase muscles (Fig. 1) included section 2 α of the adductor mandibulae complex (AM), a powerful adductor of the mandible; the protractor hyoideus

Fig. 1. Schematic diagram of the anatomical relationships between the six muscles studied and key skeletal elements in the head of the burrfish *Chilomycterus schoepfi*. Thick lines indicate attachments of the six muscles that function during the buccal-expansion and -compression phases of inflation behavior. The circled dots indicate the locations of joints between skeletal elements. Muscle abbreviations: AM, section 2 α of the adductor mandibulae; HA, hyohyoideus abductor; LO, levator operculi; LP, levator pectoralis; PH, protractor hyoideus; PP, protractor pectoralis. Other abbreviations: BR, enlarged first branchiostegal ray; CB, ceratobranchial; CL, cleithrum; HY, hyomandibula; IO, interoperculo-mandibular bone; LJ, lower jaw; NC, neurocranium; OP, opercle; UJ, upper jaw (fused maxilla and premaxilla).



Other abbreviations: BR, enlarged first branchiostegal ray; CB, ceratobranchial; CL, cleithrum; HY, hyomandibula; IO, interoperculo-mandibular bone; LJ, lower jaw; NC, neurocranium; OP, opercle; UJ, upper jaw (fused maxilla and premaxilla).

(PH), which retracts the hyoid to its resting position and forces the hyoid dorsally into the buccal cavity; and the protractor pectoralis (PP), the antagonist to the LP that protracts the pectoral girdle relative to the neurocranium.

Electromyograms were recorded using fine-wire bipolar stainless-steel electrodes constructed from paired 1.5 m insulated wires that were glued together to fix the distance between the recording tips. Electrode tips of approximately 0.5 mm were exposed, by removing the insulation with a blade under a microscope, mounted in hypodermic needles with the tips bent back to form anchoring hooks, and implanted percutaneously into the cranial muscles while the fish were under anesthesia (tricaine methanesulfonate, about 0.7 g l^{-1}). Electrodes were sutured to the skin on the dorsum immediately posterior to the left orbit and glued together into a common cable.

Electromyograms were amplified 10 000 times with Grass P-511 preamplifiers, using a signal bandpass of 100–1000 Hz. The 60 Hz notch filter was always employed. Buccal pressure and electromyograms, together with a simultaneous voice track, were recorded on a 14-channel TEAC XR-5000 FM analog recorder. Hard copies used for visual inspection of the recorded events were produced by a Graphtec thermal-array recorder.

For further analysis, the analog recorded pressure and electromyographic data were digitized using a Keithley system and a sampling rate of 8 kHz, and subsequently the same digital filter (low-pass finite infinite response filter; 100 Hz pass, 350 Hz stop) was applied to every file to reduce high-frequency electrical interference in the electromyograms. For each individual, about three inflation sequences were selected and analyzed cycle by cycle, for an average of approximately 30 cycles per individual. Each inflation cycle (Fig. 2) consisted of a period of subambient pressure, corresponding to oral and buccal expansion as water was sucked into the mouth, and a period of superambient pressure, as the buccal cavity was compressed and water was pumped into the stomach (Wainwright *et al.* 1995). A custom-designed software program was used to measure the duration of the single burst of activity in each of the six muscles (LODUR, HADUR, LPDUR, AMDUR, PHDUR, PPDUR), the integrated area under each rectified myogram (LOAREA, HAAREA, LPAREA, AMAREA, PHAREA, PPAREA) and the onset of the activity burst relative to the onset of subambient pressure at the start of the cycle (LOONS, HAONS, LPONS, AMONS, PHONS, PPONS; see Fig. 2). In addition, we calculated the intensity of activity in each muscle burst by dividing the integrated area by the duration of activity (LOINT, HAIN, LPINT, AMINT, PHINT, PPINT). Four measurements were made from the buccal-pressure curve of each cycle: minimum and maximum pressure (MINPRES, MAXPRES) and the areas under the subambient and superambient pressure curves (NEGAREA, POSAREA).

Statistical analyses

We analyzed the subambient pressure curve and EMG data from expansive-phase muscles separately from those for the

Table 1. Descriptive statistics for EMG and buccal-pressure variables measured during inflation in the striped burrfish *Chilomycterus schoepfi*

Variable	Mean	S.E.M.	Minimum	Maximum
Buccal expansion phase ($N=121$ for all variables)				
MINPRES	-2.4	0.14	-8.1	-0.3
NEGAREA	-205.3	8.11	-513.5	-4.2
LODUR	144.1	19.51	0	2 335.8
LOONS	-40.1	4.67	-306.6	72.2
LOAREA	52.4	7.45	0	420.2
LOINT	0.73	0.03	0	1.8
HADUR	205.4	8.45	0	875.8
HAONS	-56.5	4.35	-465.3	40.2
HAAREA	40.8	6.48	0	306.2
HAIN	0.2	0.03	0	0.7
LPDUR	74.3	7.12	0	1 277.5
LPONS	-49.2	5.42	-204.8	150.1
LPAREA	31.6	1.21	0	51.9
LPINT	0.1	0.01	0	0.2
Buccal expansion phase ($N=151$ for all variables)				
MAXPRES	8.4	0.66	0.6	34.0
POSAREA	2 994.8	443.51	42.1	35 719.4
AMDUR	292.4	30.67	0	2 305.4
AMONS	118.7	5.96	-22.6	486.1
AMAREA	73.1	13.35	0	1 502.4
AMINT	0.2	0.03	0	2.0
PHDUR	268.7	24.17	0	2 223.3
PHONS	204.1	10.24	13.5	798.3
PHAREA	77.8	9.63	0	10.1
PHINT	0.4	0.03	0	2.1
PPDUR	249.9	26.00	0	2 254.8
PPONS	196.6	10.98	32.9	796.6
PPAREA	78.3	7.71	0	708.5
PPINT	0.4	0.03	0	1.6

Units of measurement: onset (ONS) and duration (DUR) are in milliseconds (ms); intensity (INT) is in millivolts (mV); integrated area of EMG (INT) is in (ms mV); minimum and maximum pressure (PRES) are in kilopascals (kPa); area under pressure curve (AREA) is in (ms kPa).

See Materials and methods for abbreviations.

compressive-phase muscles and their associated superambient pressure curve. To explore patterns of association among variables, we initially constructed Pearson correlation matrices for the two data sets using data from each individual fish separately.

Our primary analytical approach was to treat the four buccal-pressure variables as dependent variables in multiple-regression models. For each of the four pressure variables, we constructed a model with EMG variables selected following our previous functional analysis of the inflation mechanism in this species (Wainwright *et al.* 1995). For example, with maximum buccal pressure as the dependent variable, our independent variables were AMONS, AMDUR, AMINT, PHONS, PHDUR, PHINT, PPONS, PPDUR and PPINT. We chose to rely on our previous understanding of the mechanism

of the buccal pump and examined only the four models that were determined *a priori* rather than searching more broadly for the combinations of independent variables that yielded the highest explanatory power in each data set.

The data from each individual fish were analyzed separately. This approach allowed us to assess directly the repeatability of the success of each model in accounting for pressure variation. Because we experienced difficulty in obtaining adequate recordings from all six muscles simultaneously from each fish, we were able to analyze both the expansive and compressive phase from only one fish (individual 4). Thus, expansive-phase data (subambient pressure and EMG variables from the LO, HA and LP muscles) are analyzed from individuals 1–4, and compressive phase data (superambient pressure and EMG variables from the AM, PH and PP muscles) are analyzed from individuals 4–8. All statistical calculations were made on \log_{10} -transformed data using Systat for Windows version 5 (Wilkinson, 1992). $P < 0.05$ was taken as the fiducial limit of significance.

Results

Inflation behavior was typified by sequences of from three to about 25 cycles of buccal expansion and compression (Fig. 2, and see Wainwright *et al.* 1995). For each individual, we combined data from three separate inflation sequences. The following descriptions are based on mean values from the overall data set. The expansion phase was generally initiated by activity of the HA, followed within 15 ms by onset of activity of the LO and LP (Fig. 2; Table 1). The onset of subambient pressure occurred about 57 ms after the onset of HA activity and reached an average value of 2.4 kPa below ambient 129 ms after the onset of subambient pressure. The compressive phase usually began with activation of the AM

119 ms after the onset of subambient pressure. The PH and PP initiated activity at 204 and 197 ms, respectively, so at least one compressive-phase muscle usually began activity before the time of minimum pressure (Table 1). Peak buccal pressure occurred 314 ms after the onset of subambient pressure. Peak positive pressure typically rose precipitously in the final two or three cycles of a complete sequence.

Values of pressure and the degree of muscle activation varied markedly among cycles (Table 1; Figs 2, 3). All six muscles ranged in activity duration from a minimum of 0 ms to a maximum of over 1 s (Table 1). Minimum and maximum pressure each ranged across an order of magnitude, minimum pressure from 0.3 kPa below ambient to 8.1 kPa below ambient and maximum pressure from 0.6 kPa to 34 kPa.

Bivariate correlations between minimum pressure and the muscle-activity variables were rarely significant (Table 2; Fig. 3). For example, for individual 4, only two out of 24 correlations between muscle activity and minimum pressure (MINPRES) or the area under the subambient pressure curve (NEGAREA) were significant. The significant correlations were between minimum pressure and LO integrated area (LOAREA) and intensity (LOINT) (Table 2). Both of these correlations were negative, indicating that greater electrical activity in the LO, the muscle primarily responsible for opening the mouth, was associated with lower buccal pressures. Muscle activity variables rarely correlated with each other. Individual 4 showed significant pairwise correlations in only six out of 66 instances (Table 2).

Correlations between the two subambient pressure variables (minimum pressure and area under the subambient pressure curve) and EMG variables varied slightly among individuals. The number of significant bivariate correlations between minimum pressure or area under the subambient pressure curve

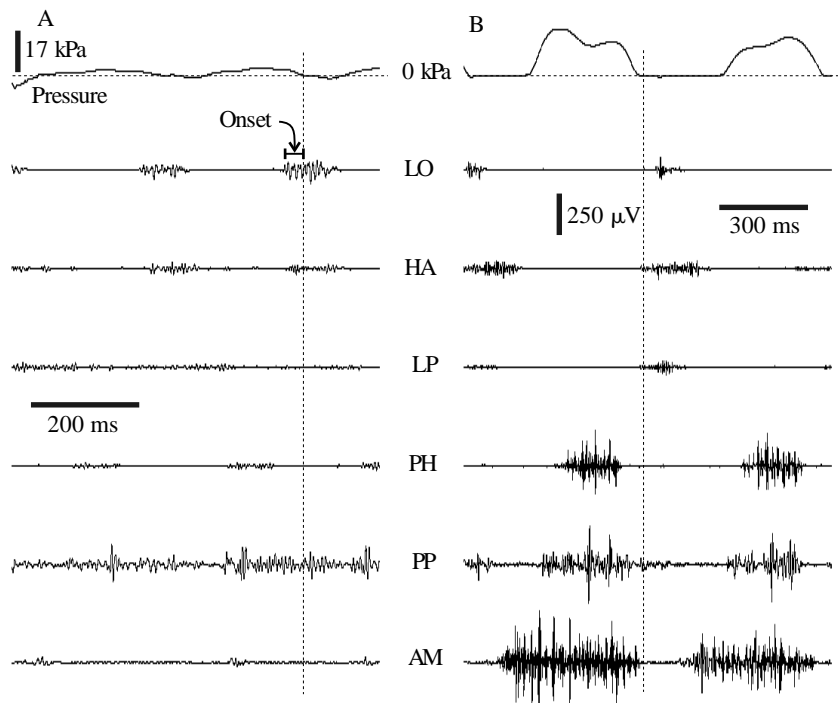


Fig. 2. Representative recordings of buccal pressure (upper trace) and electromyographic activity in six head muscles during inflation behavior in *Chilomycterus schoepfi*. A and B are recordings from the same individual (number 4) obtained a few minutes apart to illustrate the range of magnitude in buccal pressure and EMG activity that was typically seen in this study. The vertical dashed lines indicate the onset of subambient pressure at the start of an inflation cycle. This time was used as the reference for determination of relative onset of muscle activation (see A). Note that the vertical scale for the EMG and pressure records are the same in A and B. Muscle abbreviations as in Fig. 1.

and the EMG variables varied from zero out of 24 (individual 2) to two out of 24 (individuals 3 and 4). The mean number of significant correlations was 1.2 out of 24. Of the total of five significant correlations among the four fish, four involved the duration or integrated area of the LO.

Pairwise correlations between EMG variables and superambient pressure were significant more frequently than in the expansive phase data (Table 3; Fig. 3). Data from individual 4 showed significant correlations in 12 out of 24 cases in which muscle activity was being correlated with the two pressure variables (Table 3). For all three compressive-phase muscles, some variables were significantly correlated with pressure, although AM showed the strongest correlations in every individual. The burst durations of all three muscles had higher correlations with the two pressure variables than did the rectified integrated area of activity, which, in turn, had a higher correlation than did the intensity variable from the same muscle. Thus, the duration of muscle activity was more indicative of the magnitude of buccal pressure than was the amplitude of electrical activity in the burst.

Across the five individuals, the mean number of significant pressure–EMG correlations was 12.4. Among muscle-activity variables, 16 out of 66 correlations were significant for individual 4; the mean was 17.6 out of 66 possible correlations per individual.

When minimum buccal pressure was used as the dependent

variable, the multiple-regression model was significant for all four individuals (Table 4). The proportion of variation in minimum pressure explained by the model (r^2) varied from 63% to a maximum of 84% among the four individuals (mean=73%). The multiple-regression model explained slightly less of the variance in the area under the subambient pressure curve (Table 4). The model was significant for all four individuals, and the proportion of variance explained varied from 51 to 78%, with a mean of 62% (Table 4).

The proportion of variation in peak pressure explained by the regression model ranged from 76 to 95% among the five individuals (mean=88%) (Table 5). The model was highly significant for each individual. Even greater success was achieved in predicting the area under the superambient region of the pressure curve (Table 5). The r^2 values ranged from 91 to 97%, with a mean of 95%. In other words, nearly all of the variation in the area under the pressure curve was accounted for by a model composed of the variables that characterized activity of the three compressive-phase muscles.

Two analyses of variance (ANOVAs) were calculated to compare the r^2 values obtained from the multiple regressions for the various pressure variables. A one-way ANOVA contrasting the r^2 values for the regressions on minimum pressure and peak pressure revealed significantly higher values for the latter variable ($F=7.3$; d.f.=1, 7; $P=0.03$). A second ANOVA comparing the r^2 values from the regressions predicting the

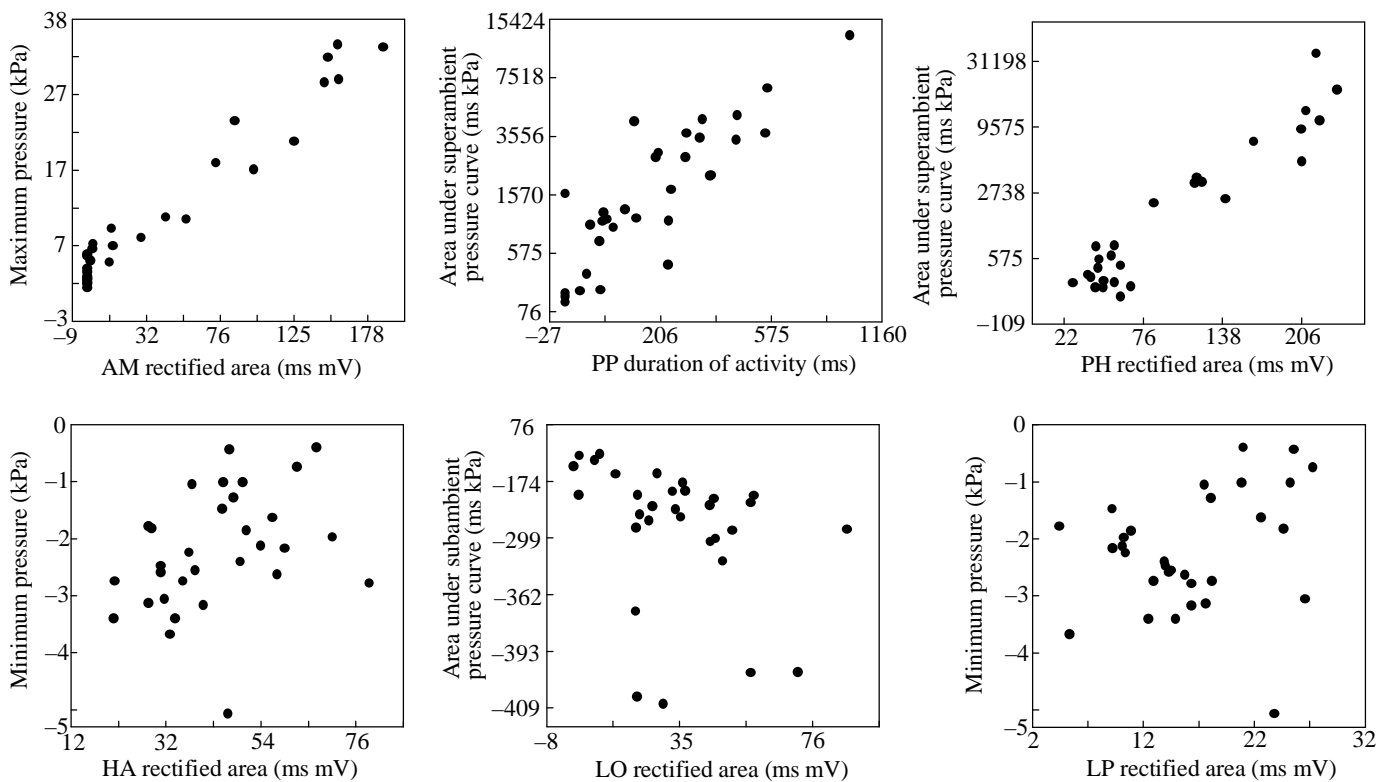


Fig. 3. Representative scatterplots of various buccal-pressure and EMG-activity variables from *Chilomycterus schoepfi* individual 4. Note the stronger relationships between EMG variables and superambient pressure from the compressive phase (three upper plots). Muscle abbreviations are as in Fig. 1.

Table 2. Correlations among variables measured during the expansive phase of the inflation cycle in one burrfish (individual 4) *Chilomycterus schoepfi*

	MINPRES	NEGAREA	LOONS	LODUR	LOAREA	LOINT	HAONS	HADUR	HAAREA	HAINT	LPONS	LPDUR	LPAREA	LPINT
MINPRES	-	-	-	-	<0.001	<0.001	-	-	-	-	-	-	-	-
NEGAREA	0.53	-	-	-	-	-	-	-	-	-	-	-	-	-
LOONS	-0.28	-0.52	-	-	-	-	-	-	-	-	-	-	-	-
LODUR	-0.41	-0.16	-0.20	-	-	-	-	-	-	-	-	-	-	-
LOAREA	-0.72	-0.34	0.08	0.57	-	<0.001	-	-	-	-	-	-	-	-
LOINT	-0.74	-0.36	0.23	0.32	0.91	0.47	-	-	-	-	-	-	-	-
HAONS	-0.54	-0.55	0.53	0.14	0.47	0.47	-0.43	-	-	-	<0.001	-	-	-
HADUR	0.52	-0.01	-0.15	-0.28	-0.36	-0.46	-0.19	0.75	<0.001	0.007	-	-	-	-
HAAREA	0.36	-0.27	0.04	-0.27	-0.31	-0.35	-0.19	0.75	0.65	0.007	-	-	-	-
HAINT	-0.03	-0.39	0.25	-0.10	-0.10	-0.05	0.16	-0.01	0.65	0.007	-	-	-	-
LPONS	-0.50	-0.51	0.43	0.19	0.41	0.40	0.74	-0.39	-0.13	0.21	-	-	-	-
LPDUR	-0.01	0.01	-0.18	-0.18	0.04	-0.02	-0.14	0.43	0.34	-0.01	-0.44	-	<0.001	-
LPAREA	0.25	0.27	-0.09	-0.32	-0.17	-0.19	-0.13	0.37	0.14	-0.21	-0.44	0.77	<0.001	-
LPINT	0.32	0.34	0.06	-0.26	-0.21	-0.18	-0.04	0.08	-0.11	-0.24	-0.18	0.17	0.75	<0.001

Values above the diagonal are Bonferroni-corrected probabilities.
N, sample size = 31; -, not significant.
 Abbreviations are defined in Materials and methods.

Table 3. Correlations among EMG and buccal-pressure variables measured during the compressive phase of the inflation cycle in one burrfish (individual 4) *Chilomycterus schoepfi*

	MAXPRES	POSAREA	AMONS	AMDUR	AMAREA	AMINT	PHONS	PHDUR	PHAREA	PHINT	PPONS	PPDUR	PPAREA	PPINT
MAXPRES	-	-	-	-	-	-	-	-	-	-	-	-	-	-
POSAREA	0.97	-	-	<0.001	<0.001	<0.001	-	<0.001	0.007	-	0.031	<0.001	-	-
AMONS	-0.16	-0.13	-	<0.001	<0.001	-	-	-	-	-	-	-	-	-
AMDUR	0.92	0.98	-0.16	-	<0.001	0.004	-	<0.001	0.002	-	0.028	<0.001	-	-
AMAREA	0.88	0.91	-0.09	0.88	<0.001	<0.001	-	0.029	-	-	0.016	0.002	-	-
AMINT	0.71	0.71	0.04	0.67	0.87	0.39	-	-	-	-	<0.001	-	-	-
PHONS	0.49	0.50	0.50	0.45	0.53	0.32	0.07	-	-	-	-	-	-	-
PHDUR	0.70	0.78	-0.32	0.82	0.60	0.32	0.10	0.79	<0.001	-	-	<0.001	-	-
PHAREA	0.58	0.65	-0.31	0.68	0.39	0.04	0.10	0.79	<0.001	-	-	0.019	0.045	-
PHINT	-0.18	-0.22	-0.04	-0.25	-0.33	-0.52	0.04	-0.26	0.33	-	-	-	-	0.003
PPONS	0.56	0.60	0.28	0.61	0.62	0.43	0.73	0.41	0.35	-0.03	0.33	-	-	-
PPDUR	0.72	0.80	-0.08	0.80	0.68	0.46	0.31	0.82	0.62	-0.31	0.25	0.49	-	-
PPAREA	0.43	0.47	0.00	0.41	0.44	0.25	0.15	0.45	0.59	0.29	0.25	0.49	-	-
PPINT	-0.34	-0.38	0.05	-0.42	-0.33	-0.37	-0.18	-0.34	0.02	0.67	-0.16	-0.49	0.46	-

Values above the diagonal are Bonferroni-corrected probabilities.
N, sample size = 31; -, not significant.
 Abbreviations are defined in Materials and methods.

rectified area under the subambient and superambient parts of the pressure curve revealed significantly higher values with the latter variable ($F=34.2$; d.f.=1, 7; $P=0.001$).

The multiple regression results for individual 4 were representative of the regression results. For the regression using minimum buccal pressure as the dependent variable, the equation (using standardized coefficients) was $\text{MINPRES} = -0.19(\text{LOONS}) - 0.19(\text{LODUR}) - 0.34(\text{LOINT}) + 0.04(\text{HAONS}) + 0.33(\text{HADUR}) + 0.09(\text{HAINT}) - 0.29(\text{LPONS}) - 0.39(\text{LPDUR}) + 0.23(\text{LPINT})$. LOINT, HADUR and LPDUR were the only variables with significant coefficients ($P < 0.05$). For the regression on area under the subambient portion of the pressure curve, the equation was: $\text{NEGAREA} = -0.34(\text{LOONS}) - 0.18(\text{LODUR}) - 0.17(\text{LOINT}) - 0.31(\text{HAONS}) - 0.36(\text{HADUR}) - 0.21(\text{HAINT}) - 0.12(\text{LPONS}) - 0.07(\text{LPDUR}) + 0.24(\text{LPINT})$. Both LOONS and HAONS had significant coefficients ($P < 0.05$). For the regression of maximum pressure, the equation was: $\text{MAXPRES} = -0.07(\text{AMONS}) + 0.34(\text{AMDUR}) + 0.48(\text{AMINT}) + 0.32(\text{PHONS}) + 0.46(\text{PHDUR}) + 0.27(\text{PHINT}) - 0.22(\text{PPONS}) - 0.15(\text{PPDUR}) - 0.09(\text{PPINT})$. AMINT, PHONS and PPONS had significant coefficients. For the regression of area under the superambient portion of the pressure curve, the equation was: $\text{POSAREA} = -0.02(\text{AMONS}) + 0.51(\text{AMDUR}) + 0.32(\text{AMINT}) + 0.21(\text{PHONS}) + 0.33(\text{PHDUR}) + 0.17(\text{PHINT}) - 0.12(\text{PPONS}) - 0.02(\text{PPDUR}) - 0.04(\text{PPINT})$. AMDUR, AMINT, PHONS, PHDUR and PHINT all had significant coefficients.

Discussion

Overall, buccal-pressure variation during inflation behavior was well characterized by the standard measures of muscle activity used in this study. Averaged across the different fish, the regression models explained 73 % of the variation in minimum pressure, 62 % of the area under the subambient pressure curve, 88 % of peak pressure and 95 % of the area under the superambient pressure curve. In every case, the multiple-regression model was highly significant (Tables 4, 5).

Table 4. Multiple-regression results using minimum buccal pressure and the area under the subambient portion of the pressure curve as the dependent variables

Individual (N)	MINPRES		AREANEG	
	Multiple r^2 (%)	P	Multiple r^2 (%)	P
1 (30)	84.2	<0.001	77.7	<0.001
2 (30)	62.9	<0.001	51.2	<0.005
3 (30)	69.7	<0.001	51.7	<0.005
4 (31)	75.6	<0.001	65.8	<0.005

Independent variables in the model for each fish were: LOONS, LODUR, LOINT, HAONS, HADUR, HAINT, LPONS, LPDUR, and LPINT.

See Materials and methods for abbreviations.

MINPRES, minimum buccal pressure; AREANEG, the subambient portion of the pressure curve.

Thus, in general, the variation in buccal pressure among cycles of inflation behavior can be accounted for by the degree of activation of the six head muscles.

Perhaps the most interesting results of the study were those in which the analyses of subambient and superambient pressure differed. Two results in particular suggest differences between the muscular bases of the subambient and superambient sections of the pressure curve during inflation behavior in the burrfish. First, the multiple-regression models were more successful in predicting superambient pressure variables than in predicting subambient pressure variables. Second, numerous bivariate correlations among EMG variables were significant and repeated in different individuals (an average of 17.6) for the superambient pressure data, whereas fewer bivariate correlations were found between EMG variables in the subambient-pressure data (an average of 4.4). Below, we discuss these differences between the expansion- and compression-phase analyses and their implications for understanding the mechanisms that underlie the generation of pressure fluctuations during inflation behavior.

EMG activity and pressure variation

The regression models were moderately successful in accounting for minimum pressure and the area under the subambient pressure curve. In contrast, almost all variation in the compressive-phase pressure variables was accounted for by the models. Indeed, the weakest compressive-phase model, peak buccal pressure for individual 6, performed about as well as the second-best expansive-phase model, area under the subambient curve for individual 1 (Tables 4, 5). An analysis of variance on the r^2 values grouped into subambient and superambient pressure variables indicated that the percentage of variance explained by each model was significantly greater in the superambient models ($F=29.8$; d.f.=1, 16; $P < 0.0001$).

At least two major factors could contribute to the difference in success between the subambient and superambient pressure

Table 5. Multiple-regression results using maximum buccal pressure and the area under the superambient portion of the pressure curve as the dependent variable

Individual (N)	MAXPRES		AREAPOS	
	Multiple r^2 (%)	P	Multiple r^2 (%)	P
4 (31)	87.2	<0.001	94.8	<0.001
5 (30)	95.4	<0.001	97.2	<0.001
6 (32)	75.9	<0.001	91.3	<0.001
7 (28)	90.5	<0.001	96.6	<0.001
8 (30)	89.1	<0.001	94.5	<0.001

Independent variables in the model for each fish were: AMONS, AMDUR, AMINT, PHONS, PHDUR, PHINT, PPONS, PPDUR, PPINT.

See Materials and methods for abbreviations.

MAXPRES, maximum buccal pressure; AREAPOS, the superambient portion of the pressure curve.

regressions. (1) The mechanisms that generate subambient and superambient pressures are different, and EMG activity may be more directly related to the latter than to the former. (2) The range of subambient pressures was not as great as the range of superambient pressures, and this reduced range in the dependent variables may have mathematically constrained the regressions. We feel that these two factors are both useful in understanding the observed differences. A third factor that should be mentioned is our exclusion of other muscles active during the expansive phase. Is it possible that we omitted muscles that contribute significantly to the generation of subambient pressure? Although there are other cranial muscles active during the expansive phase, we do not believe that they play prominent functional roles in inflation and therefore reject this as a major contributor to the poorer performance of the expansive-phase muscles in accounting for subambient pressure. Our previous work with this species indicated that two other cranial muscles function during the expansive phase, the dilatator operculi and the sternohyoideus, but neither appears to function prominently in the buccal-expansion mechanism. The dilatator operculi abduct the operculum during mandibular depression (Wainwright *et al.* 1995). In this species, the sternohyoideus is an extremely small muscle between the cleithrum and the ceratohyals that appears to function primarily as a connective-tissue linkage between the highly mobile pectoral girdle and hyoid apparatus (Winterbottom, 1974; Wainwright *et al.* 1995).

Compression of the buccal cavity is effected by protraction of the pectoral girdle, protraction of the hyoid apparatus and adduction of the jaws (Wainwright *et al.* 1995). In this study, we selected the three muscles primarily responsible for these actions, the PP, the PH and the AM, respectively. During the compressive phase, water is prevented from escaping anteriorly through the mouth by a stout, muscular oral valve that lies immediately posterior to the tooth plate of the mandible. As the buccal cavity is compressed, water is forced through the esophagus and into the stomach. Body inflation occurs as the animal repeatedly pumps water into its stomach. Thus, in this behavior, the movable parts of the skull act as components of a pump, and the magnitude of buccal pressure is a direct measure of the forces being applied by the compression muscles. Given the well-established relationship between EMG activity and muscular tension (reviewed by Basmajian and De Luca, 1985), it is not surprising that the activities of the three compressive-phase muscles studied all showed strong, significant correlations with superambient pressure and that the multiple regressions performed well (Tables 3–5).

In contrast to superambient pressure, subambient pressure may not be a simple function of muscular tension. The buccal-expansion phase is analogous to suction-feeding behavior. As the buccal cavity is expanded, subambient pressure is generated, and the mouth is opened, permitting water to enter and eliminating the pressure gradient between the inside of the buccal cavity and the ambient water. Buccal expansion is accomplished by retraction of the pectoral girdle, retraction and depression of the hyoid apparatus, and opening of the mouth (Wainwright *et al.* 1995). In this system, as with the

suction-feeding mechanism of teleost fishes, the magnitude of the subambient pressure pulse is believed not to be simply a function of the forcefulness of the expansive actions but to be influenced by several factors.

The primary determinants of the magnitude of subambient pressure are the rate of buccal expansion and the size of the aperture through which water enters the buccal cavity (Alexander, 1967; Muller *et al.* 1982; Muller, 1989). The rate of buccal expansion is a function of the rate of shortening of the expansion muscles and can be expected to be positively correlated with measures of EMG activity (Lawrence and De Luca, 1983), but the size of the oral aperture will be determined by the activity of the muscle that abducts the mandible, the LO. In *Chilomycterus schoepfi*, unlike the generalized condition of bony fishes, mandibular depression is not mechanically linked to cranial elevation or hyoid retraction. Only the LO functions in depressing the mandible. Important features of its activity might be the rectified area, indicating the force and rate of contraction, and the time of onset of the EMG burst, indicating the time of opening relative to buccal expansion. Interestingly, the LO rectified area and intensity of activity were significantly correlated with subambient pressure in three of the four individuals, whereas no LP variables and one HA variable (HADUR for individual 3) were correlated with subambient pressure. These results imply either that the rate of buccal expansion is not indicated by the EMG variables measured or that the burrfish does not alter its rate of buccal expansion to control subambient buccal pressure. Furthermore, of the factors investigated, modulation of the oral aperture appears to be the most important determinant of variation in subambient pressure.

It is noteworthy that published accounts of the relationship between subambient pressure variation and cranial muscle EMG activity from suction-feeding teleost fishes report r^2 values similar to those reported here for the subambient pressure phase of inflation in the burrfish (Lauder *et al.* 1986). It may be generally true that one cannot explain more than about 80% of variation in the magnitude of subambient pressure using EMG activity during suction feeding and analogous behaviors.

A final distinction between the analyses of buccal expansion and compression was the different ranges in the pressure variables. Minimum and maximum pressure varied across one order of magnitude, area under the subambient pressure curve across two orders of magnitude and area under the superambient pressure curve varied across three orders of magnitude (Table 1). Thus, for analogous variables, there was an order of magnitude difference in the range of the dependent variables in the regressions. Given the constant or lower variance in the expansion-phase muscle variables (Table 1), it can be anticipated that this difference in the range of the dependent variables had an impact in the direction of higher r^2 values in the compressive-phase regressions (Sokal and Rohlf, 1981). This effect should not be ignored in considering the difference in r^2 values between the expansive and compressive phases and appears to explain roughly half of the difference between the two results. When we restricted the regression analyses of

compression-phase data to cycles in which peak pressure did not exceed 12 kPa (thus, we excluded the upper two-thirds of the range of pressure values), we found the nine modified multiple regressions reduced the r^2 values by an average of 7%. The original mean r^2 values for predicting minimum and maximum pressure, respectively, were 73% and 88%. Thus, the mathematical consequences of the much greater range of superambient pressure values accounts for about half of the 15% difference between these means. The ANOVA comparing the r^2 values for minimum and maximum buccal pressure using the modified data was no longer significant ($F=1.74$, d.f.=1, 6, $P=0.23$). A similar reduction in area under the superambient pressure curve to values less than 600 ms kPa reduced the multiple regression r^2 values by an average of 8.2%. The ANOVA comparing r^2 values from the area variable regressions on sub- and superambient pressure was still significant ($F=9.1$, d.f.=1, 6, $P=0.02$). Overall, this analysis of restricted data sets indicates a general effect of the difference in range of the dependent variable between the expansive- and compressive-phase data. However, even taking this effect into account, there is still a better relationship between the EMG and superambient pressure data than between the EMG and subambient pressure data.

Patterns of correlation among EMG variables

The patterns of correlation among muscle-activity variables differed between the expansive- and compressive-phase data (e.g. Tables 2, 3). An average of 17.6 out of the 66 bivariate correlations between EMG variables were significant in the data set from each of the five individuals. This result contrasts with an average of 4.4 significant correlations out of 66 in the subambient pressure data. As with the statistical relationships between muscle activity and pressure, these differences in the correlations among EMG variables may be due to real distinctions in the associations between muscle activity or they could be an artifact of the difference in the range of EMG variables.

In this case, however, only weak evidence supports the contention that a greater range of values exists in the compressive-phase data. The six muscles do not differ substantially in their ranges of activity variables (Table 1). Furthermore, when the compressive-phase data for individual 4 are limited to cycles with maximum pressure less than 12 kPa (resulting in $N=22$), only four of the 17 previously significant correlations drop below the $P<0.05$ level. Similar results were found for each of the other four individuals. In each individual, three or four fewer of the average of 17.6 previously significant pairwise correlations were lost. The presence of fewer correlations in the expansive-phase data seems to represent a real difference between the two actions. Muscle activity was more integrated during the compressive-phase set than it was during the expansive phase.

A re-evaluation of the role of the adductor mandibulae

Within the compressive-phase data, the correlations between pressure and the extent of activity of the adductor mandibulae

were routinely higher than those for either of the other two muscles (e.g. Table 3). In all five individuals, the correlations between the superambient pressure variables and AMDUR and AMAREA were greater than 0.85. Only rarely were correlations with other EMG activity variables this high. This result suggests that the AM, the primary adductor of the lower jaw, plays a key role in generating superambient buccal pressure. The strong correlations are probably not caused by mandibular adduction contributing directly to buccal compression, because jaw adduction has a negligible impact on buccal volume in the burrfish (Wainwright *et al.* 1995). The principal movements that compress the buccal cavity are protraction of the hyoid apparatus and the pectoral girdle, which are linked by the sternohyoideus muscle and connections between the first branchiostegal and the pectoral girdle (Fig. 1; Wainwright *et al.* 1995). Furthermore, lower-jaw adduction does not appear to be an important factor in sealing the buccal cavity, a function carried out by the oral valve. Indeed, even when the jaws are fully adducted, they do not form a seal because of the uneven surfaces of the crushing plates and teeth.

The AM appears to function during buccal compression by contributing to hyoid protraction through its ventral connections to the hyoid apparatus *via* the PH (Fig. 1). The PH connects the anterior region of the ceratohyals to the posterior-ventral-most region of the mandible. As the mandible rotates during adduction by the AM, it can contribute considerably to an anterior translation of the attachment of the PH on the mandible (Fig. 1). This previously unappreciated role of the AM may account for the high correlations between the duration and integrated area of activity of this muscle and superambient pressure.

Finally, we note that a consistent pattern emerged in our compressive-phase analyses in which the activity duration variables showed higher correlations with the pressure variables than did the integrated area variables, which in turn always showed higher correlations than did the intensity variable (Table 3). This pattern was found for all five compressive-phase individuals except individual 3, in which the duration and integrated-area variables were approximately equally correlated with pressure. Because we calculated the intensity variable by dividing rectified area by burst duration, this pattern implies that duration was the component of integrated area that drove the strength of the correlations with pressure. This result is in contrast to numerous published accounts indicating that EMG amplitude is closely related to muscular tension (Bigland and Lippold, 1954; Millner-Brown and Stein, 1975; Moritani and DeVries, 1978; Lawrence and De Luca, 1983). One potentially important difference between the present study and these previous results is that burrfish inflation is associated with discrete bursts of activity that averaged approximately 0.2 s in duration, whereas the previous studies focused on behaviors that involved much longer activity periods, of the order of several seconds. The more transient nature of muscle activity and buccal pressure during inflation behavior may underlie this difference in relative significance of the intensity of muscle activity. At least in the case of burrfish inflation, the pattern suggests that the duration of the

period of muscle activation is the principal determinant of muscle tension and the strength of buccal compression.

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