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RESEARCH ARTICLE

Functional subdivision of fin protractor and retractor muscles underlies pelvic fin walking in the African lungfish *Protopterus annectens*

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ABSTRACT

African lungfish *Protopterus annectens* can produce rotational movements around the joint between the pelvis and the pelvic fin, allowing these animals to walk across benthic substrates. In tetrapods, limb rotation at the hip joint is a common feature of substrate-based locomotion. For sprawling tetrapods, rotation can involve nine or more muscles, which are often robust and span multiple joints. In contrast, *P. annectens* uses a modest morphology of two fan-shaped muscles, the pelvic fin protractor and retractor, to accomplish this movement. We hypothesized that functional subdivision, coupled with their broad insertions on the femur, allows each of these muscles to pull on the limb from multiple directions and provides a mechanism for fin rotation. To test this hypothesis, we examined the muscle activity at three locations in both the protractor and the retractor muscles during walking. Electromyograms show differences in the timing of muscle activation between dorsal and ventral regions of each muscle, suggesting that each muscle is functionally subdivided once. The subdivisions demonstrate sequential onsets of muscle activity and overlap of activity between regions, which are also features of limb control in tetrapods. These data indicate that subdivisions of protractor and retractor muscles impart functional complexity to a morphologically simple system, and suggest a mechanism that allows lungfish to produce a tetrapod-like walking gait with only two muscles. As one of few extant sarcopterygian fishes, *P. annectens* may provide important functional data to inform interpretation of limb movement of fossil relatives.

KEY WORDS: Locomotion, Biomechanics, EMG, Lungfish, Functional subdivision

INTRODUCTION

The tetrapod-like walking and bounding gaits of the African lungfish *Protopterus annectens* (Owen 1839) involve rotational movement of the pelvic limb around its hip joint (King et al., 2011) (Fig. 1). Rotation around the joint between the femur and pelvic girdle is also a feature of tetrapod hindlimb kinematics during terrestrial locomotion (Snyder, 1954; Edwards, 1977). In terrestrial tetrapods, such movements are generated by the coordinated activity of a suite of muscles (e.g. Walker, 1971; Wentink, 1976; Ashley-Ross, 1995; Gatesy, 1997). In contrast, the pelvic fin of African lungfish is actuated by two muscles, the pelvic fin protractor and retractor (Fig. 2). These fan-shaped muscles originate on the ventral and dorsal aspects of the pelvis and body wall and span the hip joint to insert on the rostral and caudal aspects of the femur, respectively (King and Hale, 2014) (see Fig. 2). It is unclear how rotational movement about this joint is produced with such a modest morphology.

One way to employ a simple muscle morphology in the performance of complex function is through physiological subdivision of the muscle. Subdivisions can reflect individual or groups of motor units (compartments) that can be activated independently of others in the muscles (e.g. English, 1984). Such subdivision allows several approaches to controlling muscle output. First, by changing the number and identity of muscle fibers activated (e.g. Lucas-Osma and Collazos-Castro, 2009), the magnitude and timing of force generation can be fine-tuned. Second, if motor units are spatially segregated or biased within a muscle of broad insertion or origin, the orientation of the force vector acting on the skeleton can vary during regional muscle contraction (e.g. Hoffer et al., 1987; Boggs and Dial, 1993). By controlling the populations of muscle fibers activated and/or the timing of their activation, an individual muscle can take on different functions in a biomechanical system (e.g. Hoffer et al., 1987).

Based on these studies of muscle subdivision and others that address tetrapod limb rotation, we proposed two hypotheses for control of a kinematic feature, fin rotation, previously identified in the lungfish (King et al., 2011). First, we hypothesized that lungfish protractor and retractor muscles are functionally subdivided, allowing muscle regions to participate in different phases of the stride cycle. King and Hale have found broad origination and insertion of these muscles (King and Hale, 2014). This morphology suggests that regional activation in each muscle could generate force in a range of directions. It has been demonstrated that functionally subdivided muscles are involved in limb rotation in several tetrapod systems (e.g. Jenkins and Goslow, 1983; Boggs and Dial, 1993; Holtermann et al., 2009) as well as in the movement of the pelvic fins of trout (*Oncorhynchus mykiss*) (Standen, 2010). Second, we hypothesized that muscle control of pelvic fin movement in walking and bounding in the lungfish involves both orderly timing of regional muscle activity onset and co-activation of multiple muscular functional subdivisions at particular phases of the stride cycle. This type of muscular coordination is common in tetrapods (e.g. Gatesy, 1997), and we suggest that the pelvic fin protractor and retractor muscles in *P. annectens* exhibit similar control strategies through the use of their functional subdivisions.

To test these hypotheses, we examined the muscle activity of the pelvic fin protractor and retractor muscles during walking in *P. annectens*. We coupled electromyograms (EMGs) from multiple electrodes per muscle with fin kinematics to determine the protractor and retractor activation patterns. Additionally, we compared the muscle activity and myology of African lungfish

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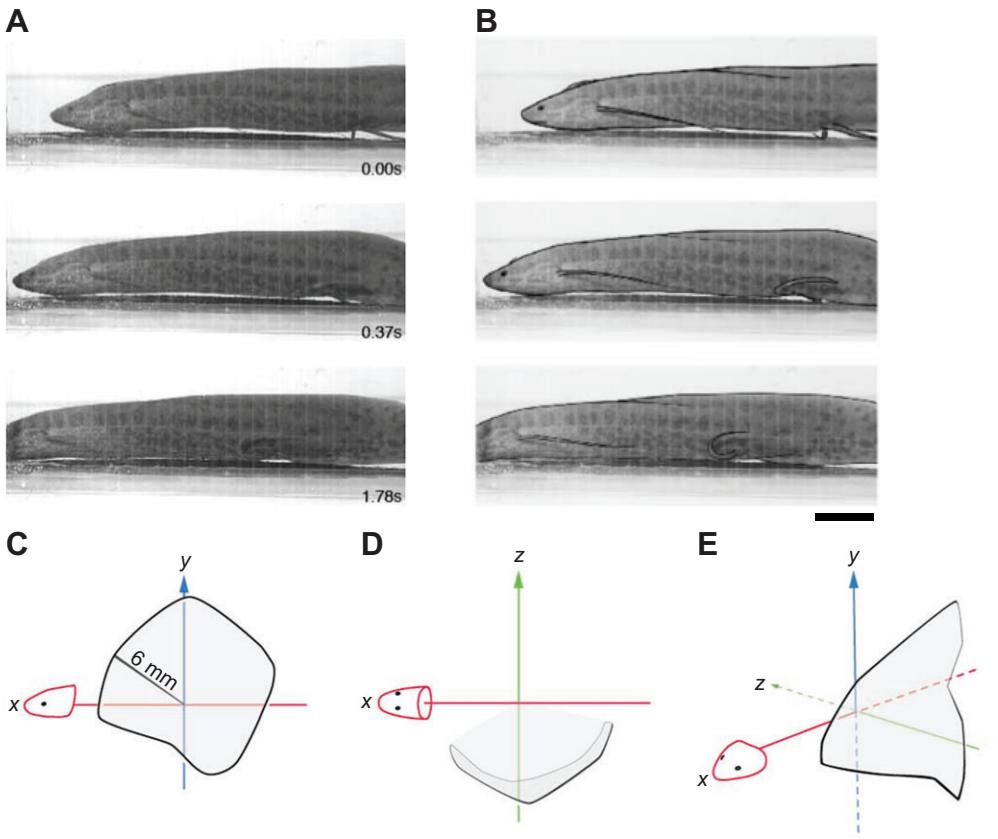

Fig. 1. Example of pelvic fin protraction in the African lungfish *Protopterus annectens*. (A) Lateral view images from a fin cycle show rotation of the left fin over the limb's joint with the hip. (B) The pelvic fin and body from the images in A are lightened and outlined in black to emphasize the location of the pelvic fin. In the top row, the fin is nearing the end of the retraction phase and can be seen ventral to its articulation with the body. The right fin can be seen in contact with the substrate ventral to the hip joint. In the middle row, the fin is in the early portion of protraction and is just dorsal to the articulation with the body. In the bottom row, the fin is ending protraction. Scale bar, 5 cm. (C–E) An example of a typical step that illustrates proximal pelvic fin kinematics in the lateral view (C), ventral view (D) and three-quarter view (E). The pelvic fin is lifted dorsal to its articulation with the body during protraction and ventral to its articulation with the body during retraction. This figure is adapted from fig. 3 in King et al. (King et al., 2011).

with published data from other vertebrates to explore alternative musculoskeletal designs capable of producing movement about the hip joint.

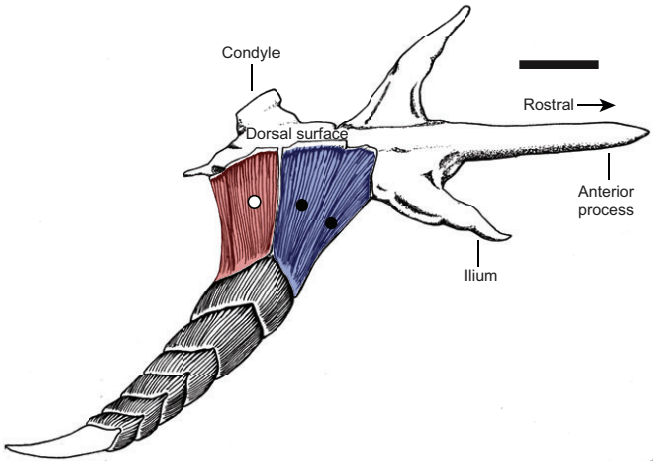

Fig. 2. Dorsolateral view of *P. annectens*' right pelvic fin musculature. The fan-shaped protractor and retractor muscles originate broadly from the ventral midline of the pelvic girdle to the dorsal aspect of the pelvic girdle. The protractor and retractor muscles insert on distal processes of the rostral and caudal aspects of the femur, respectively. The protractor muscle is highlighted in blue and marked with solid black dots and the retractor muscle is highlighted in red and marked with white dots. The black dots also illustrate where the dorsal and middle electrodes were placed in the protractor muscle. The white dot illustrates where the dorsal electrode was implanted into the retractor muscle. The out of view electrodes (MR, middle retractor; VR, ventral retractor; VP, ventral protractor) were implanted in approximately the same location along the span of the muscle in their respective regions. Rostral is to the right. Scale bar, 5 mm.

This is the first study to examine motor activity patterns in the fin muscles of a sarcopterygian fish, and one of the few to examine motor control in the pelvic fin of a fish (see also Standen, 2010). The mechanism behind pelvic fin movement in African lungfish provides an interesting comparative model for designs of rotation around a joint because the lungfish musculoskeletal architecture differs strikingly from that which is found in extant tetrapods. In addition to building our understanding of biomechanical diversity, such work may inform interpretation of fossil sarcopterygian fishes. The fossil record and trackway evidence have been used to suggest the biomechanical capabilities of extinct taxa (e.g. Niedźwiedzki et al., 2010; Pierce et al., 2012). As *P. annectens* is one of the few extant sarcopterygian fishes, data from this study may be useful in expanding our understanding of the functional capabilities of related extinct taxa, particularly in relation to the limb's soft tissue and movement.

RESULTS

Kinematics and fin cycle durations

During a stride cycle, the pelvic fin makes contact with the substrate shortly after retraction begins and maintains contact with the substrate for the majority of the retraction phase (King et al., 2011). During protraction, the pelvic fin is lifted up and over its articulation to the hip (King et al., 2011) (Fig. 1). In our study, at the end of retraction, the angle of the pelvic fin relative to the articulation with the body (measured in ventral view) was 22.31±19.02 deg (mean ± s.d.) as the fin approached the body wall caudal to the hip joint. The fin remained briefly at that angle during the transition from retraction to protraction. At the end of protraction, the angle of the pelvic fin relative to the articulation with the body was 149.20±24.22 deg as the fin extended rostrally from the hip joint. Three-dimensional rotation of the pelvic fin was confirmed in this study by visual inspection during each trial.

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Table 1. Individual morphometrics and cycle durations

	Fish morphometrics			Implanted electrodes						Cycle duration			
	Length (cm)	Mass (g)	Fin length (cm)	VR	MR	DR	DP	MP	VP	*n*	Minimum (s)	Maximum (s)	Mean ± s.d. (s)
L1	45.5	419.3	6.8	X	O	X	X	X	X	20	0.967	2.321	1.60±0.47
L2	48.0	573.0	8.0	X	X	X	X	X	X	20	1.769	3.284	2.09±0.47
L3	43.5	340.0	6.5	X	O	X	X	X	O	20	0.883	2.413	1.65±0.51
L4	44.0	468.1	6.1	X	X	X	X	O	X	20	1.190	3.284	2.20±0.73
L5	52.1	608.0	8.0	X	X	X	X	O	X	20	0.720	2.560	1.38±0.46
Summary	46.62±3.53	481.7±110.05	7.08±0.88	*N*=5	*N*=3	*N*=5	*N*=5	*N*=3	*N*=4	100	–	–	1.76±0.60

Summary values for the morphometric variables are means ± s.d.
X represents the presence of an electrode and O represents the absence of an electrode at each muscle region. *N* represents the number of individuals from which activity was recorded per muscle region; *n* is the number of fin cycles.

Fin cycle durations varied considerably among trials as well as among individuals (Table 1). The mean cycle duration across all trials from all individuals (*N*=5) was 1.76±0.60 s (*n*=100 fin cycles). The maximum and minimum cycle duration across all individuals was 3.284 and 0.883 s, respectively. The average percentage of the fin cycle dedicated to retraction, the retraction–protraction transition, and protraction was 47.58±13.18%, 17.47±14.06% and 34.95±11.20%, respectively. Both the cycle durations and ventral kinematics recorded in this study showed close correspondence with cycle durations and kinematic data recorded previously from *P. annectens* (King et al., 2011).

Regional muscle activity

The protractor and retractor muscles were active during the stride cycle, and demonstrated region-specific muscle activation patterns. There was distinct activity in the dorsal and ventral EMG electrodes in both the protractor and the retractor muscle (Fig. 3). The mean and s.d. of each region's activity onset time and duration are presented in Table 2 and illustrated in Fig. 4. Activity in the ventral region of the retractor muscle (VR) began just prior to the beginning of the fin retraction phase (VR onset: −7.63±8.24% fin cycle). VR remained active through most of the retraction phase of a given fin cycle and EMG activity ceased at approximately the end of the retraction phase (VR duration: 53.37±16.65% fin cycle). The dorsal region of the retractor muscle (DR) became active during the middle to late retraction phase (DR onset: 30.07±21.02% fin cycle). DR remained active through ~82% of the fin cycle, which includes a large portion of the protraction phase (DR duration: 52.09±20.06% fin cycle). The dorsal region of the protractor muscle (DP) usually became active just prior to the start of protraction (DP onset: 51.28±16.96% fin cycle), and remained active until very late in the protraction phase of a fin cycle (DP duration: 38.00±17.53% fin cycle). The ventral region of the protractor muscle (VP) became active during the early to middle protraction phase (VP onset: 71.52±15.19% fin cycle) and remained active through the remainder of protraction as well as the start of the subsequent fin cycle in some trials (VP duration: 27.42±13.78% fin cycle). There was considerable overlap in the activity between muscle regions, and the majority of fin cycles (88 of 100) had an onset of regional activity in the following order, anchored to the beginning of retraction in the fin cycle: VR, DR, DP, VP.

We performed linear regressions on a subset of the data (the four individuals with working electrodes in each region) to quantify the relationship between cycle duration and regional activity duration (Fig. 5, Table 3). In the four individuals examined, the cycle duration increased with the duration of activity in all four of the muscle regions. There was a significant positive correlation between cycle duration and the activity duration of each retractor region (VR and

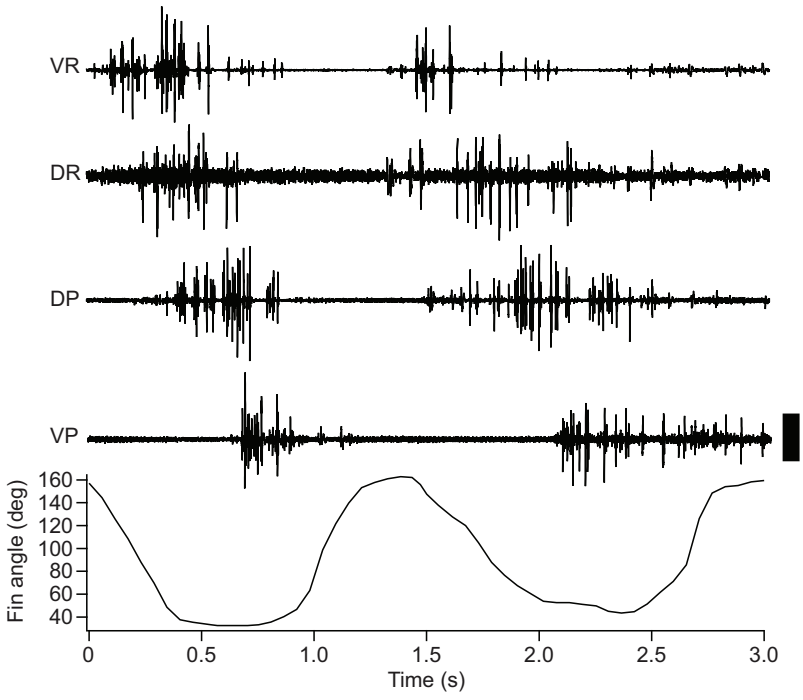

Fig. 3. Representative electromyograms (EMGs) recorded from the ventral and dorsal regions of the protractor and retractor muscles of a *P. annectens* individual during two consecutive fin cycles. The protractor and retractor muscles were active during the fin rotation cycle, and there was distinct activity in the dorsal and ventral EMG electrodes of each muscle. VR: activity in the ventral region of the retractor muscle began just prior to the beginning of the fin retraction phase and remained active through most of the retraction phase of a given fin cycle. DR: the dorsal region of the retractor muscle became active during the middle to late retraction phase and remained active through the middle of protraction. DP: the dorsal region of the protractor muscle was active throughout protraction. VP: the ventral region of the protractor muscle became active during the early to middle protraction phase and remained active through the remainder of protraction. Scale bar, 0.2 mV. The graph shows the rostral–caudal pelvic fin kinematics corresponding to the two representative fin cycles displayed.

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Table 2. Mean and s.d. of electromyographic variables for each region of the retractor and protractor muscles in *Protopterus annectens*

		Onset				Duration			
Individual	*n*	VR	DR	DP	VP	VR	DR	DP	VP
L1	20	–7.67±3.59	36.45±16.30	50.73±15.09	66.65±8.07	58.70±15.74	54.85±17.36	34.19±17.44	21.55±7.93
L2	20	–6.95±7.09	6.78±9.58	63.19±8.60	77.69±17.19	47.78±13.80	62.63±20.36	32.20±12.02	36.34±29.59
L3	20	–4.27±11.17	51.55±8.97	54.87±19.00		60.95±13.84	43.32±15.32	42.96±16.20	
L4	20	–12.65±8.47	29.46±19.22	51.52±11.67	59.57±9.23	49.15±18.10	53.56±14.76	32.03±11.03	29.37±9.83
L5	20	–5.12±12.91	23.07±22.81	34.36±18.23	92.58±7.26	44.08±16.24	39.55±22.16	50.41±22.40	34.12±15.05
Summary	100	−7.63±8.24	30.07±21.02	51.28±16.96	71.52±15.19	53.37±16.65	52.09±20.06	38.00±17.53	27.42±13.78

Numerical variables are presented as a percentage of fin rotation cycle. The onset of activity of each muscle region was measured relative to the start of a fin rotation cycle. For each variable, the mean ± s.d. onset and duration are presented. The onset of activity was always significantly different between the ventral and dorsal regions of the same muscle (P<0.05).

VR, ventral retractor; DR, dorsal retractor; DP, dorsal protractor; VP, ventral protractor; *n*, number of fin cycles.

Note: data from the middle electrode did not indicate that the middle region of each muscle had independent control from the dorsal or ventral region of that same muscle, and, thus, are not included here (see Table 4).

DR). The relationship between the activity duration of each protractor region (DP and VP) and cycle duration was also positive, but not always significant.

In all five individuals, muscle activity (onset time and duration) recordings from the middle electrode were statistically coincident with either the dorsal or ventral electrode from the same muscle in all fin cycles of a given individual (Table 4, Fig. 6). The onset of muscle activity from the middle electrode, and the region it was coincident with, were both significantly different from that of the opposite region in a given muscle. The protractor muscle of individual L3 only contained two working electrodes, the MP and DP. Therefore, without comparison to a third electrode it is not certain that this muscle activated regionally. However, the activity recorded from the MP and DP electrodes was statistically coincident (onset P=0.93; duration P=0.96).

DISCUSSION

The goal of this study was to determine how African lungfish (*P. annectens*) produce complex movements of their pelvic fin about their hip joint (King et al., 2011), given their seemingly simple morphological system (King and Hale, 2014). *Protopterus annectens* has only two muscles, the protractor and retractor, spanning the hip joint. The hip muscles originate on the dorsal and ventral surfaces of the pelvic girdle and insert broadly across the dorsal to ventral aspects

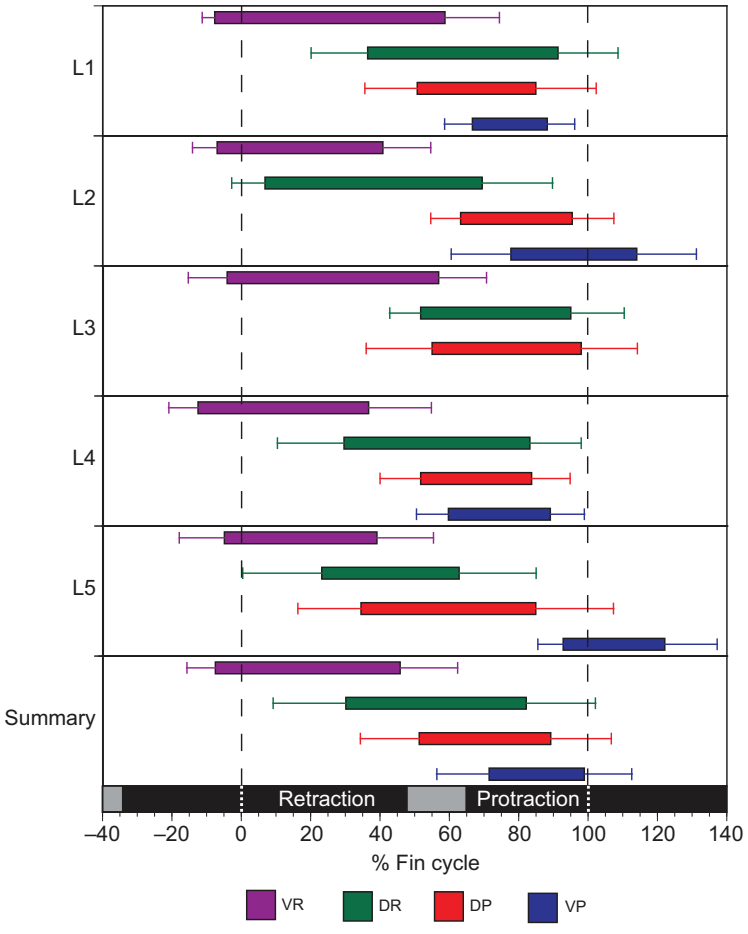

Fig. 4. Mean regional muscle activity duration per individual (L1–L5) and group summary. There was considerable overlap in the activity between muscle regions. Each individual plot represents the pooled onset and duration of muscle activity in the dorsal and ventral region of the retractor and protractor muscles for all trials examined. Overall, there was consistency in the muscles' regional activity patterns among individuals. The summary plot represents the pooled regional muscle activity from all individuals in this study. Bar length indicates the mean duration of muscle activity in the indicated region. The error bars on the left and right represent the s.d. of onset and duration times, respectively, for each region. The black and gray bar at the bottom represents the group's average proportion of the fin cycle dedicated to retraction (black), the retraction–protraction transition (gray), and protraction (black). VR, ventral retractor; DR, dorsal retractor; DP, dorsal protractor; VP, ventral protractor.

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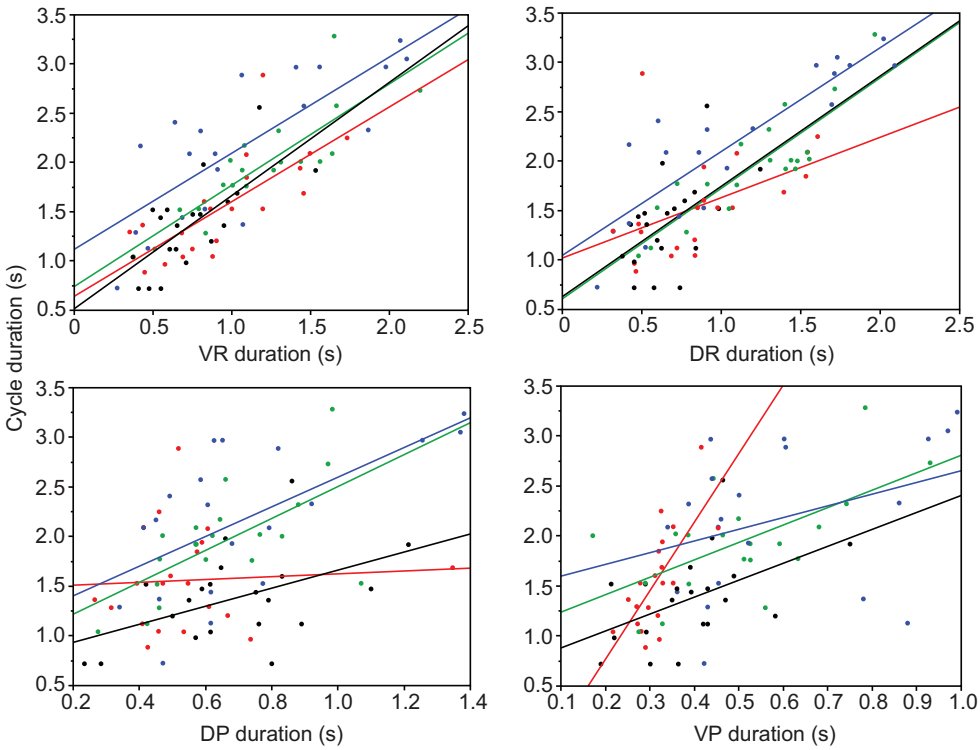

Fig. 5. Linear regressions of regional activity duration versus fin cycle duration. Regressions were performed on a subset of the individuals (L1, L2, L4, L5) for a total of 80 fin cycles. Overall, there was an increase in the fin cycle duration with increased activity duration of each muscle region (Table 3). L1, red; L2, green; L4, blue; L5, black.

of the rostral and caudal sides of the femur, respectively (King and Hale, 2014) (see Fig. 2 of the present study). Our results indicate that each muscle is functionally subdivided into two physiologically distinct regions, allowing differential activation of subdivisions to generate movement of the limb in a wide range of directions. We argue that combining this subdivided organization with coordinated and overlapping muscle activity allows this system to generate the movement of the limb about its joint with the hip including the pelvic fin rotation demonstrated by King et al. (King et al., 2011).

The presence of functionally subdivided muscles spanning a joint is not unique to African lungfish, but a common feature of many vertebrate species. Functionally subdivided muscles have been found in the tetrapod hindlimb (e.g. English and Weeks, 1987; English, 1984; English, 1990), spanning the tetrapod shoulder (e.g. Jenkins and Goslow, 1983; Boggs and Dial, 1993; Holtermann et al., 2009), in jaw muscles of tetrapods (e.g. Herring et al., 1979) and fish (Friel and Wainwright, 1999), and spanning the joint between the pelvic girdle and fin in trout (*O. mykiss*) (Standen, 2010).

In large fan-shaped muscles with broad insertions or origins, regional activation can contribute to complex movements and increase the muscle's functional capabilities (Boggs and Dial, 1993). An extreme example of this is found in the pigeon pectoralis muscle, where regional activity proceeds circularly around the large muscle, with large overlap in activity among adjacent regions (Boggs and Dial, 1993). The activity pattern seen in the pigeon pectoralis muscle is reminiscent of the activity pattern seen in the African lungfish's protractor and retractor muscles.

In the tetrapod literature, reference to the muscular mechanism of limb rotation about a joint is scarce (but see Gatesy, 1997). This likely is due to the difficulty of recording muscle activity from the full suite

Table 3. Linear regression results for cycle duration by regional muscle activity duration

Muscle region	Individual	*n*	Slope	*y*-intercept	r^2	*F*-ratio	*P*-value
VR	1	20	0.961	0.642	0.528	20.128	0.0003*
	2	20	1.029	0.742	0.726	47.795	<0.0001*
	4	20	0.976	1.119	0.611	28.334	<0.0001*
	5	20	1.149	0.516	0.507	18.497	0.0004*
DR	1	20	0.611	1.021	0.224	5.209	0.034*
	2	20	1.118	0.608	0.744	52.176	<0.0001*
	4	20	1.051	1.048	0.763	58.067	<0.0001*
	5	20	1.116	0.630	0.279	6.952	0.017*
DP	1	20	0.143	1.483	0.004	0.071	0.794
	2	20	1.607	0.898	0.407	12.333	0.003*
	4	20	1.494	1.105	0.385	11.289	0.004*
	5	20	0.910	0.754	0.228	5.317	0.033*
VP	1	20	6.861	−0.605	0.517	19.289	0.0004*
	2	20	1.743	1.065	0.376	10.830	0.0041*
	4	20	1.171	1.482	0.125	2.580	0.1256
	5	20	1.695	0.712	0.234	5.497	0.0307*

*Asterisks indicate a significant positive correlation between cycle duration and the activity duration.
VR, ventral retractor; DR, dorsal retractor; DP, dorsal protractor; VP, ventral protractor; *n*, number of fin cycles.

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Table 4. The mean onset and s.d. for middle electrodes and the adjacent electrodes within the same muscle

	L1						L2					
	Onset			Duration			Onset			Duration		
	MP	DP	VP	MP	DP	VP	MP	DP	VP	MP	DP	VP
Mean	50.6	50.7	66.7	34.2	34.2	21.5	63.4	63.2	77.7	32.6	32.2	36.3
s.d.	15.9	15.1	8.06	17.4	17.4	7.8	8.9	8.6	17.2	11.5	12.0	29.6
P-value	0.98			0.99			0.95			0.93		
P-value		0.000082			0.002			0.02			0.65	
P-value		0.0001			0.002			0.03			0.65	
	L2						L4					
	Onset			Duration			Onset			Duration		
	MR	DR	VR	MR	DR	VR	MR	VR	DR	MR	VR	DR
Mean	8.72	8.98	−6.95	62.5	62.6	47.8	−12.4	−12.6	29.4	49.4	49.2	53.6
s.d.	9.4	9.5	7.09	20.8	20.4	13.8	8.3	8.5	19.2	17.5	18.1	14.8
P-value	0.95			0.98			0.93			0.96		
P-value		0.001			0.12			2.7×10^{-7}			0.58	
P-value		0.001			0.13			2.7×10^{-7}			0.59	
	L5						L3					
	Onset			Duration			Onset			Duration		
	MR	DR	VR	MR	DR	VR	MP	DP		MP	DP	
Mean	20.6	23.1	−5.12	42.7	39.5	44.1	54.9	54.9		42.9	42.9	
s.d.	27.5	22.8	12.9	18.8	22.2	16.2	19.0	19.0		16.2	16.2	
P-value	0.82			0.72			0.93			0.96		
P-value		0.0001			0.50							
P-value		0.004			0.84							

Numerical variables are presented as a percentage of fin rotation cycle. The onset of activity of each muscle region was measured relative to the start of a fin rotation cycle.

Student's *t*-tests were run to produce each *P*-value. The shaded regions correspond to the variables used in that row's *t*-test.

DP, dorsal protractor; MP, middle protractor; VP, ventral protractor; VR, ventral retractor; MR, middle retractor; DR, dorsal retractor.

of muscles spanning a single joint; many muscles are small and deep to other muscles, muscles can be biarticular, and it can be challenging to compare muscle activity patterns recorded among electrodes (see Loeb and Gans, 1986). Tetrapods coordinate overlapping activity patterns in a suite of muscles during limb rotation. At any given moment in the cycle, some muscles act to counterbalance loads and stabilize the joint, while others drive limb movement (Gatesy, 1997; Reilly and Blob, 2003). However, discussions of why overlapping activity between muscles (or in this case, muscle regions) is a common feature of sarcopterygian locomotion and how it is used to control and smooth limb rotation about a joint are lacking in the literature. The apparent use of four functional regions to drive fin movement in the African lungfish provides an opportunity to more fully sample muscle activity around the circumference of the femur.

In the African lungfish, overlapping activity between muscles and muscle regions may be beneficial for several aspects of fin movement. One hypothesis that arises from the data is that in order to hold the body above the substrate during retraction, the VR remains active throughout most of the retraction phase, where it is able to resist femoral abduction as the DR continues to retract the fin (Figs 3, 4). This is reflected in the activity patterns of some tetrapod adductor muscles during the stance phase of a limb cycle (see Gatesy, 1997; fig. 3 in Blob and Biewener, 2001; fig. 1A in Reilly and Blob, 2003; fig. 1 in Gosnell et al., 2011). Additionally, activity overlap between adjacent muscle regions would smooth the rotation of the kinematic vector. If overlapping activity were absent, the fin would be pulled in four directions sequentially, with each direction determined by the fin's position, momentum and the contraction of the next muscle region to activate.

Muscular control of fin cycle duration

The duration of fin rotation cycles was found to be highly variable among and within individuals (King et al., 2011) (Table 1 of the present study). In general, there was a positive correlation between fin cycle duration and each muscle region's activity duration (Fig. 5). In all but two instances (DP in individual one; VP in individual four), these correlations were significant (Table 3). The significant positive correlation of regional muscle activity with cycle duration demonstrates that a considerable amount of the variation in cycle duration is controlled by neuromuscular activity and is not due to passive effects. In birds and mammals, highly rhythmic locomotion (low variation in cycle duration) has been suggested to be associated with the evolution of gamma motor neurons and large myelinated Ia sensory afferents that innervate muscle spindles (Ross et al., 2013). It is unclear whether African lungfish possess muscle spindles, but the lack of the specialized sensorimotor control system, proposed to be crucial for rhythmicity, may explain the large variability in regional muscle activity and cycle durations of this repeated movement pattern.

Evolution of a rotational joint

Walking behavior has evolved independently multiple times within actinopterygian, sarcopterygian and chondrichthyan fishes (e.g. Edwards, 1989; Pridmore, 1994; King et al., 2011) in association with variation in the muscular systems driving fin movement. As discussed above, terrestrial tetrapods rely on a large suite of muscles to produce rotation of the femur about the hip joint. For example, the salamander, *Necturus maculosus*, has 13 muscles originating from its pelvic girdle (Boisvert et al., 2013). The pectoral fin of antennariid anglerfish, which has been suggested to be the closest analog to a terrestrial tetrapod limb within the teleost clade, relies on the large posteroventral coracoradialis, abductor superficialis III, and multiple series of superficial abductor and adductor muscles to produce movement (Edwards, 1989). The epaulette shark uses three robust muscles (the levator pelvicus, an anterolaterally positioned depressor pelvicus, and adductor pelvicus) that span the pelvic girdle

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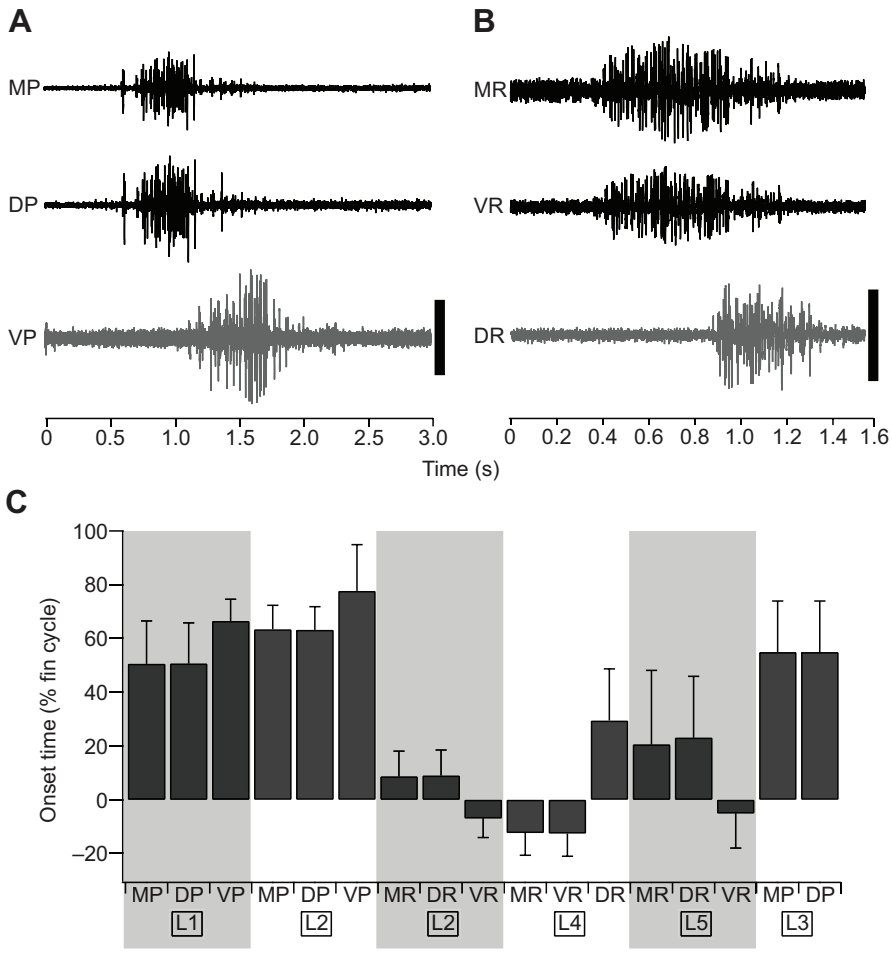

Fig. 6. Representative muscle activity recorded from the middle electrode of two individuals and during one fin cycle. (A) In L2, recordings from the dorsal and middle protractor muscle regions revealed nearly identical EMG traces, which were distinct from activity recorded in the ventral region of that same muscle. (B) In L4, recordings from the middle and ventral retractor regions produced nearly identical EMG traces, which were distinct from the activity captured in the dorsal region of that same muscle. Scale bars, 0.2 mV. (C) Mean onset and duration times of muscle activity from middle electrodes and adjacent electrodes within the same muscle per individual. Notice that in all cases muscle activity recorded by the middle electrode is nearly coincident in onset and duration with the muscle activity recorded from the dorsal or ventral electrode in the same muscle, but never both. VR, ventral retractor; MR, middle retractor; DR, dorsal retractor; DP, dorsal protractor; MP, middle protractor; VP, ventral protractor.

(Goto et al., 1999) to rotate the pelvic fin during walking (Pridmore, 1994). Future EMG studies on the hip musculature of other systems would allow us to determine whether they also have functionally subdivided muscles and inform our biomechanical understanding of alternative motor control and musculoskeletal organizations that accomplish rotation at a joint.

The architecture of the muscles spanning the hip joint in *P. annectens* may be a remnant of a more complex plesiomorphic condition. In the Australian lungfish (*Neoceratodus forsteri*), seven muscles originate from the pelvic girdle, but only two of these muscles, the deep ventral adductor depressor muscle (DVADD) and the deep ventral abductor depressor muscle (DVABD), insert on the first axial skeletal element of the fin (Boisvert et al., 2013). The DVABD and DVADD muscles in the Australian lungfish broadly originate from the anterior and posterior sides of the pelvic girdle, respectively, to insert on the distal aspect of the femur; the origins and insertions of the DVABD and DVADD muscles are similar in origin and insertion to the *P. annectens*' protractor and retractor muscles. Recent developmental work suggests that most of the muscles originating from the pelvic girdle in Australian lungfish (including the DVADD and DVABD muscles) are homologous to those originating from the pelvic girdle in the salamander (*Necturus maculosus*) (Boisvert et al., 2013). Taken together, assuming two or more plesiomorphic muscles have not become fused over evolutionary time, it is likely that the African lungfish's hip muscles are homologous to muscles spanning the hip joint in the Australian lungfish and salamanders. Thus, if Boisvert and colleagues' (Boisvert et al., 2013) comparison to salamanders holds, this would suggest that the African lungfish's hip morphology represents a secondarily derived reduced condition consisting of fundamentally conserved anatomy.

Morphological analyses alone are often insufficient to predict function (see Lauder, 1995), and this makes the inference of functional capabilities of extinct taxa challenging. The use of modeling to better understand the biomechanics of extinct taxa is becoming more common (e.g. Pierce et al., 2012). In these contexts it is useful to compare the outputs of models with the range of movements produced in extant taxa (Hutchinson, 2012). The African lungfish is one of few remaining extant sarcopterygian fishes and can help inform our biomechanical interpretation of fossils, including those on the aquatic side of species that bracket the vertebrate water to land transition. Movement of the African lungfish's pelvic fin would seem to rely on both the broad insertions of the protractor and retractor muscles (structure) and their functional subdivision (physiology). We suggest that the biomechanics of pelvic fin (hindlimb) movement in the African lungfish should be considered when developing hypotheses of the functional capabilities of related extinct taxa.

MATERIALS AND METHODS

Animals

Five African lungfish, *P. annectens*, were used in this study (body mass 0.34–0.61 kg, length 43.5–52.1 cm; Table 1). Lungfish were obtained commercially and housed in glass aquaria (56.8 l) equipped with re-circulating filters and water heaters. Fish were fed three times a week with commercial carnivore and algal food pellets (Hikari Hayward, CA, USA).

Water temperature was maintained at ~27°C and the animals were exposed to a seasonal light/dark cycle by using natural sunlight as the main source of illumination in the animal care room. All experimental procedures were carried out under University of Chicago Institutional Animal Care and Use Committee guidelines (protocol 71589 to M.E.H.).

Surgical procedures and electrode implantation

EMG electrodes were constructed using 0.005 mm diameter bifilar, insulated (nylon bond coat, annealed) stainless steel wire (California Fine Wire, Grover Beach, CA, USA) that was bared 0.5 mm on one end, fed through a 26 gauge syringe needle, and formed into a twist-hook for muscle implantation (Biewener, 1992; Loeb and Gans, 1986). Electrodes for muscle recordings were implanted into the right or left pelvic fin of each fish following published methods (e.g. Biewener, 1992; Loeb and Gans, 1986). Prior to electrode implantation, animals were anesthetized using tricaine methanesulfonate (MS-222, 0.5 g l^{-1}; Acros Organics, Geel, Belgium). Three bipolar EMG electrodes were implanted in the pelvic fin retractor and protractor muscle; an electrode was inserted in the dorsal, middle (halfway between the dorsal and ventral electrodes) and ventral region of the protractor and retractor muscles. The muscles are thin and each electrode was recording through much the muscle's depth. After implantation of EMG electrodes, hot glue was applied to bind the lead wires from all implants into one cable.

Data acquisition

EMG recordings were collected after 1–2 h of recovery. EMG signals were output to Grass digital amplifiers (model 15LT; Grass Technologies, Astro-MED Inc., West Warwick, RI, USA), and amplified by a factor of 20,000 (bandpass filtered from 30 Hz to 6 kHz, 60 Hz notch filtered). Signals were passed through an A/D converter (model PVA-16; Grass Technologies) and saved to a computer at 5000 Hz using data acquisition software (PolyVIEW16; Grass Technologies).

All data were collected while fish were filmed 'walking' in the ventral view in a 61×61 cm still tank containing a 1×1 cm squared plastic grid that was used to provide traction for the fins. The fish were filmed at 125 frames s^{-1} with a Photron APX-RS high-speed digital video camera at 1024×1024 pixel spatial resolution. Video was synchronized with EMG recordings by simultaneous data acquistion (in real time) and the application of a time stamp. Upon completion of all recordings, animals were killed in a high concentration of MS-222 solution (1.5 g l^{-1}) and the fish were dissected post mortem for verification of electrode placements.

Kinematics were recorded from ventral view videos. A lateral view required the use of a smaller tank that consequently limited the number of 'steps' a given individual could take. Fish did not behave optimally in the small tank, which was not conducive to data collection. However, we knew from previous studies that the fin was lifted dorsal to the joint during protraction and the fin was in contact with the substrate for the majority of the retraction phase (King et al., 2011). Therefore, in this study, we inferred that the pelvic fin was lifted above the joint during protraction and below the joint during retraction. Furthermore, visual inspection of each fish's movement supported our assumptions that each fish was producing rotational movements of its pelvic fins.

Electromyography and kinematic analysis

Video and EMG data from at least two consecutive fin rotation cycles were analyzed from 10 of the recorded locomotor trials per individual. Each trial included a series of at least four consecutive fin cycles. The first and last fin rotation cycles of a given series were not used in analysis. In four of five individuals, n=20 fin cycles were analyzed for each variable measured. In individual L3, data were collected from the electrodes implanted in the dorsal and ventral regions of the retractor as well as the dorsal region of the protractor. We were able to record EMGs from the middle electrode in a subset of implantations (six of 10). The implantation of the middle electrode was more difficult because the angle of insertion was restricted by the body wall, and muscle fibers in this region were originating from the body wall and the electrode was often placed too deep.

Descriptive statistics of all data are presented as means ± s.d. unless otherwise specified. To test for significant differences between regional muscle activity patterns, we performed Student's t-tests on EMG onset and duration variables recorded from all six regions surrounding the base of the pelvic fin. In a subset of data (individuals L1, L2, L4, L5), linear regressions were conducted between the duration of regional muscle activity and kinematics variables (cycle duration, retraction duration and protraction duration) to test how muscle activity patterns related to kinematics. All statistical analyses were performed in MATLAB 8.1.0 (MathWorks, Natick, MA, USA) or JMP 9.0.1 (SAS, Cary, NC, USA).

Kinematics

Pelvic fin kinematics were quantified in ImageJ (W. S. Rasband, ImageJ, US National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/, 1997–2012) using a custom-written macro (Abramoff et al., 2004). Three points were marked in every frame analyzed by digitizing the base of each fin in ventral view and a point 1 cm from the base of the fin on the body wall and the fin, respectively. Video data were used to determine the onset of the retraction and protraction phases of the pelvic fin rotation cycle. The total duration of the fin rotation cycle was calculated from the number of frames between the points of maximal protraction in consecutive pelvic fin rotation cycles. Cycles with minimal (approximately less than 5 deg in the medio-lateral plane) or no body axis undulation were used in analysis.

Electromyography

EMG data were analyzed using a multi-channel custom-written MATLAB (MathWorks) routine (R. Williams, IV and B.R.A.) to determine EMG onset (EMG_{on}), offset (EMG_{off}) and burst duration times. EMG burst duration (D_{EMG}) was calculated as the time of EMG offset minus the EMG onset ($D_{EMG}=EMG_{off}-EMG_{on}$), and measured in both absolute time (in s) and as a percentage of fin rotation cycle. EMG timing variables were then converted to percentage fin rotation cycle relative to the start of pelvic fin retraction (maximum protraction). A comparison of onset times within each fin cycle was used to determine the consistency in the order of activation. To ensure variations in onset order were not due to differences in the signal to noise ratio among channels, a difference in onset time between two electrodes less than or equal to three percent of the fin cycle was considered coincident activation.

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Competing interests

The authors declare no competing financial interests.

Author contributions

B.R.A., H.M.K. and M.E.H. designed the research; B.R.A. performed the research; B.R.A. and M.E.H. analyzed the data; B.R.A. and M.E.H. wrote the paper.

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