

Mudskipper pectoral fin kinematics in aquatic and terrestrial environments

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SUMMARY

Mudskippers use pectoral fins for their primary mode of locomotion on land and pectoral fins in conjunction with the axial musculature and caudal fin to move in water. We hypothesized that distinct pectoral fin movements enable effective locomotion in each environment. Additionally, we made three functional predictions about fin movements during locomotion on land *versus* water: the pectoral fin is depressed more on land than in water; the pectoral fin will have greater changes in fin area between propulsive and recovery phases in water *versus* land; anterior and posterior excursions will be greater on land than in water. Locomotion was recorded in each environment using a high-speed digital-imaging system and kinematic variables were calculated from digitized landmark points. Variables were analyzed using principal components analysis and matched pairs *t*-tests. Mudskippers produce distinct kinematic patterns across environments ($P < 0.003$), although only some of our predictions were supported. The magnitude of fin depression is the same across habitats. However, depression occurs during the propulsive phase on land (by -0.60 cm), whereas during the propulsive phase in water the fin is elevated (by $+0.13$ cm). We were unable to support the hypothesis that fin orientation differs between environments. Lastly, anterior extension of the fin is greater on land (1.8 cm, *versus* 1.3 cm in water), creating a larger stride length in this environment. We posit that the mudskipper pectoral fin may facilitate stability in water and thrust production on land, and suggest that the robust fin morphology of the goby lineage may predispose species within this group to terrestrial locomotion.

Key words: amphibious fishes, swimming, locomotion, crutching, *Periophthalmus*, mudskipper.

INTRODUCTION

Amphibious fishes ‘live both in and out of water as normal parts of their life history’ (Gordon, 1998; Gordon et al., 1968). Yet, although many aspects of amphibious fish physiology (i.e. respiration, nitrogen excretion, thermoregulation, fluid balance, vision) have been examined (Graham, 1997; Sayer, 2005), relatively few studies have examined how amphibious fishes move about their environments (Sayer, 2005). Many of these fishes typically use the same structures to locomote in terrestrial and aquatic environments (Sayer, 2005), however, different biomechanical demands are placed on a structure (e.g. the pectoral fin) when it is used for locomotion in water than when it is used on land. For example, when moving from an environment where viscous forces dominate (i.e. water) to one where gravitational forces dominate (i.e. land) amphibious fishes will encounter different mechanical loadings on their muscles, bones and joints. It is also likely that each environment will require different coordination and timing of structures for successful locomotion, which implies potential differences in the underlying neural control of movement in each habitat. Lastly, the direction in which these fishes will attempt to generate force must shift from being against the fluid surrounding them to against a ventrally located substrate. Thus, amphibious fishes provide an excellent opportunity to examine how differing, and potentially conflicting, locomotor demands shape the morphology and movements of locomotor structures.

Mudskippers are amphibious fishes that exemplify an extreme in amphibious behavior, with some species spending more time out of water than in (Gordon, 1998; Graham, 1997). Because they live on intertidal mudflats, mudskippers are exposed to a daily regime of alternating terrestrial and aquatic environments (Clayton, 1993). The mudskipper group comprises four genera of Oxudercinae gobies

that have skeletal and muscular morphology distinct from other gobies (Harris, 1959; Murdy, 1989). In particular, the skeletal and muscular morphology of their pectoral fins is unusual and has been hypothesized to aid in terrestrial locomotion (De and Nandi, 1984; Harris, 1959; Murdy, 1989).

The mudskipper pectoral fin differs from most actinopterygian fishes in that the radials of the mudskipper pectoral fin are elongate and protrude from the body wall (Fig. 1). This unusual morphology creates a pectoral fin with two fin segments (the radials and the rays) and two movable hinge joints: a ‘shoulder’ joint where the cleithrum meets the radials and a ‘intra-fin’ joint where the radials meet the rays (Harris, 1959). In addition, three of the four genera that comprise the mudskipper group have unusual musculature associated with the pectoral fin (Murdy, 1989). For example, the abductor superficialis muscle of the pectoral fin is divided into two sections (rather than being a single muscle, as is common with the rest of the Oxudercinae gobies) with one section inserting on the dorsal rays and the other section inserting on the ventral rays (Murdy, 1989). It has been suggested that this arrangement could provide greater control and flexibility in the movements of the pectoral fin during terrestrial locomotion (Harris, 1959; Murdy, 1989).

Mudskippers have at least two discrete types of terrestrial movement (De and Nandi, 1984; Harris, 1959; Klausewitz, 1967; Stebbins and Kalk, 1961; Swanson and Gibb, 2004). The first of these is an escape response, in which the mudskipper ‘jumps’ away from a negative stimulus using its caudal fin and axial musculature (Swanson and Gibb, 2004); mudskippers may also use this behavior to capture elusive prey (Clayton, 1993). The second type of terrestrial locomotion is used for continuous movement across the intertidal substrate (often a mudflat). This form of locomotion has been dubbed ‘crutching’ because of its superficial resemblance to

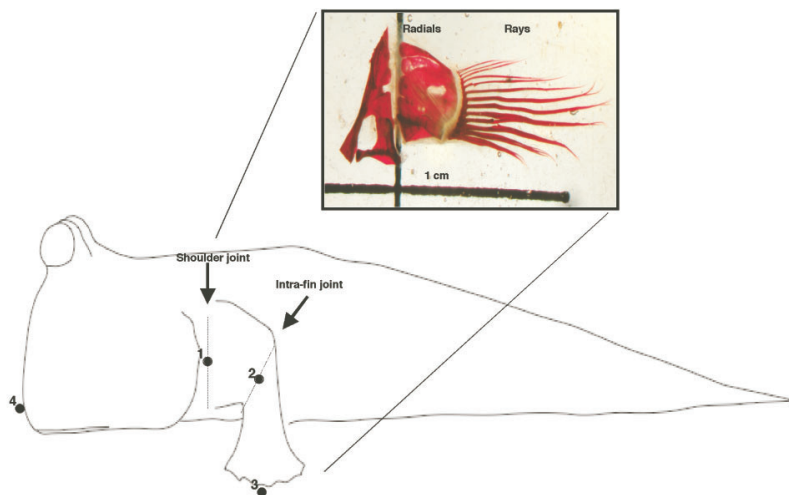


Fig. 1. Line drawing of a mudskipper and a photograph of a cleared and double stained mudskipper pectoral fin. The radials are the proximal fin segment and the rays are the distal fin segment. Points digitized for kinematic analysis are as follows: point 1 is the shoulder joint; point 2 is the fin joint; point three is the distal tip of the rays; point 4 is a reference marker on the head.

a human locomoting with crutches (Harris, 1959). While crutching, the mudskipper uses its pectoral fins synchronously; during a stride, the pectoral fins apparently lift the center of mass and allow it to swing forward. After the center of mass returns to the substrate, the pectoral fins are repositioned to initiate another stride (Harris, 1959; Stebbins and Kalk, 1961).

In aquatic environments, mudskippers, like many gobies, appear to be intermittent swimmers. Mudskippers have been reported to either 'skip' across the surface of the water or swim underwater (De and Nandi, 1984; Harris, 1959; Stebbins and Kalk, 1961), but there are no quantitative studies of either of these behaviors. Harris (Harris, 1959) noted that mudskippers use only axial undulations at high swimming speeds, but that at slower swimming speeds they also paddle using their pectoral fins. Although thrust is probably primarily generated by axial undulation, the consistent presence of pectoral fin movements during slow swimming suggests that, similar to many other fishes (Drucker et al., 2006; Lauder and Drucker, 2004) pectoral fins play a key role in aquatic locomotion.

Organisms from a broad taxonomic spectrum use different locomotor strategies in aquatic *versus* terrestrial environments (Gillis and Blob, 2001). However, to our knowledge there have been no kinematics studies on a vertebrate that not only locomotes in aquatic and terrestrial environments, but also relies solely on its anterior appendicular system (i.e. pectoral fins) to generate routine locomotor forces on land. Therefore, in this study we compare pectoral fin kinematics across aquatic and terrestrial environments to test the over-arching hypothesis that mudskippers employ distinct pectoral fin movement patterns in each habitat. We also use these kinematic variables to test specific predictions (based on our preliminary observations and those of previous researchers) about the fin kinematics during terrestrial locomotion. (1) Because terrestrial locomotion relies on ground reaction forces to generate propulsion (Biewener, 2003), we predict that the fin will be more depressed (i.e. directed more ventrally) during terrestrial locomotion to facilitate contact with the substrate, but that fin depression does not occur at the same magnitude during aquatic locomotion. (2) Because surface area and orientation of the fin to flow is important in drag-based aquatic locomotion (Webb, 1988) we predict that there will be greater changes in fin area between the propulsive and recovery phases of aquatic locomotion than during terrestrial locomotion. (3) Because the unusual pectoral fin morphology of the mudskipper may facilitate increased stride length (Harris, 1959), we predict that mudskippers have greater anterior-posterior

excursions of the pectoral fin during terrestrial locomotion relative to aquatic locomotion.

MATERIALS AND METHODS

Animals

Mudskippers, *Periophthalmus argentilineatus* Valenciennes 1937, were acquired from a commercial vendor. All mudskippers used in this study were similar in mass (2.3 ± 0.1 g) and total length (6.0 ± 0.2 cm; Table 1). Mudskippers were housed in glass aquaria containing both aquatic and terrestrial habitat, and exposed to a 12h:12h light:dark cycle. Mudskippers were fed a diet of bloodworms and commercial fish food. All experiments were conducted in accordance with NAU IACUC guidelines under protocol #02-077-R1.

Data collection and analysis

Locomotor trials were conducted in a Plexiglas tank with a mirror positioned underneath the tank at 45 deg. A Redlake Motionscope (PCI 1000s) digital-imaging system captured images from lateral and ventral (*via* the mirror) views with a single camera; images were recorded at 125 Hz. Movement of the mudskipper was induced by gently prodding the caudal fin.

Digital video images were imported into QuickImage image (a customized version of NIH Image written by J. A. Walker of University of Southern Maine) for analysis. Landmark points digitized on each mudskipper were as follows (Fig. 1): a reference point on the anterior tip of the mudskipper's head; the shoulder joint; the intra-fin joint; the most distal tip of the rays. Although these anatomical landmarks simplify the complexity of the fin rays during aquatic locomotion, they allow for a reliable comparison of appendage movements between habitats. More detailed analysis of fin movements are not practical because the fin rays tend to collapse against one another during terrestrial locomotion, which makes it impossible to identify and track individual fin rays. For each landmark, x and z coordinates were obtained from the ventral view and the y coordinate was obtained from the lateral view. In addition

Table 1. Mass, total length (TL) and fin segment lengths for each individual mudskipper

Individual	Mass (g)	TL (cm)	Radials (cm)	Rays (cm)	Total fin (cm)
1	2.2	6.37	0.317	0.826	1.143
2	2.5	5.56	0.271	0.710	0.981
3	2.1	5.96	0.384	0.804	1.188

to digitizing, the area of the fin rays was measured by drawing an outline of the rays in the ventral view of the video and using the area calculation in QuickImage.

For each mudskipper ($N=3$), five terrestrial trials and five aquatic trials were analyzed. Although this is a relatively small sample size, it has not been possible to procure more individuals of this species; in addition, significant results have been seen with similar sample sizes in other relatively recent kinematic studies (Rubenson et al., 2007; Van Wassenbergh et al., 2005). In each trial a single stroke of the pectoral fin was digitized, which was defined as the onset of fin retraction through the end of fin protraction. All of the pectoral fin strokes came from different sequences of continuous locomotion and are separate locomotor events.

Voluntary locomotor speeds were used for all aquatic *versus* terrestrial comparisons. We note that the slowest swimming speed for which a goby maintains position above the substrate while swimming continuously is faster than voluntary, steady-state, cyclic, terrestrial locomotor behavior (C.M.P., personal observation; Table 2). Because of this, velocity-matched comparisons are not possible for this study. Although it is possible that speed affects kinematics, this result would be a reflection of the natural behavior exhibited by the mudskippers. Since the duration of trials differed across environments, the duration of each stroke was transformed into a percentage (1 complete cycle=100%) so strokes of different absolute durations could be compared with one another.

The three-dimensional coordinates for each stroke were imported into a spreadsheet program and kinematic variables were calculated. Kinematic variables (Table 2) included: minimum and maximum intra-fin angle; the anterior and posterior excursion of the radials, the rays, and the entire fin; the elevation and depression of the radials, the rays and the entire fin; the area of the rays viewed ventrally halfway through the propulsive phase and halfway through the recovery phase. Movements of the radials were defined by the distal tip of the radials in reference to the shoulder joint, movements of

the rays by the distal tip of the rays in reference to the intra-fin joint, and movements of the whole fin by the distal tip of the rays in reference to the shoulder joint (Fig. 1). In addition to the kinematic variables, several timing variables were calculated: stroke duration, duration of the propulsive and recovery phases, when maximum posterior fin extension occurs, and when maximum pectoral fin elevation occurs in the stroke. Except for stroke duration the timing variables were compared as percentages of a single stroke.

Statistical analyses

In order to reduce the number of variables, a principal components analysis (PCA) was performed using the correlation matrix (McCune and Grace, 2002). Of the variables given above, ten were analyzed collectively using PCA, these variables were: minimum and maximum intra-fin angle; fin anterior and posterior excursions; fin elevation and depression; the calculated difference between the fin area seen ventrally halfway through the propulsive phase minus the fin area seen ventrally halfway through the recovery phase; stroke duration; when maximum pectoral fin posterior extension occurs; and when maximum pectoral fin elevation occurs. Axes of variation were considered significant if they had eigenvalues >1.0 (i.e. the Kaiser–Guttman criterion). Significant axes were rotated using a Varimax transformation and variables greater than ± 0.7 were considered to load ‘heavily’ on a given axis of variation.

Since the same individuals were sampled in each habitat all hypothesis testing was done using a matched pairs *t*-test to avoid violating assumptions of independence (Sokal and Rohlf, 1995). To address the general hypothesis that there are differences in pectoral fin movements between aquatic and terrestrial locomotion and to determine if habitat correlated with patterns of distribution in multivariate space, matched pairs two-way *t*-tests were performed on the scores of each rotated axis of variation. In addition, to test specific predictions associated with this general hypothesis, matched pairs one-way *t*-tests (and one two-way test on the timing of

Table 2. Means for kinematic movement and timing variables in each habitat

Variables	Terrestrial	Aquatic	Significance
Duration of stroke (s)	0.49 ± 0.04	0.26 ± 0.01	
Distance traveled per stroke (cm)	2.13 ± 0.09	3.49 ± 0.27	
Average speed (cm s ⁻¹)	4.47 ± 0.27	13.75 ± 1.05	
Duration of propulsive phase (% stroke)	45 ± 2	38 ± 2	
Duration of recovery phase (% stroke)	52 ± 2	56 ± 2	
Time of initiation of recovery phase (% stroke)	48 ± 2	44 ± 2	
Time of maximum elevation (% stroke)	67 ± 5	36 ± 2	*
Time of maximum posterior extension (% stroke)	47 ± 2	43 ± 3	
Minimum angle (deg.)	76 ± 1	121 ± 8	
Maximum angle (deg.)	178 ± 7	213 ± 4	
Elevation of radials (cm)	0.04 ± 0.03	0.01 ± 0.02	
Elevation of rays (cm)	0.05 ± 0.08	0.16 ± 0.08	
Elevation of whole fin (cm)	-0.02 ± 0.08	0.13 ± 0.09	
Depression of radials (cm)	-0.24 ± 0.02	-0.17 ± 0.04	
Depression of rays (cm)	-0.53 ± 0.03	-0.29 ± 0.02	
Depression of whole fin (cm)	-0.60 ± 0.07	-0.39 ± 0.04	n.s.
Anterior extension of radials (cm)	0.0 ± 0.01	-0.08 ± 0.03	
Anterior extension of rays (cm)	0.78 ± 0.04	0.31 ± 0.03	
Anterior extension of whole fin (cm)	0.73 ± 0.04	0.19 ± 0.02	*
Posterior extension of radials (cm)	-0.49 ± 0.03	-0.46 ± 0.03	
Posterior extension of rays (cm)	-0.66 ± 0.003	-0.74 ± 0.05	
Posterior extension of whole fin (cm)	-1.08 ± 0.02	-1.14 ± 0.07	n.s.
Ventral fin area during propulsion (cm ²)	0.21 ± 0.01	0.31 ± 0.04	
Ventral fin area during recovery (cm ²)	0.30 ± 0.02	0.46 ± 0.04	*
Difference in ventral fin area (propulsion–recovery) (cm ²)	0.08 ± 0.02	0.15 ± 0.003	n.s.

*Variables that were found to be significantly different between environments; n.s., variables that were not found to be significant at the $\alpha=0.05$ level.

maximum fin depression) were performed on selected kinematic variables (see Introduction). All statistical analyses were performed using JMP 7.02.

RESULTS

In both aquatic and terrestrial locomotion, the movement of the mudskipper pectoral fin can be partitioned into two phases. A propulsive phase, in which the fin is retracted to generate force against either the water or the ground, and a recovery phase, in which the fin is protracted so that it is repositioned to start the next stroke (Fig. 2). Additionally, in both aquatic and terrestrial locomotion a pause may occur between the propulsive and recovery phases; during this pause the pectoral fin does not move. It should be noted that during terrestrial locomotion, the pectoral fin is in contact with the ground during propulsion, but not during recovery; therefore, during terrestrial locomotion these phases are analogous to the 'stance' and 'swing' phases described for most terrestrial animals (Biewener, 2003).

Stroke duration differs across environments such that pectoral fin strokes take approximately twice as long in the terrestrial environment (Table 2). There are also small differences in the proportion of time spent in propulsive and recovery phases in each environment. In the aquatic environment propulsion occurs during the first 38% of the stroke and recovery during the last 56%, whereas in the terrestrial environment propulsion occurs during the first 45% of the stroke and recovery consists of the latter 52% (Table 2). Therefore, during terrestrial locomotion the duty cycle is 0.45, and although this term is not commonly used in analysis of aquatic locomotion, the equivalent value during aquatic locomotion would be 0.38.

Terrestrial locomotion

At the beginning of the propulsive phase, the pectoral fin is in contact with the ground, the intra-fin joint is flexed at $\sim 100^\circ$ angle, and the distal tip of the fin is anterior and ventral to the shoulder joint (Figs 3, 4 and 5). As the mudskipper begins to move forward, it uses the pectoral fins to lift its body off the substrate (Fig. 4, Table 2).

(In Fig. 4 the lifting of the body corresponds with the depression of the pectoral fin seen during the propulsive phase, that is, lifting occurs as the distance between the tip of the fin and the shoulder joint increases.) This movement appears to be generated primarily by movements of the fin rays, not the radials (Fig. 4), and causes deformation of the rays (i.e. bending) at the tip of the fin. Throughout the propulsive phase, the fin tips remain in contact with the ground while the body moves forward; thus, by the end of the propulsive phase, the fin tips are posterior to the shoulder joint. The resulting stride length is approximately 27% of the mudskippers total length (Fig. 3). While the body is lifted and moved forward during propulsion, the intra-fin joint initially flexes into an acute angle of $\sim 87^\circ$, and then extends such that the fin joint is $\sim 167^\circ$ by the end of the propulsive phase (Fig. 5).

At the beginning of the recovery phase, the entire fin is positioned posterior and ventral to the pectoral girdle and is only slightly flexed at the intra-fin joint. During recovery, the swing phase, the fin is lifted off the substrate, protracted anteriorly, and is then placed back onto the substrate. In terrestrial locomotion, the largest difference in the movements of the fin segments between propulsion and recovery (other than the direction of movement) is in the elevation of the fin. Although the fin is never elevated above the shoulder joint during the stroke, it reaches its maximum elevation during the recovery phase.

Aquatic locomotion

When the propulsive phase of aquatic locomotion begins, the pectoral fin is already abducted from the body, with the intra-fin joint bent at an angle of $\sim 130^\circ$, and the distal tip of the fin positioned just anterior and ventral to the shoulder joint (Figs 3 and 4). As the stroke begins, the fin does not initially change in either elevation or in the angle between the two segments – it is simply retracted. Approximately one-quarter of the way through the propulsive phase, the rays are elevated and the fin straightens and approaches 180° at the intra-fin joint (Figs 4 and 5). Maximum elevation occurs prior to the end of the propulsive phase, when the fin rays are elevated to a position dorsal to the shoulder joint (Table 2). Simultaneously, the

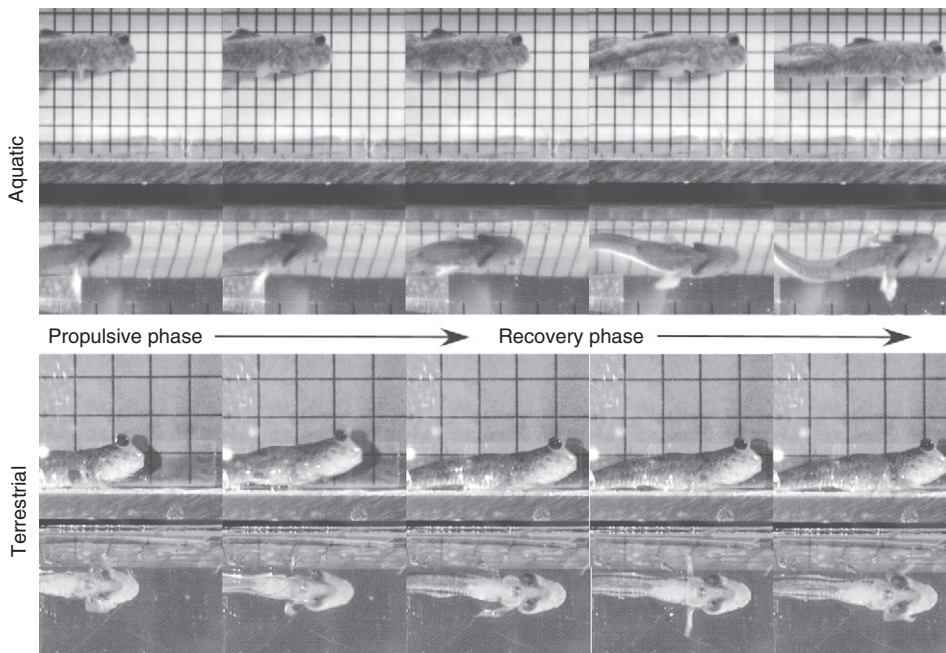


Fig. 2. Image series of a mudskipper moving in aquatic and terrestrial environments. The top image series depicts the lateral and ventral views of a single stroke in the aquatic environment. The grid at the back of the lateral view is a 0.5 cm grid and the grid in the ventral view is a 1 cm grid. The bottom image series depicts the lateral and ventral views of a single stroke in the terrestrial environment. Both of the grids in the lateral and ventral view are 1 cm grids.

fin is straightened through 180 deg. and bends in the opposite direction to form an angle of ~ 195 deg. (Fig. 5, Table 2).

The recovery phase of the pectoral fin begins with the fin fully retracted and at its most elevated position, such that the fin tips are posterior and slightly dorsal to the shoulder joint. As the fin is

repositioned, it is both depressed and protracted for the first quarter of recovery and is then simply protracted for the remainder of the recovery phase. Additionally, during recovery the rays are 'feathered' (i.e. surface area minimized relative to the flow), while during propulsion it is held more perpendicular to flow (Table 2).

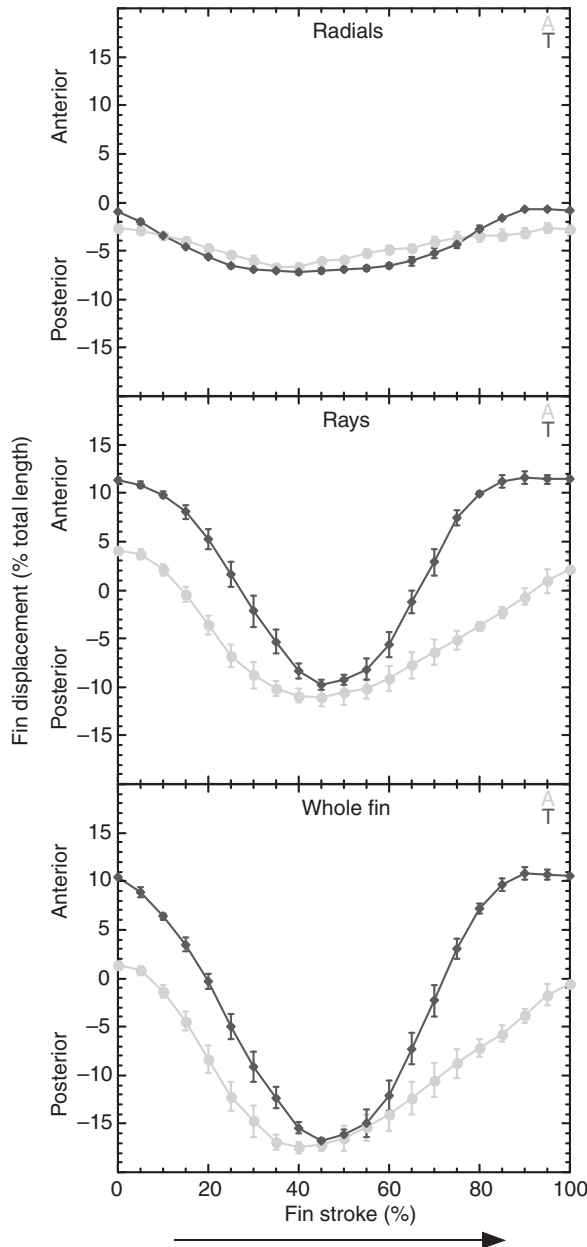


Fig. 3. Anterior and posterior fin extension. Mean kinematic profile of the anterior and posterior excursion of the radials, rays and entire fin throughout a single stroke during both aquatic and terrestrial locomotion. Aquatic profiles are in gray (A) and terrestrial trials are in black (T). All data points consist of means and s.e.m. bars. The x-axis is the percentage completion of a single stroke. The y-axis is fin displacement transformed into percentage total length. Positive values indicate that the distal end of a morphological structure is anterior to its proximal end whereas negative values indicate that it is posterior. The profile for the radials compares the distal end of the radials to the proximal end where the radials attach to the cleithrum, the profile for the rays compares the distal end of the rays to the proximal end where the rays attach to the radials, and the entire fin compares the distal end of the fin to the proximal end.

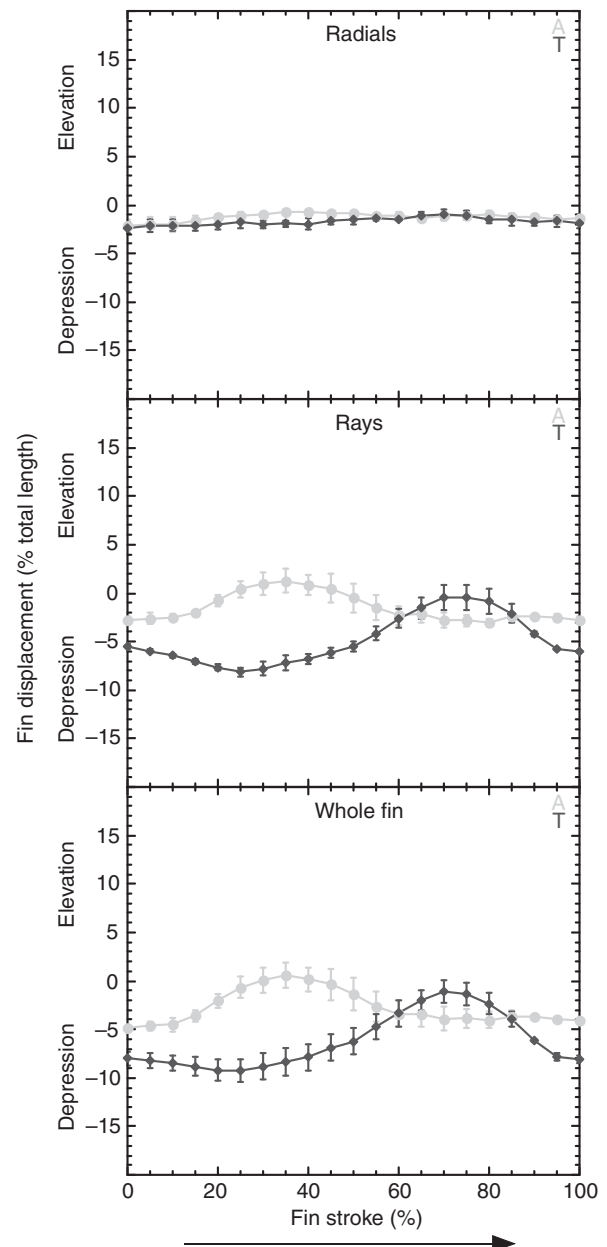


Fig. 4. Elevation and depression of the fin. Mean kinematic profile of the dorsal and ventral position of the radials, rays and entire fin throughout a single stroke during both aquatic and terrestrial locomotion. Aquatic profiles are in gray and terrestrial trials are in black. All data points consist of means and by s.e.m. bars. The x-axis is the percentage completion of a single stroke. The y-axis is fin displacement transformed into percentage total length. Positive values indicate that the distal end of a morphological structure is dorsal to its proximal end whereas negative values indicate that it is ventral. The profile for the radials compares the distal end of the radials to the proximal end where the radials attach to the cleithrum, the profile for the rays compares the distal end of the rays to the proximal end where the rays attach to the radials, and the entire fin compares the distal end of the fin to the proximal end.

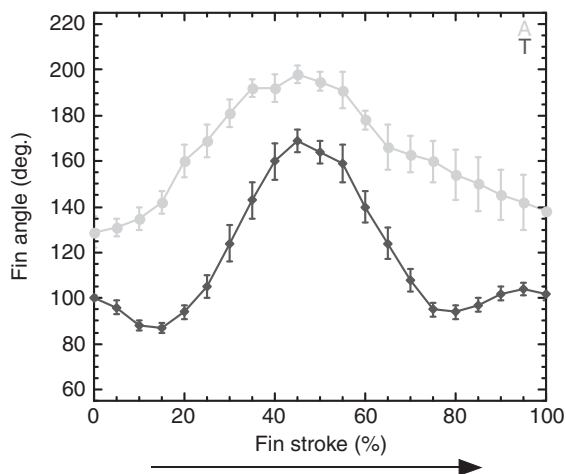


Fig. 5. Angle between fin segments. Mean kinematic profile of the angle formed between the radials and rays throughout a single stroke during both aquatic and terrestrial locomotion. Aquatic profiles are in gray and terrestrial trials are in black. All data points consist of means and s.e.m. bars. The x-axis is the percentage completion of a single stroke and the y-axis is the angle formed. Values below 180 deg. occur when the fin is bent rostrally and values greater than 180 deg. occur when the fin is bent caudally.

Comparison of aquatic and terrestrial locomotion

Principal components analysis of ten key kinematic variables (see Materials and Methods) described two axes of variation that together explained 88% of the variation. On the first axis (67% of the variation) the following six variables loaded heavily (Table 3): maximum intra-fin angle, maximum elevation of the pectoral fin, maximum depression of the pectoral fin, maximum anterior position of the pectoral, total stroke duration, and the calculated difference between the fin area seen ventrally halfway through the propulsive phase minus the fin area seen ventrally halfway through the recovery phase. On the second axis (21% of the variation) three variables loaded heavily: minimum intra-fin angle, maximum posterior position of the pectoral fin, and the time of maximum pectoral fin posterior extension. Of the ten variables used, two of the variables (minimum angle and when maximum pectoral fin elevation occurs) had strong loading values on both axes: yet, loading was slightly stronger on the second axis. Also, the timing of maximal fin elevation did not contribute with a value greater than 0.7 to either axis (Table 3).

Table 3. Factor analysis and how variables load on each significant axis after factor rotation

	Factor 1	Factor 2
% Variance explained	67.0%	21.0%
Minimum angle during stroke (deg.)	0.673	-0.726
Maximum angle during stroke (deg.)	0.973	-0.189
Maximum fin depression (cm)	0.801	-0.212
Maximum fin elevation (cm)	0.868	0.470
Maximum posterior fin extension (cm)	0.071	0.829
Maximum anterior fin extension (cm)	-0.873	0.485
Difference in ventral fin area: thrust-recovery (cm ²)	0.884	-0.122
Stroke cycle duration (s)	-0.944	0.318
% Stroke duration to maximum fin elevation	-0.661	0.685
% Stroke duration to maximum posterior fin extension	-0.251	0.850

Values in bold indicate strong loading on the axes.

A matched pairs *t*-test was done on each of the two axes (the two rotated principal components) to determine if there was a significant effect of environment. The first axis revealed a significant effect of environment (aquatic *vs* terrestrial) with $P < 0.003$. However, the second axis showed no effect of environment ($P < 0.145$; Fig. 6); we suggest that the second axis may be driven largely by differences among individuals (Fig. 6). Thus, the first axis explains the bulk of the variation, and separates fin movements based on what environment the movement was performed in. This supports the general hypothesis that mudskippers use distinct pectoral fin movements during aquatic *versus* terrestrial locomotion

Matched pairs *t*-tests were also performed on six specific kinematic variables to test *a priori* predictions about fin movements (Table 3). To determine if mudskippers depress their fin more during terrestrial locomotion (to potentially maximize ground reaction forces), we compared maximum depression across environments. Although maximum depression loaded heavily on the first PCA axis (which was different across environments), there is no significant difference in the maximum depression of the fin in aquatic *versus* terrestrial environments ($P < 0.0622$). However, it is possible that the magnitude of depression is less important than the relative timing of fin depression. Because of the nature of the fin movements in the aquatic environment (i.e. a relatively constant level of depression is maintained for over 50% of the stroke; Fig. 4), it is difficult to compare a specific time of maximum fin depression across the two environments. However, it is possible to compare the time of maximum fin elevation across the two environments. When the timing of maximal fin elevation is compared across environments we find that it occurs at different times ($P < 0.0105$). In the aquatic environment, the fin is maximally elevated at ~36% of the stroke (i.e. during the propulsive phase); in the terrestrial environment, the fin is elevated at ~67% of the stroke (during the recovery phase; Table 2). Therefore, during the propulsive phase in terrestrial environments, the mudskipper reaches maximum vertical depression, whereas during the same period in aquatic environments it reaches maximum vertical elevation (Fig. 4). This supports the hypothesis that one difference between terrestrial and aquatic locomotion is that during terrestrial locomotion fin movements are directed ventrally to interact with the ground.

To determine if there were greater changes during aquatic than during terrestrial locomotion in fin area between the propulsive and

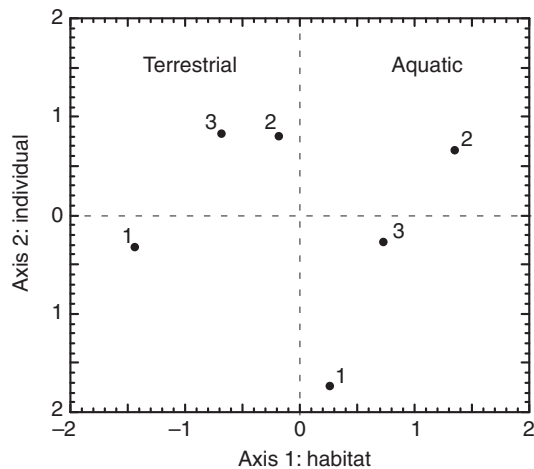


Fig. 6. Plot of two significant axes of kinematic variation derived from principal components analysis. Numbers at points on plot indicate specific individuals.

recovery phases we compared the calculated difference between the fin area seen ventrally halfway through the propulsive phase minus the fin area seen ventrally halfway through the recovery phase in each habitat. Results with large negative differences are found when a greater proportion of the fin rays can be seen ventrally during recovery than during propulsion. Although a difference of 0.15 cm^2 is seen during aquatic locomotion versus 0.08 cm^2 observed during terrestrial locomotion these results were not significant ($P < 0.069$) at $\alpha = 0.05$. (It should be noted that for this comparison and for the magnitude of total fin depression described in the preceding paragraph, that it is possibly a consequence of small sample size that these P -values approach, but do not reach, significance. We think it is probable that with a larger sample size these variables would be significantly different.) When we compare the amount of fin ray area that is feathered during recovery from the ventral view we observe more during aquatic locomotion (0.46 cm^2) than during terrestrial locomotion (0.30 cm^2 ; $P < 0.017$). Additionally, we have observed that the fin twists more at the transition points between propulsion and recovery during aquatic locomotion. We cautiously suggest that there are differences in fin area and orientation in each habitat, but concede that we have not been able to fully capture this difference with our data.

To examine stride length across environments, we compared the maximum anterior and posterior extension of the pectoral fin. Although maximum posterior extension did not differ across environments ($P > 0.1493$), maximum anterior extension did ($P > 0.0039$). In terrestrial environments, the fin was extended anteriorly, such that the tip of the fin was $\sim 0.7 \text{ cm}$ anterior to the shoulder joint; whereas in aquatic environments, the tip of the fin was $\sim 0.2 \text{ cm}$ anterior to the shoulder joint (Table 2). Terrestrially the total stride length was 1.8 cm , whereas aquatically it was 1.3 cm . This indicates that the pectoral fin has a longer stride length in terrestrial environments relative to aquatic environments because of a greater anterior extension, and supports the hypothesis that stride length increases during terrestrial locomotion.

DISCUSSION

Our comparison of mudskipper pectoral fin kinematics across aquatic and terrestrial environments reveals that the pectoral fin moves in a distinct pattern in each environment. Pectoral fins demonstrate a range of functions in aquatic locomotion: they may be used for turning, braking, stability and propulsion (Drucker and Lauder, 2003). Based on our results, the mudskipper pectoral fin probably generates forces primarily in the lateral and posterior directions during aquatic locomotion; whereas in terrestrial locomotion, the forces are probably generated in the lateral, posterior, and ventral directions. During slow swimming, pectoral fins may function as stabilizers (Weihs, 2002), and it is possible that lateral forces increase stability during locomotion in both environments because of the medially directed reaction forces they generate. For example, the center of mass of mudskippers is posterior to the pectoral girdle (Swanson and Gibb, 2004); therefore, during aquatic locomotion the pectoral fins may create medially directed reaction forces that offset the yaw generated in reaction to the undulations of the axial musculature. During the propulsive phase of terrestrial locomotion, the mudskipper appears to function as tripod, with the two pectoral fins and the tail forming three 'legs'. Medially directed reaction forces may increase stability during the stance phase of terrestrial locomotion while the center of mass is elevated above the substrate.

Typically, pectoral fins have a primary axis of movement, but the hinge joint that underlies the movement may have flexibility in

other axes (Drucker and Lauder, 2003). For example, in mudskippers the axis of the pectoral fin with the shoulder girdle creates a longitudinal hinge joint that allows the fin to move primarily in an anterior–posterior direction. In both aquatic and terrestrial locomotion the anterior and posterior excursions are greater than the dorsal and ventral excursions. However, there is sufficient flexibility in these joints that fin elements can also move in a dorsal–ventral direction. Because terrestrial movement is accomplished by generating ground reaction forces, we predicted that mudskippers would depress their fins more during terrestrial locomotion than aquatic locomotion. Interestingly, we found that whereas the magnitude of ventral depression did not differ across habitats, the timing of movements in the vertical plane did differ. During the propulsive phase of terrestrial locomotion, the mudskipper pectoral fin is depressed ventrally to interact with the ground, while during that same period in aquatic locomotion the fin is elevated. Harris (Harris, 1959) noted that in *Periophthalmus kolreuti* [now *P. barbarus*, according to Murdy (Murdy, 1989)] the dorsal radials are longer than the ventral radials, which causes a declination of the entire pectoral fin; the mudskipper species used in our study (*P. argenteolineatus*) has a similar morphology (Fig. 1). Although it is clear that a ventrally oriented fin enhances terrestrial locomotion, it is unclear if it hinders the function of the pectoral fins during aquatic locomotion. It is possible that the morphology of the radials represents a functional trade-off that enhances terrestrial locomotion at some expense to aquatic locomotion.

Animals that use drag-based propulsion (whether for propulsion or for stability and maneuvering) in aquatic environments often increase the surface area of the appendage that is perpendicular to the flow during propulsion and decrease the surface area perpendicular to the flow during recovery (termed 'feathering'). Thus, one of our *a priori* predictions was that during aquatic locomotion the fin would be more normal to flow during thrust and more feathered during recovery than in terrestrial locomotion. There was observational evidence of feathering during aquatic locomotion. However, a confounding component of terrestrial locomotion is that the fin rays 'collapse' on one another and create a fin with a small area, relative to that observed during aquatic locomotion (Table 2). During mudskipper aquatic locomotion, there appear to be benefits to maximizing surface area during propulsion and minimizing surface area during recovery, but during terrestrial locomotion the pectoral fin is oriented to maximize contact with the ground but fin surface area (*per se*) is of relatively little importance.

Terrestrial vertebrates have two options for traveling faster on land: increasing stride length or increasing stride frequency (Biewener, 2003). Given that in mudskipper terrestrial locomotion the pectoral fins appear to power movement of the entire organism (rather than assist the axial musculature, as in aquatic locomotion), it seemed unlikely that mudskippers would show an increased stride frequency on land relative to aquatic movements (in fact, the terrestrial stride lasts approximately twice as long as the aquatic stroke). Thus, one of our predictions was that during terrestrial locomotion mudskippers would increase their stride length by increasing fin extension anteriorly and posteriorly. Given that the length of the pectoral fin is approximately 19% of total length (TL) and is attached at the shoulder *via* a hinge joint, it is theoretically possible for the mudskipper fin to extend in both directions by $\sim 19\% \text{ TL}$. In both habitats the posterior extension of the pectoral fin was the same and approached the theoretical maximum of $19\% \text{ TL}$. However, in water there was almost no anterior extension of the pectoral fin, whereas on land the anterior extension was $\sim 10\% \text{ TL}$. Therefore, terrestrial stride length did increase as

predicted, but it increased in a unidirectional manner and by ~10% TL, rather than the theoretically possible 19% TL. It is noteworthy that movement of the rays, not the radials, primarily accounts for the increase in stride length (see Table 2, Fig. 3) – as it is the radials that are elongated when compared to other fishes (Harris, 1959; Murdy, 1989). It is possible that the pectoral girdle or the opercular bones inhibit the anterior extension of the radials, and manipulations of cleared and stained specimens support this hypothesis. This finding bolsters Harris's (Harris, 1959) assertion that the elongated radials are beneficial for terrestrial locomotion because they 'clear' the limb from the body wall. In other words, long radials allow an increased stride length because the rays are no longer precluded from anterior extension by other morphological features of the mudskipper, such as the opercular bones or the pectoral girdle.

Although we have described morphological features of the skeleton of the mudskipper pectoral fin that may facilitate locomotion across two environments, we cannot yet address the role of the pectoral fin musculature in generating specific locomotor movements. Harris (Harris, 1959) stimulated mudskipper pectoral fin muscles to assess the role of specific muscles in producing pectoral fin movements. He proposed that certain muscles were used primarily for terrestrial locomotion and that other muscles were used primarily for aquatic locomotion. Other researchers have also suggested that specific muscles are critical for terrestrial locomotion (Murdy, 1989). Based on our own kinematic work and studies on aquatic *versus* terrestrial locomotion with other organisms (Gillis and Blob, 2001) we also predict difference in muscle function in each habitat. However, future investigations that combine studies of muscle activity (electromyography) with kinematics are needed to elucidate the function of pectoral fin muscles in generating distinct movements across variable environments.

As the pectoral fins assist the axial musculature and caudal fin during aquatic locomotion, so the pelvic fins may assist the pectoral fins in terrestrial locomotion. However, the exact role of the pelvic fins in terrestrial locomotion remains unclear. Harris (Harris, 1959) suggested that they contribute to forward thrust, as did De and Nandi (De and Nandi, 1984). In both cases the pelvic fins were thought to move out of phase from the pectoral fins such that when the pectoral fins were in the recovery phase, the pelvic fins were in the propulsive phase. Inger (Inger, 1952) suggested that the pelvic fins move in the same manner as the pectoral fins, but contribute much less to thrust (as they are smaller). Pace and Gibb (Pace and Gibb, 2004) suggested that the pelvic fins contributed no more than 6% of thrust, and that the percentage was probably even less, as some forward movement during terrestrial locomotion in the lab was likely to result from slipping. (It should also be noted that these studies all used different species of mudskippers, so it is possible that pelvic fin movements vary across species.) Although it is probable that the pectoral fins produce most of the propulsive force in terrestrial habitats, future work will be necessary to determine how the two sets of paired fins function together during terrestrial locomotion.

It is interesting to note that mudskippers are not the only amphibious gobies; there are at least six other genera that have at least one amphibious species. In four of these genera pectoral fins are known to play a role in terrestrial locomotion (Larson, 1983; Schoenfuss and Blob, 2003; Todd, 1968; Todd, 1976). It has been suggested (Adriaens et al., 1993) that the goby pectoral fin may be important in initiating aquatic locomotor bouts. Interestingly, during

terrestrial locomotion of two Hawaiian gobies, *Lentipes concolor* and *Awaous guamensis*, pectoral fin adduction is used to initiate locomotor bouts when scaling rocks near waterfalls (Schoenfuss and Blob, 2003). Based on our assessment of the morphology of the mudskipper pectoral fin, and the convergent evolution of pectoral fin-based movements on land in this group, we posit that the robust pectoral fin, which is common to members of the goby family, is predisposed to be co-opted for pectoral-fin-based terrestrial locomotion.

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