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Author for correspondence:
Yordano E. Jimenez
e-mail: yordano_jimenez@brown.edu

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Dual function of epaxial musculature for swimming and suction feeding in largemouth bass

Yordano E. Jimenez and Elizabeth L. Brainerd

Department of Ecology and Evolutionary Biology, Brown University, Providence, RI, USA

YEJ, 0000-0001-5200-399X

The axial musculature of many fishes generates the power for both swimming and suction feeding. In the case of the epaxial musculature, unilateral activation bends the body laterally for swimming, and bilateral activation bends the body dorsally to elevate the neurocranium for suction feeding. But how does a single muscle group effectively power these two distinct behaviours? Prior electromyographic (EMG) studies have identified fishes' ability to activate dorsal and ventral epaxial regions independently, but no studies have directly compared the intensity and spatial activation patterns between swimming and feeding. We measured EMG activity throughout the epaxial musculature during swimming (turning, sprinting, and fast-starts) and suction feeding (goldfish and pellet strikes) in largemouth bass (*Micropterus salmoides*). We found that swimming involved obligate activation of ventral epaxial regions whereas suction feeding involved obligate activation of dorsal epaxial regions, suggesting regional specialization of the epaxial musculature. However, during fast-starts and suction feeding on live prey, bass routinely activated the whole epaxial musculature, demonstrating the dual function of this musculature in the highest performance behaviours. Activation intensities in suction feeding were substantially lower than fast-starts which, in conjunction with suboptimal shortening velocities, suggests that bass maximize axial muscle performance during locomotion and underuse it for suction feeding.

1. Introduction

For over 500 million years, axial musculature has been integral to the undulatory locomotion of fishes. After 200 million years of functioning as a locomotor structure [1,2], many fishes co-opted the axial musculature for high-performance suction feeding [3–5] and integrated the cranial feeding apparatus with the post-cranial musculature. Although outsourcing power to the trunk muscles enabled fishes to generate powerful cranial expansion, it placed new mechanical demands on the axial musculature since, unlike locomotion, which involves lateral flexion of the trunk and tail, suction feeding involves dorsiflexion of the head and caudoventral rotation of the pectoral girdle. So how do fish modulate contraction patterns of the axial musculature to produce both swimming and suction feeding?

One possibility is that each of the two behaviours uses different regions within the epaxial and/or hypaxial musculature. In this study, we focused on the epaxial musculature, but a parallel study of hypaxial musculature, particularly in a species that relies heavily on hypaxial musculature for suction feeding (e.g. catfishes) [6] would also be interesting. For epaxial regionalization, a common view has been that the epaxial musculature just behind the head generates power for suction feeding [7–9], while the remainder of axial musculature is used primarily for locomotion [10–12]. It was only recently discovered that the axial musculature in some fishes generates over 95% of the power for suction feeding [13,14]. This expansion power originates from muscle shortening in the cranial two-thirds of the body, which comprises over 70% of the white axial muscle mass

[15]. Consequently, swimming and suction feeding appear to use much of the same muscle mass to generate power, challenging the notion of a craniocaudal division of labour.

We reviewed prior studies on the activation of the epaxial musculature in swimming and suction feeding in largemouth bass [16,17] and synthesized their findings to develop a new hypothesis. We propose that fishes modulate muscle function by selectively activating ventral regions of the epaxial musculature for swimming and dorsal regions for suction feeding. We developed this hypothesis based on the following electromyographic (EMG) evidence. In bass, suction feeding always activated muscle in the dorsal epaxial regions between 0.15 and 0.25 body lengths (BL), while the ventral regions remained inactive [17]. By contrast, burst swimming (i.e. locomotor behaviours that activate the white musculature) always activated muscle in the ventral epaxial regions near the tail at approximately 0.75 BL [16]. Activation of dorsal epaxial regions seemed to depend on the type of burst swimming [16]. For example, fishes consistently activated all epaxial regions when performing rapid escape and predatory manoeuvres (i.e. fast-starts); however, they activated only the ventral epaxial regions for sprinting, leaving the dorsal regions inactive ([16]; also see [12] for similar results in trout). These studies speculated that differences between sprints and fast-starts produced these recruitment patterns, but they did not quantify the relationship between performance and muscle activation patterns.

These studies provide a glimpse into the potential contributions of motor control to the dual function of the epaxial musculature, but several factors preclude our ability to draw conclusions from the existing data with confidence. Firstly, the swimming studies did not measure how frequently muscle activity was observed in different regions. Secondly, Thys [17] did not measure suction feeding performance, and the swimming studies did not measure and compare performance within and between locomotor behaviours. Finally, methodological differences among these studies prevent quantitative comparisons of the intensity and spatial patterns of activation between swimming and suction feeding (e.g. electrode construction, different animals, different sampling distributions).

The goal of this study is to test our functional regionalization hypothesis by quantifying and comparing the regional contributions of the axial musculature to burst swimming and suction feeding in largemouth bass. Using electromyography, we measured electrical activity in the epaxial musculature to identify which regions are active during low- and high-performance locomotion and feeding. Electrodes were implanted in the dorsal and middle positions of the epaxial musculature in three longitudinal positions known to generate power for swimming and/or suction feeding. An additional electrode was placed in the ventralmost epaxial region of the cranialmost and midbody positions (figure 1*a*). We also quantified suction feeding performance with an intraoral pressure transducer and evaluated swimming performance with high-speed video.

2. Material and methods

(a) Animals

Bass were purchased from Hickling's Fish Farm located in Edmeston, New York. Fish were kept at Brown University and fed a mixed diet of goldfish and carnivore pellets. Standard lengths for bass 01, 02, and 04 were 245 mm, 247 mm, and 259 mm, respectively. All husbandry and experimental procedures complied with Brown University IACUC protocol #1509000157.

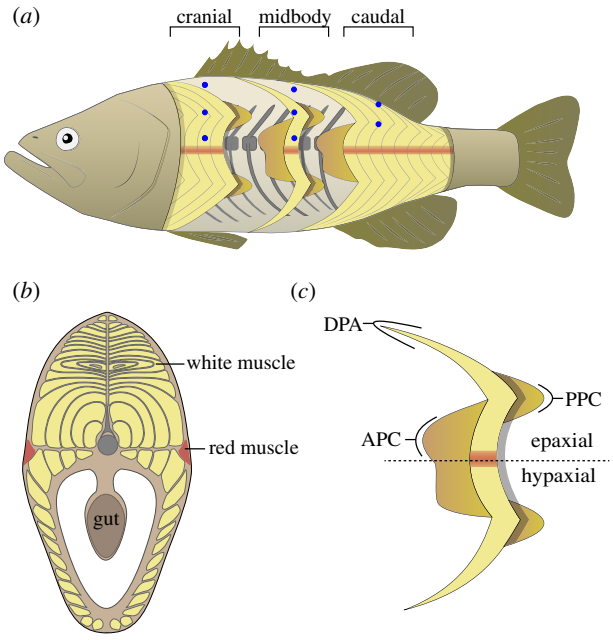

Figure 1. Anatomy of the axial musculature and electrode placement. (*a*) Lateral view of a largemouth bass showing the serially repeating segments of W-shaped myomeres of the axial musculature. Blue dots represent electrode placement in three longitudinal positions: cranial (0.35 BL), midbody (0.50 BL), and caudal (0.70 BL). (*b*) Cross-sectional view of the axial musculature, showing the relative proportions and distributions of red and white muscle fibres. (*c*) Lateral view of a myomere showing the three subregions of the dorsal epaxial region considered in this study. APC, anterior-pointing cone; DPA, dorsal-pointing arm; PPC, posterior-pointing cone. (Online version in colour.)

(b) Experimental design

To detect disparities in muscle activity related to function and performance, we recorded EMGs during swimming and suction feeding in largemouth bass at different performance intensities. To elicit variable feeding performance, we alternated between feeding pellet food and live goldfish and quantified intraoral pressure changes to measure the effects of food type on performance. We elicited three types of burst swimming—turns, sprints, and fast-starts. All three burst swimming behaviours use white muscle fibres, but they differ in their mechanics and intensities. We categorized turning and sprinting as lower-performance behaviours and fast-starting as a high-performance behaviour.

Trials were filmed from a dorsal view with a GoPro camera at either 60 or 100 frames per second. To synchronize video with EMG and pressure data, a computer monitor displaying real-time EMG and pressure recordings was positioned in the camera's field of view. Video, EMG, and pressure data for this publication have been deposited in the ZMAPortal (zmaportal.org) in the study 'Largemouth bass feeding and swimming EMG' with permanent ID ZMA17. Video data are stored in accordance with best practices for video data management in organismal biology [18].

Bass were generally fed goldfish and pellets in alternating order to ensure that differential performance was associated with food type and not with satiation. To ensure that muscle activity during suction feeding was not confounded with

locomotor activity, we used video footage to exclude trials in which any ipsilateral flexion (relative to the electrodes) of the body is concurrent with the strike. We recorded tail beat frequencies comparable to the maximum frequencies recorded in other fish species [19,20] similar in size to our bass (245 mm, 247 mm, and 259 mm), suggesting that we indeed elicited strong sprinting performance.

(c) Intraoral pressure recordings

To measure intraoral pressure, we surgically implanted a cannula onto the buccal cavity under anaesthesia with buffered tricaine methanesulfonate [21]. All fish were given a minimum one-week recovery period after surgery. On the day of experimentation, the pressure transducer was threaded into the cannula until the sensor was partially exposed to the oral cavity.

Prior studies and our pressure data justify the classification of pellet and goldfish strikes as low- and high-performance behaviours, respectively. Pellet and goldfish strikes in our study produced significantly different peak pressures, as has been previously reported in multiple studies [8,21]. Two-way ANOVA revealed no significant effect of individual on peak pressure and pooled data from all three individuals showed bass produce significantly greater ($p < 0.05$) pressure differentials for goldfish than for pellets (electronic supplementary material, figure S1). Furthermore, we elicited peak pressures comparable to those measured in bass in at least two other studies [21,22], suggesting that we elicited maximal or near-maximal suction feeding performance.

(d) Electromyography

Bipolar electrodes were constructed from 0.1 mm diameter, enamel-insulated silver wire. We glued together the last 10 cm of each wire pair along their length with cyanoacrylate and removed approximately 1 mm of insulation from the tip of each wire at the glued end. At the exposed end, we separated the last 3 mm of wire into a 'V' shape and bent them back to form recurved anchoring hooks. Each bipolar electrode was placed inside a 23-gauge hypodermic needle, with the hooked end resting on the bevelled opening of the needle. Electrodes were percutaneously implanted through the soft tissue between the scales to a depth halfway between the skin and the mid-sagittal plane. Once all the electrodes had been implanted, they were glued together to form a common cable. The common cable was secured onto the skin with sutures in two locations to relieve any tension from the electrodes and to prevent them from dislodging from the animal. Connector pins were soldered onto the exposed ends of the wire once the electrodes had been implanted and the fish had been returned to its tank.

Electrodes were implanted in a total of eight positions in each bass. The cranialmost of the longitudinal positions (0.35, 0.50, and 0.70 BL) was implanted with three electrodes corresponding to the different dorsoventral regions of the epaxial musculature—the dorsal-pointing arm (DPA), the posterior-pointing cone (PPC), and the anterior-pointing cone (APC; figure 1). The data from all body regions include values from all three bass, with the exception of both the DPA and PPC of the midbody, for which we collected data from only two out of three individuals (see electronic supplementary material, figure S2 for electrode sampling distribution). Immediately after collecting data, bass were CT (computerised tomography) scanned with an *in vivo* veterinary CT scanner to confirm electrode placement (Animage Fidex scanner; isovolumetric voxels 0.15 mm).

(e) Signal filtering and processing

Electromyograms were amplified by 10 000 (A-M systems, differential AC amplifier, model 1700, Everett, WA, USA) with low- and high-pass hardware filters set to 10 kHz and 100 Hz, respectively. A 60 Hz hardware notch filter was also used to reduce noise from ambient AC circuits. Analogue to digital conversion was done using PowerLab data acquisition hardware at a sampling frequency of 10 kHz, and EMGs were recorded in LabChart (AD Instruments, Sydney, Australia). Raw EMG signals were rectified and software-filtered using the biosignalEMG package for R-studio [23]. Raw data were processed with a butterworth filter with low- and high-pass settings of 100 Hz and 1 kHz, respectively. Using the *envelope* function in biosignalEMG, we created an envelope around the filtered data using a moving average with a window size set to 15 points. For each channel, we selected a raw voltage value for considering whether a muscle was active or inactive; muscles that exceeded this voltage value were considered to exhibit above-threshold muscle activity (ATMA). To minimize bias, threshold voltage was selected by visually comparing several (but not all) trials with different levels of muscle activity. We then selected a voltage value that seemed to represent the minimum threshold for active muscle and applied that value consistently, including trials that we did not visually examine for setting the threshold values. We calculated activation intensity by normalizing voltages for all behaviours to maximum voltage measured by each electrode across all behaviours or within each behaviour (feeding or swimming; as noted in figure legends). The highest intensities always occurred during fast-starts, so intensities within each electrode were normalized to the highest fast-start value, except when normalizing within the feeding behaviours.

(f) Statistics

Statistical analyses, including linear regressions, ANOVAs, Tukey post hoc tests, and *t*-tests were performed in R using its native functions. ANOVAs with significant results were followed up with Tukey's HSD (honest significant difference) post hoc tests. For all tests, a p-value < 0.05 was considered statistically significant.

3. Results

(a) Variation of spatial activation patterns

The presence of above-threshold muscle activity (ATMA) in the epaxial musculature at 0.35 and 0.50 BL varied with food type (figure 2*a*). The dorsal-pointing arm (DPA; the dorsal epaxial region) at the cranial position (0.35 BL) was active for 100% of goldfish and pellet strikes, indicating that muscle activity in the cranial DPA, in contrast to the midbody and caudal DPA, is obligate during suction feeding. Furthermore, the cranial and midbody anterior-pointing cones (APC; the ventral epaxial region) were active for goldfish strikes significantly more often than for pellet strikes (cranial: 79% versus 42%; midbody: 63% versus 35%; paired *t*-test: $p < 0.05$). ATMA percentages for the cranial and midbody posterior-pointing cone (PPC; the middle epaxial region) were intermediate to those of the DPA and the APC, and though not statistically different, two out of three bass recruited the cranial PPC and all individuals recruited the midbody PPC more often for goldfish strikes. Despite variation across individuals, each bass—with the exception of bass02's cranial PPC where ATMA percentages were 100% for both food types—recruited ventral muscle regions (PPC and APC) more frequently for goldfish strikes compared to pellet strikes.

In contrast to suction feeding, the relationship between burst swimming performance and regional activation was spatially inverted along the dorsoventral axis (figure 2*b*). In all three bass, burst swimming elicited muscle activity from the cranial and midbody APC for all performance levels

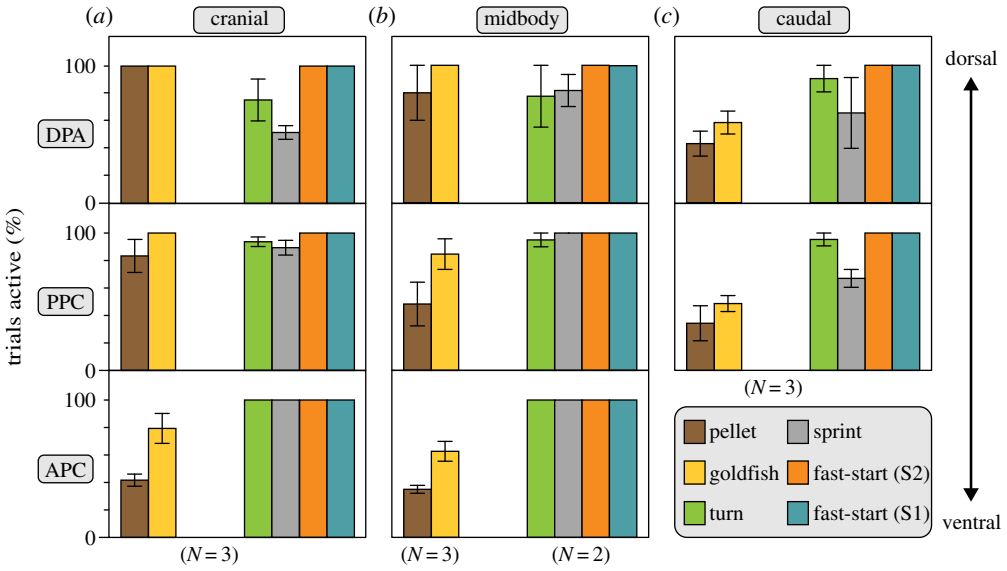

Figure 2. Per cent of trials with ATMA for different behaviours across different epaxial regions. Each plot shows a different anatomical region where the top row is dorsal and the left column is cranial. Each bar is the mean ± s.e.m. of the percentage values from all three individuals (except for swimming behaviours in the midbody region where $N = 2$). For feeding behaviours, data from successful and unsuccessful strike attempts are both included in this analysis. (*a*) Cranial region at 0.35 BL, (*b*) midbody region at 0.50 BL, and (*c*) caudal region at 0.70 BL. APC, anterior-pointing cone; DPA, dorsal-pointing arm; PPC, posterior-pointing cone; S1, stage 1; S2, stage 2. (Online version in colour.)

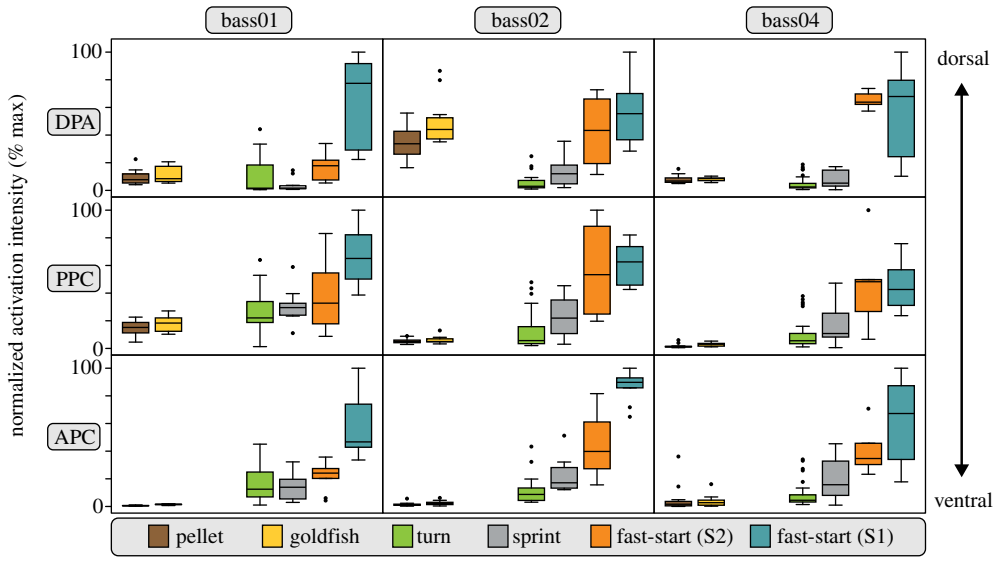

Figure 3. Normalized muscle activation intensity in three dorsoventral regions at 0.35 BL. Activation intensities were normalized to the peak fast-start voltage (the highest voltages) recorded from each electrode. Dorsoventral regions are separated by row and different individuals are separated by column. For feeding behaviours, only data from successful strikes are included in this analysis. Each coloured box includes a horizontal line (median value) that separates the second (top) and third (bottom) quartiles, while the top and bottom error bars are the first and fourth quartiles, respectively, and individual points are outliers. APC, anterior-pointing cone; DPA, dorsal-pointing arm; PPC, posterior-pointing cone; S1, stage 1; S2, stage 2. (Online version in colour.)

and behaviour types. Compared to the APC, the cranial, midbody, and caudal PPC had slightly lower ATMA percentages—though still above 75%—for turns, sprints, and stage-2 fast-starts. In the cranial, midbody, and caudal DPA, ATMA percentages for turns and sprints fell between 40 and 65% but remained at 100% for both stages of fast-starts.

Muscle activation varied with longitudinal position for feeding, but not for burst swimming (figure 2). ATMA percentages for suction feeding generally decreased craniocaudally, while ATMA percentages for burst swimming behaviours remained relatively similar. For example, goldfish strikes were similar in the cranial and midbody DPA (greater than 90%), but decreased significantly in the caudal DPA (paired *t*-test: $p < 0.05$). ATMA percentages for high-performance swimming were always 100%, indicating that the epaxial muscle was active in all longitudinal and dorsoventral positions for stage-1 and -2 fast-starts. The remainder of the results section addresses data exclusively from the cranial region at 0.35 BL.

(b) Effects of activation intensity on performance

We found a relationship between behaviour and peak muscle activation intensity, calculated as per cent of the peak fast-start voltage (the highest voltages) measured from each electrode (figure 3). Mean activation intensities in the DPA

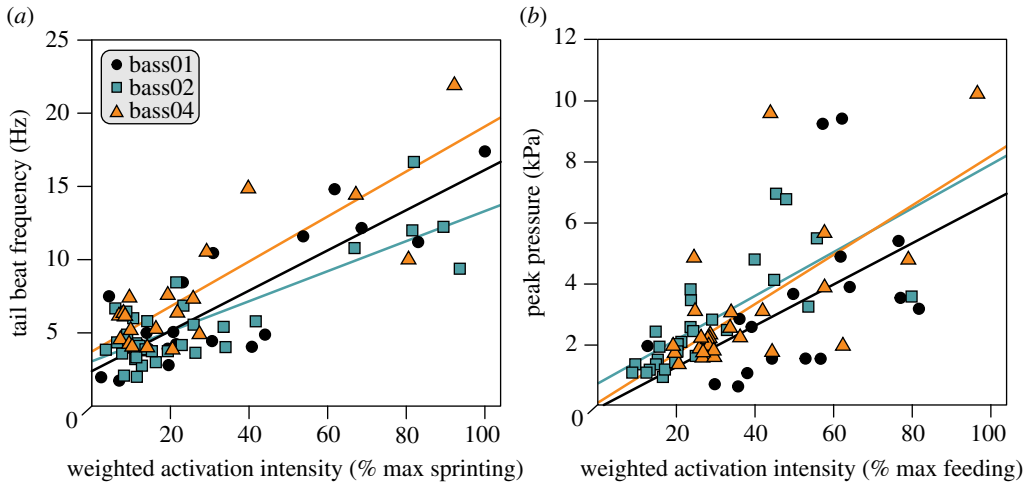

Figure 4. Effects of muscle activation at 0.35 BL on (*a*) sprinting and (*b*) suction feeding performance. Weighted activation intensity was calculated by normalizing each electrode to the max voltage measured for the corresponding behaviour (feeding or sprinting), weighing the normalized intensity from each dorsoventral region by its percentage of epaxial cross-sectional area, and adding all the weighted values. Data from successful and unsuccessful strike attempts are both included in (*b*). Circles, bass01; triangles, bass02; squares, bass04. Regression equations, R^2 values, and *p*-values in electronic supplementary material, table S1. (Online version in colour.)

were generally lowest for sprints and turning manoeuvres (5–13%; range of means across individuals), intermediate for pellet and goldfish strikes (6–58%), slightly higher in stage-2 fast-starts (17–65%), and highest in stage-1 fast-starts (56–63%). In both the PPC and APC, feeding strikes had substantially lower mean activation intensities than stage-1 (45–86%) and stage-2 (21–56%) fast-start intensities. Mean activation intensities in the PPC and APC were lowest in strikes (0–17%), intermediate in sprints and turning manoeuvres (8–30%), and highest in stage-1 and -2 fast-starts (45–86%).

We performed a multifactorial ANOVA and found that individual, muscle region, behaviour, and interactions between these factors all had a significant effect on activation intensity—with the exception of muscle region, which considered alone did not have a significant effect. Within each individual, food type had no significant effect on activation intensity. Muscle activation intensity was variable between individuals, but showed a general trend of very high recruitment for fast-starts relative to other behaviours (figure 3). In all three muscle regions for all individuals, stage-1 fast-starts used significantly higher activation intensities than sprints, turns, and suction feeding, with the exception of the DPA in bass02 where goldfish strikes and stage-1 fast-starts were not statistically different. Suction feeding (both food types) always used activation intensities statistically similar to or greater than sprints and turns.

Continuous performance variables for swimming and suction feeding were significantly correlated with weighted activation intensity, which estimates the number of active muscle fibres (figure 4 and electronic supplementary material, table S1). Weighted activation intensity was strongly correlated with the tail beat frequency of sprinting (figure 4*a*; bass01: $R^2 = 0.68$; bass02: $R^2 = 0.67$; bass04: $R^2 = 0.66$), and somewhat less strongly correlated with peak pressure for all strikes—including failed attempts (figure 4*b*; bass01: $R^2 = 0.19$; bass02: $R^2 = 0.43$; bass04: $R^2 = 0.50$: see electronic supplementary material, figure S3 for midbody feeding data). Weighted activation intensity takes the activation intensity for each electrode and multiplies it by the cross-sectional area of its corresponding region. The sum of the values was then divided by the total cross-sectional area of the epaxial muscle to generate an estimate of the percentage of muscle fibres active within the entire cross-sectional area.

4. Discussion

(a) Functional regionalization

Bass modulated function and performance by adjusting the regional distribution of muscle recruitment while also varying muscle activation intensity (figure 5). Bass recruited distinct epaxial regions depending on whether they were swimming or suction feeding (figure 5*a*,*c*), yet they remained fully capable of recruiting all three dorsoventral regions for high-performance behaviours (figure 5*b*,*d*). Bass also used a wide range of activation intensities within and among behaviours, reflecting the different mechanical demands of each behaviour and the range of intensities at which they were performed (figure 3). This was evident in sprinting and suction feeding behaviours where continuous performance variables correlated strongly with weighted activation intensities (figure 4; electronic supplementary material, figure S3). Suction feeding required contributions from more muscle fibres to produce greater peak pressures during buccal expansion [22], and sprinting required contributions from more muscle fibres to increase tail beat frequency and swim more quickly [24]. We surmise that the dual function of the axial musculature for both swimming and suction feeding depends on the ability to produce these distinct activation patterns.

The dual function of the epaxial musculature was produced primarily through dorsoventral regionalization, and these regionalized activation patterns are consistent with the neuroanatomy of this musculature. Axial muscle fibres in fishes are innervated by both primary and secondary motoneurons [25]. Primary motoneurons innervate large territories of muscle fibres and receive input from Mauthner neurons which can produce complete activation of the axial musculature [24,26–28]. In comparison, secondary motoneurons innervate smaller, overlapping territories of muscle fibres

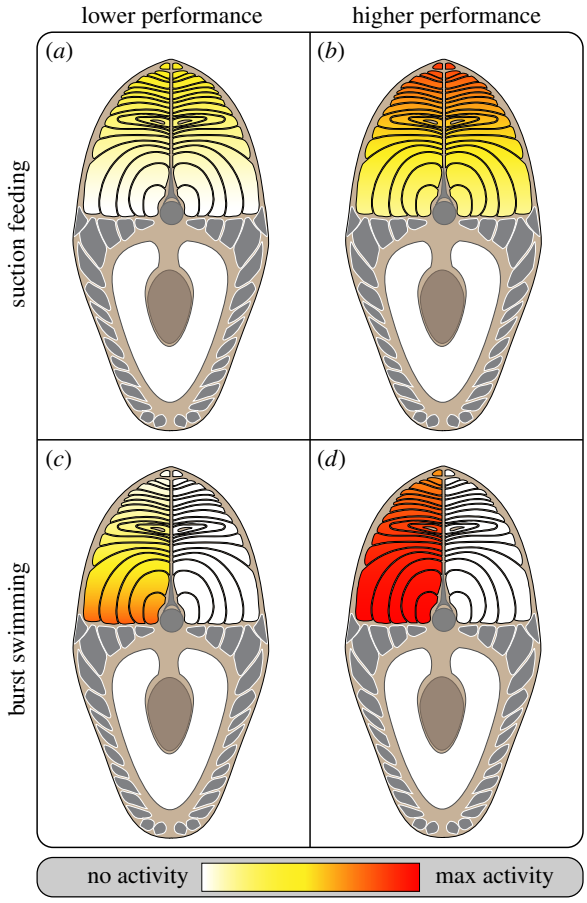

Figure 5. Diagrammatic representation of the intensities and spatial distributions of muscle activity associated with different behaviours and performance. Lower-performance feeding (*a*) uses only the dorsalmost region at moderate intensities. Higher-performance feeding (*b*) recruits the dorsal and ventral regions at high and moderate intensities, respectively. Lower-performance burst swimming (*c*; sprinting) typically uses only the ventralmost region at moderate intensities (20–40% full activation). High-performance burst swimming (*d*; fast-starts) maximally recruits the entire epaxial muscle mass. Feeding activation is expected to be bilateral (although this study used only unilateral electrode sampling), whereas swimming activation is primarily unilateral. (Online version in colour.)

that can produce graded activation of the axial musculature [24,26,28,29]. This dual innervation allows primary motoneurons to activate the entire axial musculature at higher intensities during fast-starts, while still permitting secondary motoneurons to activate the dorsal and ventral epaxial regions independently at lower intensities during non-fast-start behaviours. Interestingly, prior studies suggest that regional activation of the hypaxial musculature may mirror epaxial motor patterns. Hypaxial motoneuron distributions are similar to those of the epaxial musculature [25], and experimental data shows decoupled activity of different regions in the post-anal hypaxials of largemouth bass during locomotion [16]. Although these studies documented the independent activation of myomeric regions [12,16,17], here, we tested and confirmed their functional significance for both swimming and suction feeding.

(b) Epaxial contributions to suction feeding

Our data indicate that both dorsal and ventral epaxial regions in the cranial two-thirds of the body can contribute to high-performance suction feeding. Dorsal regions were nearly always active and used higher intensities than ventral regions (figures 2 and 3). The ventralmost region in the cranial and midbody positions was active for 70% of goldfish strikes and—despite having significantly lower activation intensities—could still contribute to power output owing to the relatively large cross-sectional area of the APC (figure 4; electronic supplementary material, figure S3). In terms of mass and cross-sectional area, the DPA was the smallest while the APC was the largest—having a cross-sectional area over twice as large as the DPA. Owing to their size and/or activation intensities, both dorsal and ventral regions appeared to contribute to high suction power during buccal expansion.

Our finding of full epaxial activation offers insight into epaxial mechanics in high-performance suction feeding. Epaxial mechanics have previously been modelled as a lever system wherein forces from the epaxial musculature act on the in-lever and forces within the buccal cavity act on the out-lever [30]. This model assumed that the fulcrum of this lever system was the post-temporal supra-cleithral (PTSC) joint, such that only force generated dorsal to the fulcrum would contribute to neurocranial elevation and force generated ventral to the fulcrum would resist neurocranial elevation. However, a more recent study found that the axis of rotation (i.e. the fulcrum) for cranial elevation is positioned at the level of the vertebral column—ventral to the PTSC joint and ventral to most of the epaxial muscle in bass [31]. Therefore, muscle positioned dorsal to the fulcrum, including the middle and ventral regions (PPC and APC and sloping portion between them), may contribute power to neurocranial elevation. Indeed, bass activated the ventralmost epaxial region (APC) significantly more often for high-performance strikes than for low-performance strikes in both the cranial and midbody positions (figure 2*a*,*b*), suggesting that APC recruitment contributes to suction performance. Furthermore, the correlation between weighted activation intensity and peak pressure in both the cranial (figure 4*b*) and midbody (electronic supplementary material, figure S3) regions shows that greater areas of muscle—including the APC—are recruited in order to produce observed changes in suction performance. However, APC muscle fibres positioned very close to the vertebral column may shorten very little and potentially contribute more toward joint stabilization than power production.

We suspect that bass maximize power output of the axial musculature for swimming (i.e. fast-starts) but underuse it for suction feeding. Maximizing power output from muscle, though determined by many other factors [32–34], generally requires meeting two conditions: all fibres within the muscle must be activated and must shorten at an approximate rate of one-third of their maximum shortening velocity [32,35]. Fast-starts met the first of these conditions by activating all epaxial regions at high activation intensities (figures 2 and 3), and though muscle shortening in swimming has not been measured in bass, other species have been shown to use optimal shortening velocities during fast-starts [10,36]. By contrast, suction feeding met neither of these strain-activation conditions, as bass used suboptimal muscle shortening velocities [13] and low activation intensities (figure 3). Consequently, the epaxial musculature generated submaximal power for most of their strikes. Interestingly, non-Mauthner-dependent behaviours like sprinting and turning often produced higher activation intensities than those used for feeding, indicating that bass could—but

did not—produce more power from the axial musculature for feeding (figure 3).

(c) Turning

The bass in our study performed turns characterized by slow, high-amplitude body flexion where the head and tail nearly touched. Because bass often used this behaviour for repositioning themselves and reversing their direction, with little translation of the centre of mass, we were surprised to find that white muscle was always active during these relatively slow manoeuvres (figure 2*a*), often at intensities comparable to those of sprinting (figure 3). We suspect that using white muscle for a relatively slow behaviour like turning may be due to the position of the muscle fibres relative to the vertebral column rather than their contractile properties as fast-twitch muscles. Although we cannot comment on the activity or inactivity of red muscle fibres during these turns, we speculate that the large distance between the superficial red muscle and the neutral axis of bending (the vertebral column) would require extreme shortening to produce considerable lateral flexion [37]. However, deeper white muscle fibres, being positioned closer to the neutral axis and oriented oblique to the long-axis of the fish, have a higher gear ratio and may be better for flexing the body with relatively less muscle fibre shortening [38,39].

(d) Evolutionary considerations of a dual-function system

The evolutionary history of the axial musculature poses a challenge: how do we best evaluate form–function relationships in this dual-function structure? The conventional approach has been to examine this muscle group from either a swimming or feeding perspective. However, adopting this strategy poses the risk of misidentifying form–function relationships, as structures that appear to underperform might actually be dual-function structures acting within the context of their secondary function. Perhaps a more appropriate framework would be one that identifies primary and secondary functions by comparing muscle performance during swimming and suction feeding. If only one function fully exerts the muscle, it is reasonable to hypothesize that selection pressure for that function may have been more important in shaping its morphology and contractile properties [40].

Although many fishes have co-opted the axial musculature for suction feeding, we are not aware of any studies that have examined whether this co-opting has created trade-offs in muscle function and performance. It is reasonable to hypothesize that trade-offs exist between high-performance undulatory locomotion and feeding, as both behaviours recruit all regions of the epaxial musculature yet require disparate types of axial flexion. These different axial motions create distinct strain gradients throughout the muscle mass—lateral flexion in locomotion [41] and dorsoventral flexion in feeding are likely to produce mediolateral and dorsoventral strain gradients, respectively. Such non-uniform strain of the muscle would likely negatively affect performance, as muscle fibres would operate at different points on their force-length and power-velocity curves, thereby decreasing power output. Prior studies have hypothesized that the complex fibre architecture of the axial musculature functions as a gearing system that homogenizes muscle fibre strain to allow for high power production in locomotion [37,38,41,42]. However, since the muscle strain gradient for suction feeding occurs on a different axis, feeding would require a different gearing system. Thus, performance trade-offs may exist at this muscle-architecture level. Given such considerations, we suggest that a dual-function framework is a strong conceptual tool for examining and comparing form–function relationships of the axial musculature in fishes with diverse locomotor and feeding strategies.

Ethics. All husbandry and experimental procedures complied with Brown University IACUC protocol #1509000157.

Data accessibility. Video, EMG, and pressure data used in this study are available on the Zoological Motion Analysis Portal upon request (zmaportal.org, Study Identifier ZMA17).

Authors' contributions. Y.E.J. and E.L.B. designed the study. Y.E.J. collected EMG, pressure, and CT scan data, analysed the data, and drafted the manuscript. All authors contributed to editing the manuscript and approved the final version.

Competing interests. We declare we have no competing interests.

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References

1. Webb PW. 1982 Locomotor patterns in the evolution of actinopterygian fishes. *Amer. Zool.* **22**, 329–342. (doi:10.1093/icb/22.2.329)
2. Fletcher T, Altringham JD, Peakall J, Wignall P, Dorrell R. 2014 Hydrodynamics of fossil fishes. *Proc. R. Soc. B* **281**, 1–9. (doi:10.1098/rspb.2014.0703)
3. Schaeffer B, Rosen DE. 1961 Major adaptive levels in the evolution of the actinopterygian feeding mechanism. *Amer. Zool.* **1**, 187–204. (doi:10.1093/icb/1.2.187)
4. Lauder GV. 1982 Patterns of evolution in the feeding mechanism of actinopterygian fishes. *Amer. Zool.* **22**, 275–285. (doi:10.1093/icb/22.2.275)
5. Longo SJ, McGee MD, Oufiero CE, Waltzek TB, Wainwright PC. 2016 Body ram, not suction, is the primary axis of suction-feeding diversity in spiny-rayed fishes. *J. Exp. Biol.* **219**, 119–128. (doi:10.1242/jeb.129015)
6. Van Wassenbergh S, Herrel A, Adriaens D, Aerts P. 2005 A test of mouth-opening and hyoid-depression mechanisms during prey capture in a catfish using high-speed cineradiography. *J. Exp. Biol.* **208**, 4627–4639. (doi:10.1242/jeb.01919)
7. Liem KF. 1978 Modulatory multiplicity in the functional repertoire of the feeding mechanism in cichlid fishes. I. Piscivores. *J. Morphol.* **158**, 323–360. (doi:10.1002/jmor.1051580305)
8. Lauder GV. 1980 The suction feeding mechanism in sunfishes (*Lepomis*): an experimental analysis. *J. Exp. Biol.* **88**, 49–72.
9. Coughlin DJ, Carroll, AM. 2006 *In vitro* estimates of power output by epaxial muscle during feeding in largemouth bass. *Comp. Biochem. Physiol. A.* **145**, 533–539. (doi:10.1016/j.cbpa.2006.08.026)
10. Franklin CE, Johnston IA. 1997 Muscle power output during escape responses in Antarctic fish. *J. Exp. Biol.* **200**, 703–712.

11. Shadwick RE, Steffensen JE, Katz SL, Knower T. 1998 Muscle dynamics in fish during steady swimming. *Amer. Zool.* **38**, 755–770. (doi:10.1093/icb/38.4.755)
12. Ellerby DJ, Altringham JD. 2001 Spatial variation in fast muscle function of the rainbow trout *Oncorhynchus mykiss* during fast-starts and sprinting. *J. Exp. Biol.* **204**, 2239–2250.
13. Camp AL, Roberts TJ, Brainerd EL. 2015 Swimming muscles power suction feeding in largemouth bass. *Proc. Natl Acad. Sci. USA* **112**, 8690–8695. (doi:10.1073/pnas.1508055112)
14. Camp AL, Roberts TJ, Brainerd EL. 2018 Bluegill sunfish use high power outputs from axial muscles to generate powerful suction-feeding strikes. *J. Exp. Biol.* **211**, 178160. (doi:10.1242/jeb.178160)
15. Camp AL, Brainerd EL. 2014 Role of axial muscles in powering mouth expansion during suction feeding in largemouth bass (*Micropterus salmoides*). *J. Exp. Biol.* **217**, 1333–1345. (doi:10.1242/jeb.095810)
16. Jayne BC, Lauder GV. 1995 Are muscle fibers within fish myotomes activated synchronously? Patterns of recruitment within deep myomeric musculature during swimming in largemouth bass. *J. Exp. Biol.* **198**, 805–815.
17. Thys TM. 1997 Spatial variation in epaxial muscle activity during prey strike in largemouth bass (*Micropterus salmoides*). *J. Exp. Biol.* **200**, 3021–3031.
18. Brainerd EL, Blob RW, Hedrick TL, Creamer AT, Müller UK. 2017 Data management rubric for video data in organismal biology. *Integr. Comp. Biol.* **57**, 33–47. (doi:10.1093/icb/icx060)
19. Bainbridge R. 1958 The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. *J. Exp. Biol.* **35**, 109–133.
20. Wardle CS. 1975 Limit of fish swimming speed. *Nature* **255**, 725–727. (doi:10.1038/255725a0)
21. Norton SF, Brainerd EL. 1993 Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *J. Exp. Biol.* **176**, 11–29.
22. Carroll AM, Wainwright PC. 2006 Muscle function and power output during suction feeding in largemouth bass, *Micropterus salmoides*. *Comp. Biochem. Physiol. A* **143**, 389–399. (doi:10.1016/j.cbpa.2005.12.022)
23. Guerrero JA, Macias-Diaz JE. 2018 biosignalEMG: tools for electromyogram signals (EMG) analysis. R package version 2.1.0.
24. Ampatzis K, Son J, Ausborn J, El Manira A. 2013 Pattern of innervation and recruitment of different classes of motoneurons in adult zebrafish. *J. Neurosci.* **33**, 10 875–10 886. (doi:10.1523/JNEUROSCI.0896-13.2013)
25. Westerfield M, McMurray JV, Eisen JS. 1986 Identified motoneurons and their innervation of the axial muscles in the zebrafish. *J. Neurosci.* **6**, 2267–2277. (doi:10.1523/JNEUROSCI.06-08-02267.1986)
26. Liu DW, Westerfield M. 1988 Function of identified motoneurones and co-ordination of primary and secondary motor systems during zebra fish swimming. *J. Physiol.* **403**, 73–89. (doi:10.1113/jphysiol.1988.sp017239)
27. Tytell ED, Lauder GV. 2002 The C-start escape response of *Polypterus senegalus*: bilateral muscle activity and variation during stage 1 and 2. *J. Exp. Biol.* **205**, 2591–2603.
28. Bello-Rojas S, Istrate AE, Kishore S, McLean, DL. 2019 Central and peripheral innervation patterns of defined axial motor units in larval zebrafish. *J. Comp. Neurol.* **527**, 2557–2572. (doi:10.1002/cne.24689)
29. McLean DL, Fan J, Higashijima S, Hale ME, Fetcho JR. 2007 A topographic map of recruitment in spinal cord. *Nature* **446**, 71–75. (doi:10.1038/nature05588)
30. Carroll AM, Wainwright PC, Huskey SH, Collar DC, Turingan RG. 2004 Morphology predicts suction feeding performance in centrarchid fishes. *J. Exp. Biol.* **207**, 3873–3881. (doi:10.1242/jeb.01227)
31. Jimenez YE, Camp AL, Grindall JD, Brainerd EL. 2018 Axial morphology and 3D neurocranial kinematics in suction-feeding fishes. *Biol. Open* **7**, bio036335. (doi:10.1242/bio.036335)
32. Marsh RL, Bennett AF. 1986 Thermal dependence of contractile properties of skeletal muscle from the lizard *Sceloporus occidentalis* with comments on methods for fitting and comparing force-velocity curves. *J. Exp. Biol.* **126**, 63–77.
33. Marsh RL. 1999 How muscles deal with real-world loads: the influence of length trajectory on muscle performance. *J. Exp. Biol.* **202**, 3377–3385.
34. Gabaldón AM, Nelson FE, Roberts TJ. 2008 Relative shortening velocity in locomotor muscles: turkey ankle extensors operate at low V/Vmax. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R200-R210. (doi:10.1152/ajpregu.00473.2007)
35. Rome LC. 2005 Principles of actuation in the muscular system of fish. *IEEE J. Ocean. Eng.* **30**, 630–646. (doi:10.1109/JOE.2005.852346)
36. James RS, Johnston IA. 1998 Scaling of muscle performance during escape responses in the fish *Myoxocephalus scorpius L. J. Exp. Biol.* **201**, 913–923.
37. Rome LC, Sosnicki AA. 1991 Myofilament overlap in swimming carp. II. Sarcomere length changes during swimming. *Am. J. Physiol. Cell Physiol.* **260**, 289–296. (doi:10.1152/ajpcell.1991.260.6.1-a)
38. Alexander RM. 1969 The orientation of muscle fibres in the myomeres of fishes. *J. Mar. Biol. Assoc. UK* **49**, 263–290. (doi:10.1017/S0025315400035906)
39. Brainerd EL, Azizi E. 2005 Muscle fiber angle, segment bulging and architectural gear ratio in segmented musculature. *J. Exp. Biol.* **208**, 3249–3261. (doi:10.1242/jeb.01770)
40. Rome LC. 1998 Matching muscle performance to changing demand. In *Principles of animal design: the optimization and symmorphosis debate* (eds ER Weibel, CR Taylor, L Bolis), pp. 103–113. Cambridge, UK: Cambridge University Press.
41. Wakeling JM, Johnston IA. 1999 White muscle strain in the common carp and red to white muscle gearing ratios in fish. *J. Exp. Biol.* **202**, 521–528.
42. Van Leeuwen JL, Van Der Meulen T, Schipper H, Kranenbarg S. 2008 A functional analysis of myotomal muscle-fibre reorientation in developing zebrafish *Danio rerio*. *J. Exp. Biol.* **211**, 1289–1304. (doi:10.1242/jeb.012336)