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Kinematics of aquatic and terrestrial escape responses in mudskippers

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Summary

Escape responses in fishes are rapid behaviors that are critical for survival. The barred mudskipper (*Periophthalmus argentilineatus*) is an amphibious fish that must avoid predators in two environments. We compared mudskipper terrestrial and aquatic escapes to address two questions. First, how does an amphibious fish perform an escape response in a terrestrial environment? Second, how similar is a terrestrial escape response to an aquatic escape response? Because a mudskipper on land does not have to contend with the high viscosity of water, we predicted that, if the same behavior is employed across environments, terrestrial escape responses should have 'better' performance (higher velocity and more rapid completion of movements) when compared with aquatic escape responses. By contrast, we predicted that intervertebral bending would be similar across environments because previous studies of escape response behaviors in fishes have proposed that vertebral morphology constrains intervertebral bending. High-speed digital imaging was used to record mudskipper escapes in water and on land, and the resulting images were used to calculate intervertebral bending during the preparatory phase, peak velocity and acceleration of the center of mass during the propulsive phase, and relative timing of movements. Although similar maximum velocities are achieved across environments, terrestrial responses are distinct from aquatic responses. During terrestrial escapes, mudskippers produce greater axial bending in the preparatory phase, but only in the posterior region of the body and over a much longer time period. Mudskippers also occasionally produced the 'wrong' behavior for a given environment. Thus, it appears that the same locomotor morphology is recruited differently by the central nervous system to produce a distinct behavior appropriate for each environment.

Key words: escape response, mudskipper, *Periophthalmus argentilineatus*, terrestrial environment, aquatic environment, intervertebral bending.

Introduction

The ability to escape predators is critical to individual fitness and is presumed to be under intense selection (Domenici and Blake, 1997; Frith and Blake, 1995; Johnston et al., 1995; O'Steen et al., 2002). The most abundant vertebrates in the world, the ray-finned fishes (Actinopterygii), typically use a 'fast-start' behavior to escape predators. In this behavior, an individual bends about its center of mass to produce a C-shape (stage 1), then rapidly straightens its body (stage 2) to produce propulsive force (see Domenici and Blake, 1997; Hale, 1999; Webb, 1978; Weihs, 1973). Fast-starts have been shown to be an effective means for fish to escape from predators including fish, birds and invertebrates (Katzir and Camhi, 1993; Seale and Binkowski, 1988; Webb, 1981).

Amphibious fishes provide an unusual opportunity to examine environmental effects on escape behavior. Voluntary amphibious behavior is widespread among bony fishes and has been documented in 11 families, 26 genera and at least 100 species (Graham, 1997). Amphibious fishes are exposed to a wide range of novel predators during terrestrial excursions, including birds, reptiles and mammals, and must perform some type of escape behavior to avoid capture by these predators (Clayton, 1993). Amphibious fishes continue to spend a significant portion of their time in an aquatic environment, where they are exposed to aquatic predators. Thus, these species must retain the ability to perform effective escape responses in the water even after they have evolved a terrestrial escape response (Harris, 1960). This raises two questions. First, how does an amphibious fish perform an escape response in a terrestrial environment? Second, how similar is a terrestrial escape response to an aquatic escape response?

Mudskippers (family Gobiidae, subfamily Oxudercinea) are an ideal group in which to study terrestrial escape responses. Most species are intertidal specialists, and many spend more than half of their time on land and can survive for several days without access to water (Clayton, 1993; Gordon et al., 1978). Field studies indicate that mudskippers have both aquatic and terrestrial predators and are a major source of food for both fish and birds in their natural habitats (Clayton, 1993; Clayton and Vaughan, 1988; Mukherjee, 1971a,b). Mudskippers also show a variety of novel behaviors that allow them to exploit terrestrial habitats, including a ‘crutching’ behavior that is used for steady locomotion and a ‘skipping’ behavior that is analogous to jumping in tetrapods. This skipping behavior is employed to avoid predation (Harris, 1960). Thus, skipping appears to be the ecological equivalent of an aquatic escape response but is performed in a terrestrial environment.

During a terrestrial escape response, mudskippers first bend the axial skeleton to move the head and tail together. This can be considered a preparatory phase (analogous to stage 1 of aquatic escapes) because the fish has started the escape behavior but the center of mass is not moving away from the threat (Weihs, 1973). During the propulsive phase (analogous to stage 2 of aquatic escapes), mudskippers use the stiffened ventral rays of the caudal fin to push off the ground as they rapidly straighten their bodies and accelerate away from a threat (Harris, 1960).

Clearly, aquatic and terrestrial environments pose different challenges for a fish attempting to move rapidly away from a predator. Although fish on land do not contend with the high density and viscosity of water, they must instead accommodate the constraints of weight and gravity. Biewener and Gillis (1999) suggested that organisms can produce movements across disparate environments *via* three non-exclusive methods. First, there may be no alteration in musculoskeletal function across environments. Second, the same locomotor muscles may be activated differently by the central nervous system. Third, different locomotor muscles may be recruited across environments.

Mudskipper terrestrial escape responses clearly employ the same locomotor structures (the axial skeleton, caudal fin and associated musculature) as aquatic escape responses and superficially appear to employ the same movement patterns. This suggests that mudskippers either do not alter musculoskeletal function across the two environments or that they modulate muscle activity patterns to create a different behavior in each habitat. However, even if there is no alteration in musculoskeletal function across environments, divergent physical conditions will have consequences for animal movement patterns.

Therefore, despite their superficial similarity, we predict that specific aspects of mudskipper terrestrial escapes are quantitatively different from aquatic escapes. For example, even if the muscluloskeletal movements that produce the escape response are the same on land as in the water, we predict that escape performance will be slower (durations of stage one and stage two, maximum acceleration and velocity) in the aquatic environment because water provides a much greater resistance to movement than does air. Therefore, mudskippers on land should achieve a greater maximum velocity during the escape response and take less time to achieve maximum velocity due to reduced drag in the terrestrial environment.

By contrast, we predict that axial bending patterns used in the response will be similar across environments. Although hydrodynamic drag will have ramifications for bending movements produced during the preparatory phase of the escape response, previous research suggests that bending kinematics for fish fast-starts are constrained by vertebral morphology (Brainerd and Patek, 1998). Therefore, although hydrodynamic resistance to bending is reduced in the terrestrial environment, we predict that intervertebral bending is ultimately limited by the mechanical design of the vertebral column and that this constraint will generate similar bending patterns across the two environments.

In the present study, we examine the escape behavior of mudskippers in the water and on land with two primary objectives. First, we describe and quantify the terrestrial escape response of the mudskipper. Second, we measure performance (e.g. maximum velocity, acceleration and timing) and kinematic (e.g. axial bending) variables for aquatic and terrestrial escape responses and use these variables to test the general hypothesis that mudskipper terrestrial escapes are quantitatively different from aquatic escapes.

In addition to the two main goals of the study, we used the mudskipper terrestrial escape response to estimate fish axial muscle power. Because escape behaviors are under intense selection, muscle power production during an escape is thought to approach maximal muscle power output (Frith and Blake, 1995). However, it is difficult to estimate muscle power production in an aquatic environment. When a fish accelerates in water, it not only moves its own mass but also the mass of the water around it (i.e. it has ‘added mass’). Thus, this added mass must be included in calculations of muscle power, and the assumptions inherent in the resulting hydrodynamic calculations are difficult to test (Frith and Blake, 1995). One way to circumvent these complications is to examine a fish out of water (Korff et al., 1996). As outlined by Alexander (1968), estimates of power are relatively simple in terrestrial jumping species because aerodynamic drag is minimal (in comparison to hydrodynamic drag) and kinetic energy can be determined using high-speed imaging (Aerts, 1998; Korff et al., 1996; Wilson et al., 2000). Thus, a third goal of this study was to use the terrestrial escape response of the mudskipper (which is analogous to jumping) to estimate power output for this species and to compare our results with published values for aquatic behaviors in other fishes and jumping in tetrapods.

Materials and methods

Animal husbandry

Seven mudskippers (mass, 1.67±0.2 g; standard length, 41.58±2.18 mm; *Periophthalmus argentilineatus* Valenciennes 1837) were obtained through the aquarium trade and were housed in glass aquaria. Emergent rock and wood substrates were provided so that the fish could leave the water voluntarily. Mudskippers were kept on ambient light–dark cycles and were fed bloodworms or tropical fish flake food every other day.

Morphometrics

Center of mass and axial muscle masses were determined using three preserved specimens. The center of mass was identified by suspending preserved specimens (three individuals) from needle probes using methods outlined by Drucker and Lauder (2003). Subsequently, both sides of the axial musculature were removed, skinned and weighed for each individual. The average of the two sides of the axial musculature for each individual was used to estimate the muscle mass for propulsion of the jump. These three individuals were also cleared and stained (Taylor, 1967). Intervertebral joints were measured on the cleared and stained specimens by taking a digital image with a Nikon coolpix 950 digital camera and measuring joint lengths with NIH Image analysis software (v. 1.62). We detected little variation in joint number among individuals. Fish used for morphometrics were similar in size and shape to those used for kinematic analyses (two of the three fish were also used for kinematics).

High-speed digital imaging

Kinematic and performance data were collected for seven individuals of *Periophthalmus argentilineatus*. Single individuals were removed from the tank, weighed and placed in an acrylic filming chamber. A Motionscope high-speed CCD camera (Redlake, San Diego, CA, USA) was mounted over the mudskipper, perpendicular to the substrate, to obtain a dorsal view for kinematic measurements. For terrestrial performance measures, the camera was placed in a lateral view to record jumping behavior. Behaviors were recorded at 250 or 500 frames s^{-1} at a shutter speed of 1/500 of a second. A blunt probe was used to elicit an escape response from the fish. Proximity to the probe was usually enough to elicit an escape response (i.e. the fish typically jumped before they were touched by the probe). Fish performed 3–4 escape responses per session, although some sessions were terminated early when the fish showed signs of fatigue.

Analysis

Digital video (AVI) files from the Motionscope camera were converted to JPEG image sequences for motion analysis. To quantify movements produced during the preparatory phase, we used two complementary metrics of axial bending. Overall axial bending was quantified using the curvature coefficient calculation proposed by Webb (1978, 1983) and subsequently modified by Brainerd and Patek (1998). The coefficient is calculated by dividing the bent vertebral chord length by the straight length; a smaller coefficient denotes more bending. Bending kinematics were analyzed using the Jayne and Lauder (1993) intervertebral bending program, following techniques detailed in that study. Briefly, maximally bent fish were outlined in a series of points using an image measurement program. Next, the program interpolated a midline through the outline and calculated intervertebral angles based on the number and length of the intervertebral segments (determined from cleared and stained specimens).

For movement calculations during the propulsive phase, the location of the center of mass was identified on the digital images as a spot just posterior to the pectoral fins. This location was converted to X and Y coordinates in consecutive frames throughout the escape response using Didge software (A. J. Cullum, 1999; Ph.D. http://biology.creighton.edu/faculty/cullum/index.html). Because calculations of velocities and accelerations from position data are subject to measurement error, data from the consecutive frames were uploaded into QuickSAND software (J. A. Walker, 1997; Ph.D. software http://www.usm.maine.edu/~walker/software.html). With this software, we used a cubic-spline algorithm and an estimated error variance to mathematically reduce the effects of digitizing error, effectively smoothing the data and removing noise. The program was used to calculate velocity and acceleration over each 4 ms frame throughout the behavior by taking the first and second derivatives of the smoothed displacement. The acceleration calculated from the program is derived from the absolute position of the fish and serves as an estimate of whole-animal performance.

To determine the acceleration produced by the axial myomeres and to estimate power production, the horizontal and vertical acceleration vectors were calculated separately. For the vertical acceleration vector, the acceleration due to gravity was added, which resulted in a larger value that reflects the effort required by the muscles to move the animal against gravity. Thus, the total acceleration produced by the axial muscles during the escape response was calculated by adding the horizontal and vertical acceleration vectors using the methods of Marsh and John-Alder (1994). For each mudskipper, total acceleration was multiplied by body mass and the velocity of the animal over the same time interval. This provided an estimate of whole-animal instantaneous power output. This value was divided by the mean lateral axial muscle mass to obtain an estimate of muscle mass-specific instantaneous power.

Statistical analysis

Intervertebral joint angles were compared between aquatic and terrestrial escape responses using a two-way analysis of variance (ANOVA). In this analysis, intervertebral angle was the dependent variable, and individual and substrate (aquatic *versus* terrestrial) were factors. A total of 29 segment angles were compared for six individuals with 2–3 trials per individual per environment (a total of 25 trials). *Post-hoc* Tukey HSD tests were used to identify differences between intervertebral joint angles along the body for the distinct behaviors. In addition, bending values, curvature coefficients and overall movement patterns were qualitatively compared with published values for other fishes performing fast-starts.

To test for potential multivariate differences in the overall performance of aquatic and terrestrial escape responses, a multivariate analysis of variance (MANOVA) was used with 18 total trials for six individuals in aquatic escapes and 17 total trials for seven individuals in terrestrial escapes. Six variables were chosen as potential indicators of individual escape response performance and included in the model. These variables were: (1) duration of stage 1 (time from first movement to maximum curvature), (2) duration of stage 2 (time from maximum curvature to straightening of body), (3) time to maximum velocity (time from first movement to peak velocity of the center of mass), (4) ratio of duration of stage 1 to duration of stage 2, (5) maximum velocity and (6) maximum acceleration. After we determined if aquatic and terrestrial escapes were different overall, we used *post-hoc* ANOVA to identify the variables contributing to differences between behaviors.

Results

Kinematics

Aquatic escape responses

It was difficult to elicit a strong aquatic escape response from mudskippers, which often simply swam slowly away from the probe. Only escape responses that appeared to be maximal efforts were included in the analysis. Fish bent rapidly around the center of mass in the preparatory phase (stage 1), then straightened to accelerate away in the propulsive phase (stage 2; see Fig. 1A). Mudskippers performing aquatic escape responses formed a stereotyped ‘C’ shape at the end of stage 1 (suggesting constant curvature along the body). Curvature coefficients during stage 1 (0.55±0.02) were similar to published values for other fishes with similar intervertebral joint numbers (0.45–0.7; Brainerd and Patek, 1998). Mean intervertebral bending angles during stage 1 were all positive (indicating uniform bending in one direction; Fig. 2). Intervertebral joint angles during stage 1 were similar at all joints along the body, which also suggests a curve of fairly constant radius. During stage 2, the fish rapidly straightened their bodies and accelerated away from the stimulus.

In a minority of aquatic escape responses, the fish appeared to perform a terrestrial escape response in the water (described in detail below). In these responses, the fish pushed off the bottom of the aquarium to accelerate out of the water. Similarly, a few of the terrestrial escape responses appeared to have similar kinematics to aquatic escape responses, with a curve of fairly constant radius at the end of stage 1. However, these responses were rare and were not included in the quantitative analysis.

Terrestrial escape responses

Terrestrial escape responses began when the fish lifted its caudal fin off the substrate (Fig. 1B). The caudal fin was then brought around to form a ‘J’ shape, in which the caudal fin lay next to the body, just behind the head and near the center of mass. Maximum bending occurred approximately two-thirds of the way down the body (Fig. 2). The anterior portion of the body, including the head, typically did not move during this phase of the response. This is the preparatory phase and roughly corresponds to stage 1 of the aquatic escape response.

The preparatory phase was followed by a slight lifting of the head, apparently produced by the pelvic and pectoral fins, and a rapid unfolding of the body, which straightened the tail. The straightening of the body occurred *via* both lateral and ventral movements of the tail (i.e. it pushed to the side and down) that lifted the center of mass off the substrate and propelled it in the direction that the fish was pointing at the beginning of the behavior. This movement roughly corresponds to stage 2 of a fish escape response (Fig. 1). The caudal fin was consistently the last part of the fish to leave the ground, and the take-off angle ranged from 27 to 59° above horizontal. Take-off angle was a good predictor of jump range, with higher take-off angles producing longer jumps (N=7, r^2=0.78), similar to predictions of a simple ballistic model of movement. However, take-off angle did not predict any other performance variables (such as maximum acceleration; N=7, r^2=0.03).

In general, mudskippers performing terrestrial escape responses formed a J-shaped curve with a very sharp bend or fold in the caudal portion of the body and a slight re-curvature in the anterior portion of the body. The values of curvature coefficients for mudskipper terrestrial escape responses were smaller (0.24±0.01; mean ± S.E.M.) than observed in aquatic escape responses (0.55±0.02), indicating greater lateral bending in the terrestrial environment. Mean intervertebral bending angles ranged from small negative values (slight bending of the anterior portion of the body away from the major axial bending of the fish) to large positive values (sharp bending along the posterior two-thirds of the fish; Fig. 2). ANOVA for intervertebral angle by location along the body showed a significant difference among intervertebral joints (29

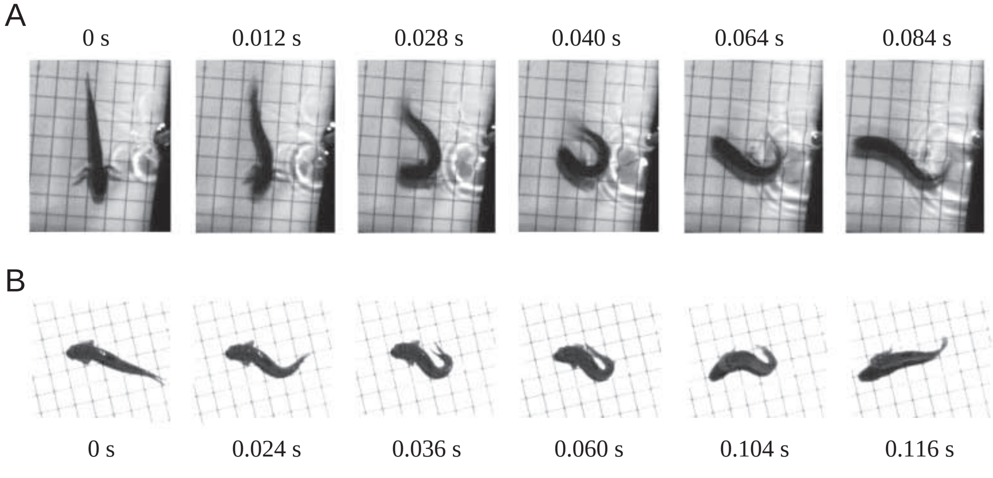

Fig. 1. Digital images of a dorsal view of a mudskipper performing an escape response in aquatic (A) and terrestrial (B) environments. In A, the fish performs a stereotyped C-start behavior in the water, with the end of stage 1 occurring at 0.040 s. In B, the fish forms a ‘J’ shape at the end of stage 1 by folding the tail to rest alongside the body in a terrestrial escape response, with the end of stage 1 occurring at 0.060 s. Time labels are from the first movement.

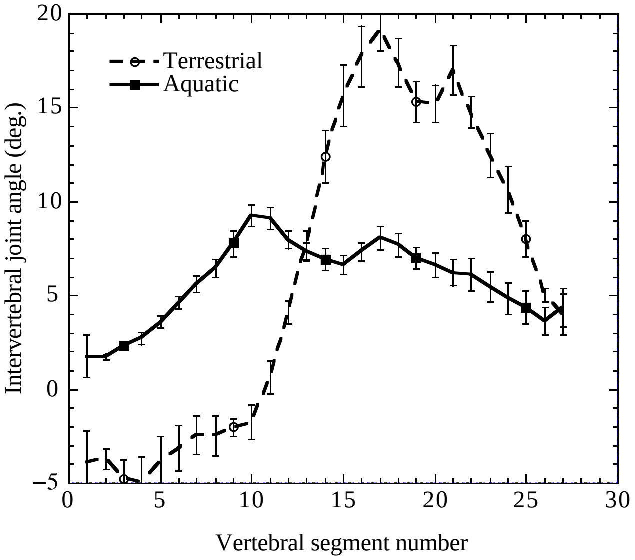

Fig. 2. Mean intervertebral joint angles ± 1 s.e.m. for aquatic and terrestrial escape responses at the end of stage 1. Segment 0 is the rostral end of the vertebral column and segment 30 is the caudal end.

intervertebral joints, 13 trials). Tukey HSD *post-hoc* tests indicated that the intervertebral segments in the anterior part of the body did not differ from one another, but segments in the posterior portion of the body differed greatly. This suggests that there is little variation in bending among anterior joints, but the caudal portion is bent to a much greater degree and does not form a constant arc (Fig. 2).

Performance

One-factor MANOVA on kinetic, or performance, variables indicated an overall significant difference between aquatic and terrestrial escape responses ($F_{8,24}=6.49$, $P<0.05$). Individual ANOVA tests for each variable indicated that duration of stage 1 ($F_{1,6}=16.05$, $P<0.05$), ratio of duration of stage 1 to duration of stage 2 ($F_{1,6}=28.04$, $P<0.05$), time to maximum velocity ($F_{1,6}=20.93$, $P<0.05$) and maximum acceleration ($F_{1,6}=15.77$, $P<0.05$) differed significantly between environments. Duration of stage 2 and maximum velocity did not differ between environments. In general, terrestrial responses took longer than aquatic escape responses (Fig. 3). In fact, it took twice as long to reach maximum velocity in terrestrial escape responses than in aquatic escape responses. This was due to stage 1 being twice the duration for terrestrial escape responses than for aquatic escape responses (see Table 1).

Instantaneous power was estimated between two consecutive digital images (a 4 ms interval). Mudskipper mass ranged from 0.4 to 2.0 g, and the mass of one set of axial myomeres (from one side of the body) averaged 20% of total body mass. Total acceleration of the body averaged 125.47 ± 11.9 m s^{-2} (mean ± s.e.m.; in calculations of muscle performance, acceleration due to gravity was included as a factor that the muscles would be required to overcome). Using

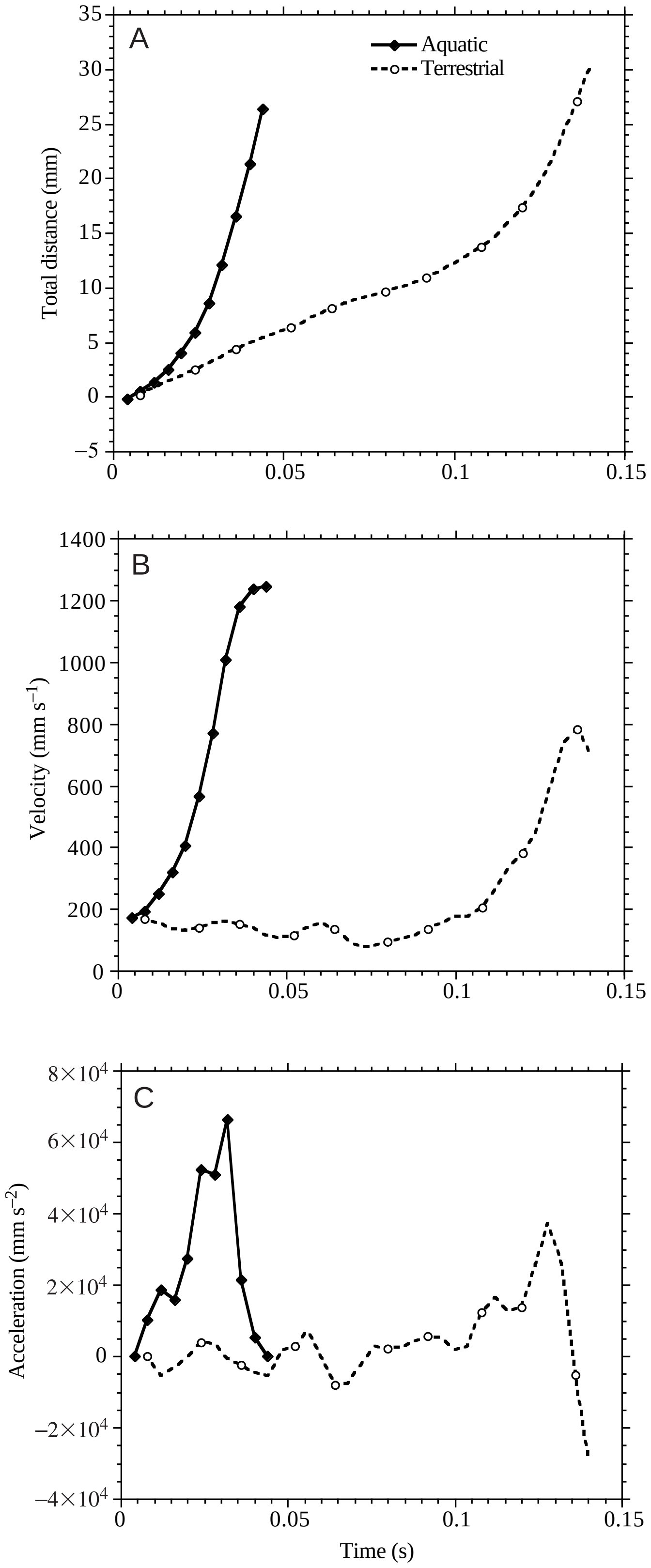

Fig. 3. A series of kinematic plots of movement of the center of mass for a representative aquatic and terrestrial escape response: (A) smoothed cumulative displacement data; (B) velocity, calculated as the first derivative of displacement, and (C) acceleration, calculated as the second derivative of displacement.

Table 1. *Means table of performance and timing variables measured in terrestrial and aquatic escape responses*

	Aquatic	Terrestrial	Statistical comparison
Maximum instantaneous velocity (m s^{-1})	1.01±0.063 (max.=1.4)	0.95±0.037 (max.=1.62)	N.S.
Maximum instantaneous acceleration (m s^{-2})	123.8±26.0 (max.=268.0)	42.9±3.3 (max.=116.0)	$P<0.05$
Maximum instantaneous acceleration taking into account acceleration due to gravity (m s^{-2})	123.8±26.0 (max.=268.0)	125.47±11.9	N.S.
Time to end of stage 1 (ms)	44.67±7.55	88.18±7.61	$P<0.05$
Time to end of stage 2 (ms)	38.56±6.25	42.12±1.68	N.S.
Ratio of stage 1 duration to stage 2 duration	1.18±0.06	2.12±0.17	$P<0.05$
Max. power (max. recorded) (W kg^{-1} muscle mass)	778		
Time to max. velocity (ms)	63±10.31	124.41±8.45	$P<0.05$

Unless stated otherwise, values are means ± 1 S.E.M. N.S. denotes no significant difference.

these values for the power calculations, muscle mass-specific power ranged from 350 to 770 W kg^{-1}.

Discussion

Few studies have examined fish locomotor behaviors across truly disparate environments. However, Gillis (1998, 2000) demonstrated that eels produce different movements during steady locomotion on land *versus* in water. Further, eels employ different activation patterns of their axial muscles when moving on land *versus* in water, and their performance (velocity) is greatly reduced relative to swimming in water. These studies, along with studies on ducks (in water and on land) and turkeys (running on flat and inclined terrain), suggest that organisms often use the same muscles and modulate their muscle recruitment patterns to produce movements in different environments. Thus, the same structure is frequently employed differently to enable movement in a new environment (Biewener and Gillis, 1999).

To escape predators, mudskippers must produce effective escape responses in aquatic and terrestrial environments. We found that these aquatic and terrestrial escape behaviors are distinct. Mudskipper aquatic escape responses are similar to those produced by other fishes and fit the stereotyped kinematic pattern of a C-start escape response (Domenici and Blake, 1997; Weihs, 1973). For example, the intervertebral joint angles at the end of stage 1 in mudskippers are similar to those reported for several species of fish (Brainerd and Patek, 1998). In addition, aquatic escapes in mudskippers are rapid behaviors, with similar durations of stages 1 and 2 of the escape response to other fast-starting fishes (Domenici and Blake, 1997).

However, the mudskipper terrestrial escape response differs from aquatic escape responses of mudskippers and those of other fishes in several ways. The escape response takes longer in the terrestrial environment, due to a twofold increase in the duration of the preparatory phase. Thus, it took mudskippers significantly longer to reach the propulsive phase and, in turn, maximum velocity when they were on land. The increased time to maximum velocity suggests that performance is 'worse' in the terrestrial environment, although we note that the maximum velocities achieved are the same across the two environments and a larger distance is covered with a single propulsive movement during terrestrial escapes. Additionally, the axial skeleton bends to a greater degree during terrestrial escape responses than during aquatic responses. The pattern of axial bending on land is also different, with flexion restricted to the posterior region of the axial skeleton.

These results are contrary to our initial predictions. We hypothesized that if the underlying musculoskeletal pattern used to produce the behavior were similar across the two environments, then reduced hydrodynamic drag in the terrestrial environment should allow improved escape performance (i.e. a quicker, higher-velocity escape response).

We also hypothesized that intervertebral bending is constrained by the morphology of the vertebral column and that decreased hydrodynamic resistance on land would not generate increased intervertebral flexion. Thus, the observed patterns are not generated as a simple consequence of employing the same behavior across two physically disparate environments.

Instead, these results suggest that mudskippers use distinct behaviors in the different environments. Several lines of evidence support this conclusion. First, aquatic responses are rapid behaviors with durations within the range of Mauthner-initiated escape responses (although it is not known if mudskippers have Mauthner neurons). By contrast, terrestrial responses appear too slow to employ this pathway (Hale, 2000). If the aquatic responses use the Mauthner cell system and the terrestrial responses do not, this would imply that a different neural pathway is used for each behavior. Second, the axial muscles appear to be recruited differentially in the different environments. In previous studies of fish escape responses, all of the myomeres in the axial musculature were activated near-synchronously (Jayne and Lauder, 1993), which produces consistent bending along the vertebral column. In mudskipper terrestrial responses, most bending occurred about a particular location on the body. The variable pattern of bending along the body in the mudskipper terrestrial response suggests that a distinct subset of myomeres is being recruited to produce the behavior (Katz et al., 1999; Wakeling and Johnston, 1999). Finally, when we attempted to stimulate aquatic escape responses, mudskippers occasionally produced what appeared to be a kinematically ‘terrestrial’ escape response in the water. All of these results suggest that the observed differences between response types are not simply passive responses to changes in the physical environment but instead reflect a novel motor pattern.

It is probable that physical differences between the two habitats necessitated the evolution of a novel escape behavior. A fish immersed in an aquatic medium must contend with the viscosity of water but is able to produce thrust along the entire lateral surface of the body (Johnston et al., 1995). By contrast, a fish performing an escape behavior on land must grapple with the novel challenges of weight and gravity and can produce thrust with only the ventral surface of the body and tail. We noted two unusual aspects of terrestrial escapes relative to aquatic escapes – terrestrial escapes require a long preparatory phase and acute posterior axial bending. We suggest that these aspects of terrestrial escapes have evolved to allow effective thrust production on land.

The long preparatory phase with a large degree of posterior axial bending may facilitate the production of a jumping, or ballistic, behavior. Many jumping tetrapods (and some invertebrates) are known to use ‘preloading’ of muscles during the preparatory phase to amplify power production during the propulsive phase (Aerts, 1998). During preloading, agonist muscles are activated by the nervous system but are prevented from shortening by the activity of antagonist muscles. When the agonists are allowed to contract (because antagonist activity is diminished), preloaded muscles produce a powerful contraction because the series elastic and contractile elements work in concert to shorten the muscle (Pilarski et. al., 2002). The long preparatory phase of mudskipper terrestrial escapes may allow a similar pre-loading of the axial muscles for ballistic propulsion, where muscles are activated contralaterally and power is amplified in the propulsive phase.

Additionally, the caudal fin must be placed close to the center of mass during the preparatory phase to lift the fish from the substrate during the propulsive phase. Placing the tail in this position requires a tight bending, or folding, of the body of the fish about a point approximately two-thirds of the way down the body. When the axial musculature straightens, it provides not only forward thrust (as in an aquatic escape response) but also the vertical thrust necessary to lift the center of mass off the ground. Again, this pattern of bending may be necessary to produce an effective ballistic movement on land.

The unusual pattern of bending observed in mudskippers also allows us to evaluate previous hypotheses about potential morphological limitations on axial bending in bony fishes. Brainerd and Patek (1998) studied the relationship between the number of vertebrae in the axial column and axial bending in several reef fish species. They found that reef fish produce approximately 8° of bending at each intervertebral joint (i.e. the angle of bending produced between two adjacent vertebrae), with only minor variation in this value present among species or joints (Brainerd and Patek, 1998; Jayne and Lauder, 1993). They suggested that increased axial bending is produced by increasing the number of vertebrae, rather than the degree of bending at each joint. Therefore, vertebral number should be a good predictor of axial flexion (because more vertebrae will generate more flexion). For instance, elongate fishes produce extreme axial flexion and form an ‘O’ shape at the end of stage 1 with the head and tail touching. This bending is produced by a large number of intervertebral joints rather than acute bending at a small number of joints (Westneat et al., 1998). Mudskippers have similar vertebral joint numbers to some of the fishes used in the Brainerd and Patek (1998) study, and mudskipper aquatic escape responses demonstrate intervertebral joint angles of approximately 8°. However, mudskipper terrestrial responses had greater bending in some regions of the vertebral column (up to 20°) than predicted by Brainerd and Patek’s model. Thus, data from the terrestrial responses suggest that vertebral morphology is not what constrains vertebral bending to 8°.

The maximum instantaneous power produced in a mudskipper jump was similar to that reported for other poikilothermic vertebrates. The values were similar to (although generally higher than) values reported for fish performing escape responses in water, which range from 100 to 500 $\mathrm{W\,kg^{-1}}$ (Frith and Blake, 1995), and similar to (although generally lower than) values reported for terrestrial jumping anurans, which range from 200 to 1000 $\mathrm{W\,kg^{-1}}$ (Marsh and John-Alder, 1994). Although the whole-animal acceleration during the aquatic escape responses was greater than that observed during terrestrial escape responses (Table 1), a re-analysis of the terrestrial escape responses, taking into account the effect of gravity, revealed that the acceleration due to the axial myomeres was actually the same in both environments. Thus, although the timing variables were quite different, the performance measures of maximum acceleration and velocity in the two different environments were not significantly different. Because aquatic mudskippers must have some added mass due to water displacement and adhesion/cohesion of water, we suggest that they actually produce more power in the aquatic environment to reach the same maximum velocity.

It is interesting to consider the ecological implications of observed performance differences in aquatic and terrestrial escape behaviors. The aquatic escape rapidly accelerates the center of mass of the fish in a variable direction (but always away from the negative stimulus). The unpredictable nature of the resulting escape trajectory, and the speed of the response, should make it difficult for a predator to anticipate a fish’s movements, or to overtake it. However, the high viscosity of the aquatic medium means that a fish must keep swimming to achieve significant displacement away from the predator (if it stops swimming, it will rapidly coast to a stop). The terrestrial escape response appears to employ a different strategy. It involves a substantially longer preparatory phase and tends to propel the fish in the same direction it was originally facing. However, because air has very low viscosity, a fish on land can use a single propulsive stroke to create a ballistic movement that will move it a great distance away from the potential predator.

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References

Aerts, P. (1998). Vertical jumping in *Galago senegalensis*: the quest for an obligate mechanical power amplifier. *Philos. Trans. R. Soc. Lond.* **353**, 1607-1620.

Alexander, R. McN. (1968). *Animal Mechanics*. London: Sidgwick and Jackson.

Biewener, A. and Gillis, G. B. (1999). Dynamics of muscle function during locomotion: accommodating variable conditions. *J. Exp. Biol.* **202**, 3387-3396.

Brainerd, E. L. and Patek, S. N. (1998). Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in tetraodontiform fishes. *Copeia* **1998**, 971-984.

Clayton, D. A. (1993). Mudskippers. *Ocean. Mar. Biol. Annu. Rev.* **31**, 507-577.

Clayton, D. A. and Vaughan, T. C. (1988). Ethogram of *Boleophthalmus boddarti* (Teleostei: Gobiidae), a mudskipper found on the mudflats of Kuwait. *J. Univ. Kuwait* **15**, 115-138.

Domenici, P. and Blake, R. W. (1997). The kinematics and performance of fish fast start swimming. *J. Exp. Biol.* **200**, 1165-1178.

Drucker, E. G. and Lauder, G. V. (2003). Function of pectoral fins in rainbow trout: behavioral repertoire and hydrodynamic forces. *J. Exp. Biol.* **206**, 813-826.

Frith, H. R. and Blake, R. W. (1995). The mechanical power output and hydromechanical efficiency of the northern pike (*Esox lucius*) fast-starts. *J. Exp. Biol.* **198**, 1863-1873.

Gillis, G. B. (1998). Environmental effects on undulatory locomotion in the American eel *Anguilla rostrata*: kinematics in water and on land. *J. Exp. Biol.* **201**, 949-961.

Gillis, G. B. (2000). Patterns of white muscle activity during terrestrial locomotion in the American eel *Anguilla rostrata*. *J. Exp. Biol.* **203**, 471-480.

Gordon, M. S., Ng, W. W.-S. and Yip, A. Y.-W. (1978). Aspects of the physiology of terrestrial life in amphibious fishes. *J. Exp. Biol.* **72**, 57-75.

Graham, J. B. (1997). *Air-Breathing Fishes: Evolution, Diversity, and Adaptation*. San Diego, CA: Academic Press.

Hale, M. E. (1999). Locomotor mechanics during early life history: effects of size and ontogeny on fast-start performance of salmonid fishes. *J. Exp. Biol.* **202**, 1465-1479.

Hale, M. E. (2000). Startle responses of fish without Mauthner neurons: escape behavior of the lumpfish (*Cyclopterus lumpus*). *Biol. Bull.* **199**, 180-182.

Harris, V. A. (1960). On the locomotion of the mud-skipper *Periophthalmus koelreuteri* (Pallas): (Gobiidae). *Proc. Zool. Soc. Lond.* **134**, 107-135.

Jayne, B. C. and Lauder, G. V. (1993). Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. *J. Comp. Physiol. A* **173**, 495-508.

Johnston, I., Van Leeuwen, J., Davies, M. and Beddow, T. (1995). How fish power predation fast-starts. *J. Exp. Biol.* **198**, 1851-1861.

Katz, S., Shadwick, R. and Rapoport, H. (1999). Muscle strain histories in swimming milkfish in steady and sprinting gaits. *J. Exp. Biol.* **202**, 529-541.

Katzir, G. and Camhi, J. M. (1993). Escape response of black mollies (*Poecilia sphenops*) to predatory dives of a pied kingfisher (*Ceryle rudis*). *Copeia* **1993**, 549-553.

Korff, W. L., Suter, R. B. and Long, J. H. J. (1996). The mechanical cost of swimming: an experimental test of hydrodynamic theory in jumping Arowana, *Osteoglossum bicirrhosum*. *Am. Zool.* **36**, 89A.

Marsh, R. L. and John-Alder, H. B. (1994). Jumping performance of hylid frogs measured with high-speed cine film. *J. Exp. Biol.* **188**, 131-141.

Mukherjee, A. K. (1971a). Food habits of water-birds of the Sundarban, 24-Parganas District, West Bengal, India-II. Herrons and bitterns. *J. Bombay Nat. Hist. Soc.* **68**, 37-64.

Mukherjee, A. K. (1971b). Food habits of water-birds of the Sundarban, 24-Parganas District, West Bengal, India-III. Egrets. *J. Bombay Nat. Hist. Soc.* **68**, 691-716.

O'Steen, S., Cullum, A. J. and Bennett, A. F. (2002). Rapid evolution of escape ability in trinidadian guppies (*Poecilia reticulata*). *Evolution* **56**, 776-784.

Pilarski, J. Q., Nishikawa, K. C. and Pierotti, D. J. (2002). Power amplification by pre-activation of semimembranosus in *Rana pipiens. Integ. Comp. Biol.* **42A**, 1294.

Seale, D. B. and Binkowski, F. P. (1988). Vulnerability of early life intervals of *Coregonus hoyi* by a freshwater mysid, *Mysis relicta*. *Env. Biol. Fishes* **21**, 117-125.

Taylor, W. R. (1967). An enzyme method of clearing and staining small vertebrates. *Proc. US Natl. Museum* **122,** 1-17.

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

Webb, P. W. (1978). Fast-start performance and body form in seven species of teleost fish. *J. Exp. Biol.* **74**, 211-226.

Webb, P. W. (1981). Responses of Northern anchovy, *Engraulis mordax*, larvae to predation by a biting planktivore, *Amphiprion percula*. *US Natl. Mar. Fish. Service Fish. Bull.* **79**, 727-735.

Webb, P. W. (1983). Speed, acceleration and manoeuvarability of two teleost fishes. *J. Exp. Biol.* **102**, 115-122.

Weihs, D. (1973). The mechanism of rapid starting of slender fish. *Biorheology* **10**, 343-350.

Westneat, M. W., Hale, M. E., McHenry, M. J. and Long, J. H. (1998). Mechanics of the fast-start: muscle function and the role of intramuscular pressure in the escape behavior of *Amia clava* and *Polypterus palmas*. *J. Exp. Biol.* **201**, 3041-3055.

Wilson, R., Franklin, C. and James, R. (2000). Allometric scaling relationships of jumping performance in the striped marsh frog *Limnodynastes peronii*. *J. Exp. Biol.* **203**, 1937-1946.