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Muscle function and power output during suction feeding in largemouth bass, *Micropterus salmoides*

Andrew M. Carroll [a,*], Peter C. Wainwright [b]

[a] *Concord Field Station, Harvard University, Old Causeway Rd., Bedford, MA 01730, USA*
[b] *Section of Evolution and Ecology, University of California, One Shields Ave., Davis, CA 95616, USA*

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Abstract

Muscle power output is thought to limit suction feeding performance, yet muscle power output during suction feeding has never been directly measured. In this study, epaxial activation and strain, hyoid depression, and intra-oral pressure were simultaneously measured during suction feeding in the largemouth bass (*Micropterus salmoides*). A mechanical model of muscle force transmission between the neurocranium and oral cavity was used to estimate muscle stress, work, and power. The epaxials shortened from rest an average of 9% of their length, with the highest efforts producing greater than 20% strain. Onset of shortening was simultaneous with or shortly after (<10 ms) onset of activation. Maximal net power for individual fish ranged from 17 to 137 W kg^{-1}. Muscle power was significantly correlated with rectified EMG area ($r=0.80$; $p<0.0001$). The power required for cranial expansion was significantly correlated with epaxial power ($r=0.81$; $p<0.0001$), and the power exponent of this relationship (~1 for 3 of the 4 fish) implies that epaxial power accounts for most of the power of cranial expansion. The limitations imposed by the kinematic requirements and loading environment of suction feeding (short delay between activation and strain, maximal stress occurring after shortening, operation at lengths shorter than resting length) may prevent maximal muscular power production.

Keywords: Suction feeding; Performance; Inverse dynamics; Muscle power; Muscle force; EMG; Muscle work; Muscle strain

1. Introduction

In aquatic suction feeding a predator rapidly expands its cranial skeleton to draw a volume of water containing a prey item into its mouth (Ferry-Graham and Lauder, 2001). In a successful feeding event this bolus of water must be large enough to entrain the prey (Werner, 1974) and move quickly enough to prevent the prey from escaping (Day et al., 2005). For a given fish, the volume and velocity of water moved, and thus the size and mobility of prey that can be taken, are ultimately limited by the power generated by suction feeding muscles (de Jong et al., 1987; Van Wassenbergh et al., 2005). It follows that a quantitative understanding of muscular power production is essential to understanding the musculoskeletal basis of suction feeding performance and addressing basic questions about its biomechanics. For instance, how does the muscular power generated during suction feeding compare to that of other power-limited behaviors, and how is muscular power generation, and thus feeding performance, affected by variation in morphology?

A quantitative understanding of muscle function during suction feeding may also shed light on our understanding of vertebrate muscle function in general. Muscle power production is critical to performance in many behaviors (Askew et al., 2001; Peplowski and Marsh, 1997; Roberts and Scales, 2002), yet mass-specific power production varies widely among these behaviors (Askew and Marsh, 2002). Many hypotheses about the basis of variation in power production remain largely untested in a comparative context. Suction feeding is performed by most members of the diverse Teleostei and the activation and kinematic patterns underlying the behavior are notably conserved (Gibb and Ferry-Graham, 2005; Lauder, 1980a). Therefore, suction feeding may be a good system in which to examine how variation in morphology and physiology results in variation in muscular performance, and how variation in

* Corresponding author. Tel.: +1 781 275 1725; fax: +1 781 275 9613.
E-mail address: acarroll@oeb.harvard.edu (A.M. Carroll).

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muscular performance results in variation in behavioral performance. Measuring muscle power production is a necessary step towards this more general research goal.

The amount of power muscles produce is determined by how they function, i.e., the time course of activation, strain, and force during a behavior (Biewener, 2002). Muscular function results, in part, from the loading regime and kinematic movement that characterize the behavior (Josephson, 1999; Lutz and Rome, 1994). The regime required by suction feeding is unlike that of most previously studied locomotor behaviors. Most locomotor behaviors involve cyclical movement allowing muscles to be stretched prior to shortening (Biewener et al., 1998; Franklin and Johnston, 1997), and involve pre-existing gravitational loads and inertial loads that peak at or before the onset of shortening (Biewener, 2002; Marsh, 1999). Suction feeding, on the other hand, begins from rest without countermovement, and loading is dominated by sub-ambient pressure inside the expanding oral cavity (Carroll et al., 2004; Van Wassenbergh et al., 2005). These forces are distinct from drag or added-mass forces that dominate other behaviors (Marsh, 1999), and result from the flow of water caused by cranial expansion, and thus develop only after onset of kinematic movement and muscle shortening (Sanford and Wainwright, 2002; Muller et al., 1982).

These unique kinematic requirements and loading environment mean that published estimates of muscular power production during other behaviors (Askew and Marsh, 2002) or from isolated teleost fascicles (Askew and Marsh, 1998; Wakeling and Johnston, 1998) may not resemble that produced during suction feeding. In particular, many aspects of muscle function known to enhance power production (such as stretching prior to shortening (Franklin and Johnston, 1997), a delay between activation and shortening (Lutz and Rome, 1994), operation over resting length (Askew and Marsh, 2002)) may not be characteristic of suction feeding. Therefore, levels of power production during suction feeding may be lower than those produced in other behaviors.

Direct measurements of muscular power required for suction feeding are not available. de Jong et al. (1987) produced a hypothetical estimate of total power expenditure based on hydrodynamic theory. Van Wassenbergh et al. (2005) produced an estimate of mass-specific muscular power production for the African catfish (*Clarus gariepinus*) based on detailed kinematics and hydrodynamic modeling. However, the estimate produced in the latter study represented a relative value, because it did not take into account the mass of all suction feeding muscle. Furthermore, in the latter study fish were not fed elusive prey. Suction feeding kinematics and performance (sub-ambient pressure generation) are known to vary widely with predator motivation and prey elusiveness (Lauder, 1980b; Nemeth, 1997a; Nemeth, 1997b; Sass and Motta, 2002), so it is possible that absolute power was underestimated.

In this study, we simultaneously measured cranial expansion, intra-oral pressure, and muscle activation and strain in the epaxial muscle mass of largemouth bass (*Micropterus salmoides*) feeding on elusive prey (live, free-swimming goldfish, *Carassius auratus*). Measured parameters were used to estimate muscular stress, work, and power; as well as the total work and power cost of cranial expansion. These data were used to address two central questions: how much power do suction feeding muscles produce relative to muscles involved in other behaviors and relative to the total power cost of feeding, and how do the kinematic requirements and loading environment of suction feeding affect muscles function and power production?

2. Materials and methods

2.1. Animals

Four largemouth bass *M. salmoides* (Lacapede) (26–29.5 cm SL) were collected from Putah creek (Yolo County, CA, USA). Fish were housed in aquaria at 22–24 °C on the campus of the University of California, Davis, and in accordance with U.C. Davis animal use and care protocols (#10211). Fish were maintained on live goldfish (*C. auratus*), earthworms (*Lumbricus* sp.), and cut squid (*Loligo* sp.) prior to surgery, with feeding discontinued 3 days prior to experimentation.

2.2. Surgery

Surgeries were performed at night allowing the fish 6–8 h of darkness in which to recover. Fish were anesthetized by exposure to 0.3 g L^{-1} of MS-222 (tricaine methane sulfonate) and moved to a surgical tray containing 0.1 g L^{-1} MS-222. Surgery lasted 35–45 min. Fish were returned to their home tanks and artificially ventilated with a pump until recovery. Data collection took place at dawn the next morning and every 4–6 h subsequently for 2–3 days. During each feeding, fish were given live goldfish (3–5 cm SL) in order to elicit a range of feeding effort. At the close of data collection, fish were euthanized in an overdose of MS-222 and fixed in a 10% formaldehyde solution for 2 weeks prior to dissection and measurement.

2.3. Estimation of muscle stress

While activation, strain, and kinematics may be easily measured through electromyography (EMG), sonomicrometry, and high-speed video, measuring muscle force in vivo is more difficult (Biewener, 2002). Methods that measure deformation of bone or tendons (Biewener and Baudinette, 1995; Biewener et al., 1998; Roberts et al., 1997) are highly invasive and especially difficult to apply to muscles with non-tendonous insertions, such as the epaxial muscle mass (Figs. 1 and 2). One non-invasive approach to estimating muscle loading is the analysis of inverse dynamics (Hatze, 2000), in which joint moments are back-calculated from externally measured skeletal loads.

One limitation with this technique is that if more than one muscle crosses a joint, joint forces cannot be resolved to stress at a single muscle (Tsirakos et al., 1997). Because the epaxial muscle group is the only muscle responsible for dorsal neurocranial rotation during suction feeding (Figs. 1 and 2), it should be possible to resolve moments acting across the joint between the neurocranium and pectoral girdle to this single

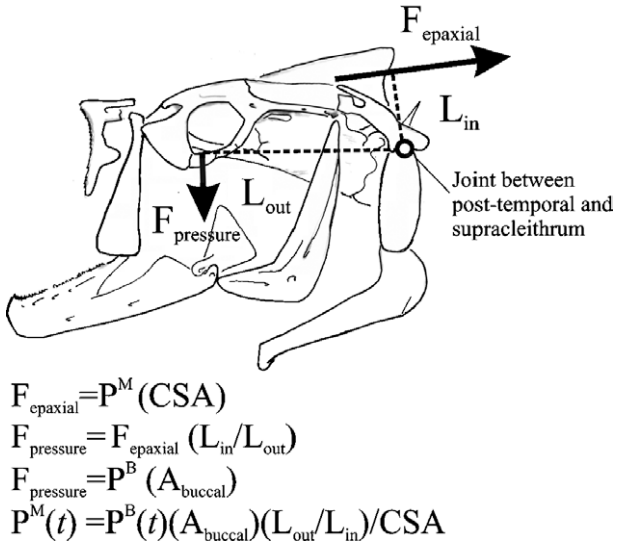

Fig. 1. Model used to calculate muscle stress. This model of force transmission between the buccal cavity and the epaxials was developed and validated in Carroll et al. (2004). Measured buccal pressure (P^B) as a function of time is used to estimate muscle stress (P^M) as a function of time. The force of the epaxial muscle ($F_{epaxial}$) is equal to the product of its stress (P^M) and its cross-sectional area (CSA). This force multiplied by its in-lever (the epaxial moment) over its out-lever (the buccal moment) is equal to the force of buccal pressure ($F_{pressure}$). The force of buccal pressure, in turn, is equal to measured buccal pressure multiplied by the area of a buccal cavity. Combining terms and measuring buccal pressure and buccal area as functions of time yields the relationship between buccal pressure and muscle stress.

muscle mass. Using a force transmission model developed in Carroll et al. (2004, Fig. 1), we estimated muscle loading based on measured intra-oral pressure, which is the dominant source of loading in suction feeding (Van Wassenbergh et al., 2005).

The model used to estimate muscle stress (Fig. 1) is described in detail in Carroll et al. (2004). Briefly, the force generated by subambient pressure in the buccal cavity is equal to the pressure magnitude (P^B) multiplied by the rectangular projected area of the buccal cavity.

$$F_{pressure} = P^B(A_{buccal}) \quad (1)$$

This force has ventral moment on the neurocranium equal to the average distance of the area from the joint (L_{out}). This torque generated by negative pressure is balanced by the force of the epaxial muscle mass ($F_{epaxial}$) acting through its moment arm (L_{in}) (Fig. 1).

$$F_{epaxial} = P^B(A_{buccal})(L_{out}/L_{in}) \quad (2)$$

The stress of a given epaxial fiber (P^M) is equal to their force divided by their physiological cross-sectional area (CSA).

$$P^M = F_{epaxial}/CSA \quad (3)$$

Therefore, as a function of time:

$$P^M(t) = P^B(t)*(A_{buccal})(L_{out}/L_{in})/CSA \quad (4)$$

Buccal pressure was measured directly; the measurement or estimation of buccal area, buccal moment, muscle moment, and muscle cross-sectional area are described below.

Note that, because the epaxials are the only muscle group capable of dorsal rotation of the neurocranium, other suction feeding muscles such as the sternohyoideus cannot effect this relationship and may be ignored when estimating epaxial stress.

2.4. Fascicle strain

The methods described here are similar to those in Carroll (2004). One mm sonometric crystals (Sonometrics Corp., London, ON, Canada) were used to measure fascicle strain in the epaxials. These crystals use sound to measure distances within the muscle tissue and are able to move freely with the fascicle to record in vivo strain (Biewener, 2002; Hoffer et al., 1989). The speed of sound in muscle tissue has been estimated at 1560 m s^{-1} (Mol and Breddels, 1982). During crystal implantation, the crest of the neurocranium was palpated beneath the middorsal skin of the fish, the first crystal was placed approximately 2 cm lateral to this crest, the second was placed 8 to 12 mm caudo-lateral to the first. Crystals were placed directly over the axis of the post-temporal supracleithral joint (Fig. 1) the axis about which the neurocranium rotates (Carroll et al., 2004) and were placed along fascicle lines but in separate myomeres (usually 3–4 myosepta apart) as in Carroll (2004).

A 2-mm incision was made through the skin to expose underlying fascicles. Fascicles were separated and the crystal was inserted between them to a depth of 4 mm. The incision was closed around the crystal wire with 5–0 suture. The suture was then tied around the crystal wire to secure it in place while ensuring enough slack to allow the crystal to move with the underlying muscle fascicles. Crystal movement was confirmed at the end of surgery and was interpreted as fascicle strain. Crystal position was confirmed post-mortem.

2.5. Activation

Fascicle depolarization was measured with bipolar electrodes fashioned from 2-m long pieces of 0.002 in. (0.051 mm)

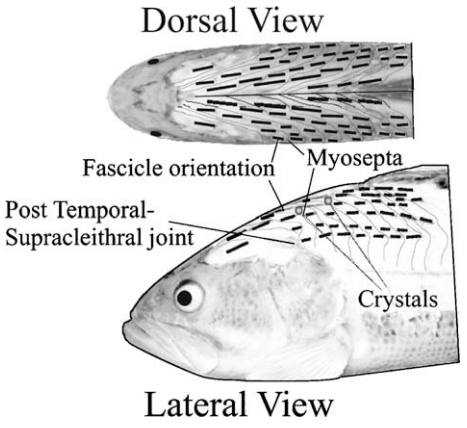

Fig. 2. Dorsal and ventral views of surface fascicle orientation in the epaxial musculature of a largemouth bass (*M. salmoides*). It was assumed for the purposes of this study that fascicles directly crossing the post temporal-supracleithral joint or inserting on the predorsal bones where capable of contributing force to neurocranial rotation (Gemballa and Roder, 2004; Thys, 1997).

dual filament stainless steel Teflon-coated wire (California Fine-Wire, Grover Beach, CA, USA) loaded into a 26-gauge hypodermic needle. The tips of the wires were stripped, spread orthogonally and bent into a hook against the shaft of the needle. The distance between stripped ends ranged from 3–1.5 mm. The needle was inserted into the skin <5 mm dorsal or ventral to the crystals and at the dept of the crystals. The electrode was held in the muscle by the hook in the wire. EMG signals were conditioned with a 4-channel differential amplifier (A-M systems, Everett, WA, USA) using a gain of 10,000, and a filter bandwidth of 3000–100 Hz. A 60 Hz notch filter was used in all recordings. This choice of gain was used to match the input range that was set on the digital conversion system.

2.6. Cranial kinematics

If the hyoid is held or retracted by the sternohyoideus muscle (Carroll, 2004), dorsal rotation of the neurocranium will result in ventral and caudal rotation of the midventral hyoid apparatus away from the neurocranium. Hyoid depression was measured by 2-mm crystals. One crystal was sutured to the basihyoid and one immediately lateral to the midline parasphenoid and caudal to the vomerine teeth. Because hyoid depression was measured in water with a speed of sound of 1490 m s^{-1}, these data were corrected in subsequent data analysis.

2.7. Intra-oral pressure

A 15-gauge needle was inserted into the fish's neurocranium rostral to the insertion of the epaxial muscles but caudal to the ascending processes of the premaxillae. The needle was inserted at the midline to avoid blood vessels and the olfactory nerves but was angled slightly laterally to emerge lateral to the parasphenoid 1–2 cm caudal to the vomerine teeth. A catheter fashioned by forming a flange on the buccal end of a 6-cm piece of PE-60 tubing was inserted into the needle. The needle was then removed leaving the catheter in place. The catheter was held in position by a sleeve of Tygon tubing glued to the catheter (Cole-Parmer, Vernon Hills, IL, USA). A Millar SPR-407 microcatheter-tipped pressure transducer (Millar Instruments, Houston, TX, USA) was threaded into the catheter; and a sleeve of smaller Tygon tubing, glued to the transducer wire, was stretched around the open end of the catheter to hold a constant position of the pressure transducer about 2 mm inside the buccal cavity.

Analog crystal signals were digitized with a TRX-8 conversion box (Sonometrics Corp.). Pressure and EMG signals were digitized and recorded on a PC running SonoView Software (Sonometrics Corp.). All data were sampled at 600 Hz.

2.8. Morphological measurements

Estimates of morphological parameters were necessary to calculate epaxial force from buccal pressure (Fig. 1) and to normalize muscle force, work, and power; and buccal work and power. As in Carroll et al. (2004), the post temporal-supracleithral joint was considered to be the axis about which the neurocranium rotated. To estimate epaxial cross-sectional area (CSA) it was necessary to include all fascicles that could potentially contribute force to neurocranial rotation, that is, all fascicles with a dorsal moment across this joint. These included both the fascicles that directly crossed the post temporal-supracleithral joint and those inserting on the pre-dorsal bones (Fig. 2, Thys, 1997). Manipulation of unfixed specimens showed that force applied to these bones was transferred to the neurocranium through the mid-ventral septum (see also Gemballa and Roder, 2004). Therefore, cross-sections of epaxial musculature were taken from caudal to the last pre-dorsal bone, rostral to the dorsal fin, and ventral to the level of the joint axis (Fig. 2). Cross-sections were cut perpendicular to the orientation of surface muscle fascicles, and were carefully removed and digitally photographed.

It should be emphasized that force generation and transmission in the epaxial muscle mass is complex and poorly understood; the cross-sectional area measured in this study may underestimate the true cross-sectional area of muscle with a moment across the joint. The area and centroid of each cut were measured on a PC using Image J software (NIH, Washington, D. C., USA). The epaxial moment (in-lever, Fig. 1) was taken as the distance from the ventral margin of the cut to the centroid of the epaxial cross-section.

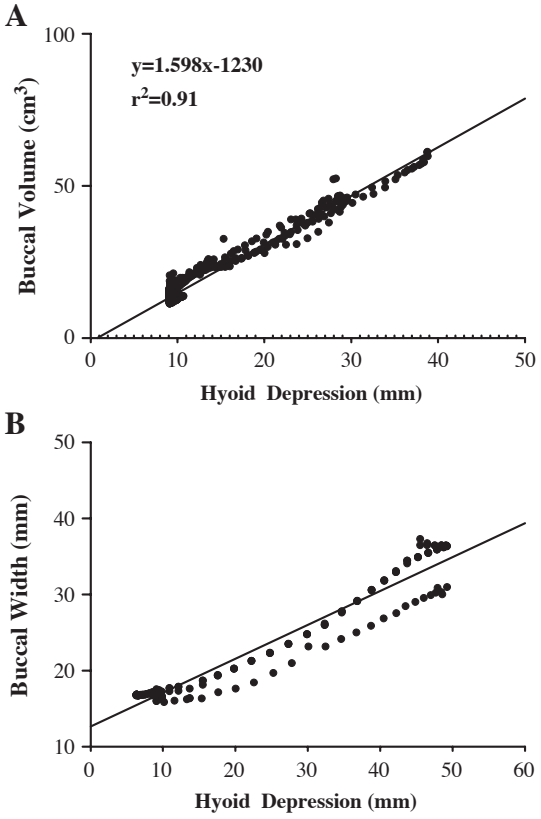

Fig. 3. Calibration of buccal volume and width from hyoid depression based on data collected by Sanford and Wainwright (2002). Hyoid depression in this study was measured from the same locations as that in Sanford and Wainwright (2002), therefore it was possible to estimate unmeasured variables of buccal volume (A) and width (B) from hyoid depression. (A) Depicts four feedings from a 26 cm bass; (B) depicts three feedings from a 26 cm largemouth bass. Maximum values for buccal volume and width were similar to those based on buccal casts (Table 1; D.C. Collar, unpublished data).

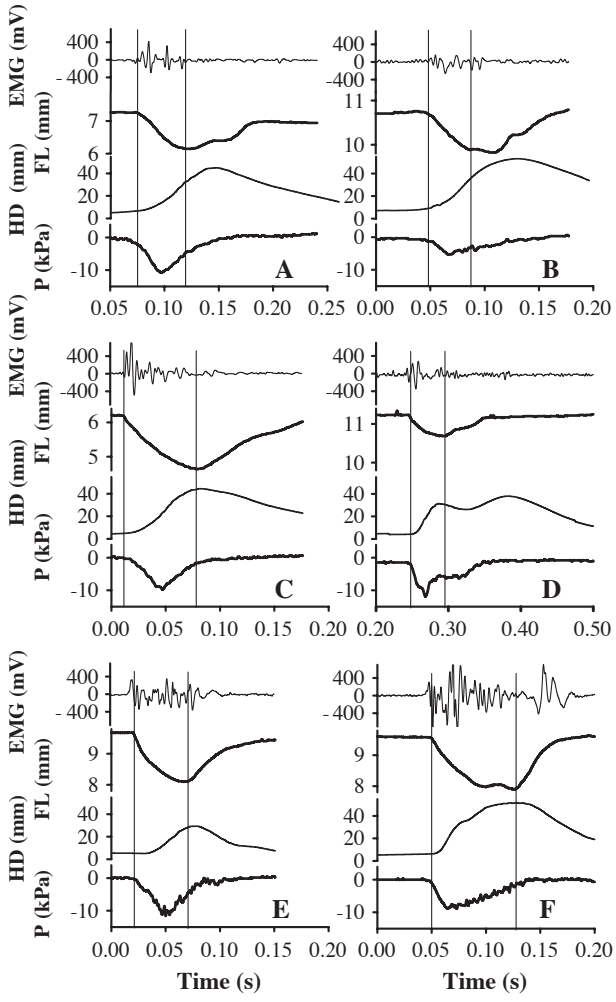

Fig. 4. Representative raw data from three fish. EMG, fascicle length (FL), hyoid depression (HD), and pressure (P) are shown for each feeding. Onset of EMG activity, fascicle shortening, hyoid depression, and pressure drop where nearly simultaneous. Peak pressure generally preceded peak fascicle strain and hyoid depression. (A) and (C) are from Bass 2, (B) and (D) are from Bass 4, and (E) and (F) are from Bass 1. Work loops from these feedings are given in Fig. 5.

The buccal moment (out-lever, Fig. 1) was estimated as the average distance of the buccal cavity from the post temporal-supracleithral joint. The length of the buccal cavity was measured directly from specimens. Buccal width could not be measured directly because it is known to expand during feeding. Therefore, this width was estimated based on hyoid depression from measurements made by Sanford and Wainwright (2002) on similarly sized fish (Fig. 3). The projected area of the buccal cavity was taken as the product of estimated width and measured length. Buccal volume as a function of time was also estimated from hyoid depression using data from Sanford and Wainwright (2002), both volume and width data were similar to those estimated from buccal casts taken from a size series of largemouth bass (D. C. Collar, unpublished data, Table 1).

2.9. Data analysis

All data were converted to ASCII text files and opened in Microsoft Excel. Onset of activation was taken as the point *before* the start of apparent depolarization in the EMG signal; therefore activation onset represents the last point that the muscle fibers were not activated. Detectable activation was defined as having an amplitude three times that of noise or more and lasting longer than 10 ms. Fascicle length was converted to strain by subtracting the length at onset (considered resting length) from each value and dividing by length at onset. The onset, peak, and return of hyoid depression, pressure, and fascicle length were unambiguous (Fig. 4).

Muscle stress (P^m) was calculated with the equation given in Fig. 1 and parameterized with measurements given in Table 1. Stress and strain measurements were used to estimate muscle work and power from onset of shortening to the end of shortening and for the duration of the muscle cycle. Buccal work and power estimations were made from measured pressure and estimated buccal volume based on hyoid depression (Fig. 3, Marsh et al., 1992). Muscle work and power were normalized by dividing the raw estimate by the mass of the muscle section from which recordings were made (i.e., fascicle resting length multiplied by cross-sectional area and converted to kilograms by assuming a muscle density of 1060 kg m^{-3}). Work and power of cranial expansion were normalized with the estimated epaxial mass based on a regression made on a size series of largemouth bass (9–30 cm, A. M. Carroll, unpublished data).

Table 1
Measured and estimated morphological parameters

	Standard length	Buccal length (mm)	Buccal moment (mm)	Epaxial moment (mm)	Epaxial Area (mm^2)	Max buccal width (mm) [a]	Max buccal width (mm) [b]	Max buccal volume (cm^3) [a]	Max buccal volume (cm^3) [b]	Epaxial muscle mass (kg) [c]
Bass 1	26	56	39.5	9.3	245	47.4	35.5	64.2	81.3	0.040
Bass 2	26.5	60	43.5	11	295	48.5	37.8	69.0	87	0.042
Bass 3	29	65	43	9.8	300	54.2	38.2	97.1	90.8	0.057
Bass 4	27.5	60	42.5	12.2	280	50.8	36	79.4	87.3	0.048

[a] Scaled, Collar, D. C., unpublished data.
[b] Based on hyoid depression, See Fig. 3.
[c] Scaled, Carroll, A. M, unpublished data.

Table 2
Relative timing of events (mean±S.E.M.)

	Activation, shortening delay (ms)	Time to peak shortening (ms)		Time to peak hyoid depression (ms)		Peak shortening, peak hyoid delay (ms)	Peak Pressure, peak strain delay (ms)	Muscle cycle duration (ms)		Hyoid cycle duration (ms)	
	Mean	Mean	Min	Mean	Min	Mean	Mean	Mean	Min	Mean	Min
Bass 1 (n=8)	7.7±1.6	51±5	29	42±4	22	8±3[a]	24±4[b]	98±12[c]	39	107+9	66
Bass 2 (n=9)	2.0±3.0[a]	53±6	29	54±5	33	17±5[a]	13±4	147±12[c]	88	138+14	91
Bass 3 (n=12)	N/A	63±4	30	61±8	38	8±5[a]	25±5[b]	163±16[c]	93	131+14	59
Bass 4 (n=20)	1.0±0.9[a]	41±3	20	54±3	22	17±3[b]	17±3[b]	180±12[c]	103	108+9	44

[a] Not significantly different from zero (p>0.01).
[b] Significantly different from zero (p<0.0001).
[c] Significantly longer than 2× time to peak shortening (p<0.001).

This regression was made by measuring all the fascicles that shortened during neurocranial rotation of unfixed specimens. These regions are also known to be active during feeding (Thys, 1997).

The work and power of cranial expansion were normalized so that they could be compared to the work and power measured from the epaxials. To test relative contribution of the epaxials to cranial expansion it was necessary to only normalize by the mass of the epaxials. Because these estimates do not include the mass of other suction feeding muscles (the sternohyoideus or dilator operculi), true normalized work and power are over-estimated in this study. However, the epaxial muscle mass in largemouth bass is much more massive than the sternohyoideus (A. M. Carroll, unpublished data); therefore, it is unlikely these values are grossly in error.

3. Results

Between eight to twenty feedings were recorded from each of the four fish. The timing and magnitude of EMG, hyoid depression, and intra-oral pressure were similar to that seen in other studies of similarly sized largemouth bass (Grubich and Wainwright, 1997; Sanford and Wainwright, 2002) suggesting that the invasive instrumentation used in this study did not reduce feeding effort. Time from onset of cranial expansion to peak cranial expansion averaged 50±5 ms across all fish, with a minimum 22 ms recorded. Minimum buccal pressure during feedings ranged from − 1 to − 20 kPa indicating that a wide range of effort was elicited.

Time from onset of shortening to peak strain averaged 51±4 ms across all fish and total muscle cycle time averaged 118±6 ms (Table 2), corresponding to a cycle frequency of 8.5 Hz, although the average minimum cycle time across all fish (80 ms) corresponds to a cycle frequency of 12.5 Hz. Average time to the full feeding cycle was longer (156±7 ms), corresponding to 6.4 Hz.

THE onset of EMG activity was simultaneous with onset of shortening in two of the three fish in which it was recorded, in the third (Bass 1) the delay averaged 8 ms (Table 2). In all fish peak strain preceded peak hyoid depression (Table 2), but this latency was only significantly different from zero in one fish. Peak pressure preceded peak strain in all fish (Table 2). Fascicle lengthening took longer than shortening in all fish (Table 2).

3.1. Fascicle strain

Fascicles shortened from and returned to resting length (Fig. 4). However, in 5 feeding events a slight (<1%) lengthening was seen prior to the onset of shortening. The magnitude and speed of shortening varied among individuals (Tables 3 and 4). Mean strain averaged 9±0.7%, although strains up to 24% where recorded (Table 3). Strain velocity averaged − 1.8±0.9 FL s^{-1} across individuals and feedings (Table 3).

3.2. Fascicle stress

Mean muscle stress averaged 44±4 kPa during muscle shortening with mean peak stress as high as 86 kPa in some feedings (Table 3). Peak stress was reached late in the strain cycle (Fig. 5), on average after 70% of total strain. Peak stress was never achieved prior to shortening. In some strikes the muscle continued to be under stress as it re-lengthened, producing negative work (Fig. 4D,F). Thus, negative buccal pressure may have aided in re-lengthening the muscle.

Table 3
Muscle parameters during shortening (mean±S.E.M.)

	Muscle strain (%)		Muscle strain rate (FL s^{-1})		Mean muscle stress (kPa)		Normalized muscle work (J kg^{-1})		Normalized muscle power (W kg^{-1})	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
Bass 1 (n=8)	−11±2	−20	−2.3±0.6	−4.3	50±13	86	5.7±1.9	13.1	129±42	330
Bass 2 (n=9)	−14±2	−24	−2.7±0.3	−3.8	31±18	61	4.3±1.1	9.3	88±27	226
Bass 3 (n=12)	−7±1	−13	−1.1±0.1	−1.8	57±28	75	4.4±1.5	8.1	69±12	135
Bass 4 (n=20)	−6±0.5	−8	−1.5±1	2.7	28±3	51	1.6±.2	4.3	36±5	62

Table 4
Muscle parameters during muscle cycle (mean±S.E.M.)

	Mean muscle stress (kPa)		Normalized muscle power (W kg^{-1})		Normalized muscle work (J kg^{-1})	
	Mean	Max	Mean	Max	Mean	Max
Bass 1 (n=8)	54±16	91	4.4±1.6	10.5	47±17	137
Bass 2 (n=9)	33±7.6	62	3.3±1.1*	8.6	27±10	79
Bass 3 (n=12)	63±10	93	3.01±0.7*	5.1	25±8	82
Bass 4 (n=20)	29±4.4	63	0.5±0.2**	3.0	3±2	17

* Significantly less than work of shortening (p<0.01).
** Significantly less than work of shortening (p<0.0001).

3.3. Muscle work and power

Mean normalized muscle work averaged 3.4±0.5 J kg^{-1} during muscle shortening and 2.2±0.4 J kg^{-1} over the muscle cycle. Maximum values for each fish ranged from 13.1 to 4.3 J kg^{-1} during shortening and 10.5 to 3.0 J kg^{-1} during the full muscle cycle (Tables 3 and 4). The lesser values across the muscle cycle result from the negative work done on the muscle during re-lengthening (Fig. 4). Individual fish varied in the degree to which including negative work of re-lengthening affected total work production. In one fish (Bass 4) cycle work was significantly less than shortening work (p<0.0001), in two fish it was less significant (p<0.01), and in one fish there was no significant difference (Table 4). In all fish for which EMG was measured, there was a significant correlation (r=0.82) between rectified EMG area (mV s) and muscle work (Fig. 6A). Mean normalized muscle power averaged 69±10 W kg^{-1} during shortening and 20±4 W kg^{-1} for the full muscle cycle. Maximum values for each fish ranged from 330 to 62 during shortening and 137 to 17 for the muscle cycle (Tables 3 and 4). There was also a significant correlation (r=0.80) between rectified EMG area (mV s) and muscle power in the three individuals for which data were available (Fig. 6B). EMG intensity (mV) was similarly correlated with work (r=0.77) and power (r=0.82).

3.4. Work and power of cranial expansion

Normalized work averaged 5.6±0.6 J kg^{-1} during cranial expansion and 4.9±0.9 J kg^{-1} over the feeding cycle (Table 5). Maximal values for each fish ranged from 13.1 to 8.8 J kg^{-1} over expansion and 13.1 to 10.6 over the feeding cycle (Table 5). Normalized buccal power averaged 103±12 W kg^{-1} during expansion and 40±5 W kg^{-1} over the feeding cycle (Table 5). Maximal values ranged from 331 to 200 W kg^{-1} during expansion and 143 to 74 over the feeding cycle (Table 5).

Normalized work and power of cranial expansion were significantly correlated with normalized muscle work and power of muscle shortening among all fish (Fig. 7A,B). The slope of these regressions represents the relative contribution of the epaxial contraction to the total cost of the strike. A slope of one indicates that the epaxial muscle mass contributed on average all the work or power of cranial expansion. In most fish the slope approximated one, but in Bass 4 it was much steeper indicating that the epaxials did not contribute as much to the work of buccal expansion in this fish. The contribution of measured muscle work to total work was also correlated with the amount of fascicle strain (Fig. 7C).

4. Discussion

This study was undertaken with the critical assumption that subambient buccal pressure represents the dominant imposed load during suction feeding. The inertia of the neurocranium and added mass of surrounding water are thought to contribute only 10–20% of total loading (Van Wassenbergh et al., 2005); and pressure measured on external cranial elements is negligible relative to intra-oral pressure (A. M. Carroll unpublished data). Furthermore, the contribution of skeletal inertia and added mass would be expected to peak at the onset of movement when buccal pressure is minimal, so the inclusion of these forces would not have increased peak estimated stress or greatly affected the overall shape of the muscle work loop. Nevertheless, our estimates of muscle force must be verified by

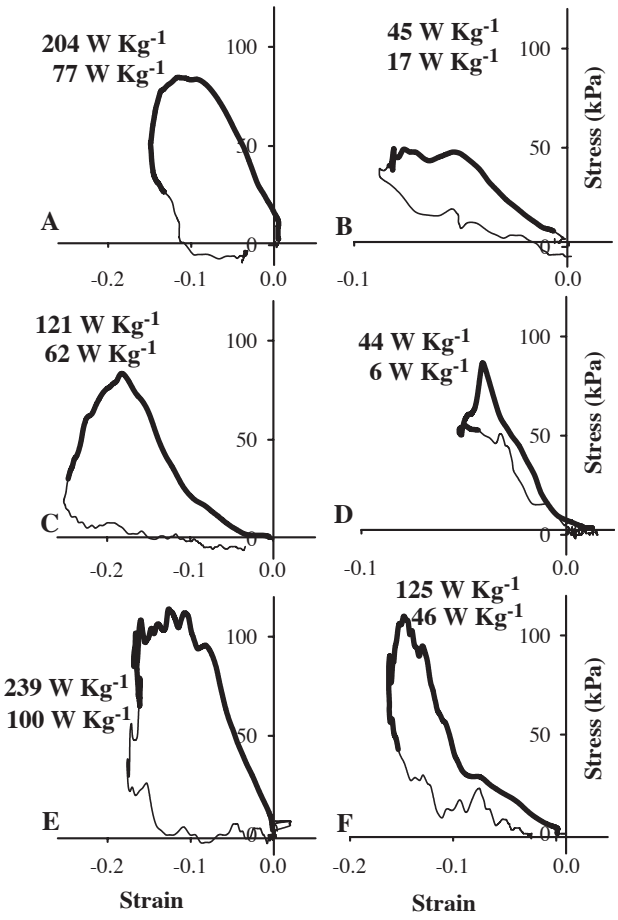

Fig. 5. Representative work loops based on data from Fig. 4. Muscle stress (kPa) is plotted against muscle strain, $(l-l_o)/l_o$. The bold regions of the work loop indicate periods when the muscle was active. Power given is for the shortening phase (greater number) and total cycle (lesser number). Unlike work loops observed in many other behaviors muscle stress reaches a maximum only after the muscle has begun to shorten.

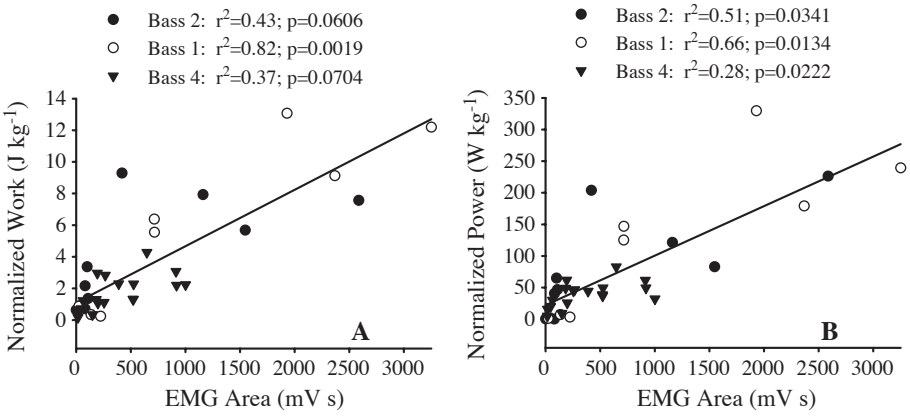

Fig. 6. Modulation of muscle work and power output. EMG area (mV s) is plotted against normalized work (J kg^{-1})and normalized power (W kg^{-1}). Data were pooled because no individual effect was found ($p=0.22$). While individual relationships were relatively weak, there was an overall trend towards increasing work or power with increasing EMG area.

comparison to in vitro work loops as has been done in several other systems (e.g., Franklin and Johnston, 1997). Still, the average and maximum mean muscle stress (44 and 86 kPa, respectively, Table 3) are within the range expected from white muscle shortening at 1 to 3 FL s^{-1}, with a maximum shortening velocity estimated at 10 FL s^{-1} (V_{max} for *M. salmoides* D. J. Coughlin, unpublished data) and with an maximum isometric stress estimated at 200 kPa (Medler, 2002).

4.1. Activation and strain

Noise in the EMG signal may have cloaked earlier low levels of activation (Fig. 4) in low motivation strikes. In maximal-effort strikes it seems probable that the muscle would be fully recruited, and that detectable levels of depolarization would be present at the onset of the behavior. In addition, there may have been passive shortening of the epaxials by other parts of the muscle. As it was recorded, observed onset of fascicle shortening occurred simultaneously with or shortly after onset of detectable activation. This delay is much shorter than the ~15 ms required to reach peak force in largemouth bass white muscle (Thys et al., 1998) and is also shorter than that seen in gravitationally loaded behaviors such as frog jumping (Lutz and Rome, 1994; Olson and Marsh, 1998) or lizard burst running (Nelson and Jayne, 2001). It was, however, similar to that seen in the preliminary ("C") bend in fish fast starts (Eaton et al., 1981). In fact, Wakeling and Johnston (1999) found an insignificant delay between activation and shortening in the common carp, *Cyprinus carpio*, and concluded that the muscle produced force as it was depolarized because it was unloaded prior to movement. Similarly, the short or simultaneous onset of activation and strain in this study suggests that suction feeding muscles are also unloaded prior to movement and shorten before full activation.

The short or minimal delay between activation and force probably prevents the muscle from reaching its maximum isometric force production during feeding (Lutz and Rome, 1994). In all feedings observed in this study, peak stress was achieved late in the strike after shortening had begun (Fig. 5). The late development of force during suction feeding does not resemble previously published work loops. In fact in most published studies of power-limited behaviors force peaks at the onset of movement (Biewener et al., 1998; Finni et al., 2000; Franklin and Johnston, 1997). Failure to reach full activation prior to shortening may have reduced muscle stress and power production in the fascicles measured in this study (Lutz and Rome, 1994).

4.2. Operating fascicle length

The epaxials spend all of their contraction cycle at lengths shorter than resting length. Epaxial fascicles shortened from rest

Table 5
Energetics of buccal expansion (mean±S.E.M.)

	Buccal expansion				Buccal cycle			
	Normalized buccal work* (J kg^{-1})		Normalized buccal power* (W kg^{-1})		Normalized buccal work* (J kg^{-1})		Normalized buccal power* (W kg^{-1})	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max
Bass 1 ($n=8$)	6.2±1.9	13.1	158±46	331	5.8±1.8	13.1	64±17	125
Bass 2 ($n=9$)	5.2±1.3	10.1	110±51	200	5.2±1.3	10.3	40±11	100
Bass 3 ($n=12$)	5.5±.06	8.8	100±20	214	7.9±1.2	17.2	53±11	143
Bass 4 ($n=20$)	3.8±0.7	9.0	76±14	203	4.3±0.7	10.6	23±4	74

*Normalized to epaxial mass only, and may overestimate actual normalized work and power.

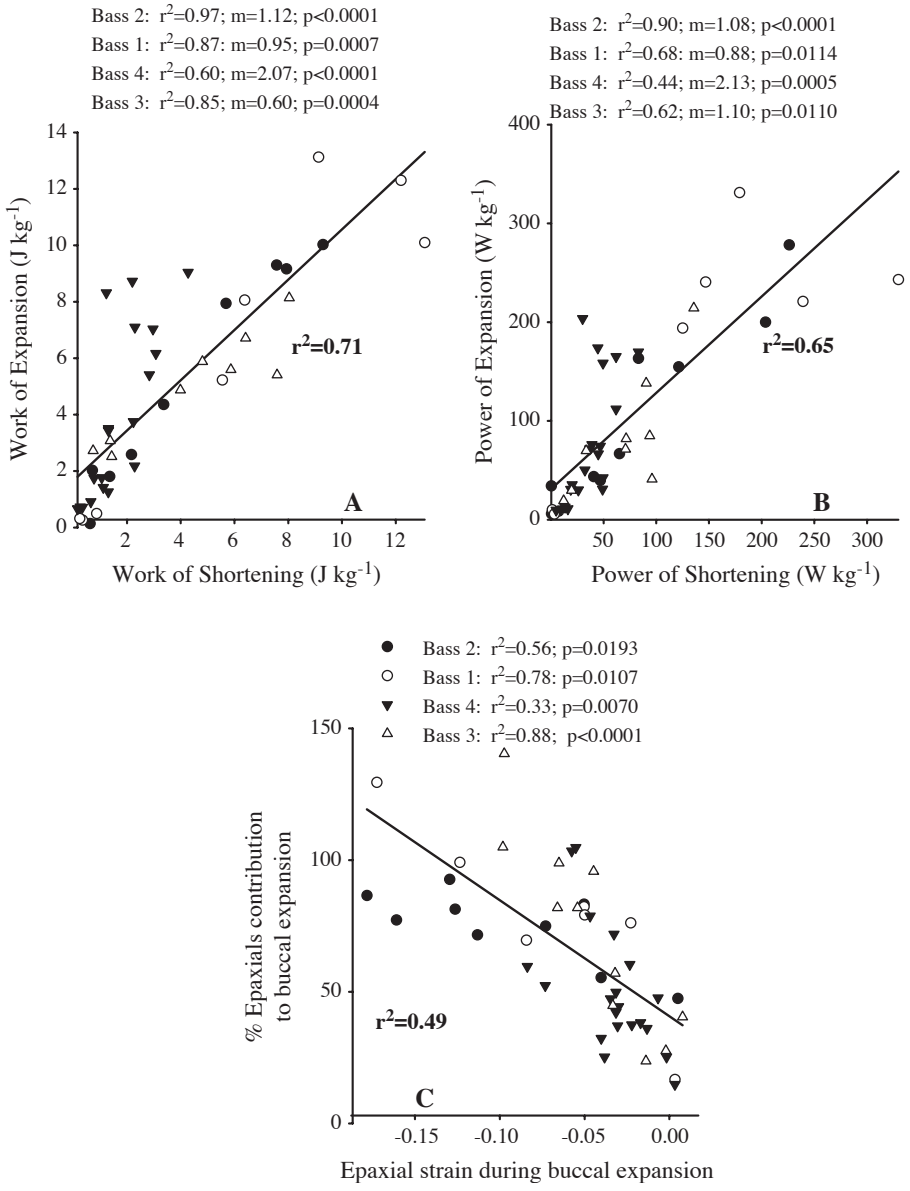

Fig. 7. Contribution of the epaxial muscle mass to the overall energetic cost of the strike. Normalized muscle work (A) and power (B) during muscle shortening are regressed against normalized buccal work and power during cranial expansion. There are significant overall correlations among and within individuals. Three individuals had slopes near one, indicating measured epaxial work and power accounted for most of the work and power of feeding. Bass 4, with a slope of 2.2 also, had the lowest mass-specific work and power production indicating that much of its work and power for feeding may come from its sternohyoideus. Across all fish the relative contribution of the epaxials to strain was weakly but significantly correlated with relative strain of the epaxials (C).

up to 24% of their length, though they averaged 9% shortening. Furthermore, peak muscle stress was not reached until the muscle had shortened from resting length. Muscles tend to operate on their sarcomeric force plateau or on the ascending curve (Burkholder and Lieber, 2001), and it is not clear whether the >20% strains observed in this study would result in a reduction in muscle force. The force-length properties of this muscle are not known. According to Rome (1998) shortening up to 20% of resting length in the common carp would result in a 15% loss of force, so it is very possible that power production during suction feeding is reduced by the kinematic demand that it shorten such a large percentage of its length from rest.

4.3. Muscle power and work production

The epaxial muscles consistently produced positive net work, although there was often significant negative work done on the muscle during re-lengthening, especially during low-motivation feedings (Fig. 5). This is in contrast to the sternohyoideus in *M. salmoides* which appears to produce negative work during the early phases of feeding in some individuals (Carroll, 2004). The negative work done during re-lengthening in the epaxials resulted in significant reduction of net power and work compared to that produced during shortening in most of the fish used in this study (Table 4). Net power output was also reduced relative to the power of

shortening by taking into account the longer re-lengthening time often seen in this study (Table 2). Lengthening times were 10% to 60% longer than shortening times in the fish used in this study making it difficult to compare the power produced by the epaxials to that produced during repeated locomotor behaviors. This is especially true given that cyclic locomotor behaviors often have asymmetrical contraction patterns in which shortening is longer than re-lengthening (Askew and Marsh, 2002).

From the above discussion, it seems possible that muscle stress is reduced during suction feeding, which would lower overall power. Although the maximal power output recorded in this study (137 W kg^{-1}) is near to that produced in isolated scorpion fish (*Scorpaena notota*) fascicles during sine-wave strain patterns (143 W kg^{-1}) at 20 C and 10.8 Hz (Wakeling and Johnston, 1998). Net power values from most individuals (Table 3) were much lower even with maximal recruitment (Fig. 6). This suggests that muscular power production during suction feeding may be limited by its kinematic requirements and loading environment. Certainly, values here were much lower than that produced during burst flight in quails, *Coturnix chinensis* (390 W kg^{-1}), thought to be the highest recorded power output yet found in vertebrates (Askew and Marsh, 2002). To fully address whether the mechanical and kinematic demands of suction feeding result in a loss of mass-specific power, work and power output under the activation and strain cycles recorded here must be compared against cycles varied to produce maximal power as has been done in several other studies (Franklin and Johnston, 1997; Josephson, 1985; Peplowski and Marsh, 1997).

de Jong et al. (1987) calculated an absolute power output of 10 W for a hypothetical fish of similar size and kinematics to the bass of this study (30 cm long with a cranial expansion time of 50 ms). Assuming the distribution of muscle in this fish was similar to that of *M. salmoides*, this power output amounts to a mass-specific power value of 78 W kg^{-1}, well within the range of values recorded in this study (Table 4). Alternatively, Van Wassenbergh et al. (2005) report mass-specific muscle power as high as 1000 W Kg^{-1}, more than twice as high as any reported from any vertebrate muscle. This discrepancy probably resulted from their failure to account for the mass of epaxial musculature.

4.4. Modulation of muscle power

In the three fish for which activation data were available, magnitude of activation was positively correlated with muscle work and power and transitively buccal work and power (Figs. 6 and 7A,B). This result answers a question hitherto unresolved in the literature: how does predator motivation translate into suction feeding performance (Grubich and Wainwright, 1997)? It appears the work and power exerted during a feeding increases linearly with magnitude of depolarization.

4.5. Work and power of cranial expansion

Epaxial work and power of shortening were significantly correlated with buccal work and shortening during expansion (Fig. 7A,B). The fact that in three of the four fish the slope of these regressions was close to 1 indicates that the epaxials were the major actuator of cranial expansion in these fish. The correlation in Bass 4 had a slope close to 2 indicating that the epaxials were not the major actuator of feeding in this fish. This result is consistent with our understanding of suction feeding which relies on two major redundant systems (the dorsal epaxial system, and the ventral sternohyoideus system). Strain data from the sternohyoideus (Carroll, 2004) suggests that there is considerable inter-individual variability in the relative contribution of each system to overall suction feeding energetics. The relative contribution of the epaxials was positively correlated with the fascicle strain (Fig. 7C). This suggests that the ventral and dorsal expansion systems, which must resist the same subambient buccal pressures, may vary the degree to which they contribute strain to kinematic displacement and overall buccal power (Fig. 7C).

4.6. Conclusions

The kinematic requirements of suction feeding and the unique loading environment in which it takes place affect the observed functional pattern of the epaxials (Figs. 4 and 5). Muscles shortened from rest with a minimal delay between activation and shortening and reached peak force after shortening. The latter characteristic is unlike that seen in other power-limited behaviors. These functional characteristics may reduce muscle force and total muscle power relative to other behaviors and the intrinsic capabilities of the muscle fibers. Finally, the epaxial muscle mass appears be the predominate actuator of maximal performance suction feeding in largemouth bass.

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References

Askew, G.N., Marsh, R.L., 1998. Optimal shortening velocity (V/V_{max}) of skeletal muscle during cyclical contractions: length-force effects and velocity-dependent activation and deactivation. J. Exp. Biol. 201, 1527–1540.

Askew, G.N., Marsh, R.L., 2002. Muscle designed for maximum short-term power output: quail flight muscle. J. Exp. Biol. 205, 2153–2160.

Askew, G.N., Marsh, R.L., Ellington, C.P., 2001. The mechanical power output of the flight muscles of blue-breasted quail (*Coturnix chinensis*) during take-off. J. Exp. Biol. 204, 3601–3619.

Biewener, A.A., 2002. Future directions for the analysis of musculoskeletal design and locomotor performance. J. Morphol. 252, 38–51.

Biewener, A.A., Baudinette, R.V., 1995. In vivo muscle force and elastic energy storage during steady-speed hopping of Tammar wallabies (*Macropus eugenii*). J. Exp. Biol. 198, 1829–1841.

Biewener, A.A., Corning, W.R., Tobalske, B.W., 1998. In vivo pectoralis muscle force-length behavior during level flight in pigeons (*Columba livia*). J. Exp. Biol. 201, 3293–3307.

Burkholder, T.J., Lieber, R.L., 2001. Sarcomere length operating range of vertebrate muscles during movement. J. Exp. Biol. 204, 1529–1536.

Carroll, A.M., 2004. Muscle activation and strain during suction feeding in the largemouth bass *Micropterus salmoides*. J. Exp. Biol. 207, 983–991.

Carroll, A.M., Wainwright, P.C., Huskey, S.H., Collar, D.C., Turingan, R.G., 2004. Morphology predicts suction feeding performance in centrarchid fishes. J. Exp. Biol. 207, 3873–3881.

Day, S.W., Higham, T.E., Cheer, A.Y., Wainwright, P.C., 2005. Spatial and temporal patterns of water flow generated by suction-feeding bluegill sunfish *Lepomis macrochirus* resolved by Particle Image Velocimetry. J. Exp. Biol. 208, 2661–2671.

de Jong, M.C., Sparenberg, J.A., DeVries, J., 1987. Some aspects of the hydrodynamics of suction feeding of fish. Fluid Dyn. Res. 2, 87–112.

Eaton, R.C., Lavender, W.A., Wieland, C.M., 1981. Identification of mauthner initiated response patterns in goldfish evidence from simultaneous cinematography and electrophysiology. J. Comp. Physiol. [A] 144, 521–532.

Ferry-Graham, L.A., Lauder, G.V., 2001. Aquatic prey capture in ray-finned fishes: a century of progress and new directions. J. Morphol. 248, 99–119.

Finni, T., Komi Paavo, V., Lepola, V., 2000. In vivo human triceps surae and quadriceps femoris muscle function in a squat jump and counter movement jump. Eur. J. Appl. Physiol. 83, 416–426.

Franklin, C.E., Johnston, I.A., 1997. Muscle power output during escape responses in an Antarctic fish. J. Exp. Biol. 200, 703–712.

Gemballa, S., Roder, K., 2004. From head to tail: the myoseptal system in basal actinopterygians. J. Morphol. 259, 155–171.

Gibb, A.C., Ferry-Graham, L.A., 2005. Cranial movements during suction feeding in teleost fishes: are they modified to enhance suction production? Zoology 108, 141–153.

Grubich, J.R., Wainwright, P.C., 1997. Motor basis of suction feeding performance in largemouth bass, *Micropterus salmoides*. J. Exp. Zool. 277, 1–13.

Hatze, H., 2000. The inverse dynamics problem of neuromuscular control. Biol. Cybern. 82, 133–141.

Hoffer, J.A., Caputi, A.A., Griffiths, R.I., 1989. Roles of muscle activity and load on the relationship between muscle spindle length and whole muscle length in the freely walking cat. Prog. Brain. Res. 80, 75–85.

Josephson, R.K., 1985. Mechanical power output from striated muscle during cyclic contraction. J. Exp. Biol. 114, 493–512.

Josephson, R.K., 1999. Dissecting muscle power output. J. Exp. Biol. 202, 3369–3375.

Lauder, G.V., 1980a. Evolution of the feeding mechanism in primitive actinopterygian fishes: a functional anatomical analysis of *Polypterus*, *Lepisosteus*, and *Amia*. J. Morphol. 163, 283–317.

Lauder, G.V., 1980b. The suction feeding mechanism in sunfishes (*Lepomis*): and experimental analysis. J. Exp. Biol. 88, 49–72.

Lutz, G.J., Rome, L.C., 1994. Built for jumping: the design of the frog muscular system. Science 263, 370–372.

Marsh, R.L., 1999. How muscles deal with real-world loads: the influence of length trajectory on muscle performance. J. Exp. Biol. 202, 3377–3385.

Marsh, R.L., Olson, J.M., Guzik, S.K., 1992. Mechanical performance of scallop adductor muscle during swimming. Nature (London) 357, 411–413.

Medler, S., 2002. Comparative trends in shortening velocity and force production in skeletal muscles. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283, R368–R378.

Mol, C.R., Breddels, P.A., 1982. Ultrasound velocity in muscle. J. Acoust. Soc. Am. 71, 455–461.

Muller, M., Osse, J.W.M., Verhagen, J.H.G., 1982. A quantitative hydrodynamical model of suction feeding in fish. J. Theor. Biol. 95, 49–79.

Nelson, F.E., Jayne, B.C., 2001. The effects of speed on the in vivo activity and length of a limb muscle during the locomotion of the iguanian lizard, *Dipsosaurus dorsalis*. J. Exp. Biol. 204, 3507–3522.

Nemeth, D.H., 1997a. Modulation of buccal pressure during prey capture in *Hexagrammos decagrammus* (Teleostei: Hexagrammidae). J. Exp. Biol. 200, 2145–2154.

Nemeth, D.H., 1997b. Modulation of attack behavior and its effect on feeding performance in a trophic generalist fish, *Hexagrammos decagrammus*. J. Exp. Biol. 200, 2155–2164.

Olson, J.M., Marsh, R.L., 1998. Activation patterns and length changes in hindlimb muscles of the bullfrog *Rana catesbeiana* during jumping. J. Exp. Biol. 201, 2763–2777.

Peplowski, M.M., Marsh, R.L., 1997. Work and power output in the hindlimb muscles of cuban tree frogs *Osteopilus septentrionalis* during jumping, vol. 200, pp. 2861–2870 (*i*).

Roberts, T.J., Scales, J.A., 2002. Mechanical power output during running accelerations in wild turkeys. J. Exp. Biol. 205, 1485–1494.

Roberts, T.J., Marsh, R.L., Weyand, P.G., Taylor, C.R., 1997. Muscular force in running Turkeys: the economy of minimizing work. Science 275, 1113–1115.

Rome, L.C., 1998. Some advances in integrative muscle physiology. Comp. Biochem. Physiol. B 120, 51–72.

Sanford, C.P.J., Wainwright, P.C., 2002. Use of sonomicrometry demonstrates the link between prey capture kinematics and suction pressure in largemouth bass. J. Exp. Biol. 205, 3445–3457.

Sass, G.G., Motta, P.J., 2002. The effects of satiation on strike mode and prey capture kinematics in the largemouth bass, *Micropterus salmoides*. Environ. Biol. Fish. 65, 441–454.

Thys, T., 1997. Spatial variation in epaxial muscle activity during prey strike in largemouth bass (*Micropterus salmoides*). J. Exp. Biol. 200, 3021–3031.

Thys, T.M., Blank, J.M., Coughlin, D.J., Schachat, F., 1998. Longitudinal variation in muscle protein expression and contraction kinetics of largemouth bass axial muscle. J. Exp. Biol. 204, 4249–4257.

Tsirakos, D., Baltzopoulos, V., Bartlett, R., 1997. Inverse optimization: functional and physiological considerations related to the force-sharing problem. Crit. Rev. Biomed. Eng. 25, 371–407.

Van Wassenbergh, S., Aerts, P., Herrel, A., 2005. Scaling of suction-feeding kinematics and dynamics in the African catfish, *Clarias gariepinus*. J. Exp. Biol. 208, 2103–2114.

Wakeling, J.M., Johnston, I.A., 1998. Muscle power output limits fast-start performance in fish. J. Exp. Biol. 201, 1505–1526.

Wakeling, J.M., Johnston, I.A., 1999. Body bending during fast-starts in fish can be explained in terms of muscle torque and hydrodynamic resistance. J. Exp. Biol. 202, 675–682.

Werner, E.E., 1974. The fish size prey size handling time relation in several sunfishes and some implications. J. Fish. Res. B 1531–1536.