

Energetic limitations on suction feeding performance in centrarchid fishes

Andrew M. Carroll^{1,*} and Peter C. Wainwright²

¹Department of Biology, University of Evansville, Evansville, IN 47722, USA and ²Department of Evolution and Ecology, University of California at Davis, Davis, CA 95616, USA

*Author for correspondence (ac204@evansville.edu)

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SUMMARY

Energetic analysis of ecologically relevant behaviors can be useful because animals are energetically limited by available muscle mass. In this study we hypothesized that two major determinants of suction feeding performance, the magnitudes of buccal volumetric expansion and subambient buccal pressure, would be correlated with, and limited by, available muscle mass. At least four individuals of three centrarchid species were studied: largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*) and green sunfish (*Lepomis cyanellus*). Buccal pressure was measured directly *via* cannulation of the buccal cavity with a catheter-tipped pressure transducer. Buccal expansion was estimated from lateral high-speed video (500 or 1000 Hz) sequences and published data on internal kinematics of largemouth bass. These estimates were calibrated from silicone casts made of the buccal cavity post-mortem. Estimated work and power were found to be significantly correlated with muscle mass over all individuals. The slopes of these relationships, estimates of mass-specific muscle work and power, were found to be $11 \pm 2 \text{ J kg}^{-1}$ and $300 \pm 75 \text{ W kg}^{-1}$, respectively. These estimates are consistent with observations made of *in vivo* and *in vitro* muscle use and with digital particle image velocimetry measurements of water flow in feeding centrarchids. A direct trade-off between mean pressure and change in volume was observed, when the latter was normalized to muscle mass. We conclude that available muscle mass may be a useful metric of suction feeding performance, and that the ratio of muscle mass to buccal volume may be a useful predictor of subambient buccal pressure magnitude.

Key words: suction feeding, *Micropterus*, *Lepomis*, performance, muscle, trade-off.

INTRODUCTION

A central issue in biology is the diversity of species and forms: what drives the diversification of forms (Darwin, 1859; Collar et al., 2009)? One possibility is that different forms confer different (and mutually exclusive) performance capabilities (Schluter, 1995; Wainwright, 1991). This possibility can be tested by linking performance capabilities for ecologically relevant tasks to individual or species morphology (Arnold, 1983). Tracing the pathway of energy from muscle to movement can link form to performance because, for animals, the mechanical energy for movement is limited by the chemical energy released from ATP by muscle myosin (Askew and Marsh, 2001; Taylor et al., 1982). Therefore, if performance in an ecologically relevant task can be expressed in terms of energetic output, it can be directly linked to the muscle volume (or mass) available to a given individual (e.g. Gray, 1936). The goal of this study is to test such a linkage in suction feeding behavior among individuals of three morphologically divergent species of centrarchid fishes.

Suction feeding is the primary means of energy acquisition in most aquatic vertebrates (Lauder, 1980a). To capture prey by hydrodynamic suction, a fish must rapidly expand its cranial skeleton causing a flow of water containing the prey to enter the expanding buccal (mouth) cavity. It can be argued that both the volume of water and the pressure inside the buccal cavity are important determinants of feeding performance (Higham et al., 2006a; Holzman et al., 2008a; Van Wassenbergh et al., 2006). The volume of water entering the mouth is important because it is the volume of water in which the prey is contained, so larger prey can be captured in a larger flow volume (Van Leeuwen and Muller, 1984), increasing energy acquired during feeding (Nyberg, 1971).

Buccal pressure relates to the velocity of the suction flow and thus the hydrodynamic force exerted on the prey item, which it must overcome to escape capture (Higham et al., 2006b; Van Wassenbergh and Aerts, 2009). As with any pump, the mechanical work exerted in suction feeding is equal to the pressure gradient across the expanding buccal cavity integrated with respect to volume, and the mechanical power is equal to the work divided by the duration of expansion (Vogel, 1994). Typically, the component of this pressure on the outside of the buccal cavity is ignored (e.g. Carroll and Wainwright, 2006; Van Wassenbergh et al., 2005; Van Wassenbergh et al., 2006), because it appears to be much lower in magnitude than that inside (A.M.C., unpublished data), as is the work required to accelerate skeletal elements themselves (Van Wassenbergh and Aerts, 2008). It is possible to estimate work and power exerted during suction feeding, and these parameters may be limited by morphology, specifically, by available muscle mass. While muscle power has been measured in a largemouth bass (Carroll and Wainwright, 2006), no attempt has been made to link muscle mass to variation among species in suction feeding performance.

Suction feeding in centrarchids is driven by ventral and dorsal expansion systems. The dorsal system is driven by the epaxial muscles (EP) and the ventral expansion system is driven by the sternohyoideus (SH) and hypaxial muscles (Lauder, 1980b). Muscle function during suction feeding has been measured in the SH and EP of largemouth bass (Carroll, 2004; Carroll and Wainwright, 2006). Both muscles actively shortened during feeding at strain rates that are expected to allow work and power production (Coughlin and Carroll, 2006). The hypaxial muscles are of much smaller mass in centrarchids (~20% of the combined epaxial and sternohyoideus

mass) (A.M.C., unpublished data), and there is no direct evidence that they contribute energy to cranial expansion in centrarchid fish [although they do in other teleost species (e.g. Herrell et al., 2005)]; therefore, they were ignored in this study.

Subambient pressure has been shown to be influenced by the lever systems through which muscle force is exerted (Carroll et al., 2004); however, lever systems cannot, in themselves, amplify work or power without violating the law of conservation of energy. Lever systems in musculoskeletal systems can do two things. First, they can exchange force for displacement and *vice versa* as required by a behavior (e.g. Westneat, 1994). Second, lever systems can influence how muscles function by setting the load placed on a muscle for a given external load. The load placed on active muscle, in turn, determines the shortening velocity through the muscle's force-velocity relationship (Hill, 1938). The amount of mass-specific work or power depends on the relative speed of muscle shortening (Lieber, 2002), so a lever system might affect mass-specific work or power if external loads are too high or low to maximize power output. Because muscle stress for a given load is determined by the muscle's physiological cross-sectional area, it is the relationship between external load, skeletal lever systems and cross-sectional area that determines how a muscle will function and how much work or power it will produce (e.g. Lutz and Rome, 1994) [for a more detailed description for suction feeding, see Wainwright et al. (Wainwright et al., 2007) or Carroll and Wainwright (Carroll and Wainwright, 2006)]. Carroll et al. (Carroll et al., 2004) found that estimates of epaxial muscle stress were similar (~70 kPa) in five species of centrarchid fish, and it was, therefore, assumed that all the fish in the present study operated over similar functional ranges of muscle stress and shortening velocity, and thus produced similar mass-specific muscular work and power.

Other factors may also alter the relationship between muscle mass and muscle power across species. These include evolutionary changes in mass-specific muscle work or power, muscle size and power amplification through passive energy storage. Carroll et al. (Carroll et al., 2009) found that mass-specific power and work production of suction feeding muscle (the epaxials, EP) increased with increasing body size in bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) but this appears to have been due to extremely low force per cross-sectional area values in small fish [also found in Van Wassenbergh et al. (Van Wassenbergh et al., 2007)]. Interestingly, no decrease in muscle unloaded shortening velocity with size was found, suggesting that myosin

ATPase activity, and thus energy production, is scale invariant in suction feeding muscle [although Van Wassenbergh et al. found such a pattern in clariid catfishes (Van Wassenbergh et al., 2007)]. Thus, scale effects may not influence the relationship between muscle mass and suction feeding performance.

Power amplification, through the rapid release of stored energy, has been demonstrated in several animal behaviors (e.g. Patek et al., 2007), including the rapid and highly specialized cranial rotation of pipefishes (Van Wassenbergh et al., 2008). However, the very short <20ms delay between muscle activation and skeletal movement in suction feeding in centrarchids (Carroll and Wainwright, 2006) appears to preclude the use of energy storage in passive structures that characterizes power amplification (Lappin et al., 2006).

It can be argued, then, that suction feeding muscle mass may be a reliable predictor of the work or power exerted on water during suction feeding among centrarchid fishes. To test this possibility we measured buccal pressure during high performance feeding events while recording high-speed video of external kinematics in three morphologically divergent species of centrarchid fish: largemouth bass *M. salmoides*; bluegill *L. macrochirus*; and green sunfish *L. cyanellus*. These parameters were combined with post-mortem measurements of internal buccal dimensions of individual fish to estimate the work and power output during feeding. These parameters were then regressed against measured mass of the SH and the portions of the EP that appear capable of actuating suction feeding (Thys, 1997) (Fig. 1). We hypothesized that muscle mass would predict work and power output. We further hypothesized that the slope of this relationship, an estimate of normalized muscle work or power, would be in the range of estimates of maximal normalized muscle work and power from other studies (e.g. Coughlin and Carroll, 2006).

Fishes are often grouped into 'suction' and 'ram' ecomorphs (Norton and Brainerd, 1993) based on their morphology and performance capabilities (for a review, see Wainwright et al., 2007). The latter are described as having a large mouth (and therefore larger volume change) and generate weaker buccal pressure gradients whereas the former have smaller mouths (and less volume change) but generate larger pressure gradients (Higham et al., 2006a). These differences can be partially explained in terms of lever mechanics of the EP muscles and projected area of the mouth cylinder in the frontal plane (see Carroll et al., 2004). Because work is the product of buccal pressure and volume change, a fuller explanation for these morphological patterns may lie in the trade-off between pressure and

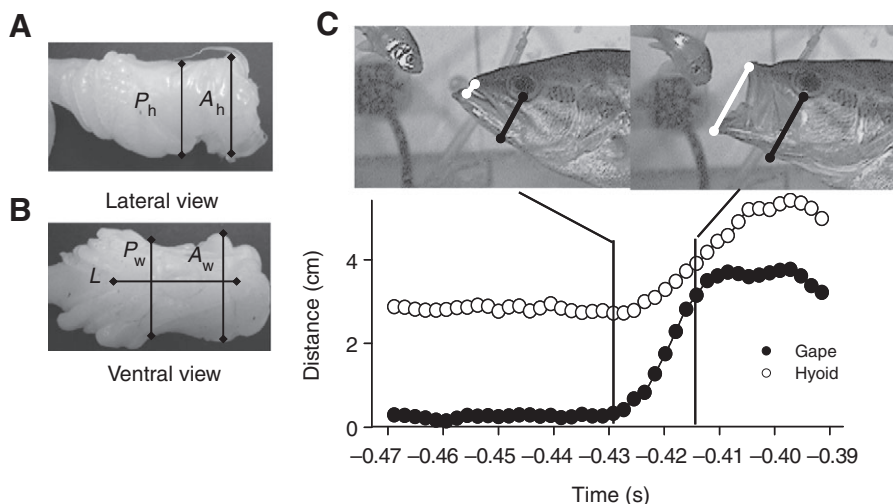


Fig. 1. Method for estimating internal buccal volume. External kinematics and buccal casts were used to estimate change in buccal volume. Length (L), anterior and posterior widths (A_w , P_w), and anterior and posterior height (A_h , P_h) were measured from buccal casts and used to estimate the volume at peak external kinematic displacement. The distance between the internal margins of the upper and lower jaws (gape, closed circles) and between the ventral margin of the eye and the hyoid (hyoid, open circles) were measured from lateral-view high-speed video (500 or 1000 Hz). The ventral margin of the eye is located near the dorsal margin of the buccal cavity in the frontal plane, making it a useful landmark. The algorithms used to convert these measures to volume at each time are given in the methods.

Table 1. Morphological parameters of individual fish for which performance variables were measured

Species	SL (cm)	Mass (g)	EP mass (g)	SH mass (g)	L (cm)	A _h (cm)	A _w (cm)	P _h (cm)	P _w (cm)	Vol. (cm ³)
<i>Micropterus salmoides</i>	20.5	180.0	19.5	0.9	3.8	3.0	2.5	2.8	2.6	21.8
<i>M. salmoides</i>	16.2	75.0	8.6	0.3	3.3	2.5	2.4	2.5	2.3	15.1
<i>M. salmoides</i>	22.0	190.0	22.3	0.7	4.0	2.6	3	3.4	3.7	31.6
<i>M. salmoides</i>	21.5	175.0	19.9	0.7	4.1	2.7	3.1	3.2	3.8	33.4
<i>Lepomis macrochirus</i>	15.5	140.0	15.9	0.7	2.8	1.5	1.2	1.4	1.4	4.1
<i>L. macrochirus</i>	8.9	24.0	2.2	0.0	1.8	0.8	0.6	0.7	0.7	0.7
<i>L. macrochirus</i>	12.0	60.0	5.4	0.1	2.5	1.2	0.9	1.2	1.1	2.4
<i>L. macrochirus</i>	14.3	90.0	9.2	0.0	3.2	1.1	1.2	1.1	1.4	3.5
<i>Lepomis cyanellus</i>	10.4	47.0	5.3	0.2	2.7	1.6	1.6	1.5	1.8	5.6
<i>L. cyanellus</i>	12.0	50.0	5.4	0.3	3.3	1.6	1.5	1.6	2.0	7.2
<i>L. cyanellus</i>	10.4	31.0	4.0	0.1	2.8	1.2	1.6	1.5	1.5	4.6
<i>L. cyanellus</i>	12.1	48.0	5.4	0.3	3.6	1.6	1.7	1.7	1.8	8.2
<i>L. cyanellus</i>	11.6	44.0	5.2	0.2	3.2	1.6	1.8	1.6	1.8	7.3

SL, standard length; EP, epaxial muscle; SH, sternohyoideus; L, buccal length; A_h, anterior height; A_w, anterior width; P_h, posterior height; P_w, posterior width.

volume for a given capacity to produce work, measurable as suction feeding muscle mass. In order to normalize for muscle mass and to produce a linear relationship, we divided the total muscle mass capable of driving suction feeding kinematics by buccal volume change (as estimated from post mortem buccal casts and external kinematics), and regressed mean subambient buccal pressure against this ratio. We hypothesized that individuals capable of generating large pressure magnitudes would be able to do so because of higher ratios of muscle mass to volume change and *vice versa*.

MATERIALS AND METHODS

Animals

The three species, largemouth bass (*Micropterus salmoides* Lacepede), bluegill (*Lepomis macrochirus* Rafinesque) and green sunfish (*Lepomis cyanellus* Rafinesque) were chosen to represent morphological disparity in centrarchid fishes. Largemouth bass exemplify a 'ram' morphology (Norton and Brainerd, 1993), bluegill a 'suction' morphology (Higham et al., 2006a; Norton and Brainerd, 1993) whereas green sunfish have relatively large mouths compared with other *Lepomis* species and are predicted to produce lower subambient pressure magnitudes (Collar and Wainwright, 2006), and therefore represent an intermediate morphology. Despite these differences in morphology all species are known to exhibit high performance suction feeding on elusive prey such as freshwater shrimp, nymphs, crayfish and smaller fish (Etnier and Starnes, 1993). Although fish species may vary in the amount of ram vs suction used to capture prey (Norton and Brainerd, 1993), ram-feeding teleosts still typically use hydrodynamic suction to draw prey into their buccal cavity during prey capture (Wainwright et al., 2001). Thus, the assumption was made that variation in feeding strategy should not affect the energetics of cranial expansion.

The size ranges of fish for which pressure and volume were measured are given in Table 1, and additional specimens were used to scale morphological variables (Table 2; Fig. 2). Fish were collected from Putah Creek, Yolo County, CA, USA (bluegill and largemouth bass) and Pleasant Creek, Solano County, CA, USA (bluegill and

green sunfish). Fish were housed in aquaria at 22–24°C on the campus of the University of California, Davis, CA, USA, and in accordance with U.C. Davis animal use and care protocols (#10211). Fish were maintained on live goldfish (*Carassius auratus*), earthworms (*Lumbricus* sp.), shrimp (*Palaeomonetes* sp.) and cut squid (*Loligo* sp.). Feeding discontinued at least 3 and as many as 6 days prior to surgery.

Surgery

A Millar SPR-407 microcatheter-tipped pressure transducer (Millar Instruments, Houston, TX, USA) was implanted in the buccal cavity of each fish under anesthetic. Surgery was performed at night, allowing fish to recover overnight, with experiments performed early the next day. Fish were anesthetized by exposure to 0.3 g l⁻¹ of KOH buffered MS-222 (tricaine methane sulfonate) and moved to a surgical tray containing freshwater. In no instance did surgery last long enough for the fish to recover from anesthetic before being returned to its home tank. To cannulate the buccal cavity, a 15-gauge needle was inserted into the fish's neurocranium rostral to the EP muscles but caudal to the ascending process of the premaxilla. The needle was entered at the midline to avoid the olfactory tract and associated vasculature but was inserted at a slight lateral angle, so that it entered the buccal cavity lateral to the vomer and parasphenoid bones. Care was taken to assure a similar position in each cannulated individual. The cannula itself was fashioned from PE-60 (smaller fish) or PE-90 (larger fish) tubing. This was heated with a soldering iron to form a wide flange on its distal (buccal) end. After the small piece of bone and tissue left in the needle was pushed out with a wire, the cannula tubing catheter was inserted dorsally through the needle, which was subsequently removed to leave the catheter behind, held in the buccal cavity by its flange on the dorsal surface of the cranium by a small piece of Tygon tubing (Cole-Parmer, Vernon Hills, IL, USA) glued on with cyanoacrylate. Thus attached, this cannula could be left in place for several days.

The pressure transducer had been previously passed through a smaller piece of Tygon tubing, just small enough to stretch tightly

Table 2. Scaling of morphological variables with respect to log-body mass

	Log epaxial mass					Log sternohyoideus mass					Log buccal volume				
	Slope	s.e.m.	Intercept	s.e.m.	P	Slope	s.e.m.	Intercept	s.e.m.	P	Slope	s.e.m.	Intercept	s.e.m.	P
<i>Micropterus salmoides</i> (N=9)	1	0.08	-0.97	0.17	<0.001	1.04	0.10	-2.49	0.22	<0.001	0.80	0.13	-0.38	0.29	<0.001
<i>Lepomis macrochirus</i> (N=13)	0.82	0.08	-0.64	0.14	<0.001	1.43	0.22	-3.34	0.41	<0.001	1.10	0.16	-1.71	0.30	<0.001
<i>Lepomis cyanellus</i> (N=14)	1.02	0.06	-0.99	0.09	<0.001	1.49	0.19	-3.14	0.31	<0.001	1.50	0.20	-1.78	0.31	<0.001

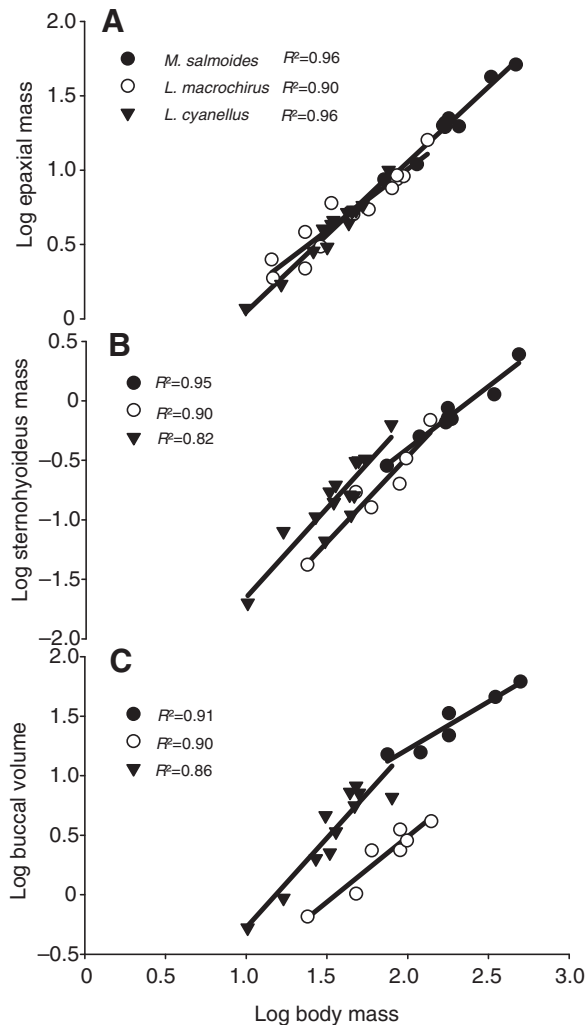


Fig. 2. Scaling of log sternohyoideus (SH) mass (g), log epaxial (EP) mass (g) and log buccal volume (cm^3) to body mass. All variables were significantly correlated ($P < 0.05$) with log mass within species. Scaling differences among species are described in the Results section and in Table 2.

around PE-60 or PE-90 tubing, and the proximal half of this tubing was filled with silicone sealant using a syringe. This arrangement both secured the transducer cable and sealed the tubing. The location of this tubing was such that when the transducer was threaded into the cannula, its sensing tip emerged into the buccal cavity. Prior to threading the transducer into the cannula, the cannula was filled with water using a syringe. Transducer signals were digitized using one channel of a TRX-8 conversion box (Sonometrics, London, Ontario, Canada) and recorded on a PC running SonoView Software. An additional channel recorded trigger voltages to the high-speed video camera and was used to synchronize pressure signals to video. All data were sampled at 600 Hz, and later values were interpolated using a spline-fitting function in a custom MatLab script (Mathworks, Natick, MA, USA) so they could be re-sampled (or interpolated) to the camera frequency (500 Hz or 1000 Hz).

Kinematics

External kinematics were recorded during feeding using a high-speed video system (NAC Memrecam ci, Tokyo, Japan). Videos were recorded at 500 Hz for larger fish and 1000 Hz for smaller, faster fish.

Only fully lateral sequences were analyzed. Square-pulse voltage from a custom post-trigger was used to save a sequence, and the voltage from this trigger was recorded alongside the pressure trace so that video and pressure signals could be synchronized. Every effort was made to elicit and record maximal performance, by using relatively large elusive prey (goldfish and shrimp), and by recording only the first few (<4) feedings in a 12 h period as performance dropped off precipitously with satiation (Lauder, 1980b).

Kinematic measurements were produced by digitizing the upper and lower jaws on their distal, inner margins, the lower margin of the eye and the hyoid (Fig. 1). Because the hyoid is not visible in the early phases of the strike, the spot on the lower jaw from which it would emerge was digitized. Gape distance is defined as the linear distance from the upper to lower jaw, and hyoid distance defined as the linear distance from the eye to the hyoid (Fig. 2). Kinematic measurements were scaled to maximum gape measured post-mortem. Digitization was accomplished using a MatLab script written by and available from Tyson Hedrick (<http://www.unc.edu/~thedrick/software1.html>, University of North Carolina, Chapel Hill, NC, USA).

Morphological measurements

Following data collection fish were killed with an overdose of MS-222. Fish were weighed to the nearest gram and standard length (SL) was measured to the nearest tenth of a centimeter. To estimate the amount of EP mass capable of dorsally rotating the head (and thus of contributing to suction feeding), fish were skinned on one side to expose the muscle, the head was then repeatedly rotated dorso-caudally while the myomeres of the EP were carefully observed. All fascicles that were observed to shorten were considered to have the potential to contribute to cranial rotation, although fascicles that inserted through the skin itself would have been missed. Fascicles found to shorten with rotation included those of the dorsal anteriorly projecting arm of the EP, extending caudally from the neurocranium to half the length of the soft dorsal fin. This estimation is consistent with regions found to be electrically active during feeding (Thys, 1997); however, muscle being active is not necessarily an indication that it produces work, and it is not clear from Thys that more caudal EP muscles are not involved (Thys, 1997). Some of these fibers appear to contribute to cranial rotation *via* the horizontal septum (Gemballa et al., 2003). Nevertheless, each half of the EP mass was estimated and weighed to the nearest tenth of a gram and these weights were combined. The mass of the SH was also weighed to the nearest tenth of a gram and these weights were summed to estimate combined individual suction feeding muscle mass.

After masses were measured, the buccal cavity of each fish was filled with commercial silicone caulk (cf. Norton, 1995) and allowed to cure. The maximal dimensions of the buccal cavity were measured from landmarks impressed in the silicon cast (Fig. 1). These were the anterior height [$A_{h(\text{cast})}$, from upper jaw to lower jaw], posterior height [$P_{h(\text{cast})}$, basihyal to roof of the buccal cavity], anterior width [$A_{w(\text{cast})}$, anterior-most width], posterior width [$P_{w(\text{cast})}$, width at approximate location of the interhyal/symplectic joint] and buccal length [$L_{(\text{cast})}$, from fifth or caudal-most basibranchial to front of the mouth] (Fig. 1).

Volume estimation

These buccal measurements were used to estimate the absolute internal kinematics of the buccal cavity. The first assumption made was that external movements of a skeletal structure reflected its internal movement. A second set of assumptions was based on Sanford and Wainwright (Sanford and Wainwright, 2002). Their

study used sonomicrometric crystals to measure the vertical distance between the upper and lower jaws, the rotation and translation of the hyoid, and the lateral expansion of the buccal cavity in largemouth bass. The results of this study were cautiously generalized to all species in this study to estimate two key unknowns: the temporal relationship between buccal height and buccal width, and the relative starting position of the hyoid. The anterior and posterior buccal widths were estimated to begin at 2/3 their maximum and expand throughout the strike to reach a maximum concurrent with maximum gape and maximum hyoid displacement, respectively (Sanford and Wainwright, 2002) (and A.M.C., unpublished data). The hyoid was assumed to begin at 21% of its maximum value (Sanford and Wainwright, 2002). Prior to becoming visible the hyoid was assumed to start with and expand in proportion to gape (Sanford and Wainwright, 2002). After the hyoid became visible, it was assumed to expand in proportion to its maximum displacement as measured from video.

Thus, the equation for anterior height for time t was given by:

$$A_h(t) = [\text{gape}(t) / \text{gape}_m] \times A_{h(\text{cast})}, \quad (1)$$

where $A_h(t)$ is the estimated buccal anterior height, $\text{gape}(t)$ is the measured gape from the video, $A_{h(\text{cast})}$ is the measured anterior height of the buccal cavity from the cast and gape_m is the maximum measured distance from the upper and lower jaws at time. Likewise, the posterior height prior to the hyoid becoming visible was given by:

$$P_h(t) = [\text{gape}(t) / \text{gape}_m] \times P_{h(\text{cast})} + 0.21 \times P_{h(\text{cast})}, \quad (2)$$

where $P_h(t)$ is the estimated posterior height at time t , $P_{h(\text{cast})}$ is the measured height of the buccal cavity from the cast. After the hyoid became visible the following formula was used:

$$P_h(t) = [\text{hyoid}(t) / \text{hyoid}_m] \times P_{h(\text{cast})}, \quad (3)$$

where $\text{hyoid}(t)$ was the measured hyoid depression at time t and hyoid_m is the maximum hyoid displacement measured from the video. The anterior and posterior widths [$A_w(t)$ and $P_w(t)$, respectively] were given by the following equations:

$$A_w(t) = [\text{gape}(t) / \text{gape}_m] \times A_{w(\text{cast})} \times 1/3 + 2/3 \times A_{w(\text{cast})} \quad (4)$$

$$P_w(t) = [\text{gape}(t) / \text{gape}_m] \times P_{w(\text{cast})} \times 1/3 + 2/3 \times P_{w(\text{cast})}. \quad (5)$$

Any changes associated with buccal length, for instance those associated with jaw protrusion, were ignored because it was not clear how they would contribute to the energetic cost of suction feeding as water incorporated into the buccal cavity by rostral translation of the upper and lower jaw is not moved in the fish's frame of reference.

From the preceding equations means of buccal height and buccal width at each frame were calculated and these were used to calculate buccal volume [$\text{Vol.}(t)$] with the following equation of general elliptical cylindrical volume:

$$\text{Vol.}(t) = L_{(\text{cast})} \times [\text{mean height}(t) / 2] \times (\text{mean width}(t) / 2) \pi, \quad (6)$$

where $L_{(\text{cast})}$ is the measured length of the buccal cast (Fig. 1). The final volume in all cases should be equal to the dimensions of the cast. Therefore, these equations depend on a given fish achieving peak buccal dimensions in each measured strike. Volumes were given in cubic centimeters for heuristic convenience but were converted to cubic meters in work and power calculations.

Suction feeding work and power

Mechanical work (J) was calculated by numerical integration of pressure with respect to volume for each time point, from the onset

of gape opening until approximately peak volume (Fig. 3). Although pressure may remain below ambient after peak volume is achieved (Fig. 3), no work can be done if volume does not change. Work was divided by duration to generate power (W) and both values were divided by total suction muscle mass to estimate normalized work and power (J kg^{-1} and W kg^{-1}). It should be noted that the work and power estimated produced by these methods are only valid for maximal work and power because they rely on maximal buccal volume measured post mortem. The potential for energetic modulation could not be investigated by these methods, as it was in Carroll and Wainwright (Carroll and Wainwright, 2006).

Statistical analyses

Three feedings were selected and digitized for each fish, based on quality of video, kinematic excursion and pressure magnitude. The means of these three feedings for each individual were analyzed as a single data point per individual. Individuals were treated as independent observations, and regressions among all individuals were generated for all traits except morphological data (for which there were many more individuals available) (Fig. 2). Species effects and non-independence of species might have affected the results due to differences in mass-specific work or power output [which were not detected between bluegill and largemouth bass *in vitro* (Carroll et al., 2009)], differences in internal dimensions or differences in muscle functional patterns.

Regression analysis was used to determine the relationship between muscle mass and suction feeding work and power and to test for trade-offs between mean pressure and volume change. Regressions were performed in JMP 4.0 (SAS Institute, Cary, NC, USA). In order to assess differences in morphological traits among species, EP mass, SH mass and buccal cast volume were log-transformed and analyses of covariance (ANCOVAs) were performed with log-transformed body mass as a covariate and species as a fixed categorical factor. Tukey HSD *post-hoc* tests with a starting α level of 0.05 were used to detect differences among species in all parameters.

RESULTS

Morphological measurements

SH mass was much smaller than EP mass for all individuals and species. For largemouth bass it was only $3.9 \pm 0.3\%$ of EP mass, for bluegill it was $3.0 \pm 0.2\%$ and for green sunfish it was $4.0 \pm 0.4\%$ (mean of individual means \pm s.e.m., unless otherwise noted) with no significant differences found among species in ANCOVA ($P > 0.05$).

SH and EP mass were found to scale with body mass (Fig. 2A,B; Table 2). In largemouth bass the scaling of the EP and SH did not differ from isometry. Bluegill showed significant negative allometry in the EP and positive allometry in the SH. Green sunfish showed isometry of the EP and positive allometry of the SH. After ANCOVA was used to account for the effects of body size, the EP and SH were found to differ in mass between species ($P < 0.05$ for the EP; $P < 0.01$ for the SH), with green sunfish having significantly larger EP than bluegill (but not bass) in *post-hoc* tests.

Buccal volume scaled with body mass (Fig. 2C) in all species but there were differences in scaling pattern. Bass were found to display a slight negative allometry in this parameter. Bluegill displayed an isometric scaling pattern, and green sunfish displayed a positive allometric pattern (Table 1). When ANCOVA was used to account for the effects of body size, species was found to have a significant effect on buccal volume ($P < 0.001$), and bluegill were found to have a significantly smaller volume than green sunfish and largemouth bass in *post-hoc* tests.

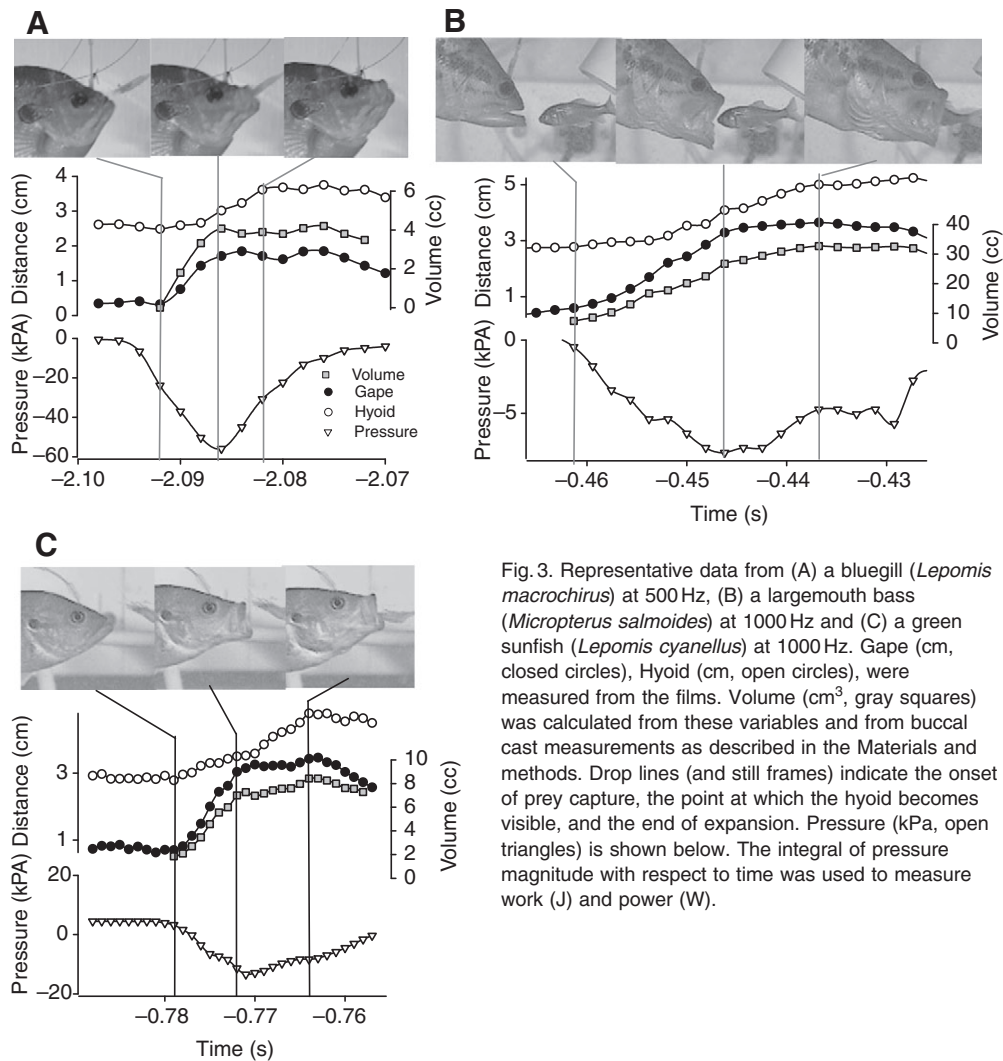


Fig. 3. Representative data from (A) a bluegill (*Lepomis macrochirus*) at 500 Hz, (B) a largemouth bass (*Micropterus salmoides*) at 1000 Hz and (C) a green sunfish (*Lepomis cyanellus*) at 1000 Hz. Gape (cm, closed circles), Hyoid (cm, open circles), were measured from the films. Volume (cm³, gray squares) was calculated from these variables and from buccal cast measurements as described in the Materials and methods. Drop lines (and still frames) indicate the onset of prey capture, the point at which the hyoid becomes visible, and the end of expansion. Pressure (kPa, open triangles) is shown below. The integral of pressure magnitude with respect to time was used to measure work (J) and power (W).

Total muscle mass was found to be $11.8 \pm 0.3\%$ of body mass in largemouth bass, $10.4 \pm 0.5\%$ in bluegill and $12.1 \pm 0.3\%$ in green sunfish. However, statistical differences were only detected between green sunfish and bluegill in *post-hoc* tests. The ratio of buccal volume to combined suction feeding muscle mass varied among species. Largemouth bass had significantly higher volume per muscle mass ($1.27 \pm 0.11 \text{ cc g}^{-1}$) than green sunfish ($0.86 \pm 0.10 \text{ cc g}^{-1}$) and both were significantly higher than bluegill ($0.3 \pm 0.03 \text{ cc g}^{-1}$).

Individual performance

Representative profiles of gape and hyoid distances, measured pressure and measured volume are given in Fig. 3. Species means (mean of individual maximal peak pressures \pm s.e.m.) were $41 \pm 6 \text{ kPa}$ for bluegill, $18 \pm 2 \text{ kPa}$ for green sunfish and $17 \pm 4 \text{ kPa}$ for largemouth bass (see Table 3 for full data set). Maximum peak pressures were similar to those described in other studies (Carroll, 2004; Grubich and Wainwright, 1997; Lauder, 1980b), and, thus, it appears that maximal performance was achieved in this study.

Work and power

Combined individual muscle mass was significantly correlated with suction feeding work output among individuals ($P < 0.001$; $R^2 = 0.77$) (Fig. 4A) as was suction feeding power ($P < 0.01$; $R^2 = 0.59$) (Fig. 4B). The slope of the regression between muscle mass and

work output was estimated at $11 \pm 2 \text{ J kg}^{-1}$. The slope of the regression between muscle mass and power output was estimated at $300 \pm 75 \text{ W kg}^{-1}$. The link between suction feeding muscle mass and energetic output appears to be driven by the relationship between suction feeding muscle mass and change in volume ($P < 0.001$; $R^2 = 0.71$) (Fig. 5A), because there was no relationship between muscle mass and mean subambient pressure magnitude ($R < 0.01$) (Fig. 5B). Power appears to have been decreased in larger individuals due to an increase in kinematic duration with muscle mass ($P < 0.05$; $R^2 = 0.49$) (Fig. 5C).

Trade-offs between pressure and volume change

There was a weak and non-significant trade-off ($P = 0.08$; $R^2 = 0.24$) (Fig. 6A) between volume change and mean pressure. Such a direct trade-off is only predicted, however, for fish with the same muscle mass and thus the same work output. In addition, the predicted relationship is inverse rather than linear. If work output is equal to the change in volume multiplied by mean pressure and is proportional to muscle mass:

$$\text{muscle mass} = \mathbf{k} \times \Delta \text{volume} \times \text{mean pressure}. \quad (7)$$

In that case, a predicted trade-off curve for a given muscle mass can be plotted if muscle work output per unit mass (\mathbf{k}) is estimated at, for instance, $\sim 10 \text{ J kg}^{-1}$. Plotting such isoclines for 0.002 and

Table 3. Performance variables estimated for all individual fish used in this study

Species	Mass (g)	Muscle mass (g)	Δ vol. (cm ³)	Mean pressure (kPa)	Mean peak pressure (kPa)	Max. peak pressure (kPa)
<i>Micropterus salmoides</i>	180.0	21.3	22.4	9.1	12.2	24.3
<i>M. salmoides</i>	75.0	9.2	17.7	3.1	7.5	7.5
<i>M. salmoides</i>	190.0	23.7	32.8	7.9	17.4	23.6
<i>M. salmoides</i>	175.0	22.2	31.8	8.9	15.0	22.3
<i>Lepomis macrochirus</i>	140.0	17.0	3.6	23.7	42.8	55.3
<i>L. macrochirus</i>	24.0	2.3	0.6	20.9	40.1	47.2
<i>L. macrochirus</i>	60.0	5.7	2.4	12.0	25.1	33.7
<i>L. macrochirus</i>	90.0	9.2	3.5	12.6	21.0	29.8
<i>Lepomis cyanellus</i>	47.0	5.6	5.6	9.0	14.5	18.0
<i>L. cyanellus</i>	50.0	6.0	7.1	7.4	12.4	17.9
<i>L. cyanellus</i>	31.0	4.2	5.8	12.7	18.3	19.0
<i>L. cyanellus</i>	48.0	6.0	7.1	7.3	10.0	12.4
<i>L. cyanellus</i>	44.0	5.5	7.2	9.5	16.6	22.6

0.02 kg total muscle mass (Fig. 6A) illustrates how the relationship between volume and pressure might depend on muscle mass.

Nevertheless, it would be useful to fit a linear relationship to the relationship between volume change and mean pressure and to compare among individuals. Therefore, the ratio of muscle mass to volume change (kg l⁻¹) was calculated and mean pressure was regressed against this parameter for each individual. It was found that predicted mean pressure (kPa) changed among individuals ($P < 0.001$; $R^2 = 0.67$) (Fig. 6B). A weaker relationship was found between mean pressure (kPa) and the ratio of muscle mass to change volume over time (kg s l⁻¹) ($P < 0.05$; $R^2 = 0.64$). Thus, a strong, direct trade-off between buccal subambient pressure and volume change per time was found when muscle mass was taken into account.

DISCUSSION

The results of this study are consistent with the hypothesis that suction feeding energetic output, including both work and power, is limited by suction feeding muscle mass. Muscle work (subambient buccal pressure magnitude integrated with respect to volume change) was significantly correlated with muscle mass (Fig. 4A) as was suction feeding power (Fig. 4B). The muscle mass-specific work and power predicted by this relationship were found to be $11 \pm 2 \text{ J kg}^{-1}$ and $300 \pm 75 \text{ W kg}^{-1}$, respectively. These relationships appear to be driven more by variation in volume change than in mean pressure among the individuals used in this study. Finally, when volume change was normalized to muscle mass (by dividing muscle mass by volume change) it was found to significantly predict mean pressure. Thus, for a given muscle mass, there is a direct trade-off between volume change and muscle mass.

This study suggests some interesting implications of design in suction-feeding fishes. First, fish that feed on elusive prey must

devote muscle mass to cranial expansion. This need not necessarily reduce mass devoted to, or otherwise compromise, locomotor performance (Wakeling and Johnston, 1998) because much of the dorsal EP appears to serve a role in both feeding and C-starts (Thys, 1997). Second, a large amount of axial muscle (~10% of total mass) (Fig. 2A) appears to be devoted to cranial rotation in centrarchid fishes, and much of this mass is located extrinsic to the cranium. Intrinsic cranial muscles, such as the levator arcus palatini and SH, although active in feeding (Grubich and Wainwright, 1997), are not of sufficient mass to power suction feeding alone. Finally, suction-feeding fish, having committed mass to suction feeding, can use that mass either to produce volume change or subambient pressure but cannot simultaneously maximize both.

The results of this study should be cautiously interpreted in light of the absence of internal kinematics in bluegill and green sunfish. The equations used to estimate volume are based on measurements from largemouth bass (Sanford and Wainwright, 2002). Variation in the relative starting point and movement of the hyoid structure, and lateral displacement of the suspensoria might affect the relative change in volume and increase or decrease work and power output. Of these, the calculations of power are more likely to be error-prone because they are based on the estimated time-course of internal buccal dimensions. Work is more reliably estimated because it depends only on the accurate measurement of pressure and estimation of the starting and final buccal volumes, the latter of these we expect to be equivalent to buccal cast volume for maximal performance strikes, which were measured for each individual. Finally, inclusion of the hypaxial muscle mass would have significantly lowered work and power estimates to $\sim 9.5 \text{ J kg}^{-1}$ and $\sim 250 \text{ W kg}^{-1}$, if hypaxial mass is ~20% of other muscle mass.

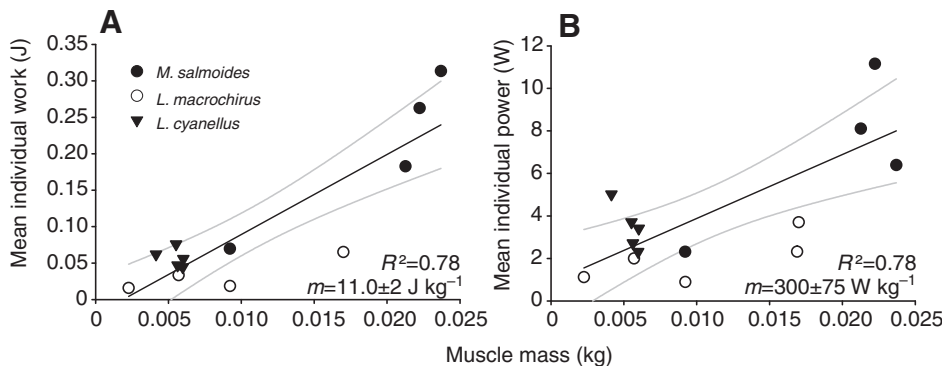


Fig. 4. The relationship between suction feeding muscle mass (kg) and mean work (Joules, A) and mean power (Watts, B) for individual fish. Data points represent means of at least three presumed-maximal strikes from each fish. Largemouth bass are indicated by closed circles, bluegill by open circles and green sunfish by closed triangles. Both energetic variables were strongly related to muscle mass with slopes ($11 \pm 2 \text{ J kg}^{-1}$ and $300 \pm 75 \text{ W kg}^{-1}$) that are realistic with respect to muscle physiology and suction feeding hydrodynamics. Nevertheless, the limited species overlap suggests that the data should be interpreted cautiously.

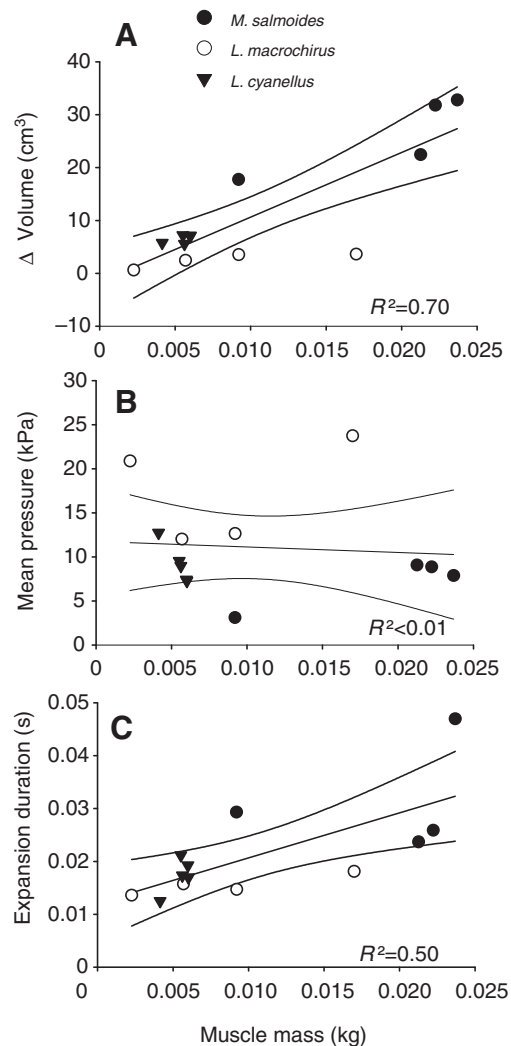


Fig. 5. Determinants of work and power output for individual fish in this study. Mean volume change (cm^3 , A), pressure (kPa, B) and expansion duration (s, C) for each fish were regressed against muscle mass. No relationship was found between pressure and muscle mass ($P>0.5$), suggesting that volume change (in which a significant relationship was found, $P<0.001$) drove the relationship between muscle mass and work output. There was an increase in duration of expansion with muscle mass ($P<0.001$) that would have lowered relative power output in larger animals.

Determinants of work output

Consistent with other studies (e.g. Carroll et al., 2004), considerable interspecific variation in subambient pressure generation capacity was observed in this study (Fig. 5B). However, this variation was not correlated with muscle mass and thus does not appear to influence the relationship between muscle mass and suction feeding work or power output. Rather, variation in buccal volume change with size appears to drive observed variation in work output. Some of this variation is a consequence of change in body size, so it would be informative to have interspecific overlap in body size, buccal volume and muscle mass.

Scaling of muscle energetic capacity

In this study bluegill relative mouth volume increased with size while EP volume increased (Table 2). This increase in buccal volume per muscle mass would be expected to decrease subambient pressure

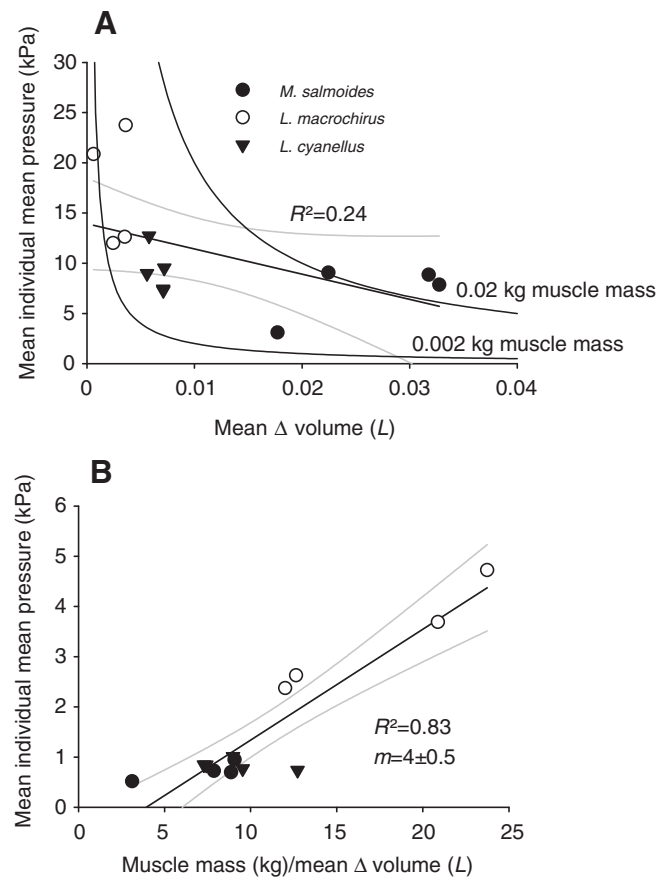


Fig. 6. Trade-off between pressure and volume. In (A) mean individual mean buccal subambient pressure magnitude (kPa) is regressed against change in volume (L). Although a weak relationship is observed between pressure and volume ($P=0.08$), a tight linear relationship is not necessarily expected because of differences in muscle mass and the hyperbolic relationship between pressure and volume of a pump (see text for explanation). To illustrate, lines indicating expected pressure vs volume relationship for muscle mass are shown. These lines are generated by assuming a mass-specific work of 10 J kg^{-1} , and represent realistic minimum (2 g) and maximum (20 g) muscle mass found in the individuals of this study (Table 1). These lines illustrate the need to take muscle mass into account when comparing pressure and volume as is done in (B). In B mean pressure magnitude was regressed against the ratio of muscle mass to change in volume ($m/\Delta \text{vol.}$). This relationship was found to be both linear and highly significant ($P<0.001$).

with size (Fig. 6B). (Due to its small relative size, Table 1, scaling of the SH is ignored in this discussion.) Carroll et al. found a decrease in measured peak pressure with size in bluegill (Carroll et al., 2004). Over a broader range of size, however, predicted pressure and peak flow velocity (which is correlated with pressure) (Higham et al., 2006b) were found to increase with size in bluegill (Holzman et al., 2008b). It is possible that a broader size range of buccal pressure measurement might resolve these discrepancies in bluegill.

Largemouth bass have been generally found to scale isometrically in many aspects of their feeding system (Richard and Wainwright, 1995). In this study, the buccal volume showed a slight negative allometry that would be expected to increase suction pressure with size. However, no such trend in pressure has been found over a broad range of sizes (Carroll et al., 2004). This trend might be explained if there were a subtle increase in the actual buccal volume change (as opposed to that of the cast) with size. Again, a broader

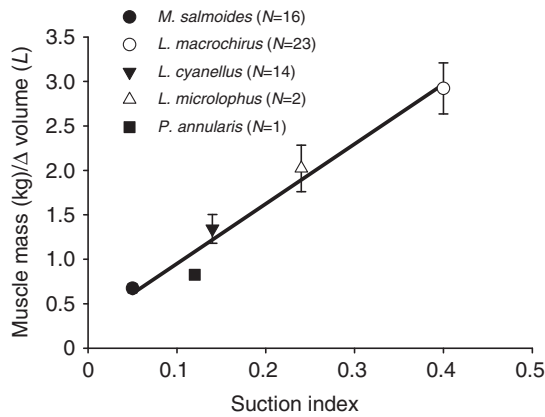


Fig. 7. Relationship between ratio of muscle mass to volume change ($m/\Delta vol.$) and suction index, an independent predictor of mean pressure for individuals of five species of centrarchid fish. Values of suction index are taken from Collar and Wainwright (Collar and Wainwright, 2006). Species and numbers of individuals are indicated on the graph. These data suggest a strong relationship between suction index and $m/\Delta vol.$ among species and supports the suggestion (Fig. 6) that $m/\Delta vol.$ is also a predictor of subambient buccal pressure generation. *M. salmoides*, *Micropterus salmoides*; *L. macrochirus*, *Lepomis macrochirus*; *L. cyanellus*, *Lepomis cyanellus*; *L. microlophus*, *Lepomis microlophus*; *P. annularis*, *Pomoxis annularis*.

range of buccal cast and muscle mass measurements is needed to address these discrepancies. To our knowledge, no scaling data exists for green sunfish with which to compare our results.

Mechanical power output appears to have been reduced as muscle mass increased due to slowing of kinematics (Fig. 5C). This trend is likely due to a general decrease in kinematic speed with body size among suction feeding fish (e.g. Wainwright and Shaw, 1999). This trend is consistent with a general slowing of muscle with size that is observed in other behaviors (e.g. Altringham and Johnston, 1990) but contradicts direct measurements of scaling of muscle kinetics in largemouth bass and bluegill (Carroll et al., 2009), in which no slowing of muscle shortening velocity with size was found in the regions of EP muscles involved in feeding and measured in the present study. Slowing of kinematics with size may be explained in bluegill by an increasing ratio of EP input lever length to output lever length (Holzman et al., 2008b), leading to more force and less speed transfer with size (also increasing pressure in the buccal cavity). In the more isometric largemouth bass, slowing kinematics could result from slowing relative fascicle velocity with speed, which would increase muscle stress (Hill, 1938) and pressure output. However, no such increases in pressure with size were observed in largemouth bass over a relatively large size range (Carroll et al., 2004). No scaling data on muscle functional variables *in vivo* is currently available for green sunfish.

Trade-off between volume and pressure

One of the strongest results of this study is the trade-off between pressure and volume change predicted by the energetic limitation on suction feeding hydrodynamics (Eqn 7) and borne out by the data (Fig. 6B). The existence of a 'ram' feeding ecomorph, exemplified by largemouth bass, and a 'suction' feeding morph, like bluegill, has been observed in numerous studies (e.g. Norton and Brainerd, 1993; Werner et al., 1977; Winemiller, 1991). Carroll (Carroll, 2004) suggested that this trade-off had a basis in the force balance during neurocranial rotation: fish with wider mouths could exert less

pressure for a given cross-sectional area of muscle and lever ratio. The current study extends these earlier observations and suggests a more comprehensive explanation. Because work equals the integral of pressure with respect to volume change, work capacity can be used to generate pressure or volume change but both cannot be simultaneously maximized (Vogel, 1994). Furthermore, the trade-off between pressure and volume is not obvious when work generation capacity (i.e. muscle mass) is not taken into account (Fig. 6A). In other words there is no absolute trade-off between pressure and volume. A fish could increase volume change without reducing pressure generation by simply growing (isometrically) or devoting more muscle cross-sectional area to feeding. Rather, the trade-off is between pressure and the volume change for a given muscle mass-specific work. This effect is expressed by the relationship between the ratio of muscle mass to volume change (henceforth $m/\Delta vol.$) and mean pressure (Fig. 6B).

Carroll et al. found a relationship between peak pressure and the ratio of the product of EP muscle cross-sectional area and lever ratio (in-lever/out-lever) to buccal cross-sectional area (Carroll et al., 2004). If muscle stress is constant this morphological parameter, currently termed Suction Index (SI), will predict suction pressure. If the relationship between $m/\Delta vol.$ found in the current study is valid, it ought to be consistent with that predicted by SI. SI was reported for all centrarchid species (Collar and Wainwright, 2006), and using those values we regressed mean species $m/\Delta vol.$ against mean species SI (Fig. 7). This comparison was made using several individuals of the species used in this study, as well as additional species of redear sunfish (*Lepomis microlophus* Guenther) and white crappie (*Pomoxis annularis* Rafinesque) (Fig. 7). The significant ($P < 0.001$) relationship between SI and $m/\Delta vol.$ suggests a strong agreement between these independent estimates of buccal subambient pressure.

The relationship between SI and buccal pressure is based on the stress produced by muscles. This stress depends on how muscles function and, in particular, the ratio of contraction velocity to maximal or unloaded velocity, which also changes the mass-specific power muscle can produce (Hill, 1938). Thus, changes in muscle function would change the relationship between SI and the ratio of muscle mass to volume change. For instance, a fish with EP muscles that contract isometrically (without shortening) would be able to produce a much greater peak pressure for a given cross-sectional muscle area but there would be no relationship between the ratio of $m/\Delta vol.$ and pressure because mass-specific muscle work (for the EP) is zero.

Validation of work and power values

One of the limitations of the current study is that the lack of broad intraspecies variation and interspecies overlap (e.g. Fig. 4) means that the larger largemouth bass may have a disproportionate leverage on the slope of estimated work and power curves. Therefore, it would be informative to compare the estimated work and power values ($\sim 11 \text{ J kg}^{-1}$ and $\sim 300 \text{ W kg}^{-1}$) with published values. As this study purported to measure maximal performance, these results should be compared with maximal values in other studies. The differences between maximal and mean values for suction feeding performance can be quite profound (Carroll and Wainwright, 2006) and depend on the number of feedings recorded in an experimental session (Lauder, 1980b). For instance, mean EP muscle work during shortening for an individual largemouth bass was found to be as low as 5.7 J kg^{-1} (129 W kg^{-1}) but maximal power was found to be as much as 13.1 J kg^{-1} (330 W kg^{-1}) (Carroll and Wainwright, 2006). As can be seen, though, the maximal values from their study are

consistent with the estimates obtained in the present study. Maximal power values are reported for *in vitro* muscle for isolated largemouth bass EP muscle fascicles ($\sim 280 \text{ W kg}^{-1}$) (Coughlin and Carroll, 2006) and are similar to maximal values found for bluegill EP muscle fascicles (Carroll et al., 2009). Thus, *in vivo* and *in vitro* maximal values for centrarchids broadly agree with those reported in this study.

Several digital particle image velocimetry (DPIV) studies have reported values of pressure and flow velocity for both bluegill and largemouth bass. If mouth area is estimated these values can be used to estimate power output as the product of mouth area, mean pressure and mean flow velocity, assuming a constant mouth area. For instance, Higham et al. measured peak flow and peak pressure for both largemouth bass and bluegill (Higham et al., 2006b). Bluegill in Higham et al. (Higham et al., 2006b) exceed the size range found in the present study, but for a 18 cm largemouth bass maximal values of peak pressure and flow in this study were $\sim 10 \text{ kPa}$ and 2 ms^{-1} , respectively. (Flow values are necessarily reported at the mouth aperture.) If the radius of the mouth is assumed to be 1.6 cm (Table 1), the area of the mouth will be $8 \times 10^{-4} \text{ m}^2$. If total muscle mass is estimated at 12 g (Table 2), and it is assumed that mean pressure values are $\sim 1/2$ peak values (Table 3), then the estimated power during the strike is estimated at 334 W kg^{-1} . This value is quite similar to the mean normalized mass-specific power reported here.

Overall, despite the relative simplicity of the assumptions made in this study (particularly the estimation of muscle mass and change in buccal volume), the estimated intraspecific work and power values (Fig. 4) appear to be reasonable in light of published data on centrarchid suction feeding muscle physiology (*in vivo* and *in vitro*) and suction feeding hydrodynamics.

Muscle mass as a predictor of suction feeding performance

If suction feeding performance is related to change in volume and pressure gradient (Fig. 4), then it may be possible, based on the results of this study and its hydrodynamic underpinning, to use muscle mass as a predictor of suction feeding performance and, by extension, a predictor of species or individual performance in an ecological context. Fish that feed on elusive or evasive prey, like those in this study, would be expected to have higher ratios of muscle mass to body mass than those who require less pressure or volume change to feed. By measuring buccal volume as well, it would be possible to further evaluate a predator's ability to generate volume change or buccal subambient pressure.

LIST OF ABBREVIATIONS

$A_{h(\text{cast})}$	anterior height of the buccal cavity from the cast
$A_h(t)$	estimated anterior height at time t
$A_w(t)$	estimated anterior width at time t
$A_{w(\text{cast})}$	anterior width of the buccal cavity from the cast
EP	epaxial muscles
gape_m	maximum distance from the upper and lower jaws from video
$\text{gape}(t)$	gape at time t from video
hyoid(t)	hyoid depression at time t from video
hyoid $_m$	maximum hyoid displacement from video
$L_{(\text{cast})}$	buccal length
$P_{h(\text{cast})}$	posterior height of the buccal cavity from the cast
$P_h(t)$	estimated posterior height at time t
$P_w(t)$	estimated posterior width at time t
$P_{w(\text{cast})}$	posterior width of the buccal cavity from the cast
SH	sternohyoideus
SI	suction index
SL	standard length
t	time
$\text{vol.}(t)$	estimated buccal volume at time t

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