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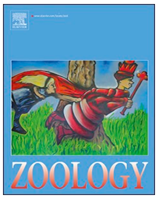

A walking behavior generates functional overland movements in the tidepool sculpin, *Oligocottus maculosus*

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ABSTRACT

Tidepool sculpins (*Oligocottus maculosus*) have been observed moving overland in the rocky intertidal, and we documented the terrestrial walking behavior that they use to accomplish this. We quantified the terrestrial movements of *O. maculosus* and compared them to (1) their aquatic locomotion, (2) terrestrial locomotion of closely-related subtidal species (*Leptocottus armatus* and *Icelinus borealis*), and (3) terrestrial movements of walking catfishes (*Clarias* spp.). We recorded sculpin movements (210 fps) on a terrestrial platform and in a water tank and tracked body landmarks for kinematic analysis. The axial-appendage-based terrestrial locomotion of *O. maculosus* is driven by cyclic lateral oscillations of the tail, synchronized with alternating rotations about the base of the pectoral fins, a behavior that appears similar to a military "army crawl." The pectoral fins do not provide propulsion, but act as stable points for the body to rotate around. In contrast, individuals of *O. maculosus* use primarily axial undulation during slow-speed swimming. The army crawl is a more effective terrestrial behavior (greater distance ratio) than the movements produced by *L. armatus and I. borealis*, which use rapid, cyclic oscillations of the tail, without coordinated pectoral fin movements. Relative to *Clarias* spp., *O. maculosus* rotated the body about the base of the pectoral fin, rather than the tip of the fin, which may cause *O. maculosus* to have a lower distance ratio. Since *O. maculosus* lack major morphological adaptations for terrestrial locomotion, instead relying on behavioral adaptations, we propose behavioral adaptations may evolutionarily predate morphological adaptations for terrestrial locomotion in vertebrates.

1. Introduction

Fishes that live in the marine intertidal zone can be stranded in isolated, poorly-oxygenated pools, or even in pools that dry out completely. Some fish species are able to move overland in response to these environmental challenges. Indeed, the ability to move effectively on land has been documented in a variety of teleost species that live at the water's edge, including killifishes (Cyprinodontiformes), walking catfishes (*Clarias* spp.), *Cryptotora thamicola*, and mudskippers (*Periophthalmus* spp.; Bressman et al., 2018, 2016; Flammang et al., 2016; Gibb et al., 2011, 2013; Pace and Gibb, 2009, 2014). Fishes display a variety of terrestrial locomotor behaviors that are achieved using a combination of propulsive systems, including the axial body and tail, pectoral fins, pelvic fins, or a combination of these and other body elements (Bressman et al., 2016; Flammang et al., 2016; Gibb et al., 2011, 2013; Pace and Gibb, 2009, 2014; Pronko et al., 2013; Standen et al., 2014). Terrestrial locomotor behaviors that superficially resemble tetrapod walking have also been termed "walking" in fishes (Johnels, 1957; Flammang et al., 2016; Pace and Gibb, 2009, 2014; Standen et al., 2014); these behaviors involve the axial body working in concert with the paired appendages to produce axial-appendage-based locomotion (*sensu* Pace and Gibb, 2014). Fishes that use an axial-appendage based method of moving on land, such as the blind cavefish, *Cryptotora thamicola*, and the walking catfishes, *Clarias spp.*, coordinate side-to-side, whole-body bending movements with alternating appendage movements (Flammang et al., 2016; Pace and Gibb, 2014). These fishes tend to have robust pectoral fins and a flattened ventral surface of the body, which allow them to elevate the head above the substrate when moving on land.

Sculpins and poachers (Superfamily Cottoidea, *sensu* Smith and Busby, 2014) are an example of a lineage of marine teleosts for which many species can be found in the temperate intertidal zone. Intertidal

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sculpins tend to be hypoxia tolerant (Davenport and Woolmington, 1981; Mandic et al., 2009) and some species can respire through the skin when on land (Knope and Scales, 2013). Because sculpin species live at the water's edge, they may be occasionally compelled to move onto land to avoid predators, to escape an unfavorable environment, or to gain access to new prey items. The tidepool sculpin, *Oligocottus maculosus* Girard 1856, is commonly found in rocky tidepools throughout the intertidal zone, from the Sea of Okhotsk and the Bering Sea to Los Angeles, California (Eschmeyer et al., 1983; Horn et al., 1999; Lamb and Edgell, 2010; Nakamura, 1976). *Oligocottus maculosus* have among the greatest hypoxia tolerance (Knope and Scales, 2013; Martin, 1991) and largest temperature range reported for any sculpin (Mandic et al., 2009; Nakamura, 1976), and are known to be capable of tide pool homing — defined as the ability of an individual to return to a particular pool after it has been displaced (Green, 1971). Because *O. maculosus* can move between tide pools, it is probable that they perform this behavior in order to access pools that contain better resources. Individuals of *O. maculosus* have robust pectoral fins and a flattened ventral surface (Kane and Higham, 2012), which suggests that they may be capable of substrate locomotion using their pectoral fins in aquatic and terrestrial environments. Subtidal sculpin species, including *Leptocottus armatus* and *Icelinus borealis*, have smaller pectoral fins and relatively deeper bodies. The Pacific staghorn sculpin, *Leptocottus armatus,* occupies the intertidal Pacific Northwest at early life history stages (Eschmeyer et al., 1983; Horn et al., 1999; Lamb and Edgell, 2010), but moves to subtidal habitats at larger sizes. The northern sculpin, *Icelinus borealis,* is a medium-sized sculpin that has similar body shape to *O. maculosus* and overlaps in range with this species, (Eschmeyer et al., 1983: Lamb and Edgell, 2010), but is typically found in the subtidal zone (9 m–310 m).

Because *Oligocottus maculosus* have several key anatomical and ecological features associated with amphibious behaviors, we hypothesized that they are able to produce effective terrestrial movements to support amphibious behaviors. We asked: (1) how do *Oligocottus maculosus* move on land? (2) is this behavior distinct from the aquatic locomotor behaviors of *O. maculosus*, or are these fish simply "swimming" on land? and (3) how similar is terrestrial locomotion in *O. maculosus* to the terrestrial locomotion of closely-related primarily-subtidal sculpin species and (4) to other, more distantly-related amphibious fishes? To answer these questions, we quantified the kinematics of aquatic and terrestrial locomotion of *O. maculosus* to characterize previously-undescribed terrestrial behaviors and to determine if locomotor kinematics on land are distinct from swimming behaviors. We also compared the terrestrial movements of *Oligocottus maculosus* to the terrestrial movements of two primarily subtidal species (*L. armatus* and *I. borealis*) to determine if *O. maculosus* move more rapidly and in a more linear manner when on land, relative to the two subtidal sculpin species. To determine if the locomotor pattern of *O. maculosus* is distinct from other "walking" amphibious fishes, we also compared terrestrial locomotion of *O. maculosus* to the terrestrial movements of the "walking" catfishes, *Clarias spp*., because they are similar in body shape and their terrestrial locomotion has been well-studied.

2. Materials and Methods

2.1. Animals

Oligocottus maculosus and *Leptocottus armatus* were collected by dip net from tidepools at Deadman's Bay, San Juan Island, Washington (48°30'47.6" N 123°08'48.5" W) and by seining at Jackson Beach, San Juan Island, Washington, (48°31'13.0" N 123°00'35.1" W, $N_{tidepool} = 50$; $N_{staghorn} = 10$). *Icelinus borealis* were collected by bottom trawl conducted around Orcas Island, Washington (N = 4). All sculpins studied were within a similar size range (SL = 4.5–8 cm). Experimental animals were housed in flow-through sea-tables at Friday Harbor Laboratories with ambient lighting and an approximate water temperature of 11 °C. Fishes were fed a diet of live mysid shrimp twice each week. At the conclusion of the experiments, some individuals were euthanized for morphological study using MS-222, according to IACUC guidelines. Center of mass (COM) was determined for individuals before preservation by balancing the fish on the edge of a sheet of acrylic to determine the center of balance, which approximates the center of mass. However, while the body bends during locomotion, the true COM may shift, so we used this as an estimation for COM during locomotor behaviors. All experiments and procedures were conducted in accordance with UW IACUC protocol #4238-03.

2.2. Data Collection and Analysis

During terrestrial locomotion trials, *Oligocottus maculosus* individuals (N = 6) were placed on a damp, flat terrestrial platform (a piece of acrylic covered with a moist paper towel) positioned within a glass aquarium. A homogenous substrate was chosen in place of their natural, heterogeneous substrate (Fig. S1), to allow for consistent kinematic analyses and comparisons. One end of the platform provided access to water, which served as incentive for the fish to move across the experimental arena. High-speed video was recorded at 210 frames per second (fps) with two Casio Exilim FH-20 cameras simultaneously from above the tank (a dorsal view of the fish) and from the side (a lateral view of the fish). In the terrestrial trials, the fish were allowed to move voluntarily without human stimulation or intervention. If an individual did not move for over 10 minutes on the terrestrial platform for any trials, it was not included in the analysis. One successful sequence of locomotion was recorded for each individual. The same individuals were also filmed during short bouts of swimming in water so that we could compare terrestrial locomotor movements with aquatic locomotor movements. For aquatic locomotor trials, the terrestrial platform was removed from the glass aquarium, which was then filled with seawater to a depth of approximately 7.5 cm. If a fish would not swim voluntarily, swimming was initiated by either tapping on the outside of the aquarium or by chasing the fish with a net. Some of these individuals would not swim when placed in the water, so three additional *O. maculosus* were filmed moving through the water to increase sample size (N = 6). Using the same platform and animal handling protocol that we used for terrestrial trials of *O. maculosus*, the terrestrial behaviors of *L. armatus* (N = 5) and *I. borealis* (N = 4) were also recorded using a Casio Exilim FH-20 at 210 fps from an overhead view.

To quantify the kinematics of locomotor behaviors from the trials, seven points on the *O. maculosus* were manually tracked in the top-down view using the DLTdv3 application (Hedrick, 2008) in MATLAB: (1) tip of snout, (2) caudal peduncle, (3) COM, (4) tip of left pectoral fin, (5) base of left pectoral fin, (6) tip of right pectoral fin, and (7) base of right pectoral fin. For sculpins from the lower intertidal and subtidal, *L. armatus* and *I. borealis,* no pectoral fin movements were observed during terrestrial locomotion, so only points 1, 2, and 3 were tracked for these species. These three points were also tracked during the aquatic trials of *O. maculosus*. The lateral high-speed videos were used for a qualitative gait analysis to determine which parts of the fish were in contact with the substrate during locomotion in the terrestrial trials and the duration of contact with the substrate.

For the three points measured for all species (including the *O. maculosus* aquatic trials), displacement versus time was plotted to determine movement of various regions of each fish. Wave amplitudes, curvature coefficients, pectoral fin stride lengths, and stride frequencies were also measured using MATLAB and ImageJ. The curvature coefficient is a ratio of the distance between the snout and peduncle when the tail is at the maximum amplitude to the SL (modified from Brainerd and Patek,1998) and is used to quantify how far anteriorly the fishes move their tails. Wave amplitude is defined as the maximum amplitude of the head, COM, and caudal peduncle in terms of % standard length (SL) and is used to measure how far laterally the snout, COM, and peduncle move during strides. Stride frequency is a measure of how rapidly fishes displace the tail from side-to-side. Similar to the stride definition by Ashley-Ross and Lauder (1997), a single stride was defined as the lateral movement of the caudal peduncle from maximum curvature to the maximum curvature again on the same side of the body. We also used ImageJ to measure the angle of the pectoral fins in relation to the body of *O. maculosus* at each frame. The angles of the pectoral fins were measured at the maximum abduction of each half-stride.

For the three sculpin species (including the *O. maculosus* aquatic trials), a "distance ratio" was calculated for the snout, peduncle, and COM. The distance ratio is a measure of the sinuosity of overall movement and defined as the net displacement of the points over a series of full strides divided by the gross displacement (Pace and Gibb, 2014). The distance ratio served as a proxy for the linearity of the path traveled, providing a measure of how effective each locomotor strategy was for producing forward movement in the species compared. Before calculating this ratio for each point, we first filtered the raw digitized data, because the distance ratio metric is particularly sensitive to digitizing noise. Using the *signal* package in R (Ligges et al., 2015), we used a low-pass butterworth filter with a frequency of 0.2 (using the *butter* function), and we filtered both forwards and backwards to avoid the phase shift that is typical of butterworth filters (using the *filtfilt* function).

2.3. Statistical Analysis

Statistical analyses were performed with R using the standard statistics package (R Core Team, 2016). Welch's Two-Sample t-tests were used to compare the distance ratios and wave amplitudes at the head, COM, and tail between the aquatic and terrestrial locomotion of *O. maculosus*. ANOVAs were performed to compared the distance ratios and wave amplitudes at the head, COM, and tail between the terrestrial locomotion of *O. maculosus*, *L. armatus*, and *I. borealis*. Posthoc Tukey-Kramer tests were performed to determine which groups were significantly different in the ANOVAs. The *p*-values expressed in the results section reflect the probabilities that the disparities between data groups were observed only due to random chance, as determined by the respective statistical tests, with an alpha level of 0.05. Standard errors (SE) were calculated by dividing standard deviations for each measurement by the number of successful trials for each species.

3. Results

When an *Oligocottus maculosus* individual is placed on a substrate, it will adopt an upright posture, wherein the ventral surface of the body is in contact with the substrate. The fish then proceeds forward via synchronized, cyclical movements of the pectoral fins and trunk to achieve forward displacement of the center of mass (Figs. 1–3; Video 1); we term this motion the 'army crawl' because it appears similar to movements produced by soldiers adopting a low-profile while crawling along the ground. During this behavior, *O. maculosus* individuals rotate the center of mass about the base of a stationary pectoral fin (ventral pectoral fin rays are in constant contact with the same spot on the substrate; Fig. 2), while pushing against the substrate with the tail (Fig. 1). We define the "base" of the pectoral fin as approximately where the radials insert into the pectoral girdle. While the fish do appear to push against the substrate with their tail (Video 2), we did not measure ground reaction forces, so we use the term "push" as a descriptive term. During this movement, the fish also rolls about the anteroposterior axis, which allows the fish to lift the mid-body above the substrate when they seemingly push down with their stationary pectoral fin on one side and on the tail on the contralateral side (Fig. 3, Video 2). Typically, a fish will subsequently use the contralateral pectoral fin as a pivot point and swing the tail to the opposite side to produce alternating cycles of movement (left fin, tail to the right of the body, right fin, tail to the left of the body, etc.; Fig. 3). The rotation about the base of the stationary pectoral fin swings the body away from the fin, protracting the fin (Fig. 2). Once this rotation is complete, the fish retracts and adducts the fin. While *O. maculosus* have one pectoral fin in a stance position against the substrate, they swing the tail towards the midline from the contralateral side of the body, pushing off the substrate as the tail approaches and crosses the midline to the ipsilateral side (Figs. 1–3). With fins analogous to forelimbs and a tail analogous to two hindlimbs (depending on which side of the body the tail is), *O. maculosus* has a diagonal-like gait and footfall pattern that is similar to terrestrial salamander walking. However, since diagonal couplet gaits are based on quadrupedal locomotion, *O. maculosus* does not use a true diagonal gait. While one pectoral fin/forelimb is in a stance position, the tail/foot pushes against the substrate on the contralateral side, rotating the anterior body forward around the stationary fin/forelimb (Figs. 2, 3).

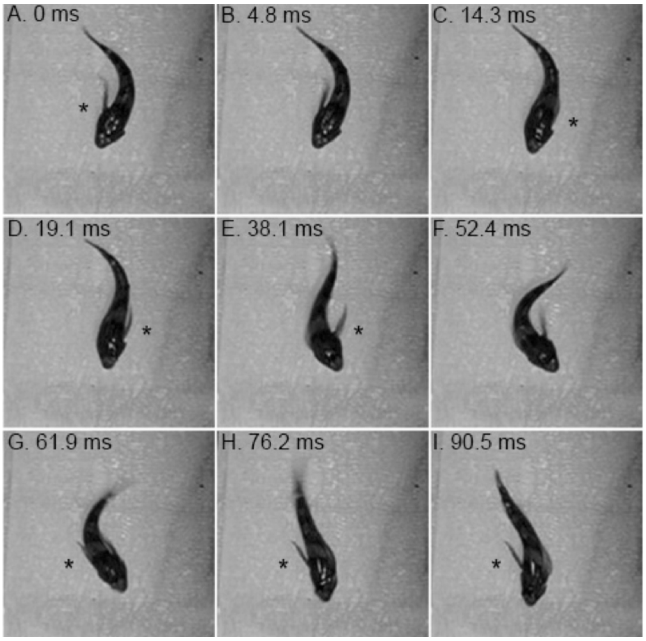

Fig. 1. *O. maculosus* alternatingly rotate about their pectoral fins while using their tail to power their movements during an army crawl.
A sculpin can initiate an army crawl starting at any stage depicted in this storyboard. While this storyboard depicts a standard army crawl cycle, there are often variations in the amplitude of the pectoral fins and tail. An "*" denotes which pectoral fin is in contact with the ground and when. The tail is in contact with the ground throughout the stride and provides the power for anterior locomotion. The pectoral fins do not contribute to their terrestrial locomotor power, rather they serve as pivot points. A) While resting on its ventral surface, the sculpin has its right pectoral fin (RP) protracted, its left pectoral fin (LP) fully retracted, and its posterior body curved so that its tail extends towards the right of its body. B) With its LP planted firmly on the substrate, the sculpin begins to retract its RP. C) The RP is fully retracted. The sculpin rolls partially around its long axis towards its planted LP, while rotating its body around the base of the planted pectoral fin. The sculpin also begins to swing its tail towards its midline, pushing its caudal peduncle against the substrate to lift its body and drive rotation. D) The LP protracts as the body moves anteriorly. E) The LP is fully protracted and the tail has crossed the midline. F) The sculpin plants its RP against the substrate, while retracting its LP. The tail is at maximum amplitude. G) The sculpin rolls around its long axis towards its RP, while swinging its tail towards the midline while pushing it against the substrate. Its LP is fully retracted. H) The RP protracts as the anterior body continues to rotate about the RP. The tail crosses the midline of the body and continues moving towards the right. I) The RP is fully protracted and the tail has reached maximum amplitude. The sculpin has moved anteriorly, completing a single army crawl stride.

As they move overland, *O. maculosus* oscillate the tail with an average frequency of ~9.9 Hz for short bursts (average duration = 0.417 s; Table 1) and use a pectoral fin stride length of ~0.25 % BL to produce a terrestrial velocity of 1.6 BL/s (where standard length (SL) is

Fig. 2. Sequential outlines of an *O. maculosus* army crawl half-stride.
Using sequential frames from high-speed video of the *O. maculosus* army crawl, this series of outlines emphasizes how the body rotates anteriorly around a relatively stationary pectoral fin (the right pectoral fin in this half-stride), while the caudal body moves from the contralateral to the ipsilateral side of the stationary fin. The sequence progresses from red to blue outlines.

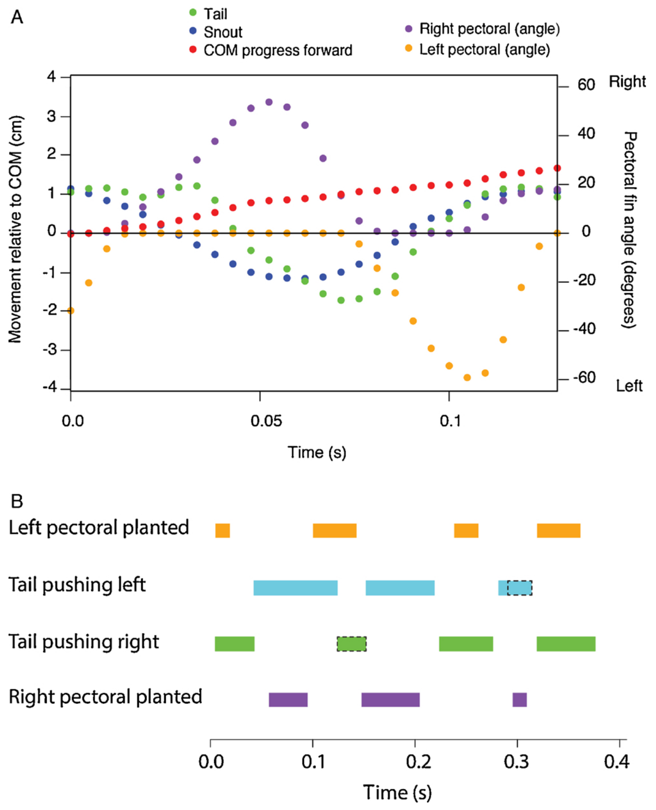

Fig. 3. Sample terrestrial "footfall" pattern and sequence of *Oligocottus maculosus*.
A) This is a representative terrestrial army crawl stride for *O. maculosus* (at 210 fps) using tracked points, where the sculpin is moving to the right of the screen. The pectoral fins are completely out of phase with each other, and the snout is out of phase with the protracted pectoral fins. However, the fins are neither completely in phase nor out of phase with the tail. While a pectoral fin is in a stance position (indicated by increasing angle), the tail is swinging from the contralateral side, crossing over the midline of the body to the ipsilateral side.
B) During terrestrial locomotion, a tide pool sculpin alternatingly anchors a pectoral fin against the substrate, rotates about the anchored fin, and then adducts that fin. While one pectoral fin is anchored or adducting, the opposite pectoral fin is tucked against the body. Additionally, while a pectoral fin is planted on one side of the body, the tail pushes against the substrate on the contralateral side (as seen in the lateral view; Video 3), causing the body to rotate about the pectoral fin. Dotted outlines indicate that the tail is swinging, but making little contact with the ground, resulting in very little rotation of the body.

used as body length; Table 1). *O. maculosus* protract their pectoral fins ~55° (N = 45 strides of 5 individuals, SD = 12.9), with a maximum protraction of ~82° observed during this movement. The terrestrial walking behavior of *O. maculosus* is produced by oscillation of the body that generates a cycle of bending that is similar to a standing wave, with the lowest wave amplitude near the COM, and much larger wave amplitudes experienced by the head and tail (Fig. 4). When changing the overall direction of travel, an individual of *O. maculosus* will increase the amplitude of the tail to one side, a movement that also increases rotation of the head to the contralateral side. The fish can then continue on its new heading, or use another increased-amplitude tail-movement on the ipsilateral (same) side to continue to rotate.

Because *O. maculosus* are benthic, they initiate swimming from a resting position (prone) on the substrate. When swimming, they produce a cyclic, undulatory, axial bending behavior with a wave amplitude that increases along the body from snout to tail (Fig. 4A; Video 3). To initiate a bout of swimming from a prone position, *O. maculosus* individuals will rotate the body about the center of mass to produce a C-shaped lateral bend (Fig. 5), then swing the caudal fin back through the midline to produce tail-beat to the opposite side. These movements are superficially similar to an aquatic fast-start, but are too slow to be Mauthner-cell initiated (Eaton and Hackett, 1984); we term this behavior a "swimming start". After using the body bend + tail beat to accelerate, *O. maculosus* individuals then transition into cycles of slow swimming. During slow swimming, *O. maculosus* oscillate their tails at an average frequency of 15.4 Hz, but some individuals achieved frequencies of 27.4 Hz (Table 1). There are a few key similarities between the kinematics of the terrestrial and aquatic locomotion of *O. maculosus*. The initial phases of the terrestrial army crawl and swimming start involve similar tail beat amplitudes, and fish rotate about their pectoral fin during both forms of locomotion to a similar degree (~55.2° and ~56.3°, respectively).

Leptocottus armatus, a sculpin species typically found in the shallow subtidal and low intertidal, employ a similar body + caudal fin movement to that of *O. maculosus*, but do not produce cyclic motions of the pectoral fins (Fig. 7; Video 4). Their pectoral fins do not appear to contribute to forward motion, as *L. armatus* achieve very little anteriorly-directed movement of the COM. While lying on their ventral surface, individuals of *L. armatus* oscillate the tail from side-to-side with greater amplitude than *O. maculosus* ($p = 0.002$, $F = 15.8$, $df_1 = 2$, $df_2 = 12$; Fig. 4A). *L. armatus* move their tail on land with an average frequency that is slower than that of *O. maculosus,* 7.0 Hz ($p = 0.013$; $t = 3.1$; Table 1). They also have a slower terrestrial velocity than *O. maculosus*, 0.5 BL/s ($p < 0.001$, $T = 10.2$; Table 1).

In a manner similar to that exhibited by *L. armatus*, *Icelinus borealis* oscillate the axial body from side to side, but produce little anteriorly-directed movement when on land. In addition to cyclic oscillations, individuals of *I. borealis* sometimes move the tail slowly towards one side, and then, as the tail approaches maximum curvature, rapidly accelerate the caudal peduncle and head towards each other to form a tight C-shape (Fig. 8; Video 5). During this behavior, the center of the body remains in full contact with the substrate, but the head and tail may lift off the substrate. When moving on land, individuals of *I. borealis* oscillate their tail at a higher frequency than *O. maculosus,* 13.6 Hz

Table 1
Intertidal sculpins are more effective at terrestrial locomotion than subtidal sculpins. In addition to the average terrestrial data recorded for stride frequency, locomotor sequence duration, velocity, and curvature coefficient (CC) and the associated standard errors (SE), the slow swimming (SS) and fast swimming (FS) stride frequencies were recorded for *O. maculosus*. Standard lengths were used for body lengths (BL).

Species	Stride Frequency (Hz)	SE	Sequence Duration (s)	SE	Velocity (BL/s)	SE	CC (bent length/BL)	SE
O. maculosus	9.92	0.089	0.417	0.068	1.56	0.094	0.739	0.050
L. armatus	7.03	0.134	0.307	0.065	0.46	0.053	0.580	0.044
I. borealis	13.61	0.352	0.535	0.057	0.40	0.166	0.325	0.091
O. maculosus SS	15.38	0.128	0.277	0.069	-	-	-	-
O. maculosus FS	27.39	0.466	0.073	0.022	-	-	-	-

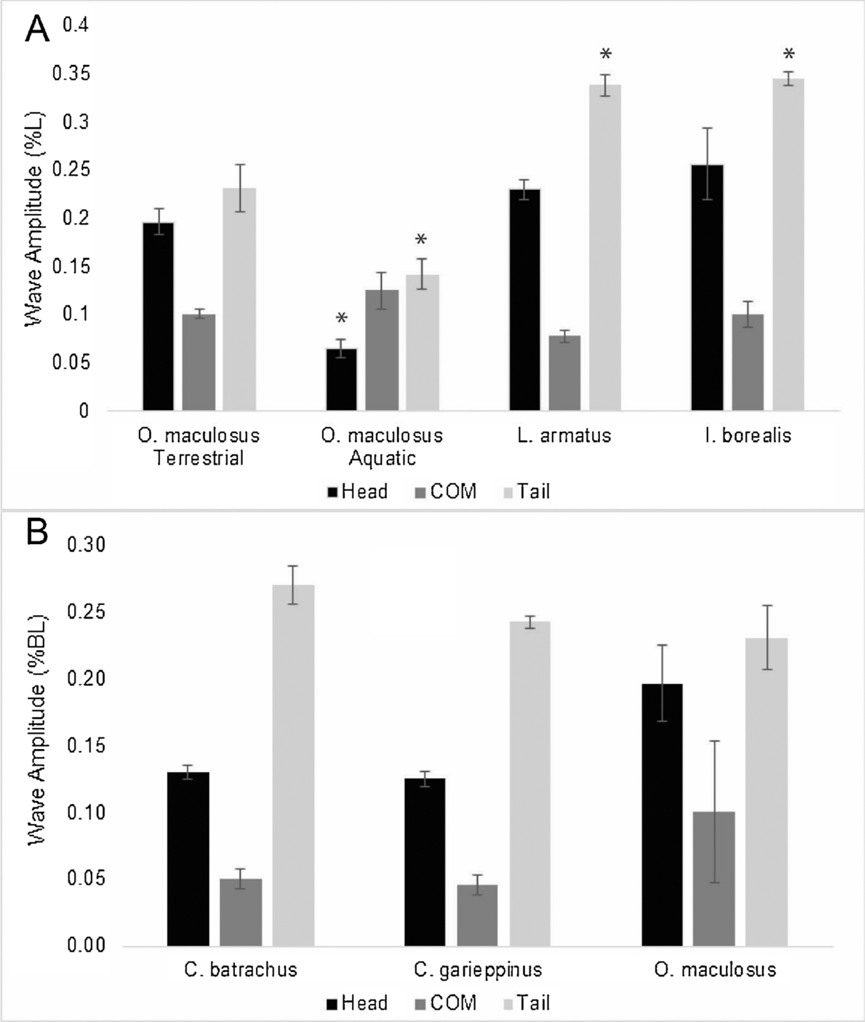

Fig. 4. Wave amplitudes of sculpins and amphibious fish. The wave amplitudes for the snout (head), COM, and caudal peduncle (tail), as %SL, are shown for the terrestrial locomotion of *O. maculosus*, *L. armatus*, and *I. borealis* (N = 6, 5, 4, respectfully) and the aquatic locomotion of *O. maculosus* (N = 6) in A. An "*" denotes a significant difference from the terrestrial *O. maculosus* values. The wave amplitudes of *O. maculosus* and two other species of fish that use axial-appendage-based terrestrial locomotion, the Asian walking catfish, *Clarias batrachus* (N = 4), and the African walking catfish, *C. gariepinus* (N = 5), are shown in B as % body length (BL). Error bars represent SE.

($p = 0.022$, t = 3.5; Table 1) and produce a terrestrial velocity of 0.4 BL/s (Table 1), which is slower than *O. maculosus* ($p = 0.004$, t = 5.2). During terrestrial movements, individuals of *I. borealis* do not achieve substantial rotation or linear displacement of the COM. However, if the body and tail rotate such that the lateral side of the body makes contact with the substrate, the fish will use a rapid acceleration of the tail to push off of the ground and flip or roll tail-over-head into a "summersault" behavior.

Distance ratios, or DR (a measure of sinuosity in forward movement, where movement is considered more effective as DR approaches 1), for the movement of *O. maculosus* are greater during aquatic locomotion than during terrestrial locomotion at the head ($p < 0.001$, t = 17.2), COM ($p = 0.007$, t = 10.7), and tail ($p = 0.015$, t = 5.6). *O. maculosus* individuals move using larger distance ratios, relative to *L. armatus* and *I. borealis* at the head ($p < 0.001$, F = 31.2, $df_1 = 2$, $df_2 = 12$), COM ($p < 0.001$, F = 66.9, $df_1 = 2$, $df_2 = 12$), and tail ($p < 0.001$, F = 17.9, $df_1 = 2$, $df_2 = 12$; Fig. 6A). *L. armatus* has a greater COM distance ratio than *I. borealis*, as supported by posthoc ANOVA and Tukey-Kramer tests ($p < 0.001$, F = 66.9, $df_1 = 2$, $df_2 = 12$).

The wave amplitudes produced during swimming by *O. maculosus* are lower than the terrestrial wave amplitude for both the head ($p < 0.001$, t = 7.2) and tail ($p = 0.026$, t = 2.8), but not for the COM ($p = 0.395$, t = 1.1). *O. maculosus* also has significantly smaller tail wave amplitudes than *L. armatus* and *I. borealis* ($p = 0.001$, F = 12.7, $df_1 = 2$, $df_2 = 12$), but there is no significant difference between head ($p = 0.159$, F = 2.2, $df_1 = 2$, $df_2 = 12$; Fig. 4A) and COM wave amplitudes for all three species ($p = 0.090$, F = 4.6, $df_1 = 2$, $df_2 = 12$). The wave amplitudes and distance ratios of individuals of *O. maculosus* are similar to those produced by the two walking catfish species studied by Pace and Gibb (2014), particularly of the African walking catfish, *Clarias gariepinus* (Figs. 4B, 6 B). The pectoral fin stride length of *O. maculosus* (0.252 % BL) is also similar to the stride length of *C.*

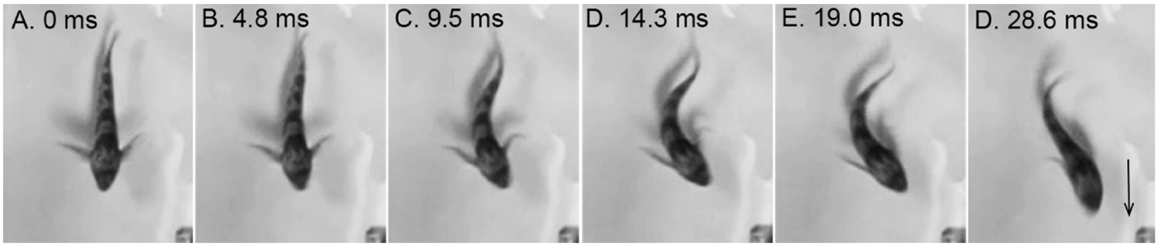

Fig. 5. Initiation of swimming in *O. maculosus*.
A) To initiate a bout of swimming while at rest, an *O. maculosus* will start with its body oriented linearly with its pectoral fins protracted. B) The snout and tail begin to move towards each other on the left side of the body, while the base of the left pectoral fin appears to maintain a stable position relative to the water. C) The snout and tail continue to move towards each other. The base of the left pectoral fin maintains its position, but the tip begins to curve away from the body. D) The snout and tail are at their maximum amplitude. The left pectoral fin begins to retract. E) The caudal fin straightens and the tail begins to move towards the midline. Both pectoral fins are partially protracted. The head starts moving anteriorly. F) Both pectoral fins are retracted and the fish starts undulatory swimming. The arrow denotes the direction of travel.

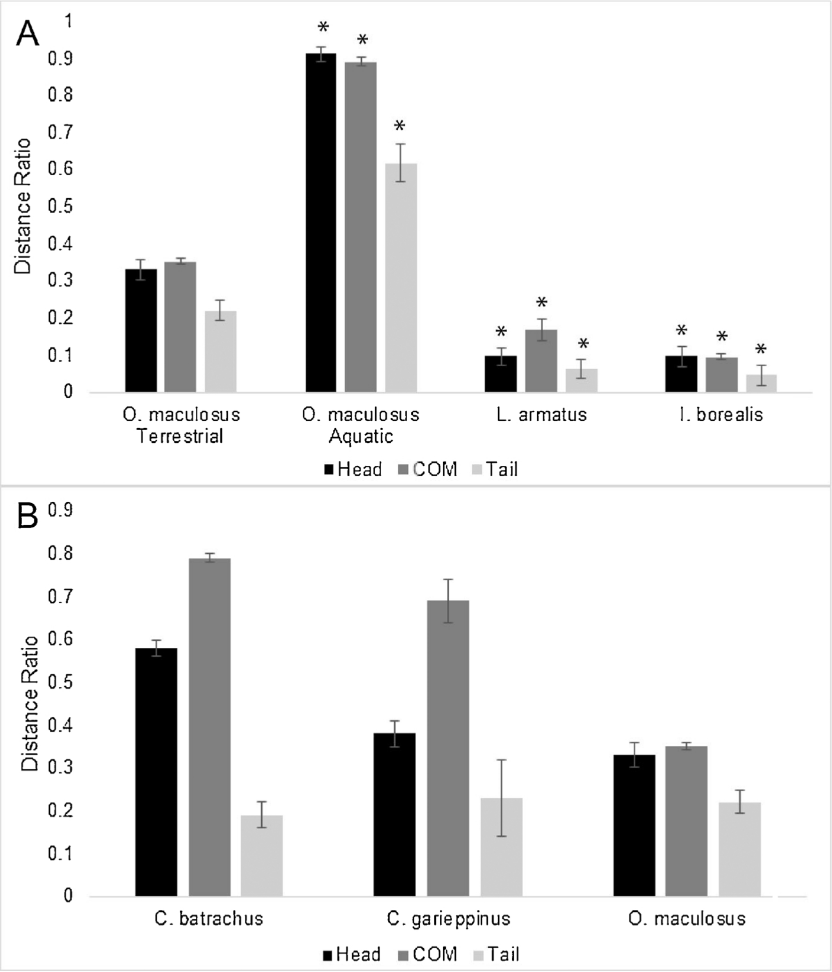

Fig. 6. Distance ratios of sculpins and amphibious fish.
The distance ratios for the head, COM, and tail, are shown for terrestrial locomotion of three sculpin species (*O. maculosus* N = 6, *L. armatus* N = 5, *I. borealis* N = 4) and the aquatic locomotion of *O. maculosus* (N = 6) in A. An "*" denotes a significant difference from the terrestrial *O. maculosus* values (α = 0.05). The distance ratios of *O. maculosus*, *C. batrachus* (N = 4), and C. gariepinus (N = 5), are shown in B. Errors bars represent SE.

gariepinus (0.278 % BL). However, the COM distance ratio is much greater for *Clarias* spp. than *O. maculosus*.

4. Discussion

Individuals of *Oligocottus maculosus* are more effective at moving over land than their subtidal relatives, *Leptocottus armatus* and *Icelinus borealis*, likely because *O. maculosus* move using an "army crawl" behavior, a form of axial-appendage-based terrestrial locomotion. Individuals of *O. maculosus* move over land more quickly, in a more linear path, and with reduced tail movements, relative to individuals representing the two subtidal sculpin species considered here. Similarly, terrestrial blennies are able to move more quickly and with greater stability than more aquatic blennies (Hsieh, 2010) *O. maculosus* individuals produce the army crawl behavior by coordinating the movements of their caudal fin with their pectoral fins (Figs. 1–3), accompanied by rolling in the anteroposterior axis, which elevates the COM up above the substrate (Video 2). This rapid behavior likely allows

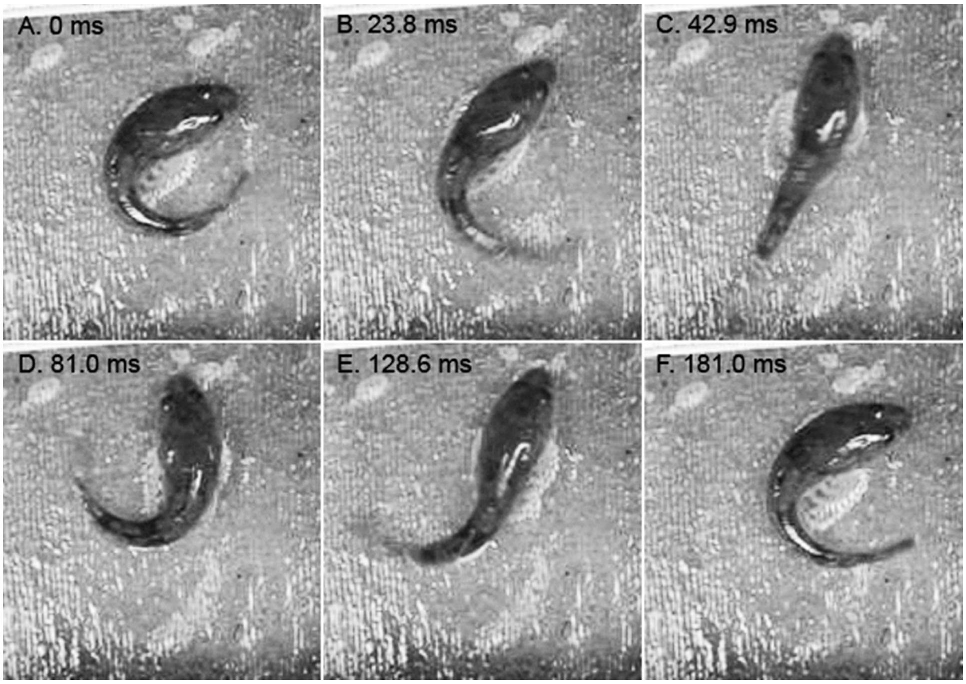

Fig. 7. *L. armatus* oscillate on land and produce little-to-no anterior displacement.
While on land, the pectoral fins of *L. armatus* maintain constant contact with the substrate with a relatively constant degree of protraction. Bubbles under the paper towel can be used as reference points between the panels. A) While at rest propped upright on its pectoral fins, an *L. armatus* has its body curved so that its tail is near its head on the right side of its body. B) The snout and tail begin to move laterally away from each other towards the left side of the body. C) The snout and tail cross the midline and continue moving towards the left side of the fish's body. D) The snout and tail are at maximum amplitude on the left side of the body. E) The snout and tail begin to move towards the right side of the body. F) The snout and tail are at maximum amplitude on the right side of the body.

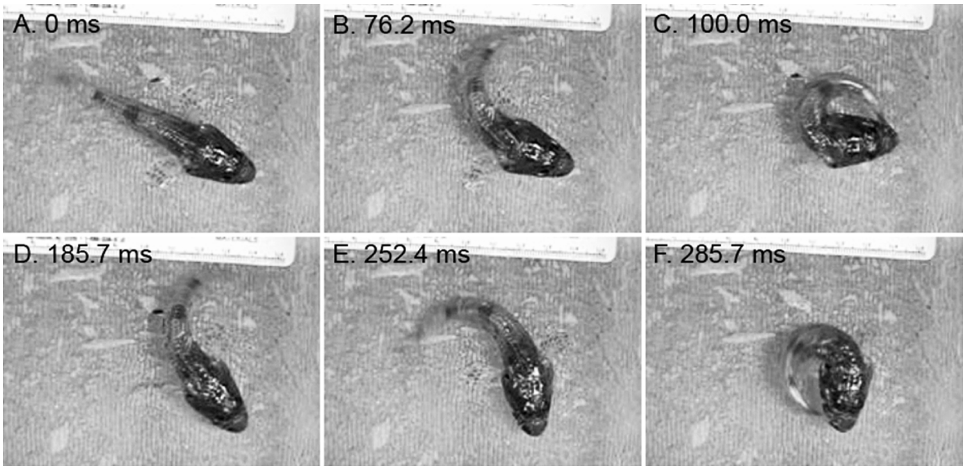

Fig. 8. *I. borealis* produces an oscillatory motion when on land that achieves little-to-no displacement.
While on land, the pectoral fins of *I. borealis* maintain a fixed position relative to the body, and make little contact with the substrate. Bubbles under the paper towel can be used as reference points between the panels. A) Starting with its body in a linear orientation and its pectoral fins fully protracted, B) the fish moves its snout and tail laterally towards the left side of its body at a constant rate. C) The sssnout and tail accelerate towards each other in a jamming-motion until they make contact. D) The snout and tail move away from each other and towards the midline. E) The snout and tail cross the midline and continue moving towards the right side of the body. F) The snout and tail move rapidly towards each other in a jamming motion until they make contact.

them to be able to return to water more quickly when stranded and more easily avoid possible predation, desiccation, and hypoxia, *O. maculosus* individuals also appear to have the ability to easily alter their course when locomoting on land by producing asymmetrical strides. By decreasing the angle of one-half stride and increasing the angle of the subsequent half-stride on the contralateral (opposite) side of the body, *O. maculosus* can quickly alter their direction of travel during overland movements.

In a manner similar to many other teleosts, *O. maculosus* use their pectoral fins for braking, steering, and propulsion during swimming. However, on land, pectoral fins are used as pivot points and do not provide propulsion: they are static during anterior movement. *O. maculosus* are able to move much more quickly in water than on land (Fig. 6A), which suggests that their pectoral fins and caudal fin are more effective as aquatic propulsors than they are as terrestrial propulsors. However, swimming fishes experience only minimal effects of the forces of gravity, which undoubtedly influences differences in velocity between the two environments. *O. maculosus* individuals have pectoral fin adaptions that may allow them to perform better on land than other sculpins, including longer pectoral fins with reduced webbing on the ventral surface, relative to *L. armatus* (as described by Kane and Higham, 2012). Reduced ventral webbing improves aquatic substrate contact in the longhorn sculpin (*Myoxocephalus octodecimspinosus*; Taft, 2011; Taft and Taft, 2012), and may improve substrate contact during terrestrial locomotion of *O. maculosus*. Reduce ventral webbing also improves terrestrial walking in other amphibious fishes (mudskippers), at the detriment to terrestrial climbing (Wicaksono et al., 2016). The long pectoral fins of *O. maculosus* may also improve purchase by increasing area in contact with the substrate, reducing slippage in terrestrial environments. They also have smaller and more vertical pectoral fin insertions relative to *L. armatus* (Kane and Higham, 2012); this may enable *O. maculosus* to produce a greater degree of anterior rotation about the pectoral fin base by having the pectoral fin more perpendicular to the anteroposterior axis, similarly to rowing labriform swimmers (Thorsen and Westneat, 2005).

The kinematics of *O. maculosus* are qualitatively similar to salamander walking due to the diagonal gait, large lateral oscillations of the body, and the use of the hindlimbs for propulsion and forelimbs for stabilization (Kawano and Blob, 2013). While the terrestrial locomotor patterns of *O. maculosus* resemble the footfall patterns produced by salamanders moving on land (Ashley-Ross et al., 2009), they do not use a true diagonal gait. They are not quadrupedal, and therefore they instead use their tail in place of two hind limbs during army crawls, which may not have a tetrapodal analog. Additionally, *O. maculosus* have a similar terrestrial behavior to that of the walking catfishes, which also use axial-appendage-based locomotion. However, individuals of *O. maculosus* move the tail while the pectoral fin is maintained in a stance position — thus, the pectoral fin does not move relative to the substrate (Figs. 1, 2) when the tail is moving. In addition, *O. maculosus* rotate about the base of their pectoral fins, whereas *Clarias* spp. rotate around the tips of the pectoral spines (Johnels, 1957; Pace and Gibb, 2014). Both of these fish use parts of their pectoral fins as the fulcrum in class two levers. However, by having the load (COM) farther away from the fulcrum, *Clarias* spp. are able to move their COM a greater net distance than *O. maculosus*, even if they rotate the same degree about the fulcrum, which could account for their greater distance ratio — suggesting more effective overland movements. While *O. maculosus* produce more lateral movement (as quantified by the distance ratio), their stride frequency of ~10 Hz is almost 20x greater than that of *Clarias* spp., which move at only about 0.5 Hz (Johnels, 1957). However, the difference in stride frequency between *O. maculosus* and the catfishes may be a result of size differences; because the *Clarias* spp. that was studied by Johnels (1957) is larger (~40 cm), it is expected to move at a slower frequency due to simple scaling principles. The terrestrial locomotor behavior of *O. maculosus* is also similar to that of *Polypterus senegalus,* which also uses axial-appendage-based locomotion. Both *P. senegalus* and *O. maculosus* have flexible fins compared to catfish, and both species produce movement by rotating about the base of the pectoral fins with their fin rays in contact with the substrate (Standen et al., 2014, 2016). However, *P. senegalus* incorporate a great degree of snakelike undulatory motions into their terrestrial locomotor behaviors (Standen et al., 2016) that are not seen in *O. maculosus*.

In contrast with *O. maculosus,* individuals of *L. armatus* and *I. borealis* were ineffective at producing forward movement when stranded on land. However, when they oscillated the tail from side to side on wet, terrestrial surfaces, *L. armatus* and *I. borealis* did impart sufficient momentum to move forward across a thin film of water, likely due to the lower friction coefficient than dry land. On an incline, oscillating the posterior body and caudal fin from side to side, in combination with the forces of gravity, may create sufficient force to overcome static friction, thus allowing fish to convert potential energy to kinetic energy and move downhill (Birn-Jeffery and Higham, 2014). In addition, the rapid tail oscillations of I. *borealis* sometimes caused the fish to roll down the hill like a tire; this behavior is analogous to the terrestrial rolling behavior of *Gambusia affinis*, but employs yaw (movement around the dorsoventral axis) instead of roll (movement around the anteroposterior axis, see Boumis et al., 2014).

As we learn more about how amphibious fishes move over land, we develop a clearer understanding of the mechanical and physiological challenges of exapting aquatic propulsors for use in terrestrial locomotion. These challenges were faced not only by recent ancestors of modern amphibious fishes, but also by ancestral tetrapods as they transitioned from aquatic to terrestrial habitats during the Devonian Period (Shubin et al., 1997). For example, some early tetrapodamorphs, such as *Icthyostega*, may have used modes of terrestrial locomotion similar to the pectoral fin-based crutching of *Periophthalmus* (Harris, 1960; Pierce et al., 2012), with associated pelvic fin movements (Wicaksono et al., 2017). Others, like *Panderichthys*, may have used axial-appendage-based locomotion like *Clarias* (Boisvert, 2005) or *O. maculosus*. It may be possible that, in a manner similar to *Periophthalmus* and *O. maculosus,* some early tetrapodamorphs may have used their tails, in coordination with their pectoral fins, to effectively move onto and across the land (McInroe et al., 2016). We also note that, as new fossil evidence is discovered, the timeline of first vertebrates on land may be pushed farther back in time (Niedźwiedzki et al., 2010). We suggest that osteichthyan fishes may have had the capacity to locomote on land before the rise and terrestrial success of lineages that demonstrate robust limbs or other obvious physical adaptations for life on land (e.g. sarcopterygians). Rather, early land colonist may have relied on behavioral adaptions like *O. maculosus*. Modified behavioral patterns used to create terrestrial movement would leave no anatomical fossil record, but could potentially create trackway fossil evidence (Falkingham and Horner, 2016).

5. Conclusions

The tidepool sculpin, *Oligocottus maculosus,* uses an effective axial-appendage-based form of terrestrial locomotion, which we describe as an “army crawl.” The army crawl involves oscillations of the axial body, paired with alternating rotations about the base of planted pectoral fins. The kinematics of this behavior are distinct from the aquatic locomotion of *O. maculosus*, which is more undulatory than oscillatory. This behavior exhibits similar kinematics to the axial-appendage-based terrestrial locomotion of walking catfishes, *Clarias* spp. While subtidal sculpins, like *Leptocottus armatus* and *Icelinus borealis*, have similar body and fin shape to *O. maculosus* and use oscillatory motions of the body on land, they do not coordinate their pectoral fins with the oscillations of the body, achieving little-to-no net displacement while on land. This suggests that primarily behavioral adaptations, rather than morphological adaptations, allow *O. maculosus* to effectively locomote in a terrestrial environment.

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Competing interests

The authors declare no competing or financial interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.zool.2018.10.003.

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