A kinematic analysis of prey-capture prone jumping by the barred mudskipper (*Periopthalmus argentilineatus*)

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Abstract

Mudskippers are amphibious fish that have developed multiple effective means of terrestrial locomotion. One of these modes of locomotion is the “prone jump”, a jumping motion in which the fish launches itself forwards by coiling and rapidly extending its tail against the ground. Mudskippers utilize this jumping motion to accomplish tasks such as prey-capture and traversal of the environment. To move and hunt effectively they must have some control over the direction in which they are jumping. Here, I define the kinematics of the prey-capture prone jump and then determine how mudskippers adjust the kinematics to produce different jump trajectories. To test how mudskippers control jump trajectory, highspeed cameras were used to capture the three-dimensional movement of mudskippers performing prey-capture prone jumps for food held in various positions. First, initial body position, food position and resultant jump direction are related. Mudskippers were found to rapidly adjust multiple aspects of their initial body position relative to the food and their tail coiling side before the jump. Second, mudskippers were found to increase the maximum speed and acceleration of their jumps (primarily in the vertical direction) when food was positioned farther away or higher above the horizon. Lastly, mudskippers were occasionally observed changing body conformation in the air. These movements were hypothesized to contribute to jump accuracy by altering course trajectory in the air. However, all the jumps analyzed in this study were successful and had initial ground reaction forces and resultant take-off angles that resulted in a ballistic trajectory that did not require aerial course correction to hit the food target. Future analysis of failed jump attempts would be required to confirm the consequence of aerial course correction in these animals.

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Contents

Abstract ii

Acknowledgments iii

List of Figures vi

List of Tables viii

Chapter 1: Literature review 1

Amphibious fish 1

Fish terrestrial locomotion 1

Goal-directed movement and the neural control of movement 2

Goal-directed locomotion 3

Mudskippers 4

Mudskipper morphology and adaptations 6

Mudskipper terrestrial movements 10

Chapter 2: The coordination of a prey-capture prone jump 18

Introduction 18

Methods 24

Results 43

Discussion 68

Chapter 3: Future directions 79

Force measurement (kinetics) 80

Biomimetic mudskipper robot 81

Aerial movement analysis 83

Comparative analyses 83

Conclusion 85

References 87

Appendix 96

List of Figures

Figure 1 | Phylogeny of mudskippers and allies. 6

Figure 2 | The Atlantic mudskipper (*Periophthalmus barbarous*) crutching across different substrates and inclinations. 12

Figure 3 | Illustrations of a mudskipper producing a prone jump. 15

Figure 4 | Mudskipper filming setup illustration. 25

Figure 5 | Digitized loci from the lateral view (top) and ventral view (bottom). 27

Figure 6 | Food being suspended at different positions relative to the mudskipper. 28

Figure 7 | Illustration of how food position variables change relative to the fish. 31

Figure 8 | Suspension method for determining center of mass. 34

Figure 9 | The real trajectory (blue) and predicted trajectory (red) of the aerial phase. 35

Figure 10 | A prey-capture prone jump sequence with 3 phases (preparatory, propulsive, aerial) from the lateral and ventral view. 44

Figure 11 | Ventral view of the pectorals fins before (**A**) and after (**B**) planting on the ground. 45

Figure 12 | Pelvic girdle trajectory path during aerial and non-aerial prey-capture prone jumps from the start of the propulsive phase until food capture. 47

Figure 13 | Relationship between tail-pelvic girdle distance and food position at the end of the preparatory phase. 50

Figure 14| Logistic regression curve showing the relationship between tail planting side and lateral food angle. 52

Figure 15 | Relationship between head pitch and food position at the end of the preparatory phase. 53

Figure 16 | Relationship between pelvic girdle height and head pitch at the end of the preparatory phase. 54

Figure 17 | The difference in contralateral and ipsilateral fin distance relative to the pelvic girdle at the end of the preparatory phase. 56

Figure 18 | Relationship between initial 30ms vertical trajectory angle and body position at the end of the preparatory phase for prone jumps with end of preparatory phase food distances of 0.66 body lengths or less. 58

Figure 19 | The relationship between tail plant side and initial 30ms lateral trajectory angle. 59

Figure 20 | Relationship between pelvic girdle maximum velocity and food position. 61

Figure 21 | Relationship between the average acceleration to max velocity and food position at the end of the preparatory phase. 63

Figure 22 | Visualizing the predicted trajectory (red) of the pelvic girdle next to the realized trajectory (blue) for all aerial trials. 65

Figure 23 | Comparing the distance to the food of the real trajectory and the predicted trajectory of the fish. 66

List of Tables

Table 1 | Summary of hypotheses and predictions. 23

Table 2 | Total trials filmed and analyzed for each fish and their recorded length. 29

Table 3 | Variable specification for food position, body positioning, trajectory, velocity and acceleration variables. 32

Table 4 | Variable specification related to the real and predicted trajectory of the fish during the aerial phase. 37

Table 5 | Specification of random intercept linear mixed effects models. 40

Table 6 | Food position at end of preparatory phase for aerial and non-aerial jumps. 48

Table A1 | Total trials filmed of mudskippers post pectoral fin amputation and their recorded length at the end of the post amputation trials. 96

Table A2| All trials collected from the mudskippers and the number of good trials observed. 97

Table A3 | Summary of final models' statistics. 99

Table A4 | Correlation of fixed effects included in the final models. 100

Chapter 1: Literature review

Amphibious fish

Fishes generally have body types and locomotion styles that are optimized for aquatic life. Despite their aquatic optimization, numerous fish species willingly spend part of their lives on land and others find themselves stranded on land regularly (Sayer and Davenport, 1991). Fish that naturally spend part of their life histories on land do so for a variety of biotic and abiotic factors and are referred to as “amphibious fish” (Gordon, Malcom et al., 1969; Sayer & Davenport, 1991). Amphibiousness in fish has evolved independently across diverse taxa and there is a large degree of interspecific variation in their terrestrial ability (e.g. movement performance, emersion tolerance) (Lutek et al., 2022; Ord & Cooke, 2016; Turko et al., 2021). These amphibious fish often have a higher degree of terrestrial adaptations compared to fully aquatic fish such as the ability to breathe air, resist desiccation, excrete or store waste products, and the ability to move effectively on land (Sayer and Davenport, 1991).

Fish terrestrial locomotion

Water and land are vastly different force environments; therefore, most fish cannot simply translate swimming movements into productive terrestrial locomotion. Fish capable of navigating land have coopted their muscles, fins, and spine to produce a continuum of terrestrial movements that range in effectiveness and use (Gibb, Ashley-Ross and Hsieh, 2013). These amphibious fish use their bodies and fins in very different ways between aquatic and terrestrial environments (Swanson and Gibb, 2004; C. M. Pace and Gibb, 2009; Pace and Gibb, 2011; Standen *et al.*, 2016). For example, the amphibious mudskippers use their pectoral fins as undulating paddles in water and as stiff crutches on land. Some amphibious fish also have highly specialized anatomy that facilitates functional performance across environments (i.e. mudskipper, climbing gobies etc.), while others use their existing 'aquatic' morphology in similar or novel ways (i.e. walking catfish, *Monopterus*, *Polypterus*)(Pace and Gibb, 2011; Standen *et al.*, 2016; Bressman, Morrison and Ashley-Ross, 2021).

The types of locomotion fishes use on land have been categorized into several modes that impact performance in the terrestrial environment (Gibb, Ashley-Ross and Hsieh, 2013; Mehta *et al.*, 2021). Locomotor modes are patterns of body movement with specific propulsive elements. Amphibious fish terrestrial locomotor modes include axial-based locomotion (e.g. sinusoidal body undulation, oscillations and jumping), appendage-based locomotion (e.g. crutching), appendage-axial locomotion (e.g. crutching on deformable substrates) and underwater walking (Lutek et al., 2022).

Goal-directed movement and the neural control of movement

Certain amphibious fish terrestrial tasks such as foraging, escaping predators and traversing obstacles naturally require the ability to move effectively towards a goal. These goal-directed movements (i.e. locomotion, reaching with appendages, eye movements, balancing, etc..) are critical to the survival of all animals regardless of their environment and have been the subject of a great deal of research concerning their neural control within vertebrates (Grillner & El Manira, 2020; Lutek et al., 2022). To produce goal-directed movements animals perceive the outside world (exteroception), perceive their body position and orientation (proprioception), and integrate this information to produce the correct pattern and magnitude of muscle activation that will result in the movement of their body towards their goal.

The neural patterns that control muscle activation are the output of neural circuits comprised of brain centres, central pattern generators (CPGs) in the spine, sensory neurons and motor neurons. These neural patterns are constantly being modified by sensory input via the brain and proprioceptive feedback to account for changes in body position, orientation, and the environment or goal properties (Grillner & El Manira, 2020). The forebrain is responsible for selecting the correct behaviour(s) for a given stimulus and the chosen behaviour(s) will depend on the properties of the goal (eg. Location, movement, type of stimulus) and the movement repertoire of the organism (Grillner & El Manira, 2020). It is hypothesized that amphibious fish co-opt pre-existing or modified aquatic neural circuits or use novel neural circuits for terrestrial locomotion (Lutek et al., 2022). For example, mudskippers producing aquatic and terrestrial escape responses (aquatic C-starts and terrestrial prone jumps) use similar locomotor morphology but the kinematic patterns of the two movements are different and the fish sometimes produce the wrong type of movement for their given environment (Swanson & Gibb, 2004). These findings suggest that the terrestrial escape response uses a novel neural circuit despite recruiting the same locomotor morphology and performing a similar task as the aquatic escape response (Swanson & Gibb, 2004).

Goal-directed locomotion

Translating goal-directed movements of the body into terrestrial locomotion requires generating the ground reaction force and torque necessary to propel the body in a desired direction. The forces and torques that propel the fish are dependent on the biomechanical and physiological properties of the organisms and the properties of the substrate. Many biomechanical and physiological properties can affect a fish's ability to produce force in a terrestrial environment such as size, shape, tissue plasticity, strength, and anatomical specializations (Lutek et al., 2022). Also, amphibious fish often move between very different force environments (e.g., substrate types, levels of emersion, inclination, etc.) and have been shown to change their locomotory behaviours based on the physical properties of the substrate such as inclination and substrate type (Naylor & Kawano, 2022; Standen et al., 2016). While it is not always clear how modifying gait type or changing their locomotory behaviours gives the fish a functional advantage, it does indicate that there is locomotor flexibility in amphibious fish that responds to changing physical environments. Goal-directed locomotory behaviours are also dependent on the physical position and movement of the goal and the type of stimulus (e.g. prey-capture, escape response, courtship). For example, a fish running from a predator and a fish chasing prey will have different stimulus-dependent contexts that can influence the choice of locomotory mode, the rate at which the fish will move and the direction the fish will move.

Mudskippers

Mudskippers (Gobiidae: Oxudercinae) are a group of amphibious fish that inhabit (and sometimes skip across) mudflats and brackish coastal regions within the indo-pacific and the west coast of Africa (Parenti & Jaafar, 2017). The group consists of 10 genera and 45 species (Figure 1) (Steppan et al., 2022), many of the mudskipper species are amphibious and emerse onto land at least once a day (Murdy, 1989). While on land these fish demonstrate fascinating behaviours like terrestrial prey-capture, burrowing, fighting over territory, and elaborate courtship displays (Clayton, 2017; Clayton & Townsend, 2017; Yin Hui et al., 2019). Terrestriality has evolved independently at least two times across the mudskipper phylogeny, once within the *Periophthalmus*/*Periophthalmodon* lineage and again in the *Boleophthalmus* lineage (Figure 1) (Steppan et al., 2022). Many physiological, behavioural, and morphological/structural adaptations allow amphibious mudskippers to exploit both aquatic and terrestrial environments (Chew & Ip, 2017; Clayton, 2017; Clayton & Townsend, 2017; Ishimatsu, 2017; Kuciel et al., 2017; Martin & Ishimatsu, 2017; C. Pace, 2017). The adaptations that affect their terrestrial modes of locomotion and the locomotory modes themselves are discussed below.

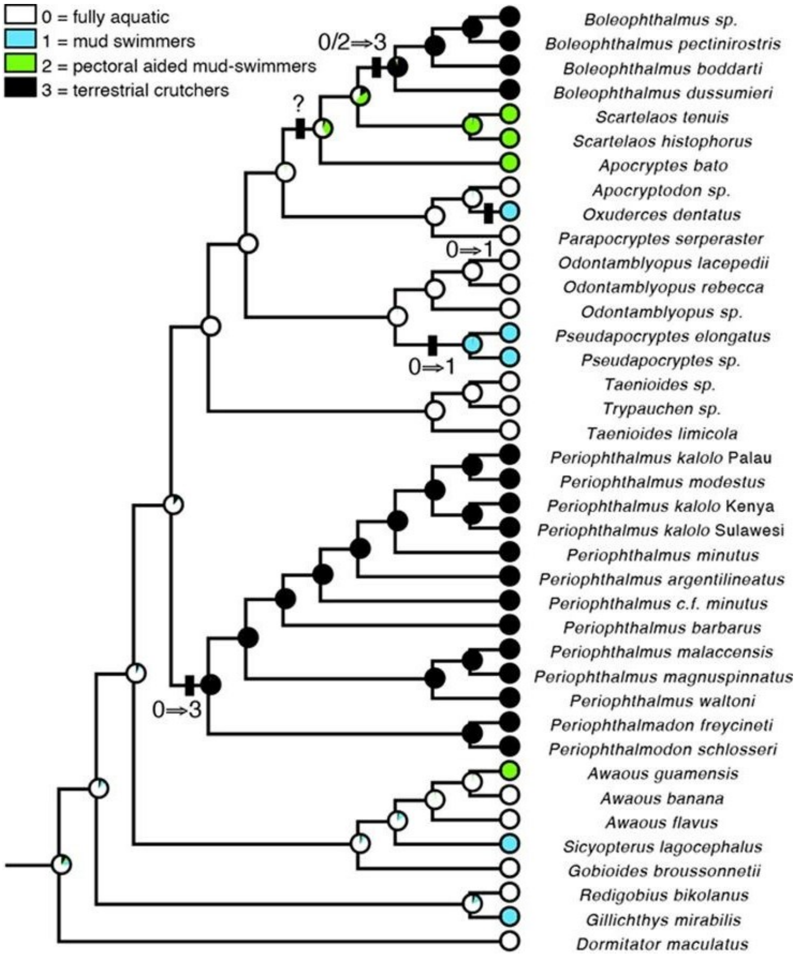

Figure 1 | Phylogeny of mudskippers and allies. (Figure used with permission from Steppan et al., 2022)

Mudskipper morphology and adaptations

Mudskippers like other gobies are generally short, cylindrically shaped, and slightly dorsoventrally flatted (Zander, 2011). Being dorsoventrally flattened helps the fish remain upright as it lowers their center of mass (CoM) relative to their base of support. In comparison, a more mediolaterally flattened fish such as a sunfish would flop onto its side when placed on land due to its high CoM relative to its base of support (Lutek et al., 2022). Being upright is not essential for terrestrial locomotion as fish can produce effective terrestrial locomotion even when on their sides (see killifishes and climbing perch) (Davenport & Matin, 1990; Gibb et al., 2011). However, being upright is essential for mudskipper terrestrial locomotion (ie. crutching and prone jumping) and does appear to provide some advantages concerning goal-directed locomotion such as being able to perceive the environment with both eyes and control movement direction via their axial anatomy and pectoral fins. Short body length is also a common trait among terrestrial jumping fish and therefore could be important for the biomechanics of jumps (Lutek et al., 2022). However, the relationship between body length and jump performance has not yet been studied.

Appendicular anatomy

The appendicular anatomy of mudskippers is essential for terrestrial locomotion and includes both the pectoral and pelvic fins and their associated girdles. Their pelvic fins are located on the ventral surface of the mudskipper and can be extended or retracted against the body while on land but are usually extended during locomotion. When the pelvic fins are extended on land they support the head region of the fish. Mudskippers like other gobioid fish can have unfused pelvic fins or fused pelvic fins that form a disc (Zander, 2011). Fusion and morphology of the pelvic fins varies between and within mudskipper genera (Murdy, 1989). Differences in the morphology and fusion of the pelvic fins combined with other differences between species could impact terrestrial locomotor performance (Hidayat et al., 2022; Wicaksono et al., 2016). For example, *Periopthalmus variabilis,* a tree climbing mudskipper, has unfused flexible pelvic fins with a large pelvic fin surface area to underbody surface ratio, an abundance of mucus-secreting cells and a small body size which altogether likely aid in adhesion during climbing (Hidayat et al., 2022; Wicaksono et al., 2016). On the other hand, *Periophthalmus boddarti*, a mudskipper incapable of climbing steep or vertical inclines, has fused pelvic fins, a smaller pelvic fin surface area ratio, fewer mucous-secreting cells and a large body size that likely limits their climbing ability (Hidayat et al., 2022; Wicaksono et al., 2016). Harris (1960) noted that the unfused pelvic fins and shorter and stiffer pelvic rays of *Periopthalmus barbarous* are likely better at supporting the weight of the mudskipper during crutching and jumping compared to mudskippers like *Periophthalmdon schlosseri* and *Periophthalmus chrysospilos* that have long fused fins and flexible rays used as suckers for climbing. Interestingly, both fused and unfused pelvic fins were attributed to climbing ability by Harris (1960) and Wicaksono et al. (2016). Therefore, it is unclear whether fused or unfused are better for climbing.

Some mudskipper genera (*Boleophthalmus, Periophthalmodon, Periophthalmus, and Scartelaos*) have a unique pectoral fin morphology that distinguishes them from other gobies and ray-finned fishes. Specifically, they have elongated proximal radials that protrude from the body wall (Harris, 1960; Murdy, 1989; C. M. Pace & Gibb, 2009; Zander, 2011). This elongation allows the fins to rotate anteriorly past the operculum which would normally limit such movements in fish with shorter radials (Harris, 1960; C. Pace, 2017; C. M. Pace & Gibb, 2009). This increased range of motion allows for greater stride lengths during crutching (C. M. Pace & Gibb, 2009). There are two hinge joints responsible for this range of motion; one hinge is positioned where the radials meet the cleithrum and allows for primarily anteroposterior movement, while the other hinge is positioned where the radials meet the rays and allows the rays to bend forwards and backwards at the end of the radials (Harris, 1960; C. M. Pace & Gibb, 2009). *Periopthalmus barbarous and argentilineatus* have a slight declination of the pectoral fin axis due to a longer dorsal proximal radial compared to the ventral proximal radial (Harris, 1960; C. M. Pace & Gibb, 2009). More terrestrial mudskipper species tend to have longer pectoral fin lengths relative to body length, longer and wider bones, more robust cleithra (ie. increased ossification and muscle attachment points) and larger intraradial muscles that likely facilitate force and lift production by the pectoral fins during terrestrial locomotion (Harris, 1960; Zander, 2011; Zhou et al., 2023).

Axial anatomy

Mudskippers use their tails to propel themselves during jumps and to stabilize their bodies while crutching on deformable (e.g. sand) and inclined substrates (Naylor & Kawano, 2022; Swanson & Gibb, 2004). Terrestrial mudskipper species tend to show a greater degree of vertebral flexibility and ossification compared to less terrestrial or aquatic species, likely facilitating tail-driven terrestrial movements such as jumps where the fish pushes off the ground with their tail (Tran et al., 2023). The ossification and tail flexibility of terrestrial mudskipper species is greatest in the caudal region of the tail (Tran et al., 2023). Many mudskipper species also have some degree of flexibility around the intersection of their skull and vertebrae that allows for elevation and depression of the head like a neck (Tran et al., 2022). Changing head pitch is likely important for terrestrial goal-directed locomotion since it allows the mudskipper to keep the region of their eye with higher visual acuity pointed towards their target (Takiyama et al., 2016).

Eyes and sensory abilities

Seeing in water and air is a challenge for amphibious fish since the refractive indices of water and air change the way light is focused onto the back of the retina, such that an eye adapted to vision in water would be short-sighted or myopic in air (Sayer, 2005). Many terrestrial mudskipper species have overcome this challenge to some extent since they display many behaviours that rely on vision (e.g. prey-capture, courtship, territorial defence) (Clayton & Townsend, 2017; Sayer, 2005; Stebbins & Kalk, 1961; Yin Hui et al., 2019). Their ability to see outside of water has been attributed to modifications of the eye such as a curved cornea in association with a spherical lens that is partly flattened (Kuciel et al., 2017; Sayer, 2005; Stebbins & Kalk, 1961).

Mudskippers are also capable of moistening their eyes, removing debris and protecting their eyes from physical damage via a blinking motion where each eye descends into a fluid-filled cup just under the eye (Aiello et al., 2023). Analysis of the retinal cell topography in *Periopthalmus modestus* showed the fish have a greater visual acuity in the lower frontal field of their vision. This is supported by observations that the fish orient this area of their field of view towards suspended prey before jumping at them (Takiyama et al., 2016).

Mudskipper terrestrial movements

The adaptive pressures of living on land have made mudskippers experts in terrestrial locomotor behaviours, demonstrating at least four modes of terrestrial locomotion (crutching, climbing, tail-flip jump and the prone jump) (De & Nandi, 1984; Gibb et al., 2013; Harris, 1960;

Stebbins & Kalk, 1961; Swanson & Gibb, 2004; Van Dijk, 1960; Wicaksono et al., 2016). These movements facilitate a wide range of behaviours such as hunting, burrowing, terrestrial escape responses and more (Clayton & Townsend, 2017; De & Nandi, 1984; Yin Hui et al., 2019). Their ability to produce these movements can be attributed at least partially to their specialized anatomy (Harris, 1960; Wicaksono et al., 2016). Studies on the kinematics and dynamics of these movements reveal how mudskippers utilize their locomotor morphology to produce effective terrestrial locomotion and have also shed light on the origin of early tetrapod locomotion (Kawano & Blob, 2013; Mo et al., 2020; Pierce et al., 2012), new adaptations to living on land (Harris, 1960), and differences in movement control between aquatic and terrestrial environments (C. M. Pace & Gibb, 2009). The kinematics and dynamics of four of their primary forms of terrestrial locomotion are summarized below.

Crutching

Crutching is a sustained locomotory mode commonly used by mudskippers when traversing their terrestrial environment (Figure 2). The kinematics of the “crutching” movements produced by *Periophthalmus barbarus* and *Periopthalmus argentilineatus* are generally well understood (Kawano & Blob, 2013; Naylor & Kawano, 2022; C. M. Pace & Gibb, 2009; Quigley et al., 2022). Crutching involves the synchronous movement of the pectoral fins to lift the CoM and swing it forwards, followed by anterior repositioning of the pectoral fins (like humans using crutches) (Kawano & Blob, 2013; C. M. Pace & Gibb, 2009). During repositioning of the pectoral fins, the mudskipper is supported by its extended pelvic fins and tail. The mudskipper pectoral fins are the primary propulsive force generators, producing forces in the lateral, posterior, and ventral direction while crutching (C. M. Pace & Gibb, 2009). The pelvic fins likely do not produce any propulsive force during crutching (C. Pace, 2017). Mudskippers can modify the kinematics of the crutching movement to compensate for inclined and deformable substrates as well as recruiting the tail to provide additional propulsive force and prevent slipping (Figure 2)(McInroe et al., 2016; Naylor & Kawano, 2022).

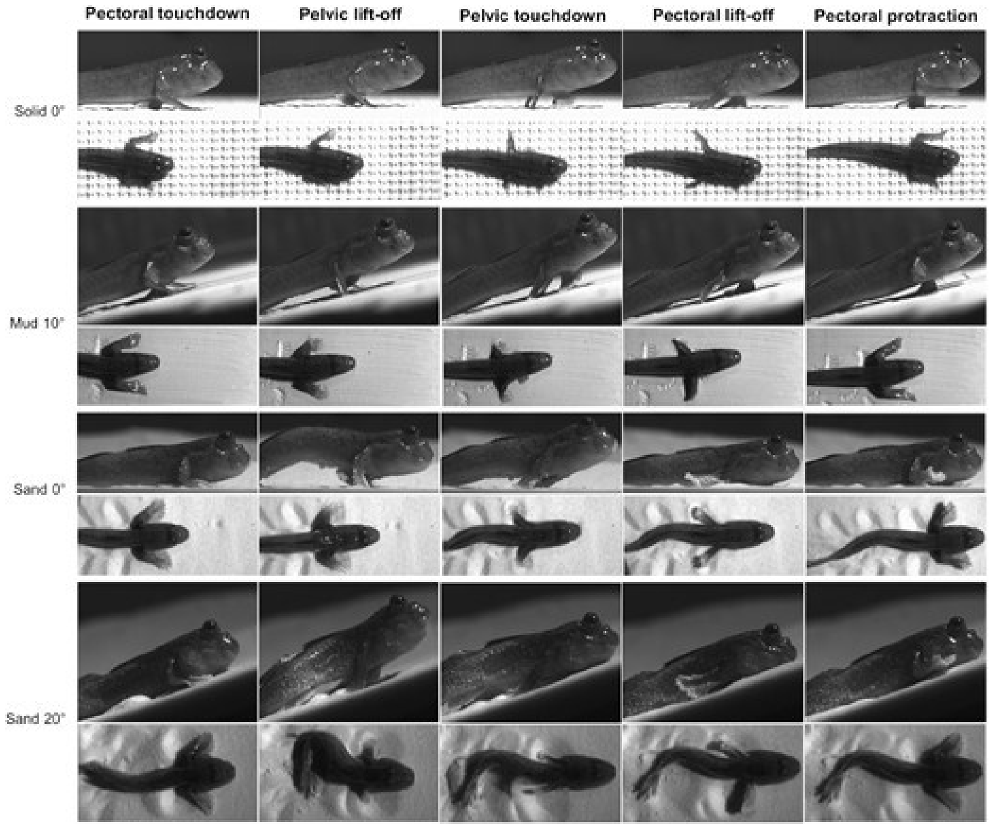

Figure 2 | The Atlantic mudskipper (*Periophthalmus barbarous*) crutching across different substrates and inclinations. (Figure used with permission from Naylor and Kawano, 2022).

Climbing

Climbing is used by many mudskipper species within the *Periophthalmus* genus to climb up vertical or steeply inclined surfaces such as the roots and trunks of mangrove trees during high tide (Harris, 1960; Polgar & Crosa, 2009). Relatively little is known about the kinematics of climbing behaviours, but the motion of the pectoral fins has been described as similar to those of crutching (Harris, 1960; Hidayat et al., 2022; Wicaksono et al., 2016). In that, the movement appears to be driven by the simultaneous movement of the pectoral fins to pull the mudskipper up the substrate. The pelvic fins are also an essential component of the climbing motion since they aid in adhesion to the substrate (Hidayat et al., 2022). It is unclear whether the pelvic fin is lifted from the substrate or whether it stays adhered to the surface during the entirety of the climbing motion. It is also unclear when mudskippers transition from a modified crutching gait on inclined surfaces to a climbing gait on very inclined surfaces. Variation in climbing ability between mudskipper species has been attributed to changes in the morphology of the pelvic fin (Hidayat et al., 2022; Wicaksono et al., 2016).

Tail-flip jump

The tail-flip jump is a jumping motion driven by the axial skeleton (tail) that launches the fish in the caudal direction. During a tail-flip, the fish first curls its head over its tail and then rapidly extends its tail contralaterally into the substrate to launch itself into the air. Tail-flip jumps have been studied extensively within cyprinodontiformes (such as killifishes) but have not yet been studied in mudskippers. The current resources available on the tail-flip jump produced by mudskippers are limited to a supplementary video published by Gibb et al. (2013)

in which a mudskipper is observed producing a tail-flip jump as an escape response. One notable difference between the tail-flip jump produced by the mudskipper in this video and those of killifish is that mudskippers start the jump from an upright position while killifish start tail-flip jumps on their side. Otherwise, the jump appears similar to those produced by other tail-flip jumpers.

Prone Jumping

Lastly, mudskippers produce a jumping motion called “prone jumping” (also sometimes referred to as “skipping”) (Figure 3), so called because the fish remains in ventral contact with the ground while pushing off the ground with its tail. This jumping movement is driven mainly by the tail and can be quite powerful, sometimes launching the fish several body lengths in the cranial direction (Harris, 1960; Swanson & Gibb, 2004). Multiple prone jumps can be strung together in rapid succession of one another, allowing the mudskipper to cover a considerable distance in a very short period (Van Dijk, 1960). This jumping motion is used in a variety of circumstances such as terrestrial prey-capture, escape responses, territorial behaviours, courtship behaviours and general traversal of the terrestrial environment (Clayton & Townsend, 2017; De & Nandi, 1984; Sponder & Lauder, 1981; Swanson & Gibb, 2004; Takiyama et al., 2016; Van Dijk, 1960).

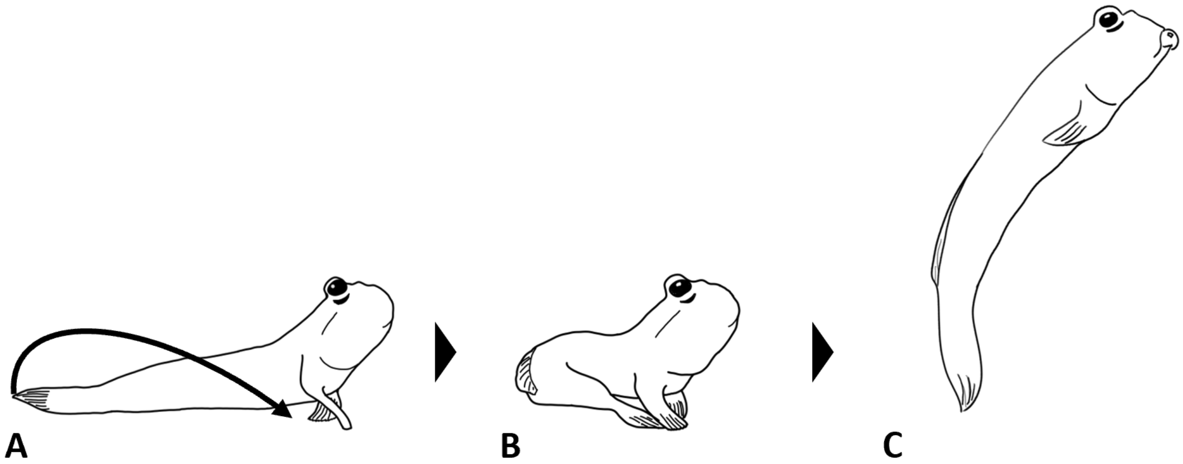

Figure 3 | Illustrations of a mudskipper producing a prone jump. (**A**) The preparatory phase of the prone jump consists of anterior coiling of the tail and then planting of the caudal fin, pectoral fins and pelvic fins on the ground. (**B**) The mudskipper at the end of the preparatory phase and before the propulsive phase. The propulsive phase starts when the mudskipper rapidly extends its tail against the ground to propel itself forward and into the air. (**C**) The aerial phase of a prone jump consists of the mudskipper in the air.

The only study to quantify the kinematics of prone jumps was conducted by Swanson and Gibb (2004) via the analysis of high-speed imagery of prone jumps produced as escape responses. Although there are other descriptive accounts of the kinematics of prone jumps, these accounts are somewhat conflicting and do not use high-speed imagery while describing the very quick prone jumps. Thus, the following description of prone jumps kinematics is derived largely from the work of Swanson and Gibb (2004).

Swanson and Gibb (2004) split the prone jump into three phases: a preparatory phase, a propulsive phase, and an aerial phase. During the preparatory phase, the fish bends its tail anteriorly and then plants its tail on the substrate, the paired fins (pectoral and pelvic fins) support the anterior/head region of the body during this coiling motion (Swanson & Gibb, 2004). Maximum tail bending occurs approximately two-thirds down the body towards the posterior end (Swanson & Gibb, 2004). During the propulsive phase the tail rapidly uncoils, causing the mudskipper to launch in the cranial direction. The head of the mudskipper also raises slightly at the start of the propulsive phase (Swanson & Gibb, 2004). Finally, during the aerial phase of the prone jump, the fish is no longer in contact with the ground.

It was suggested that mudskippers can anticipate their prone jump landing site (Gibb, Ashley-Ross and Hsieh, 2013). This is an accurate presumption as prone jumps are employed during prey-capture events that require the anticipation of the trajectory and landing site. Thus, mudskippers must have some ability to control the trajectory of their prone jumps via changing prone jump kinematics. There is also evidence that indicates prone jumps produced for terrestrial escape responses are different than the prone jumps produced for terrestrial prey-capture. Specifically, *Periopthalmus modestus* have been found to raise their heads before the propulsive phase of prey-capture prone jumps to target their prey, while the mudskippers *Periopthalmus argentilineatus* have not been found to raise their heads before the propulsive phase of terrestrial escape response prone jumps (Swanson & Gibb, 2004; Takiyama et al., 2016).

Targeting prey is likely an important step in producing terrestrial prey-capture prone jumps. There are likely other kinematic differences between the two behaviours. For example, terrestrial prey-capture, especially of moving prey, requires the ability to precisely control trajectory and intercept their prey whereas a terrestrial escape response would benefit from maximal jumping distance, speed, and randomness to avoid predation.

The primary objective of this research is to quantify how, during prey-capture behaviours, mudskippers alter the kinematics of their prone jumping movement to produce different jump trajectories. To study the coordination of prey-capture prone jumping I will first describe and define the three phases of a prey-capture prone jump. This is important because prone jumps performed during prey-capture are not necessarily the same as those described in the literature that were produced as escape responses. In each phase, I will calculate kinematic variables that allow us to test hypotheses defined in the second chapter about how changing body and fin position/movement influence jump trajectory. Prey-capture prone jumps will be elicited from fish using food incentives and high-speed video will be used to capture the three-dimensional movement of each animal.

Chapter 2: The coordination of a prey-capture prone jump

Introduction

Amphibiousness in fish has evolved independently numerous times across the actinopterygian taxa (Wright & Turko, 2016). These fish emerse for a variety of biotic and abiotic reasons and often possess many terrestrial adaptations, including the ability to move effectively on land (Sayer, 2005; Sayer & Davenport, 1991). The ability to locomote on land is essential to the survival of many of these species as they must accomplish terrestrial tasks such as traversing obstacles, foraging, escaping predators, courtship and effectively returning themselves to the water. To locomote on land amphibious fish use their existing aquatic morphology (e.g. fins, axial skeleton and associated musculature) and sometimes specialized morphology (e.g. modified pectoral fins of the mudskipper) to produce the ground reaction forces and torque necessary to propel themselves in a desired direction. The modes of locomotion vary considerably between amphibious fish species and have varied effectiveness and usages. One group of amphibious fish that are highly effective at producing terrestrial locomotion is the mudskippers (Gobiidae: Oxudercinae).

Mudskippers are a group of amphibious fish that inhabit the mudflats and brackish coastal regions of the Indo-Pacific and the west coast of Africa (Parenti & Jaafar, 2017). The adaptive pressures of living on land have made mudskippers experts in terrestrial locomotor behaviours, demonstrating at least four modes of terrestrial locomotion: crutching, climbing, tail-flip jumping and prone jumping (De & Nandi, 1984; Gibb et al., 2013; Harris, 1960; Stebbins & Kalk, 1961; Swanson & Gibb, 2004; Van Dijk, 1960; Wicaksono et al., 2016).

Crutching behaviour is an appendage-driven movement where the mudskipper synchronously uses both pectoral fins to lift and propel its CoM forwards, similar to how humans use crutches (Pace & Gibb, 2009). The climbing gait has been described similarly to that of crutching, in that it is driven by the synchronous movement of the pectoral fins, but it is different because the mudskipper must also adhere itself to the inclined substrate both during and between strides to resist slipping (Hidayat et al., 2022; Wicaksono et al., 2016).

Unlike the crutching and climbing behaviours that are driven by the appendicular skeleton, both jumping movements are primarily driven by the axial skeleton. During a tail-flip jump, the head is curled towards the tail and then the tail is rapidly extended into the ground, launching the fish into the air in the direction of the tail (Gibb et al., 2013). During a prone jump, the tail is curled towards the head and the fish is launched in the cranial direction by the tail pushing of off the ground.

The prone jump consists of 3 phases: a preparatory phase, a propulsive phase, and an aerial phase (Swanson & Gibb, 2004). The preparatory phase involves the fish's movements before propelling itself towards the food, this includes axial and appendicular movements such as coiling of the tail and placement of the paired fins (pelvic and pectoral fins) (Harris, 1960; Swanson & Gibb, 2004; Takiyama et al., 2016; Van Dijk, 1960). During prey-capture prone jumps, mudskippers also orient their heads toward their prey before jumping (Takiyama et al., 2016). At the end of the preparatory phase, the mudskipper is curled in a c-shape body conformation with its tail, pectoral fins and pelvic fins planted on the ground. The propulsive phase starts when the mudskipper rapidly unfolds its body, which pushes the tail against the ground and launches the fish in the direction it is facing. The fish then enters the aerial phase which is aptly named because the mudskipper is in the air.

It was suggested that mudskippers can anticipate their prone jump landing site (Gibb, Ashley-Ross and Hsieh, 2013). This was likely an accurate suggestion since mudskippers employ prone jumps during certain behaviours (e.g. terrestrial prey-capture and the general traversal of the terrestrial environment) that require some degree of anticipation of the landing site or trajectory (Clayton & Townsend, 2017; De & Nandi, 1984; Sponder & Lauder, 1981; Swanson & Gibb, 2004; Takiyama et al., 2016; Van Dijk, 1960). For example, observations of terrestrial prey-capture events indicate that the mudskippers adjust the inclination of their jump trajectory to reach the prey.

Although the general kinematics of prone jumps in the context of escape response (ie. leaving an area) have been quantified and described by Swanson and Gibb (2004), how mudskippers adjust the kinematics of their prone jumps to produce different jump trajectories towards a target is not yet explored. There is evidence that escape response jumps and prey-capture jumps are different. For instance, mudskippers orient their heads to look at their target before jumping during prey-capture (Takiyama et al. 2016), a behaviour not reported for jumps elicited as escape responses (Swanson and Gibb 2004). Following the work of Swanson and Gibb (2004), this thesis will first define the prey-capture prone jump in three phases. This will add detail to the 3 phases originally described by Swanson and Gibb (2004) and define prone jumps in the context of a prey-capture stimulus. The position and movement of the fish will be quantified during the 3 phases to assess what characteristics of a prone jump are responsible for the ultimate prey-capture performance. The kinematic analysis will focus on the final body position of the fish during the preparatory phase, the maximum velocity and average acceleration of the fish during the propulsive phase and course correction during the aerial phase. The hypotheses present in this thesis are discussed below and summarized in Table 1.

The first hypothesis is that the fish adjust tail position relative to food position. I predict that steeper food angles will result in the fish planting their tail closer to their center of mass (CoM) since this will direct ground reaction forces (GRFs) underneath their CoM. I also predict fish will coil their tail to the side of their body opposite the food. In this way, as the tail uncoils against the ground it will push the fish up and towards the food position.

The second hypothesis is that mudskippers change the position of their pectoral fins relative to their tail coiling side to resist slipping and rolling. I predict that the pectoral fin opposite the tail coiling side (contralateral to the tail) will be planted farther from the CoM compared to the fin on the same side (ipsilateral to the tail). In addition to potentially stabilizing the fish, the pectoral fins can lift the CoM (e.g. during crutching). Based on this knowledge, the third hypothesis is that fish alter the height of their CoM relative to the food position and by doing so change the direction of ground reaction forces acting on the CoM. My prediction is that steeper food angles will result in an increased CoM height.

The fourth hypothesis is that fish orient their heads in the direction of the target, with a prediction that head pitch will increase with the vertical food angle. This prediction is supported by the finding that the mudskipper *Periopthalmus modestus* positions targets within a region of greater visual acuity in the eye before prey-capture prone jumps (Takiyama et al., 2016).

The fifth hypothesis is that the tail position and CoM position effect trajectory direction. I predict that the initial vertical trajectory angle has a negative relationship with tail to CoM distance and a positive linear relationship with CoM height. The initial lateral trajectory angle will also be directed towards the left for right-planted tails and towards the right for left-planted tails.

On a gross scale, the maximum speed and the average acceleration to maximum speed are two performance metrics that quantify the fishes' movement and force production during the propulsive phase. The sixth hypothesis is that the mudskippers adjust their jump speed and acceleration relative to the target position. I first predict that as food distance at the end of the preparatory phase increases, so will the maximum speed of the fish. To reach these higher maximum speeds the fish must accelerate faster by increasing force production and/or the time spent accelerating. My second prediction is that fish jumping for food that is positioned further away will have a higher average acceleration.

Although most control of jump performance is assumed to occur in the propulsive phase, the aerial phase may also contribute to jump trajectory. Prone jumping mudskippers can be observed changing their posture midflight to position their mouth closer to the food. The seventh hypothesis is that mudskippers do this to course correct bad trajectories in which the original ballistic trajectory of the mouth would not have intersected the food. I predict that the predicted jump trajectories will not be close enough to the food for food capture to occur. I also predict that jump trajectories calculated using the initial position and velocity at the start of the aerial phase will differ from the actual trajectories realized by the fish. As a result, predicted jump trajectories will be less accurate (be further from the food) than the actual trajectory of the fish.

By using high-speed video of barred mudskippers jumping for food items I will test these seven hypotheses.

Table 1 | Summary of hypotheses and predictions.

Phase	Hypotheses	Predictions
Preparatory	H_1: Fish adjust tail position relative to food position.	P_{1a}: Tail to CoM distance decreases as vertical food angle increases. P_{1b}: The tail is planted contralateral to the food.
	H_2: The pectoral fins resist unwanted lateral movement and rolling.	P_2: The contralateral pectoral fin is planted further away from the CoM than the ipsilateral fin.
	H_3: The fish raise or lower their CoM relative to food position.	P_3: CoM height increases with vertical food angle.
	H_4: The fish orient their heads towards the food.	P_4: Head pitch increases with vertical food angle.
	H_5: Tail position and CoM position effect trajectory direction.	P_{5a}: Initial vertical trajectory angle has a negative linear relationship with Tail to CoM distance and a positive linear relationship with CoM height. P_{5b}: The initial lateral trajectory angle will be directed towards the left for right planted tails and towards the right for left planted tails.
Propulsive	H_6: The fish adjust speed and acceleration relative to food position.	P_{6a}: Max speed increases with end of preparatory phase food distance. P_{6b}: Average acceleration to max speed increases with end of preparatory phase food distance.
Aerial	H_7: The fish course correct in the air.	P_{7a}: Predicted jump trajectories will not be close enough to the from for food capture to occur. P_{7b}: Predicted jump trajectories will be further from the food then the real jump trajectories.

Methods

Animals

Barred mudskippers (*Periophthalmus argentilineatus* or *P.arg*) were obtained from *Below Water*, Montreal, QC, Canada. The mudskippers were housed in a saltwater recirculating tank system (salinity = 15-25ppt, 28-29℃, 1-3 fish/tank, fed once daily either blood worms or shrimp; lights on from 7am-7pm). Each tank was fitted with a lid to stop the fish from climbing out, and gravel islands that allowed the fish to exit the water when desired. Five fish were used in this experiment, the sex was not able to be determined. Four of the five fish (Fish ID = 1,2,3 and 4) were initially group housed (3 fish per tank) but were isolated into their own tanks following filming to ensure that filmed fish were not mixed. The fifth fish (Fish ID = 5) was housed with another mudskipper following filming that was not included in the jumping trials. All work with the mudskippers was done under the approval of the University of Ottawa animal care protocol BL3257.

Filming

The fish were filmed in a clear acrylic tank with etched 0.5 cm grid marks on the bottom surface (Figure 4). The etched grid marks increased the friction of the otherwise smooth acrylic ground and served as ground reference marks during the calibration of the cameras. At one end of the tank just outside of the camera's field of view was a trough of water with a heater set to 28C and a gravel ramp. The gravel ramp was provided to help the fish leave the water, but the fish did not have trouble leaving the water without the ramp. A lid was placed on the tank whenever the fish were not supervised to prevent the fish from climbing out of the tank. Fishes lived in the filming tank for the duration of the filming period.

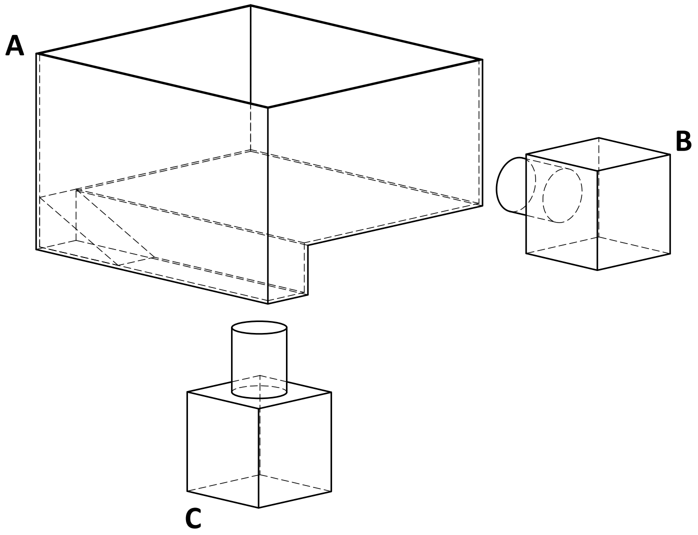

Figure 4 | Mudskipper filming setup illustration. **A.** Clear acrylic tank with water trough/ramp. **B.** Lateral high-speed video camera. **C.** Ventral high-speed video camera. Cameras: Photron Fastcam Mini; Lenses: Sigma 24mm F1.4. Etched 0.5cm grid marks are not shown on the bottom surface of the tank illustration. Drawing not to exact scale.

Two high-speed videos (Cameras: Photron Fastcam Mini; Lenses: Sigma 24mm F1.4) of the prone jumps were recorded from a lateral view and a bottom view of the filming tank (Figure 4). The videos were initially taken at 500fps, however, following initial analysis the frame rate was increased to 1000fps to reduce motion blur. The area seen by both cameras was the “capture volume”, prone jumps within the capture volume could be tracked in 3D space and analyzed. Camera calibration was done via wand calibration (wand length tip to tip equalled 7.7cm) and the wand calibration tool easyWand (version 5.7.7, Theriault et al., 2014). Approximately 50-100 wand tip points from both sides of the wand were used for all the calibrations. Three points forming a right angle and lying on the plane of the ground were used to define the origin and direction of the x,y and z axis. This allowed us to easily make the x and y axis coplanar with the ground of the filming tank and the positive z axis perpendicular to the ground plane. The videos were digitized in DLTdv8 (version 8.2.5, Hedrick, 2008), a MATLAB app used for positional tracking in 2D/3D space of multi-camera setups.

Several loci were tracked throughout each video, these were the: food, mouth (pre-maxilla), pectoral fin tips, pelvic girdle, vent and the tail /caudal fin tip (Figure 5). The 3D (x,y,z) and 2D (x,y) coordinates of these loci were used to calculate the kinematic variables (Table 3). These loci were selected due to their visibility in both the lateral and ventral views throughout the video. The vent locus did not end up being used in the analysis.

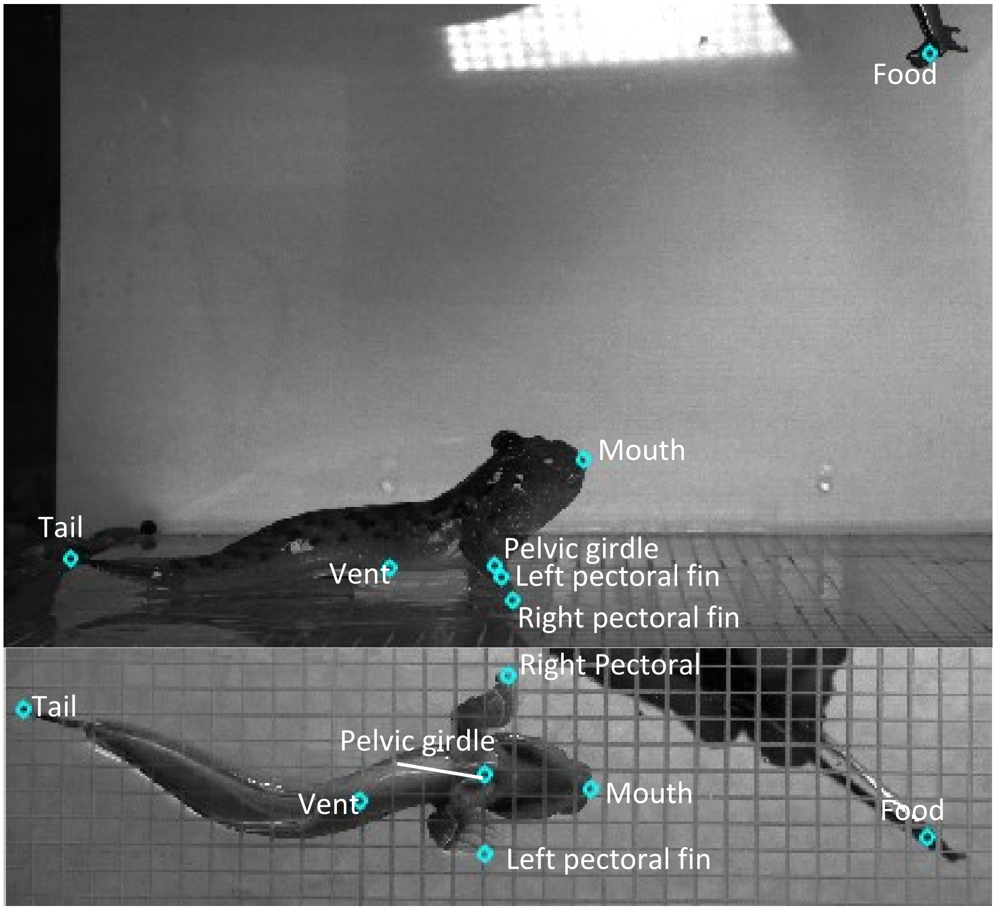

Figure 5 | Digitized loci from the lateral view (top) and ventral view (bottom).

Eliciting behaviour

Prone jumps were elicited by suspending thawed bloodworms with tweezers in front of the mudskippers or by placing live crickets in the tank. The bloodworms were held in front of the fish until the fish would move to grab the food from the tweezers with its mouth. The food was suspended at varied heights and distances from the fish to promote a range of prone jump trajectories (Figure 6). The food would be reintroduced to the fish several times or gradually brought closer to the fish until the fish showed interest in the food (pitching the head and turning toward the food, approaching the food, and jumping) and performed a prey-capture prone jump. I attempted to elicit prone jumps for the widest range of prey positions relative to the fish as this would increase the variation in jump kinematics. Live crickets were placed in the tank in an attempt to elicit prey-capture prone jumps from fish that did not show interest in blood worms. The crickets were either placed in view of the camera on the floor of the tank or on a stick that was suspended at varied heights. The stick had two end caps made of smooth plastic to prevent the cricket from climbing off the stick. Note that no data from cricket-elicited jumps is used in this thesis.

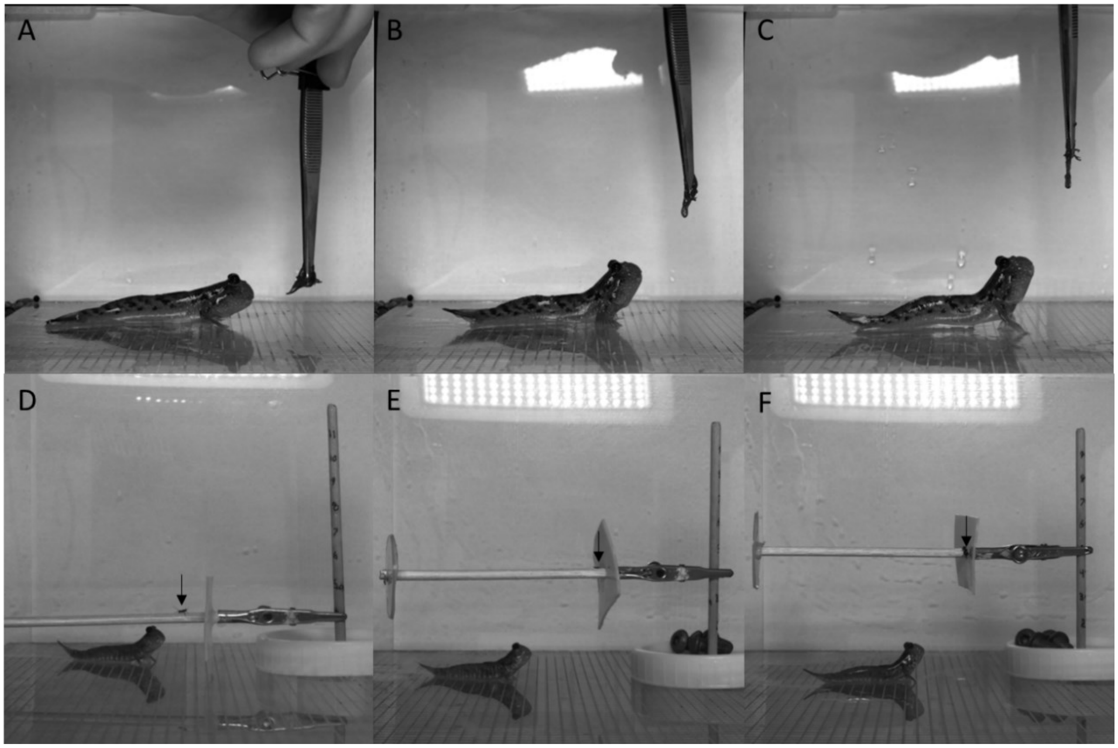

Figure 6 | Food being suspended at different positions relative to the mudskipper. The food was used to elicit prey-capture prone jumps. (**A-C)** Bloodworms were held with tweezers at different heights and distances relative to the fish to promote varied jump trajectory angles. Photos were captured from highspeed footage just before the start of the preparatory phase.

(**D-F**) Live crickets were placed on a stick that was clamped at different heights on a stick (D= 2cm, E = 4cm, F=5cm). The crickets are indicated with black arrows. Photos were captured just before the start of the preparatory phase.

To decrease stress from handling, the mudskippers were allowed to acclimate in the filming tank at least 1 night before filming and were kept in the filming tank for several days until the fish had completed its prey-capture behaviour trials for a variety of prey positions or until the fish showed no signs of becoming interested in the suspended food. Fish lengths and weights were recorded on the last day of multiple-day filming sessions to avoid stressing the fish (Table 2). Length was recorded by placing a ruler next to live fish while they remained approximately straight and stationary; length was measured from the tip of the caudal fin to the tip of the mouth.

Table 2 | Total trials filmed and analyzed for each fish and their recorded length.

Fish ID	**Trials analysed (worms only)**	**Trials recorded Worms**	**Trials recorded Crickets**	**Weight (g)**	**Length (cm)**
1	9	19	0	1.36,1.16 [a]	5.2
2	10	17	0	1.14,1.09 [a]	5,5.2[a]
3	18	19	0	0.97	5.2
4	12	33	0	5.48	8.4
5	11	19	0	1.1	4.95
Total	60	107	0		

[a] Fish 1 and 2 had length measurements for two separate filming periods.

Variables:

Trials were only analyzed if the fish successfully captured the food and did not slip during the propulsive phase of the jump (Table 2). For each fish, I selected trials with the widest range of prey positions (Figure 7). The 3D coordinate data was smoothed using a gaussian-weighted moving average filter with a 5-frame window to reduce digitizing error. The kinematic variables were calculated with the coordinates of digitized loci (Tables 3 and 4). Many of the observed jumps did not include an aerial phase (non-aerial jumps) since the fish did not lose contact with the ground. These jumps appeared kinematically identical to aerial jumps so they were analyzed together, except for the analysis of course correction that focused solely on jumps with aerial phases. Body position variables and food position variables were measured at the end of the preparatory phase/start of the propulsive phase for aerial and non-aerial jumps. The end of the preparatory phase/start of the propulsive phase is the first frame where the tail can be seen visibly pushing into the ground (splaying of the caudal fin rays against the ground). The initial trajectory of the jump was taken as the motion of the CoM (pelvic girdle) during the first 30ms of the propulsive phase. The instantaneous and maximum velocity of the CoM (pelvic girdle) of the fish was calculated over the entire propulsive phase.

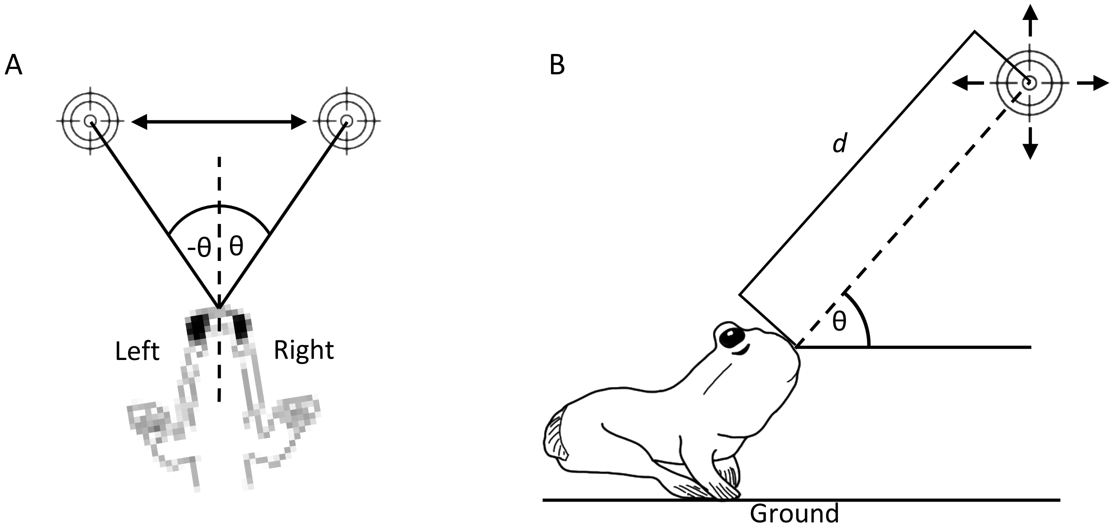

Figure 7 | Illustration of how food position variables change relative to the fish. (**A**) Lateral food angle measured at the end of the preparatory phase. Lateral food angle is the acute angle between the mid-sagittal plane of the fish's head and a line connecting the food to the mouth. This angle lies within the plane of the ground and can be measured as a negative (left) or positive (right) angle with an angle of 0 being in line with the mid-sagittal plane of the fish's head. (**B**) Food distance and vertical food angle measured at the end of the preparatory phase. Distance (*d*) is the distance between the mouth of the fish and the food. Vertical food angle is the acute angle between the plane of the ground and the line intersecting the fish's mouth and the food. The target ⌖ represents the food suspended in the air.

Table 3 | Variable specification for food position, body positioning, trajectory, velocity and acceleration variables. All the anatomical reference points specified below refer to the coordinates of the loci illustrated in Figure 5.

	Variable	Units	Specification
Food position	Food distance	Body lengths	Distance between the fish mouth and the food (Figure 7B). Measured during the last frame of the preparatory phase.
	Vertical food angle	Degrees	Acute angle between the plane of the ground and the line intersecting the mouth and the food[a] (Figure 7B). Measured during the last frame of the preparatory phase.
	Lateral food angle	Degrees	Acute angle between the mid-sagittal plane of the fish and a line intersecting the mouth and the food. (Figure 7A). Measured during the last frame of the preparatory phase.
Body position	Head pitch angle	Degrees	Acute angle between the line intersecting the mouth and pelvic girdle and the line intersecting the pelvic girdle and a point that lies underneath the mouth at the height of the pelvic girdle. Measured during the last frame of the preparatory phase.
	Tail-pelvic distance	Body lengths	Distance between tail tip and the pelvic girdle in the ground plane [a]. Measured during the last frame of the preparatory phase.
	Tail plant side	Left or right	The tail is planted on the left or right side of the fish during the preparatory phase. The tail plant side refers to the final tail placement side prior to the propulsive phase. Measured during the last frame of the preparatory phase.
	CoM (Pelvic girdle) height	Body lengths	The perpendicular distance between the pelvic girdle and the ground in the z direction. Measured during the last frame of the preparatory phase.
	Ipsilateral and contralateral fin distance	Body lengths	Distance between the pectoral fin on the same side of the coiled tail (ipsilateral) and the pelvic girdle and the distance between the pectoral fin on the opposite side of the planted tail (contralateral) to the pelvic girdle. Measured in the ground plane [a]. Measured during the last frame of the preparatory phase.
Initial trajectory	Initial 30ms vertical trajectory angle	Degrees	Acute angle between the line formed by the pelvic girdle trajectory (pelvic girdle point at the start of the propulsive phase and 30ms later) and the plane of the ground.
	Initial 30ms horizontal trajectory angle	Degrees	Acute angle between the mid-sagittal plane of the fish (line between mouth and pelvic girdle in the ground plane) at the beginning of the propulsive period and the initial pelvic girdle trajectory (line between pelvic girdle point at the start of the propulsive phase and 30ms later). [a]
Velocity and acceleration	Instantaneous velocity	m/s	The frame by frame velocity of the pelvic girdle during the propulsive phase.
	Max velocity	m/s	Max instantaneous velocity of the fish during the propulsive phase.
	Average acceleration	m/s^2	Average acceleration from when the fish starts moving towards the food during the propulsive phase until the fish reaches max velocity.

[a] The ground plane is coplanar with the XY plane, and the Z axis extends upwards from the ground plane.

The center of mass position was determined by the “suspension method” to be 0.33 body lengths posterior to the tip of the mouth or 0.125 body lengths posterior to the pelvic fin loci of the fish (Figure 8). The suspension method involves suspending a preserved fish from a string threaded through a point on the dorsal surface of the fish within the sagittal plane (Macaulay et al., 2017). When suspended from a string, the CoM will fall directly beneath the string assuming there are no additional forces other than gravity and the tension of the string acting on the fish. Resuspending the fish from another point on the dorsal surface within the sagittal plane produces a second image where the CoM is in line with the string. By overlaying the two images such that the fish body is perfectly aligned, the trajectory of the two hanging strings will cross and mark the approximate location of the fish’s CoM. The centre of mass is located just dorso-posteriorly of the pelvic girdle locus (Figure 8). It is important to note that the true center of mass changes as the fish moves its axial and appendicular skeleton so CoM calculations are always approximations. The pelvic girdle locus of the fish was used to approximate the position of the center of mass, due to its proximal location to the center of mass and its visibility in the video images.

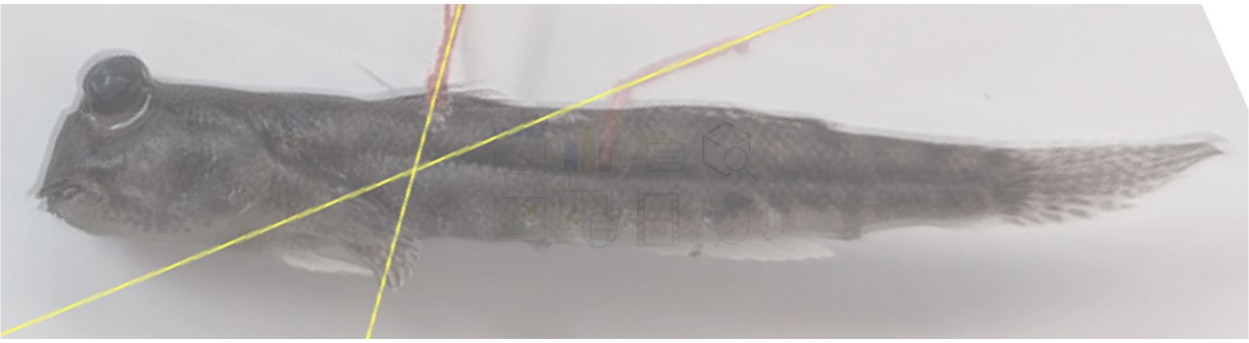

Figure 8 | Suspension method for determining center of mass. A photo was taken of the fish suspended from a string in one position then the fish string was attached to another part of the fish for a second photo. The string was threaded through a section of skin on the dorsal side of the fish in the sagittal plane. The fish in both photos were rotated and positioned such that the fish overlayed each other. The yellow lines were added after the fact to extrapolate the line of the string into the body of the fish. Assuming the fish weighs the same either side of the sagittal plane then the CoM lies within the sagittal plane of the fish where the two lines intersect. The center of mass position is located posterior to the pectoral fin, within the sagittal plane and approximately midway dorsoventrally.

Predicting aerial trajectory:

The initial position and velocity of the fish were used to calculate and plot the predicted trajectory of the fish during the aerial phase for trials with aerial phases (Figure 9). During aerial phases, the fish were no longer in contact with the ground. Fish were assumed to be point masses concentrated at their pelvic girdle and air resistance was assumed to be negligible. The functions describing the instantaneous displacement (x_t, y_t, z_t) of the fish throughout its predicted trajectory are:

$$x_t = x_0 + v_{x0}*t$$

$$y_t = y_0 + v_{y0}*t$$

$$z_t = z_0 + v_{z0}*t + 0.5g*t^2.$$

Where $g = -9.81\text{m/s}^2$, (x_0, y_0, z_0) are the initial 3D coordinates of the pelvic girdle and v_{x0}, v_{y0} and v_{z0} are the initial velocity of the fish in the x,y and z directions respectively. The initial velocity of the fish was measured during the last 6ms of the propulsive phase. The predicted trajectory started at the beginning of the aerial phase (t_0) and ended when the predicted trajectory intercepted the ground at the end of the jump. The beginning of the aerial phase was the first frame where the tail no longer appeared to be contacting the ground.

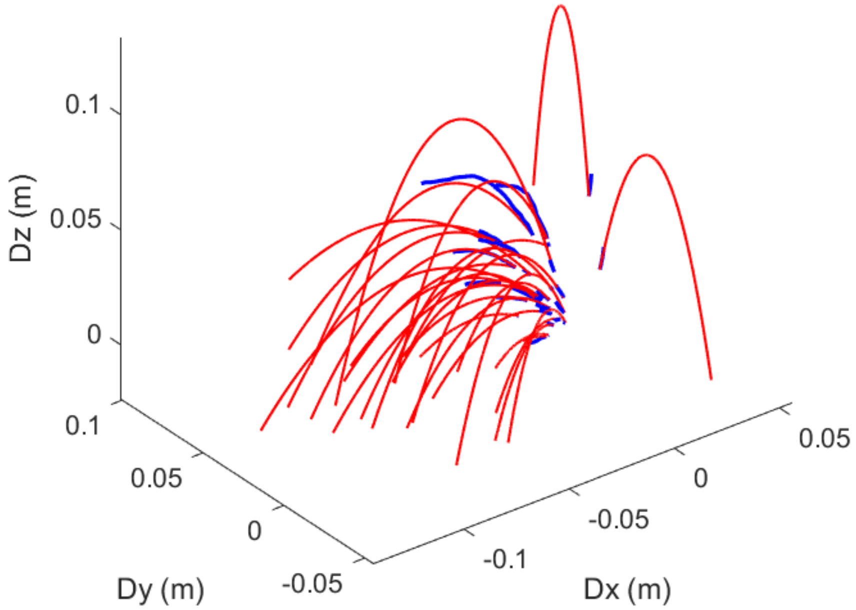

Figure 9 | The real trajectory (blue) and predicted trajectory (red) of the aerial phase. The predicted trajectory stops when it intersects with the ground and is displayed for all trials with aerial phases. n = 29

My goal was to determine if fish corrected their jump trajectory once they were airborne. To do this I measured the distance of the pelvic loci to the food at the point of food capture (fish accuracy) as well as the duration of the jump from the start of the aerial phase to food capture. Then, based on the initial trajectory, velocity and duration of the real jumps I calculated the predicted trajectory and found the minimal distance between the predicted pelvic loci and the food after the same duration of jump (predicted accuracy). The predicted accuracy was measured twice, once relative to the initial position of the food (predicted accuracy to initial food position) and a second time relative to the final position of the food (predicted accuracy to final food position).

The predicted accuracy to final food position was compared with fish accuracy. If fish accuracy was smaller than predicted accuracy to final food position, I could assume real fish are altering their trajectory mid-flight to minimize their distance to the food.

The predicted accuracy of the jump based on its initial trajectory and velocity (predicted accuracy to initial food position) was compared with the distance between the fish's mouth and its pelvic girdle (mouth to pelvic girdle distance). If the predicted accuracy to initial food position was larger than mouth to pelvic girdle distance, then I could assume the predicted trajectory was not directed at the food since the fish would not have been able to catch the food from this distance.

Finally, aerial food distance, was measured at the start of the aerial phase from the pelvic girdle of the fish to the food. Aerial food distance was used to illustrate how far each fish travels during the aerial phase. The variables are specified in more detail in Table 4.

Table 4 | Variable specification related to the real and predicted trajectory of the fish during the aerial phase.

	Variable	Specification
Predicted aerial trajectory	Aerial food distance	Distance between the pelvic girdle and the food at the start of the aerial phase. Measured at the first frame where the fish was no longer in contact with the ground.
	Fish accuracy	Distance between the pelvic girdle and the food at the point of capture for actual fish jumps.
	Predicted accuracy to initial food position	Minimum distance between the pelvic girdle predicted trajectory and the initial food position. For this variable, the predicted trajectory starts at the beginning of the aerial phase and ends when it has flown for the same duration as the fish did from takeoff to food capture.
	Predicted accuracy to final food position	Minimum distance between the pelvic girdle predicted trajectory and the final food position. For this variable, the predicted trajectory starts at the beginning of the aerial phase and ends when it has flown for the same duration as the fish did from takeoff to food capture.
	Mouth to pelvic distance	Distance between the mouth of the fish and its pelvic girdle.
	Minimum predicted distance	Minimum distance between the pelvic girdle predicted trajectory and the initial food position over the entire predicted trajectory. Measured when the predicted trajectory is closest to the food regardless of the real flight duration.

Statistical analysis:

Statistical analysis was carried out in *R* 4.3.1 (https://www.r-project.org/) via the *Rstudio* IDE (https://posit.co/, Version: 2023.06.1+524).

Linear models comparing kinematic variables, food position and trajectory

Linear mixed effects models (LMM) were fit to data comparing the linear relationship between predictor and response variables as these models allow us to account for repeated measures among fish by including fish as a random effect. The intercept of these linear models was allowed to vary based on fish ID. Before the fitting of linear mixed effects models, kinematic variables (e.g tail-pelvic girdle distance, head pitch, max velocity) were first plotted against food position variables (e.g. food distance, vertical food angle) or trajectory variables to assess linearity of the relationship between response and predictor variables. No predictor variables were sufficiently collinear that they warranted removal from the final LMMs (Table A4). Then, relevant linear mixed effects models were fit to the data using maximum likelihood method (ML) in the *r* package *lmerTest* (https://cran.r-project.org/web/packages/lmerTest/index.html) and compared via second-order Akaike information criteria scores (AICc) via the *AICcmodavg r* package (https://cran.r-project.org/web/packages/AICcmodavg/index.html) (Table 5). If models were within five AICc values they were considered very similar. Very similar models were compared using likelihood ratio tests to assess whether more complex models were significantly better. The model with the lowest AICc value was validated for model assumptions by visually assessing the variance of the residual error vs fitted values, and assessing the normality of the residuals. No major violations of the final model assumptions were found. It should also be noted that linear mixed modes are generally robust to violations of model assumptions (Schielzeth et al., 2020). The best fit model's parameter estimates were recalculated via REML as ML may underestimate the variance of the random effects. All fitted models, AICc values and models can be found in Table 5, model statistics can be found in Table A3. *Johnson-Neyman* plots were plotted via the *r* package *interactions* (https://cran.r-project.org/web/packages/interactions/readme/README.html) to visualize the significant range of slopes for linear mixed models with interactions. Conditional ($R^2_{LMM(m)}$) and marginal ($R^2_{LMM(c)}$) coefficient of determination values were obtained using the *r* package *performance* (https://cran.r-project.org/web/packages/performance/index.html). The marginal coefficient of determination corresponds to the proportion of variance in the response variable accounted for by variation of the fixed effects while the conditional coefficient of determination refers to the variation of the response variable accounted for by variation in the fixed and random effects (Nakagawa et al., 2017). I evaluate the significance of the linear mixed effects models with p-values obtained from the *lmerTest* package and confidence intervals obtained from *R*; since P-values alone are not reliable indicators of significance in LMMs (Luke, 2017).

Table 5 | Specification of random intercept linear mixed effects models. See appendix Table A3 and A4 for model statistics.

	Response variable [Hypotheses, Prediction]	**Best model**	**AICc[a]**	**Linear mixed effects models[b]**
a.	**Tail-pelvic girdle distance (*TPd*)** **[H_1:** Fish adjust tail position relative to food position.**, P_{1a}:** Tail to CoM distance decreases as vertical food angle increases.**]**		-225.8	_~ VFA + (1|FishID)_
			-200.6	_~ d + (1|FishID)_
			-229.8	_~ VFA + d + (1|FishID)_
		X	-243.5	_~VFA + d + VFA*d + (1|FishID)_
b.	**Distance between pectoral fin tip and pelvic girdle[c]** **[H_2:** The pectoral fins resist unwanted lateral movement and rolling., **P_2:** The contralateral pectoral fin is planted further away from the CoM then the ipsilateral fin.**]**			_~Fin side + (1|FishID) + (1|FishID:Trial)_[d]
c.	**CoM (Pelvic girdle) height (*Ph*)** **[H_3:** The fish raise or lower their CoM relative to food position., **P_3:** CoM height increases with vertical food angle.**]**		-402.1	_~ VFA + (1|FishID)_
			-394.3	_~ d + (1|FishID)_
		X	-410.7	_~ Hp + (1|FishID)_
			-409.3	_~ Hp + VFA + (1|FishID)_
			-409.8	_~ Hp + d + (1|FishID)_
			-403.0	_~ VFA + d + (1|FishID)_
			-408.2	_~ VFA + d + Hp + (1|FishID)_
			-401.1	_~VFA + d + VFA*d + (1|FishID)_
d.	**Head pitch (*Hp*)** **[H_4:** The fish orient their heads towards the food., **P_4:** Head pitch increases with vertical food angle.**]**		374.4	_~ VFA + (1|FishID)_
			465.7	_~ d + (1|FishID)_
		X	372.8	_~ VFA + d + (1|FishID)_
			373.9	_~VFA + d + VFA*d + (1|FishID)_
e.	**Initial vertical trajectory angle** **[H_5:** Tail position and CoM position effects the trajectory direction., **P_{5a}:** Initial vertical trajectory angle has a negative linear relationship with Tail to CoM distance and a positive linear relationship with CoM height.**]**		370.6	_~ TPd + (1|FishID)_
			364.4	_~ Ph + (1|FishID)_
		X	352.1	_~ TPd + Ph + (1|FishID)_
			353.8	_~TPd + Ph + TPd*Ph + (1|FishID)_
f.	**Initial lateral trajectory angle (*ILT*)[c]** **[H_5:** see above, **P_{5b}:** The mean initial lateral trajectory angle will be directed towards the left (negative *ILT*) for right planted tails and towards the right (positive *ILT*) for left planted tails.**]**			_~Tail plant side + (1 |FishID)_
g.	**Max speed** **[H_6:** The fish adjust speed and acceleration relative to food position., **P_{6a}:** Max speed increases with end of preparatory phase food distance.**]**		-16.8	_~ VFA + (1|FishID)_
			-15.2	_~ d + (1|FishID)_
			-43.1	_~ VFA + d + (1|FishID)_
		X	-46.2	_~VFA + d + VFA*d + (1|FishID)_
h.	**Max vertical velocity** **[**No hypothesis, No prediction**]**		-3.97	_~ VFA + (1|FishID)_
			32	_~ d + (1|FishID)_
			-28.9	_~ VFA + d + (1|FishID)_
		X	-40.0	_~VFA + d + VFA*d + (1|FishID)_
i.	**Max horizontal velocity** **[**No hypothesis, No prediction**]**		-51.4	_~ VFA + (1|FishID)_
			-51.2	_~ d + (1|FishID)_
			-52.3	_~ VFA + d + (1|FishID)_
		X	-58.2	_~VFA + d + VFA*d + (1|FishID)_
j.	**Average acceleration until max velocity**		372.5	_~ VFA + (1|FishID)_

	[H_6: see above, **P_{6b}:** Average acceleration to max speed increases with end of preparatory phase food distance.**]**		390.1	*~ d + (1|FishID)*
		X	365.5	*~ VFA + d + (1|FishID)*
			363.9	*~VFA + d + VFA*d + (1|FishID)*
k.	**Mouth to pelvic distance and predicted accuracy to initial food position**[c] **[H_7:** The fish course correct in the air., **P_7:** Predicted jump trajectories will not be close enough to the food for food capture to occur.**]**			*~ Category* [e] *+ (1 |FishID) + (1|FishID:Trial)*[d]
l.	**Distance between pelvic girdle and final food position (*dpf*)**[c] **[H_7:** The fish course correct in the air., **P_7:** Predicted jump trajectories will be further (higher mean *dpf*) from the food then the real jump trajectories.**]**			*~Trajectory + (1 |FishID) + (1|FishID:Trial)*[d]

[a] Models within 5 AICc values were compared via likelihood ratio tests.

[b] *VFA* = vertical food angle at the end of the preparatory phase, *d* = distance between the mouth and the food at the end of the preparatory phase, *TPd* = horizontal distance between the tail locus and pelvic girdle at the end of the preparatory phase, *Ph* = height of the pelvic girdle at the end of the preparatory phase, *Hp* = head pitch, Fin side = ipsilateral or contralateral, Tail plant side = left or right, Trajectory = predicted or real, *dpf* = distance between the pelvic girdle and the food for predicted trajectories to initial food position and real jump trajectories.

[c] Only one model was fit to data. No AiCc value is included.

[d] Trial is a nested random effect within *FishID*. This pairs fin distance measurements from the same trial.

[e] This model has a continuous response variable and a categorical fixed effect with two levels. The first level of the categorical fixed effect is Distance from mouth to pelvic girdle and the second category is distance from predicted jump trajectory to initial food position. The response variable is the distance associated with each level of the categorical fixed effect.

Mixed effect logistic regression model for comparing Coiling side and lateral food angle

A mixed effect logistic regression (MELR) model with a binomial distribution (left or right planted tail) was used to evaluate the effect of lateral food angle on tail coiling side. The intercept of the model was allowed to vary based on fish ID. The model was fit to the data using the function *glmer* from the *r* package *lme4* (https://cran.r-project.org/web/packages/lme4/index.html) and parameter estimates were fit by maximum likelihood. The model (ID = M_{H1e}) was specified as:

Tail plant side ~ *Lateral food angle* + (1|*FishID*) (Model ID: m)

[Table 5, **H_1:** Fish adjust tail position relative to food position**.**, **P_{1b}:** The tail is planted contralateral to the food (increasing lateral food angle increases the probability of left planted tails, decreasing lateral food angle increases the probability of a right planted tail).]

Model parameter estimates were fit via the maximum likelihood method (Table A3). Conditional ($R^2_{LMM(m)}$) and marginal ($R^2_{LMM(c)}$) coefficient of determination values were obtained using the *r* package *performance* (https://cran.r-project.org/web/packages/performance/index.html). Model fit was assessed using the *r* package *DHARMa* (https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html#binomial-data). The Model parameter estimates can be found in Table A3 of the appendix.

T-tests comparing food distance and vertical food angle between aerial and non-aerial jumps

Paired t-tests were used to compare the mean food distance between aerial and non-aerial jumps and the mean vertical food angle between aerial and non-aerial jumps. Food distance and vertical food angle (Figure 7, Table 3) were measured at the end of the preparatory phase. I calculated the mean food distance and vertical food angle for aerial and non-aerial jumps across all the trials from a given fish. Such that each fish would only have one distance and one vertical food angle measurement for aerial jumps and another value for non-aerial jumps. I then compared the mean value from all 5 fish between the aerial and non-aerial jumps in a paired t-test.

Results

Prey-capture prone jumps description

Preparatory phase:

During the preparatory phase (Figure 10A-B), fish pitched their head towards the food. “Pitching” in this case refers to the rotation upwards or downwards of the head within the sagittal plane. The pectoral fins were occasionally repositioned after initial head pitching while the pelvic fins always remained planted on the ground to support the head region of the fish. Repositioning of the pectoral fins while the head remained pitched towards the food indicated that the pectoral fins were not needed to maintain head pitch. Head pitching occasionally occurred while the fish was approaching the food (via crutching or prone jumps, before the prone jump preparatory phase). Head pitching was accompanied by anterior curling of the tail, planting of the pectoral fins and planting of the pelvic fins on the ground. The pectoral fins were always planted before the start of the propulsive phase and the splaying of the pectoral fin rays against the ground indicates that they exert some downwards force against the substrate (Figure 11). The fish would curl their tails anteriorly until the caudal fin was planted flat on the ground close to their pectoral girdle. During tail coiling, the caudal fin, and the tail region proximal to the caudal fin were lifted off the ground and the body was supported by the paired fins and mid-body. As the tail curled and planted, the mid-body of the fish would lift off the ground and rotate slightly over top of the tail. Occasionally the fish stopped tail curling or switched curling direction after the start of curling. The preparatory phase ended with the fish upright, facing the food, the tail coiled and planted against the ground, and the pectoral and pelvic fins planted against the ground (Figure 10B).

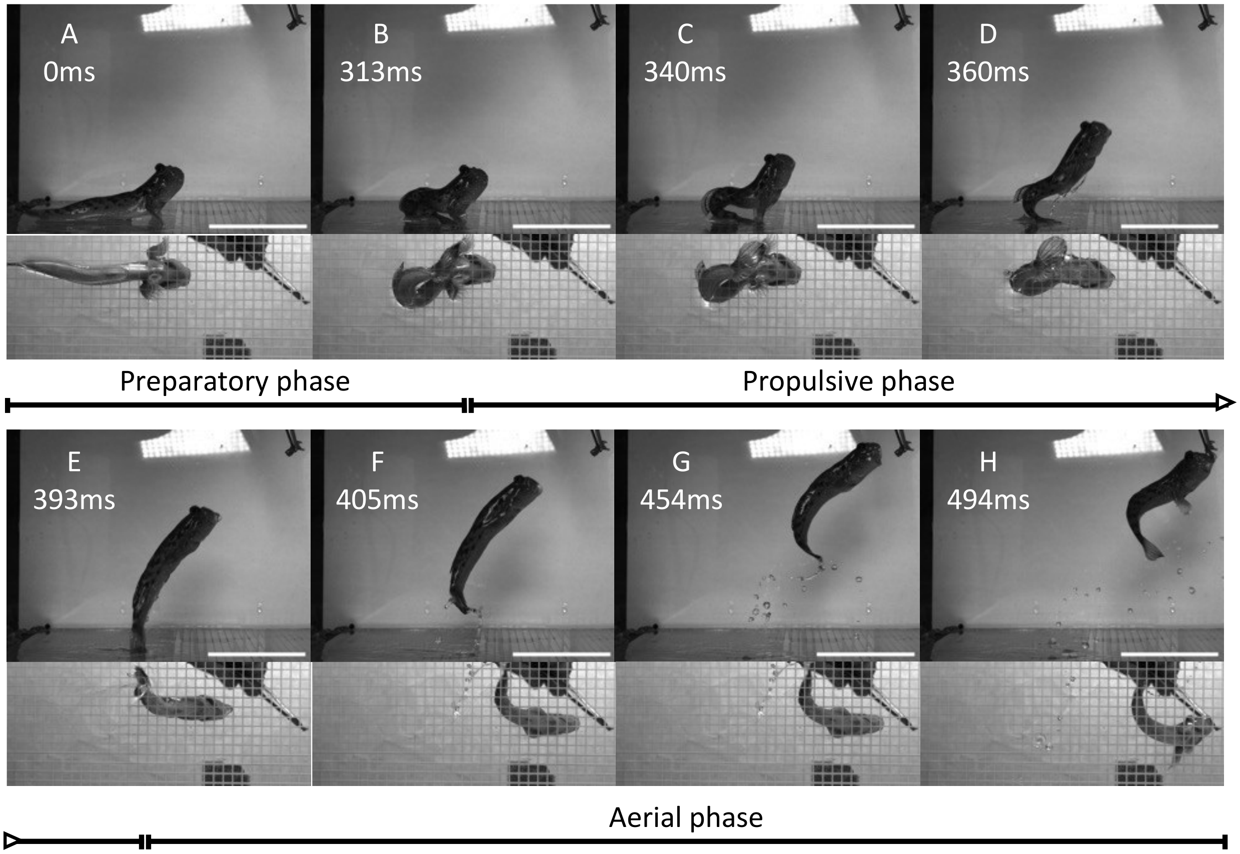

Figure 10 | A prey-capture prone jump sequence with 3 phases (preparatory, propulsive, aerial) from the lateral and ventral view. Images were chosen to demonstrate key moments during the jump. (**A**) The paired fins are planted before tail coiling and the head is oriented towards the food. (**B**) The tail and paired fins planted on the ground at the end of the preparatory phase. (**C**) The tail pushes off the ground and the anterior body begins to lift off the ground. (**D**) The anterior body is lifted off the ground, the tail continues to push off the ground and the paired fins adduct medially against the body. (**E**) The last moment of the tail contacting the ground and the end of the propulsive phase/start of the aerial phase. (**F**) The fish is no longer in contact with the ground and the paired fins remain adducted against the body wall. (**G**) The fish opens its mouth to catch the food and coils its tail. (**H**) The fish captures the food in its mouth and

abducts the pectoral fins. The time starts when the pectoral fins were planted on the ground prior to tail coiling. White scale bar represents 5cm.

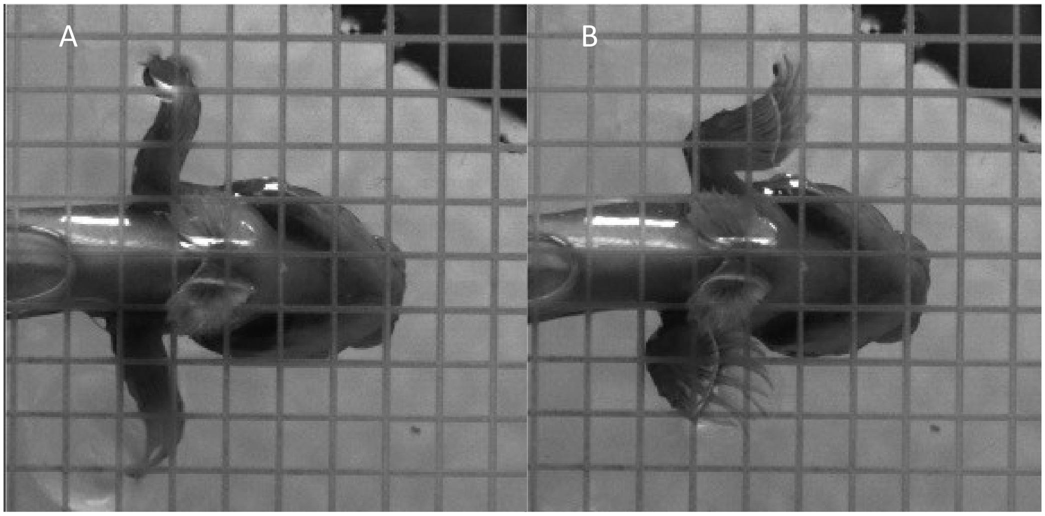

Figure 11 | Ventral view of the pectorals fins before (**A**) and after (**B**) planting on the ground.

Propulsive phase:

The propulsive phase started shortly after the caudal fin was planted on the ground and continued until the fish had stopped pushing off the ground (upon food capture or loss of ground contact; Figure 10B-E). The start of the propulsive phase was followed by rapid extension/straightening of the tail against the ground and adduction of the pectoral and pelvic fins. It appears as though the pectoral fins propel the anterior body upwards off the ground during the initial moments of the propulsive phase. The pelvic fins immediately adduct against the body of the fish and do not appear to propel the fish. As the tail begins to extend (Figure 10B), the pelvic fins were the first part of the fish to leave the ground (Figure 10C), followed shortly by the pectoral fins (Figure 10D) and then the caudal fin (Figure 10E; assuming the fish launched into the air). During jumps without aerial phases, the fish would remain in contact with the ground with their caudal fin or posterior body region. In addition, they abducted their pectoral fins upon food capture.

Aerial phase:

The aerial phase starts when the caudal fin loses contact with the ground (Figure 10E). Occasionally there were movements of the axial and appendicular skeleton during the aerial phase of the prone jump before food capture (Figure 10E-H). Axial movements included laterally, ventrally, and dorsally directed axial bending, resulting in varying degrees of ‘C’ or ‘S’ shape body conformations before the fish had reached the food. These movements appeared to be an attempt to course correct for jump trajectories that would not reach the food and were relatively rare. The paired fins would lay flat against the body wall during the start of the aerial phase (Figure 10E) and then abduct during food capture (Figure 10H). Upon food capture the fish would close its mouth around the food.

Control of Jump Direction

The relationship between body position and food position

Many prey-capture prone jumps did not include an aerial phase (the fish did not leave the ground; Figure 12, Table 6). The mean distance to the food was significantly shorter for non-aerial prone jumps than it was for aerial prone jumps (Table 6; T-test: t = -3.00, df = 4, p-value = 0.0399), but the mean vertical food angle was not significantly different between aerial and non-aerial jumps (Table 6; T-test: t = 0.828, df = 4, p-value = 0.454). Non-aerial jumps appeared kinematically identical to jumps with aerial phases. Thus, the kinematics of these jumps will be analyzed together with aerial jumps except for the analysis of course correction which will focus solely on jumps with aerial phases.

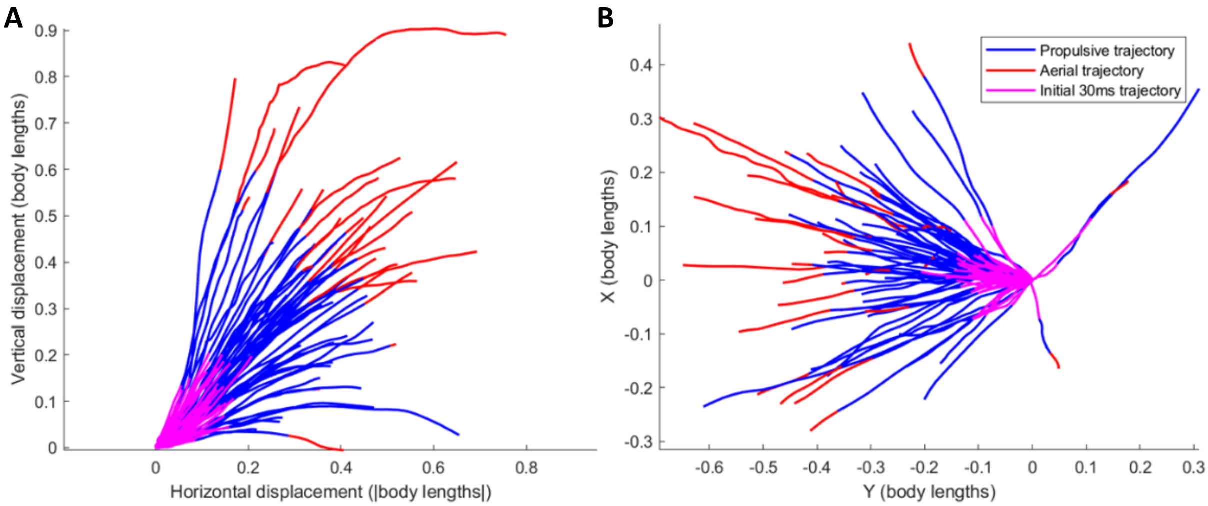

Figure 12 | Pelvic girdle trajectory path during aerial and non-aerial prey-capture prone jumps from the start of the propulsive phase until food capture. **(A)** Pelvic girdle vertical displacement relative to absolute horizontal displacement. (**B**) Top-down pelvic girdle displacement. All trials start positions have been translated to the 0,0 point by subtracting the initial position of the fish from all displacement measurements. The pelvic girdle approximates the position of the CoM. Many of the jumps did not include an aerial trajectory ($n_{\text{non-aerial}}$ = 31, n_{aerial} = 29). The direction of the top-down trajectory has not been rotated.

Table 6 | Food position at end of preparatory phase for aerial and non-aerial jumps. Values in the bottom row of non-aerial and aerial prone jumps represent the mean value across all 5 fish, treating each fish as a replicate. The SD in the bottom row of the aerial and non-aerial jumps is the SD associated with the mean calculated from the mean value for each fish (n=5). T-tests comparing the mean jump distance and vertical food angle of non-aerial and aerial jumps are included. $n_{non\text{-}aerial} = 31$, $n_{aerial} = 29$.

Non-aerial prone jumps

	Food distance (body lengths)				Vertical food angle (degrees)				Lateral food angle (degrees)				
Fish ID	**Min**	**Max**	**μ_{Fish}**	**SD**	**Min**	**Max**	**μ_{Fish}**	**SD**	**Left max**	**Right max**	**μ_{Fish}**	**SD**	**n**
1	0.356	0.460	0.389	0.037	14.0	58.5	41.2	16.3	-10.3	9.82	-3.81	7.61	7
2	0.238	0.542	0.390	0.099	25.0	56.8	43.4	12.0	-12.4	1.76	-6.07	4.69	8
3	0.249	0.608	0.480	0.111	0.559	65.8	36.7	25.7	-23.3	13.4	1.62	14.4	7
4	0.224	0.438	0.343	0.109	15.9	66.1	48.4	28.1	-6.57	0.88	-3.48	3.88	3
5	0.203	0.463	0.385	0.094	4.07	71.3	36.3	24.4	-10.7	3.13	-3.88	5.21	6
$\mu_{non\text{-}aerial}$			0.397	0.05			41.2	5.03			-3.15	2.54	

Aerial prone jumps

	Min	**Max**	**μ_{Fish}**	**SD**	**Min**	**Max**	**μ_{Fish}**	**SD**	**Left max**	**Right max**	**μ_{Fish}**	**SD**	**n**
1	0.506	0.558	0.532	0.037	44.9	49.2	47.1	3.02	-6.60	6.72	0.06	9.42	2
2	0.319	0.442	0.381	0.087	25.3	27.9	26.6	1.87	-6.43	2.53	-1.95	6.34	2
3	0.361	0.921	0.665	0.137	11.3	82.9	56.2	18.9	-18.2	9.77	-0.01	7.43	11
4	0.470	1.126	0.720	0.200	31.0	74.0	53.3	12.8	-14.2	14.8	-0.77	8.34	9
5	0.515	0.807	0.680	0.121	33.8	64.3	47.5	11.2	-9.68	9.77	1.23	7.48	5
μ_{aerial}			0.595	0.139			46.1	11.6			-0.29	1.05	

Paired T-test comparing $\mu_{non\text{-}aerial}$ and μ_{aerial}

Df	**T-stat**	**p-value**	**α**	**Df**	**T-stat**	**p-value**	**α**
4	-3.00	0.0399	0.05	4	0.828	0.454	0.05

When considering the relationship between tail-pelvic girdle distance and food position at the end of the preparatory phase, there was a significant interaction between food distance and vertical food angle (Figure 13, Table 5a and A3a). The slope of tail-pelvic girdle distance with vertical food angle was significant and negative for food distances smaller than 0.66 body lengths and significant and positive for food distances greater than 1.06 body lengths (Figure 13B). Despite being significant, the slope was not interpreted above 1.06 body lengths since there was a lack of observed food distances (lack of common support) and relatively wide confidence intervals at this range. The negative linear relationship seen during low food distance jumps supports my prediction that the fish plant their tails closer to their center of mass as the vertical food angle increases. However, I had not predicted that as food distance increased the slope would become flatter or non-significant. The coefficients of determination ($R^2_{LMM(m)}$ = 0.559, $R^2_{LMM(c)}$ = 0.701) indicate that the model accounted for a moderate amount of the variation in tail-pelvic distance between trials.

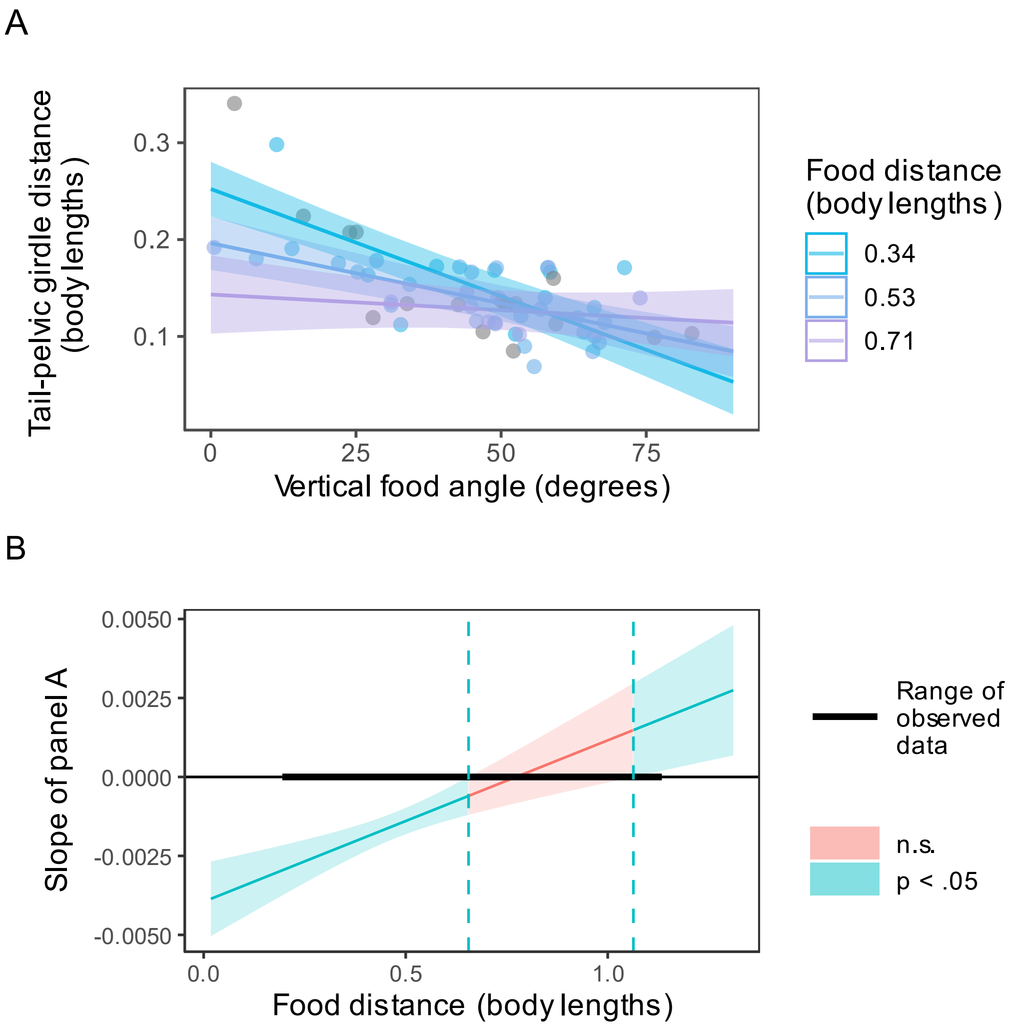

Figure 13 | Relationship between tail-pelvic girdle distance and food position at the end of the preparatory phase. (**A**) Interaction plot for the linear mixed model (Table 5a and A3a) comparing how vertical food angle and food distance at the end of the preparatory phase affect the horizontal distance between the planted tail and the pelvic girdle. (**B**) Jonhson-Neyman plot demonstrating the significant range of slopes in panel A and the range of observed data. The pelvic girdle locus approximates the position of the fishes CoM. The regression lines and 95% confidence intervals are shown for a mean value of the moderator variable (food distance) and

the mean value ± 1SD. (n = 60 jumps, samples per fish $n_1 = 9$, $n_2 = 10$, $n_3 = 18$, $n_4 = 12$, $n_5 = 11$; $R^2_{LMM(m)} = 0.559$, $R^2_{LMM(c)} = 0.701$).

Food position relative to the mid-sagittal plane of the fish had a significant impact on the side the tail was planted on (Figure 14: Table A3m, P = 8.16e-4, $CI_{95\%} = [-0.705,-0.215]$). Lateral food angle moving from the left side to the right side of the fish was associated with a decrease in the probability of the fish planting their tail on the right side (or an increase in the probability of a left planted tail; Figure 14). This supports my prediction that fish plant their tails contralateral to the food. However, there was also a region of overlap around 0 degrees (directly in front of the fish) where left or right planted tails could occur, indicating that there was some flexibility in tail coiling side if the food was in front of the fish. The model accounted for a high amount of variation in the response variable with and without the inclusion of Fish ID as a random effect ($R^2_{LMM(m)} = 0.823$, $R^2_{LMM(c)} = 0.898$).

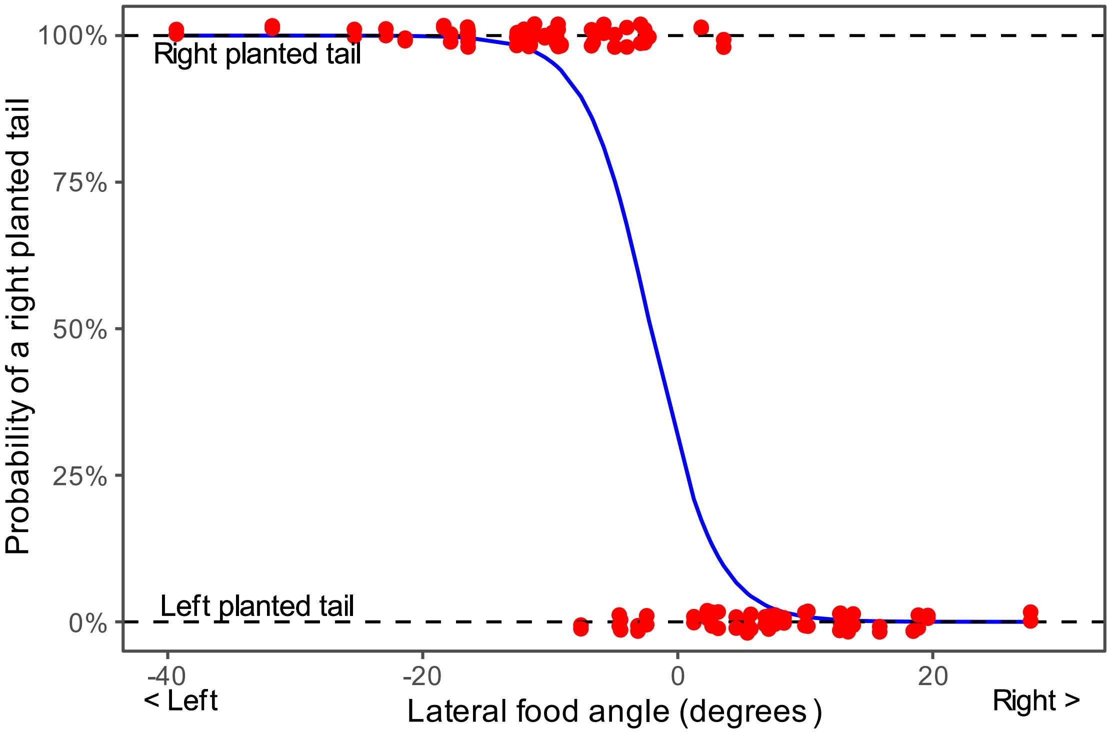

Figure 14| Logistic regression curve showing the relationship between tail planting side and lateral food angle. The blue curve represents the probability of a right planted tail. Each point represents the occurrence of a left (y = 0) or right (y = 1) planted tail and the lateral food angle from that trial. Lateral food angles below 0 degrees indicate that the food was to the left of the fish and angles above 0 degrees indicate the food is to the right of the fish. Estimates were obtained from a mixed effect logistic regression model (MELR) with lateral food angle as the sole continuous predictor and Fish ID as a random intercept (Table A3m). Lateral food angle was a significant predictor of tail planting side (MELR: β_1 = -0.393, $P_{\beta 1}$ = 8.16e-4, $CI_{95\%, \beta 1}$ = [-0.705,-0.215]), indicating that moving the food from the left to the right side of the fish causes the fish to plant its tail on the right (and vice-versa as the food moves to the left). n = 60, samples per fish: n_1 = 9, n_2 = 10, n_3 = 18, n_4 = 12, n_5 = 11; $R^2_{LMM(m)}$ = 0.823, $R^2_{LMM(c)}$ = 0.898.

There was a significant positive linear relationship between head pitch and vertical food angle, supporting my prediction that the fish orient their heads towards the food (Figure 15A; Table 5d and A3d, P_{slope} = 2e-16, $CI_{95\%, slope}$ = [0.48,0.619]). Head pitch also had an almost positive linear relationship with food distance (Figure 15B, P_{slope} = 0.0548, $CI_{95\%, slope}$ = [0.104,15.2]). The model accounted for most of the variation in the response variable ($R^2_{LMM(m)}$ = 0.788, $R^2_{LMM(c)}$ = 0.85).

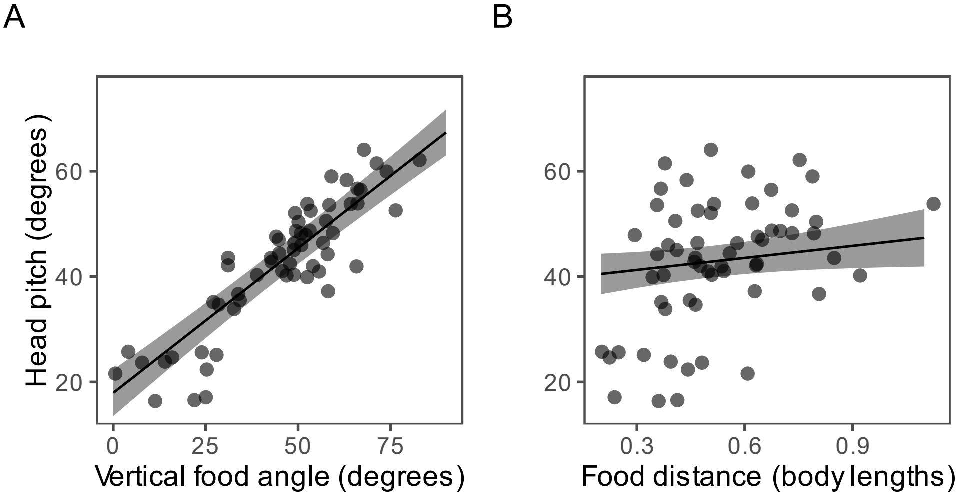

Figure 15 | Relationship between head pitch and food position at the end of the preparatory phase. (**A**) Linear relationship between head pitch and vertical food angle (*VFA*) at the end of the preparatory phase. (**B**) Linear relationship between head pitch and food distance (*d*) at the end of the preparatory phase. The regression lines (*head pitch* = 13.9 + 0.549**VFA* + 7.61**d*) and 95% confidence intervals for both fixed effects are shown with a shared y-axis. Estimates were obtained from a linear mixed effect model (Table 5d and A3d) including vertical food angle and food distance as well as random intercepts for Fish ID. n = 60 jumps, samples per fish: n_1 = 9, n_2 = 10, n_3 = 18, n_4 = 12, n_5 = 11;. $R^2_{LMM(m)}$ = 0.788, $R^2_{LMM(c)}$ = 0.850; $P_{intercept}$ = 1.32e-05,

P_{VFA}= 2e-16, P_d = 0.0548; $CI_{95\%,\ intercept}$ = [8.92,19.0], $CI_{95\%,\ VFA}$ = [0.48,0.619], $CI_{95\%,\ d}$ = [0.104,15.2].

Because head pitch and vertical food angle are correlated, they both impact pelvic girdle height. The model with head pitch as a fixed effect best explained the variation in pelvic girdle height (Table 5c). There was a significant positive linear relationship between pelvic girdle height and head pitch (Figure 16, Table 5c and A3c, P_{slope} = 1.78e-06, $CI_{95\%,\ slope}$ = [2.70e-4,5.85e-4]; $R^2_{LMM(m)}$ = 0.293, $R^2_{LMM(c)}$ = 0.463). My initial prediction was that pelvic fin height would increase with vertical food angle, which it does, however, the head pitch of the animal explains more of the variation in pelvic girdle height (Table 5c).

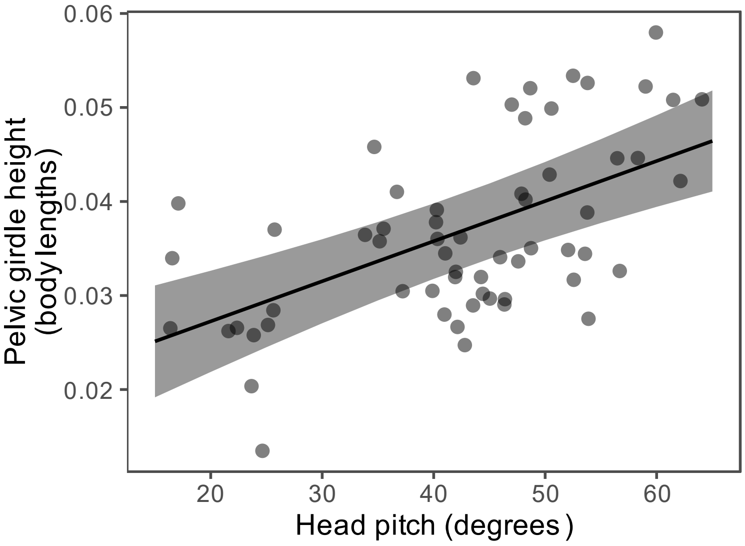

Figure 16 | Relationship between pelvic girdle height and head pitch at the end of the preparatory phase. The pelvic girdle approximates the position of the CoM. The regression line (*Pelvic girdle height* = 1.87e-2 + 4.26e-4**head pitch*) and 95% confidence intervals are shown.

Estimates were obtained from a linear mixed effect model (Table 5c and A3c) including vertical food angle as well as random intercepts for Fish ID. n = 60 jumps, samples per fish: n_1 = 9, n_2 = 10, n_3 = 18, n_4 = 12, n_5 = 11; $R^2_{LMM(m)}$ = 0.293, $R^2_{LMM(c)}$ = 0.463; $P_{\text{intercept}}$ = 3.72e-05, $P_{head\ pitch}$ = 1.78e-06; $CI_{95\%,\ \text{intercept}}$ = [0.0111,0.0264], $CI_{95\%,\ head\ pitch}$ = [2.70e-4,5.85e-4].

There was a significant difference between the mean distance of ipsilateral and contralateral fins relative to the pelvic girdle (Figure 17; Table 5b and A3b; P =. 8.04e-06, $CI_{95\%}$ = [0.00904,0.0213]; $R^2_{LMM(m)}$ = 0.11, $R^2_{LMM(c)}$ = 0.465). The mean ipsilateral fin distance was 0.0152 body lengths farther from the pelvic locus than the contralateral fins. This does not support my predictions as I predicted the contralateral fins would be placed further from the pelvic girdle than the ipsilateral fins.

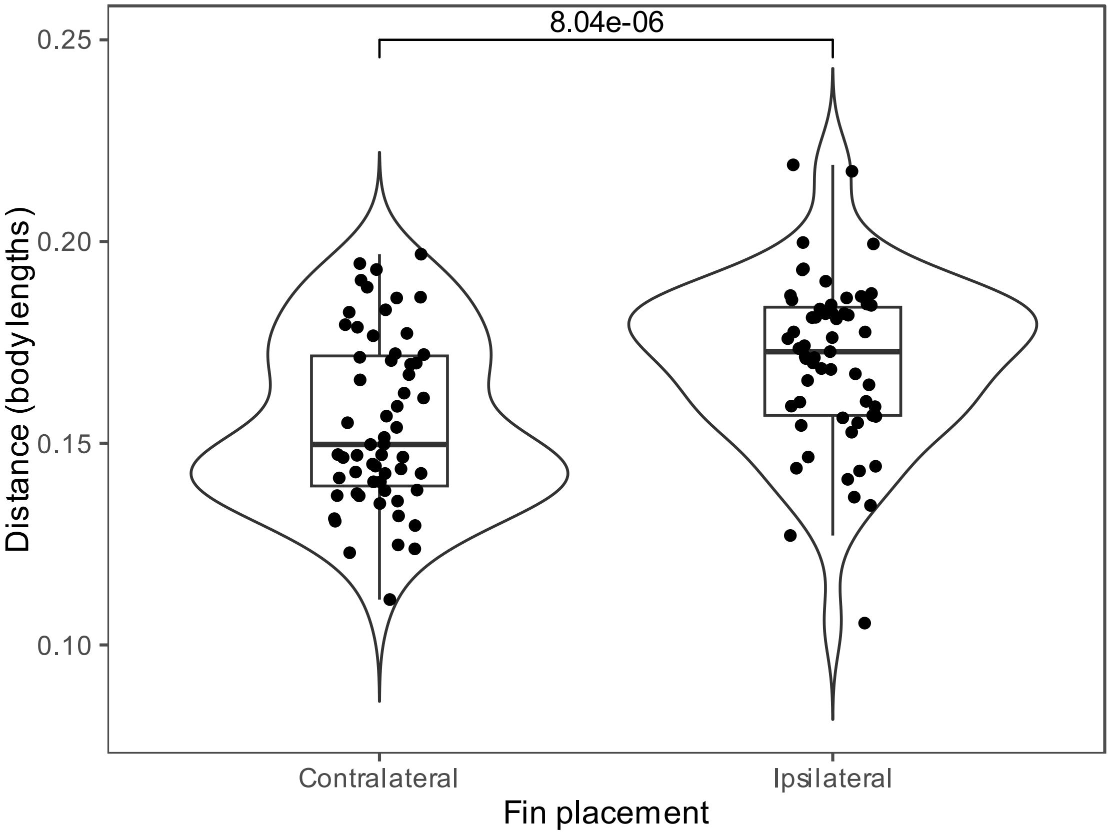

Figure 17 | The difference in contralateral and ipsilateral fin distance relative to the pelvic girdle at the end of the preparatory phase. Contralateral fins are fins planted on the opposite side of the body relative to the planted tail while ipsilateral fins are planted on the same side. Distance is measured in the ground plane between the pectoral fin locus and pelvic fin locus. The central line demonstrates the median value and the whiskers represent the minimum and maximum values (1st or 3rd quartile ± 1.5IQR). Estimates were obtained from a linear mixed effects model with distance as the sole continuous predictor, Fish ID as a random intercept and trial number nested within Fish ID (Table 5b and A3b). The first parameter estimate was significant (LMM: $\beta_1 = 0.0152$, $P_{\beta 1} =$. 8.04e-06, $CI_{95\%, \beta 1} = [0.00904, 0.0213]$), indicating that the mean ipsilateral fin distance is 0.0152 body lengths greater than the mean ipsilateral distance

(mean = 0.155). IQR = Interquartile range. n = 60 jumps, samples per fish: n_1 = 9, n_2 = 10, n_3 = 18, n_4 = 12, n_5 = 11; $R^2_{LMM(m)}$ = 0.11, R^2_{LMMI} = 0.465.

Evaluating the effect of body position on initial trajectory angle

When evaluating the effect of body position on initial 30ms vertical trajectory angle there was a significant effect of both pelvic girdle height (Figure 18A; Table 5e and A3e ;$CI_{95\%, slope}$ = [329,939], P_{slope} = 2.18e-04) and tail-pelvic girdle distance (Figure 18B; Table 5e and A3e, $CI_{95\%, slope}$ = [-189,-84.8], P_{slope} = 5.72e-06). The initial vertical trajectory angle had a negative relationship with tail-pelvic girdle distance and a positive relationship with pelvic girdle height, supporting my prediction. The $R^2_{LMM(m)}$ of the model was 0.432 and the R^2_{LMMI} was 0.705, indicating that the model accounted for a good amount of the variation in initial vertical trajectory angle.

Tail plantings side also had a significant effect on the mean lateral trajectory angle (Figure 19; Table 5f and A3f; P = 1.18e-05, $CI_{95\%}$ = [-13.9,-5.19]), such that trials with right planted tails had a mean initial 30ms lateral trajectory angle (mean = -7.411) that was to the left of jumps with left planted tails (mean = 2.45 degrees). The fish jumping opposite their tail planting side supports my prediction. There was also a region of overlap around 0 degrees between the initial lateral trajectory angle for left and right planted tails that suggests that the fish could direct their jump trajectory in front of them with right or left planted tails. There was one jump with an initial lateral trajectory angle of -56 degrees that appears to be an outlier. However, the lateral trajectory angle for this trial appears to be accurate based on a review of the digitization and videos from this trial. The model accounted for a little to moderate amount of the variation in the response variable ($R^2_{LMM(m)}$ = 0.283, R^2_{LMMI} = 0.389).

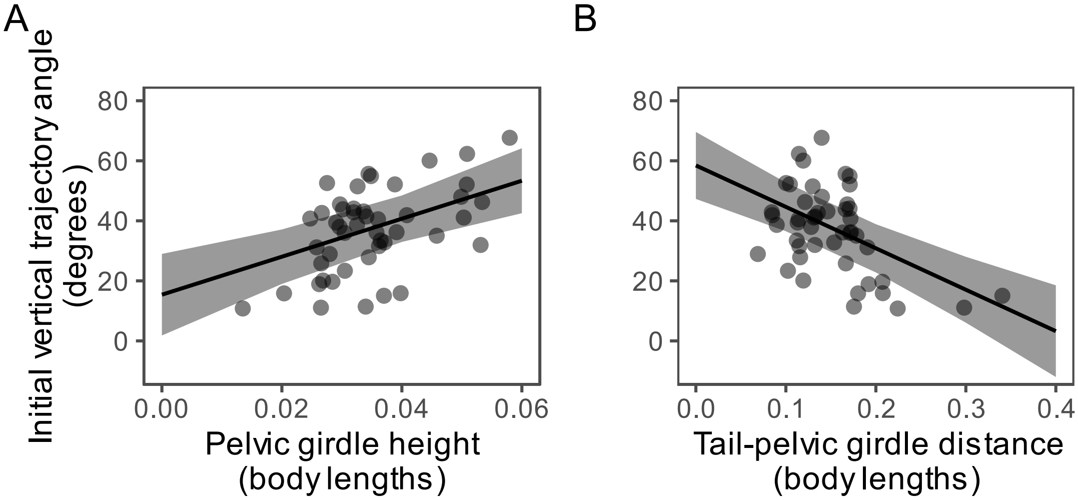

Figure 18 | Relationship between initial 30ms vertical trajectory angle and body position at the end of the preparatory phase for prone jumps with end of preparatory phase food distances of 0.66 body lengths or less. (**A**) Linear relationship between initial 30ms vertical trajectory angle and pelvic girdle height (*Ph*). (**B**) Linear relationship between initial 30ms vertical trajectory angle and tail-pelvic girdle distance (*TPd*). The pelvic girdle approximates the position of the CoM. The regression lines (*initial vertical trajectory angle* = 36.2 + 633*Ph* – 138*TPd*) and 95% confidence intervals are shown with a shared y-axis. Estimates were obtained from a linear mixed effect model (Table 5e and A3e) including tail-pelvic girdle distance and pelvic girdle height as well as random intercepts for Fish ID. n = 47 jumps, 5 fish, 7-12 jumps per fish. $R^2_{LMM(m)}$ = 0.432, $R^2_{LMM(c)}$ = 0.705 ; $P_{intercept}$ = 1.68e-04, P_{Ph} = 2.18e-04, P_{TPd} = 5.72e-06 ; $CI_{95\%, intercept}$ = [19.6,52.8], $CI_{95\%, Ph}$ = [329,939], $CI_{95\%, TPd}$ = [-189,-84.8].

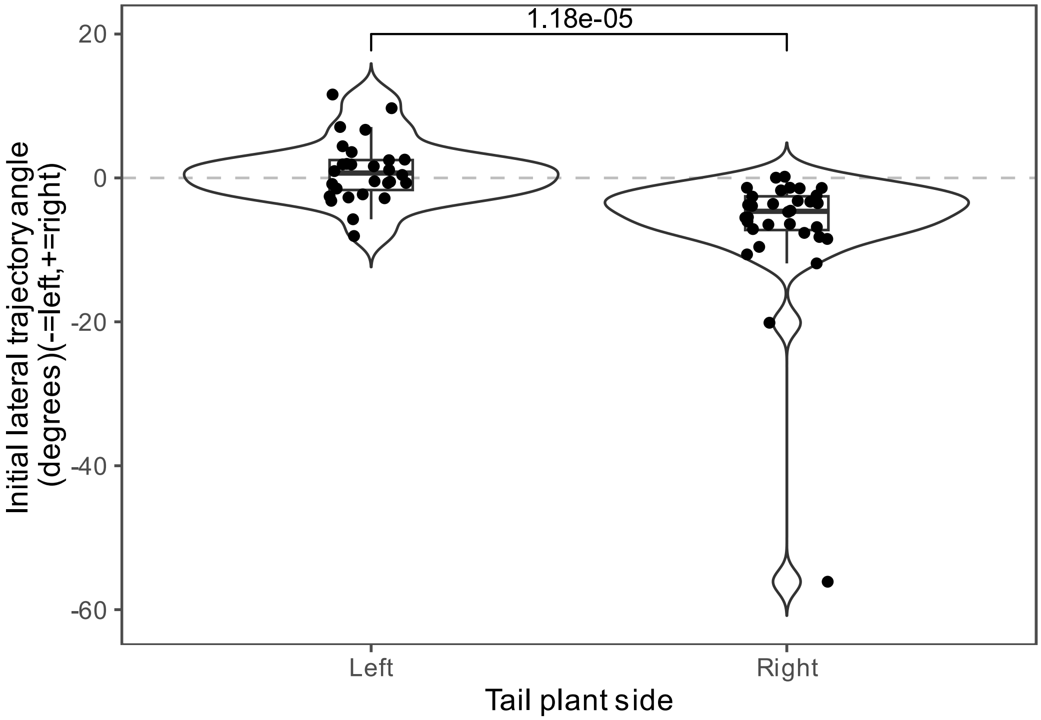

Figure 19 | The relationship between tail plant side and initial 30ms lateral trajectory angle. Negative angles indicate the trajectory direction is to the left of the mudskipper and positive angles indicate the trajectory direction is to the right of the mudskipper. The dashed line represents a lateral trajectory angle directly in front of the mudskipper. Negative initial 30ms lateral trajectory angles indicate the trajectory was to the left of the fish and positive angles indicate the trajectory was to the right. Estimates were obtained from a linear mixed effects model (Table 5f and A3f) with initial lateral trajectory angle as the sole continuous predictor and Fish ID as a random intercept. The first parameter estimate is significant (LMM: β_1 = -9.86, $P_{\beta 1}$ =. 1.18e-05, $CI_{95\%,\ \beta 1}$ = [-13.9,-5.19]) indicating that right-planted tails have a mean initial lateral trajectory angle that is 9.86 degrees to the left of left-planted tails. N = 28 left plants, n = 32 right plants. $R^2_{LMM(m)}$ = 0.283, R^2_{LMMl} = 0.389.

Max Jump velocity and acceleration relative to food position

When considering the relationship between max speed and end of preparatory phase food position, there was a significant interaction between the food distance and vertical food angle (Figure 20A; Table 5g and A3g). The slope for max speed vs vertical food angle was significant and positive for jumps greater than 0.25 body lengths in distance. This model had a moderate/high $R^2_{LMM(m)}$ of 0.657 and $R^2_{LMM(c)}$ of 0.728. The interaction suggests that increasing vertical food angle and food distance had a larger effect on maximum speed than the sum of the effect of these two variables alone. This supports my predictions that increasing vertical food angle and food distance increase the maximum speed of the fish, even though I did not predict that there would be an interaction between the two food position variables. The two component vectors of the maximum velocity (vertical velocity and horizontal velocity) also had a significant interaction with food position (Figure 20B and 20C; Table 5h and 5i; Table A3h and A3i). The vertical velocity had a similar relationship to the overall max speed of the fish with food position and a higher $R^2_{LMM(m)}$ of 0.776 and $R^2_{LMM(c)}$ of 0.851. The slope of this relationship was significant and positive for food distances greater than 0.15 body lengths. Horizontal velocity also had an interaction with food distance and vertical food angle but instead had a negative linear relationship with vertical food angle for food distances greater than 0.48 body lengths. This relationship became increasingly more negative as food distance increased. This model has a much lower $R^2_{LMM(m)}$ of 0.169 and $R^2_{LMM(c)}$ of 0.331 compared to the other two models. The higher coefficients of determination for the vertical velocity component indicate that the fish adjusted the overall velocity of their jumps relative to food position primarily by increasing their vertical velocity for higher food distances and vertical food angles.

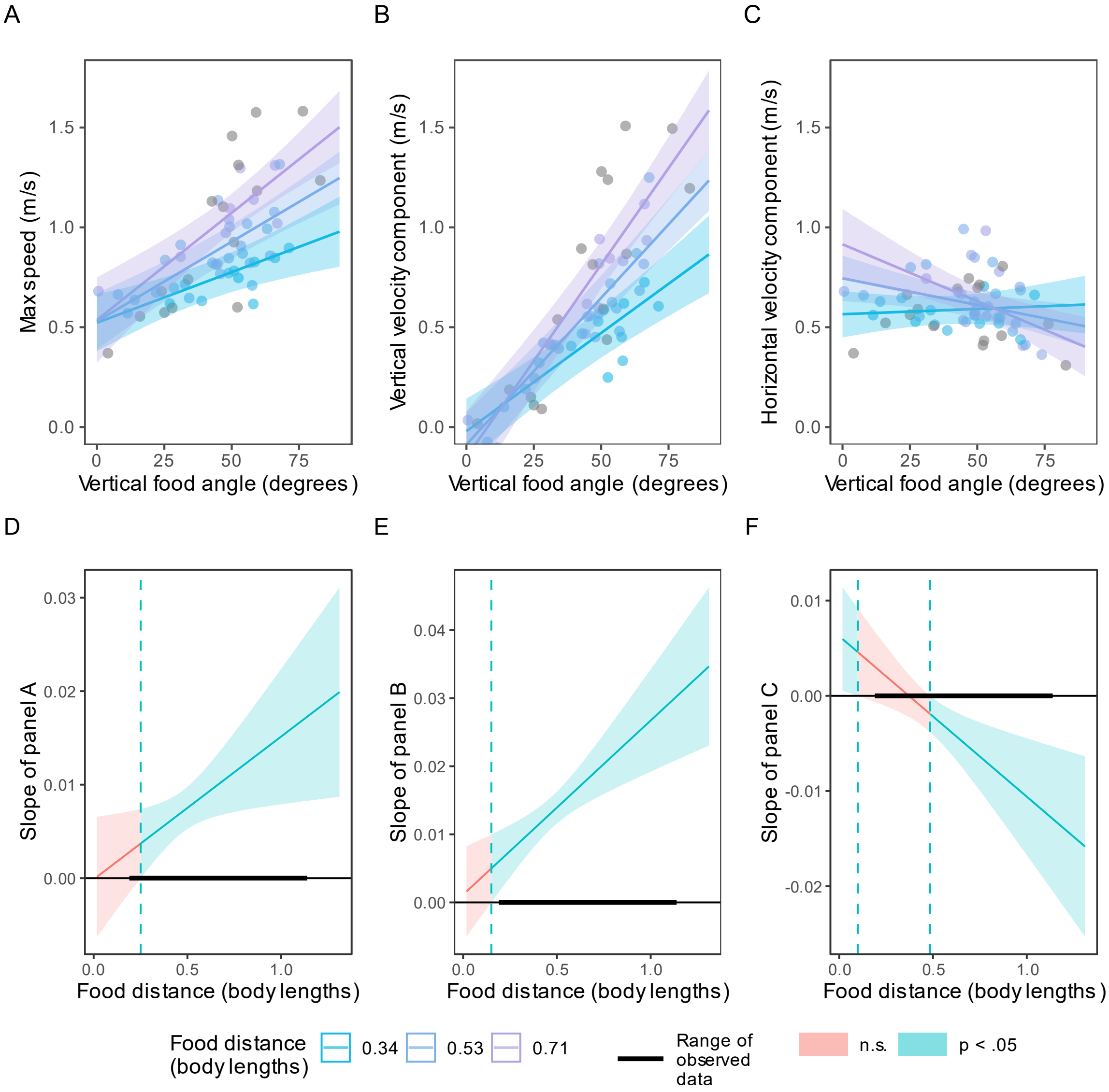

Figure 20 | Relationship between pelvic girdle maximum velocity and food position. (**A-C**) Interaction plots for the linear mixed models comparing how vertical food angle and food distance at the end of the preparatory phase affect the max velocity of the fish and the vertical and horizontal components of the max velocity. (**D-F**) Jonhson-Neyman plots demonstrating the significant range of slopes in panels (A-C) and the range of observed data. The regression lines and 95% confidence intervals are shown for a mean value of the moderator variable (food

distance) and the mean value ± 1SD. Estimates were obtained from 3 separate linear mixed effect models (Table 5g, 5h, 5i, A3g, A3h and A3i) including an interaction between vertical food angle and food distance as well as random intercepts for Fish ID. For further explanation please consult the methods. n = 60 jumps, samples per fish: $n_1 = 9$, $n_2 = 10$, $n_3 = 18$, $n_4 = 12$, $n_5 = 11$; $R^2_{LMM(m)\text{ of A}} = 0.657$, $R^2_{LMM(c)\text{ of A}} = 0.728$, $R^2_{LMM(m)\text{ of B}} = 0.766$, $R^2_{LMM(c)\text{ of B}} = 0.851$, $R^2_{LMM(m)\text{ of C}} = 0.169$, $R^2_{LMM(c)\text{ of C}} = 0.331$.

Average acceleration (up to maximum velocity) had a significant positive linear relationship with both end of preparatory phase vertical food angle and food distance (Figure 21; Table 5j and A3j, $P_{\text{vertical food angle}} = 4.66\text{e-}07$, $P_{\text{distance}} = 0.0301$, $CI_{95\%,\text{ vertical food angle}} = [0.134,0.273]$, $CI_{95\%,\text{ distance}} = [4.15,18.1]$). The model had a moderate $R^2_{LMM(m)}$ of 0.514 and no $R^2_{LMM(c)}$ since the random effect (FishID) did not account for any variation in the response variable. This model suggests that the fish accelerate faster as the vertical food angle increases and food distance increases. However, the effect of food distance on acceleration appears to be lower. This supports my prediction that the fish increase acceleration relative to food distance, although, the relatively low coefficient of determination indicates that food position only accounts for approximately 51% of the variation in acceleration.

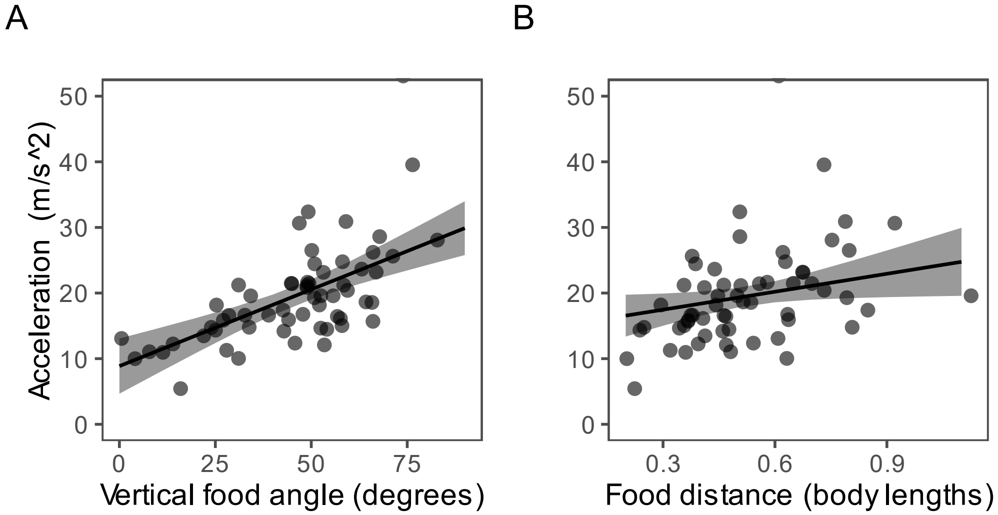

Figure 21 | Relationship between the average acceleration to max velocity and food position at the end of the preparatory phase. (**A**) Linear relationship between acceleration and vertical food angle (*VFA*) at the end of the preparatory phase. (**B**) Linear relationship between acceleration and food distance (*d*) at the end of the preparatory phase. The regression lines (*acceleration* = 4.51 + 0.203**VFA* + 11.1**d*) and 95% confidence intervals are shown with a shared y-axis. Estimates were obtained from a linear mixed effect model (Table 5j and A3j) including vertical food angle and food distance as well as random intercepts for Fish ID. n = 60 jumps, samples per fish: $n_1 = 9$, $n_2 = 10$, $n_3 = 18$, $n_4 = 12$, $n_5 = 11$; $R^2_{LMM(m)} = 0.514$. $P_{\text{intercept}} = 0.0361$, $P_{\text{VFA}} = 4.66\text{e-}07$, $P_{\text{d}} = 0.0301$; $\text{CI}_{95\%,\ \text{intercept}} = [0.431,8.58]$, $\text{CI}_{95\%,\ \text{VFA}} = [0.134,0.273]$, $\text{CI}_{95\%,\ \text{d}} = [4.15,18.1]$.

Course correction

Of the 60 trials digitized, only 29 of these trials had an aerial phase where the fish left the ground for at least 1 frame before capturing the food (Figure 22). The distance these fish travelled during the aerial phase was generally low, with a mean aerial food distance of 0.368

body lengths, a minimum range of 0.183 body lengths and a maximum range of 0.814 body lengths (Figure 23A). In fact, 6 trials had aerial food distances less than 0.255 body lengths (mean distance between mouth to pelvic girdle) when the fish left the ground, indicating that food was close enough to the pelvic girdle for the mouth to capture it (Figure 23A).

The predicted accuracy of the fish to the initial food position was significantly shorter than the mouth to pelvic girdle distance (mean predicted accuracy =0.229 body lengths; mean mouth to pelvic girdle distance = 0.255 body lengths) suggesting that the initial trajectory and velocity for the jumps analyzed in this thesis were on target (Figure 23C; Table 5k and A3k; P = 0.0203, $CI_{95\%}$ = [-4.74e-2,-4.69e-3]) . The accuracy of the fish (mean fish accuracy = 0.231 body lengths) and the predicted accuracy to final food position (mean = 0.239 body lengths) did not significantly differ (Figure 23B and 23D; Table 5l and A3l, P = 0.196, $CI_{95\%}$ = [-2.05e-2,4.14e-3]). This does not support my prediction that the real fish trajectory (fish accuracy) would be more accurate than the predicted trajectory. Of the 29 aerial jumps, 28 had predicted trajectories that get within food capture range at some point during the trajectory (Figure 23D).

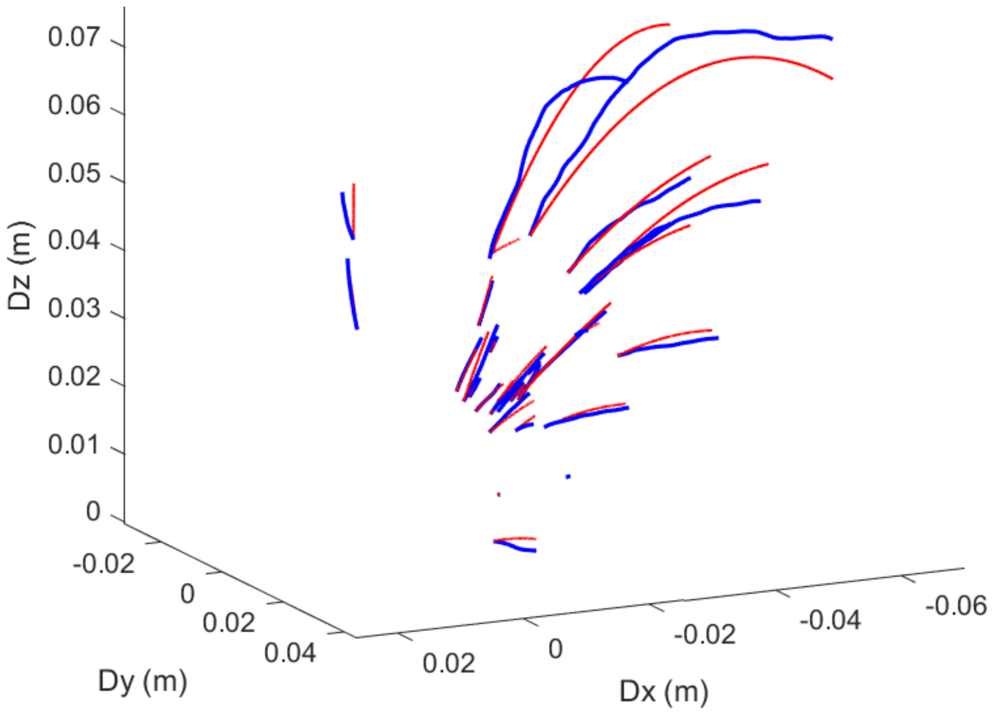

Figure 22 | Visualizing the predicted trajectory (red) of the pelvic girdle next to the realized trajectory (blue) for all aerial trials. The pelvic girdle point approximates the position of the CoM. Real trajectories are plotted from the start of the aerial phase until food capture. Predicted trajectories are plotted from the start of the aerial phase until the real time it took the fish to capture the food. n = 29.

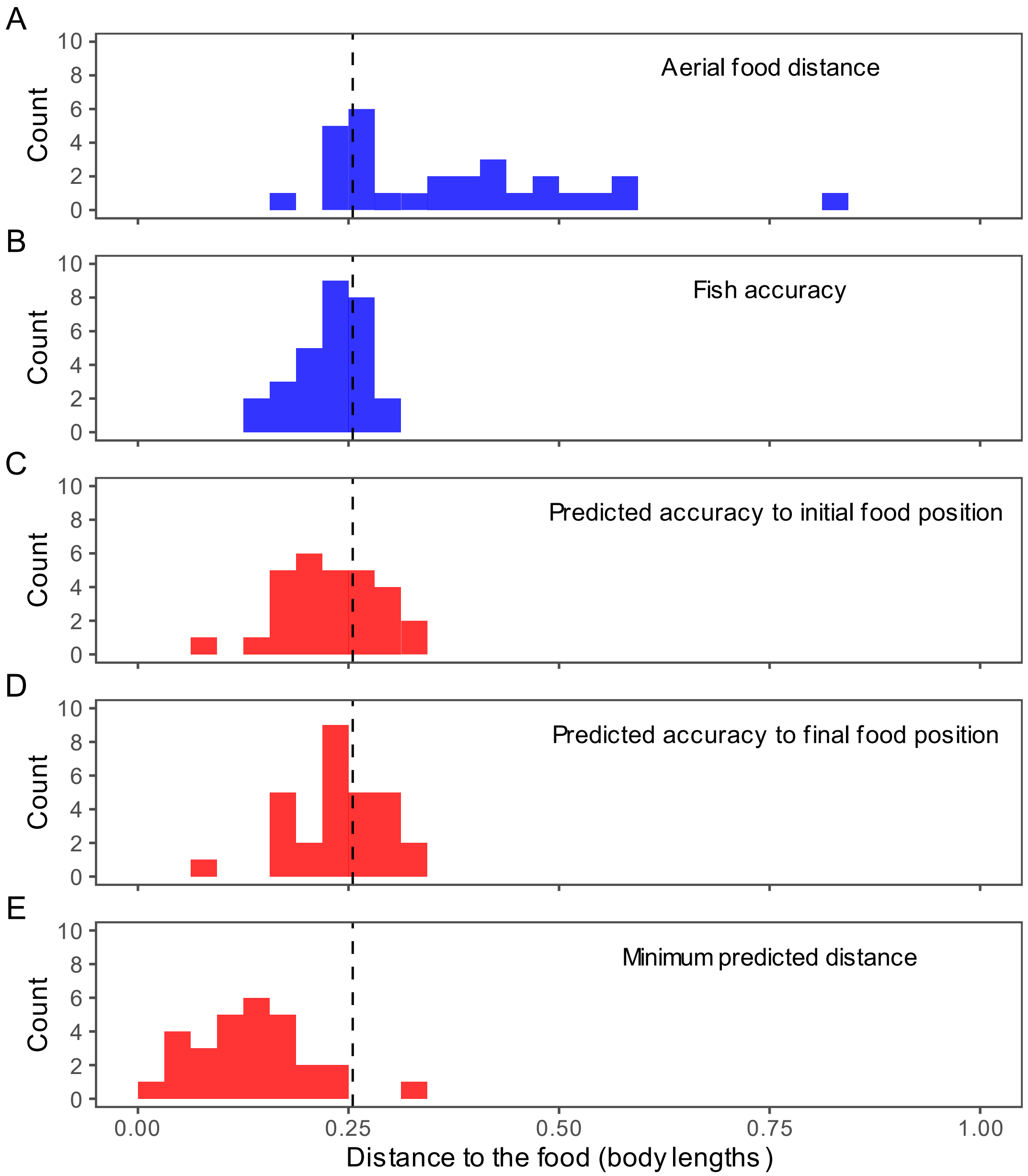

Figure 23 | Comparing the distance to the food of the real trajectory and the predicted trajectory of the fish. (**A**) Distance between the pelvic girdle and the food at the first frame of the aerial phase. (**B**) Distance between the pelvic girdle and the food during food capture of the real fish jumps. (**C**) Distance between the predicted fish path and the foods initial position when the predicted fish path had flown for the same duration as the real fish. (**D**) Distance between the predicted fish path and the foods final position when the predicted fish path had flown for

the same duration as the real fish. (**E**) Minimum distance between the predicted aerial trajectory of the pelvic girdle and the food when the predicted fish path goes until ground contact. The pelvic girdle approximates the position of the CoM. The dashed vertical line indicates the mean distance between the mouth locus/mouth of the mudskipper and the pelvic girdle for all the mudskippers (0.255 body lengths). Colours: blue = distance between two real loci, red = distance between a predicted locus position and the food locus. n = 29.

Discussion

Defining a prey-capture prone jump

Prey-capture prone jumps appear to follow the same general kinematic patterns as the escape response prone jumps described by Swanson and Gibb (2004), suggesting a general constraint on jump kinematics. Although, prey-capture prone jumps did have two specific characteristics that have not been observed during escape response prone jumps. First, fish oriented their head towards the prey before the propulsive phase (Figure 15A and Takiyama et al. (2016)). Orienting the head towards the food was likely important because it positions the food within the region of higher visual acuity in the mudskipper eye, allowing the fish to perceive the prey position and adjust the kinematics of the jump accordingly (Takiyama et al., 2016). The second characteristic of prey-capture prone jumps was the lack of a consistent aerial phase; fish did not always leave the ground if the food was positioned close to the mudskipper. Although remaining in contact with the ground during the entirety of the jumping motion would make these not a true “jump” these non-aerial jumps appeared kinematically similar to aerial prone jumps during the preparatory phase and propulsive phase. Despite these two unique characteristics the overall similarity of prey-capture versus escape response prone jumps suggests functional constraint in jump kinematics.

Within the literature on prone jumps, there are three thoughts on the body parts that control force production during a prone jump and the direction in which those body parts generate force (Harris, 1960; Swanson & Gibb, 2004; Van Dijk, 1960). The first thought by Harris (1960) was that the tail can only produce force horizontally (parallel to the plane of the ground) and that the vertical force was most likely produced by the pelvic fins and potentially the pectoral fins. However, in addition to generating horizontal force, I found that the tail likely does produce vertically directed force since the fish adjusted their tail position relative to the vertical angle of their target (Figure 13) and tail positioning impacted vertical jump trajectory (Figure 18). The second thought by Swanson and Gibb (2004) was that the pectoral fins and pelvic fins lift the head during the initial moments of the propulsive phase and that the tail was also responsible for lifting the CoM and propelling it forwards via vertical and horizontal force production. My findings are consistent with those of Swanson and Gibb (2004) concerning the tail and add to their data by showing that by adjusting tail planting side fish used their tail to contribute both lateral and forward horizontal forces during the jump (Figure 14 and 19). My data contradicts Swanson and Gibb only slightly as in the present data it did not appear as though the pelvic fins produced force that lifted the head during the initial moments of the propulsive phase. The third thought by Van Dijk (1960) was that the direction of force production was controlled by the degree of tail bending before jumping and that unequal force production by the pectoral fins controlled the lateral trajectory of the jump. Their thoughts on the degree of tail bending controlling the direction of force production are consistent with my first hypothesis on how the position of the planted tail changes relative to the food position. This hypothesis was supported when the food was close to the fish and is discussed in detail further in the discussion. Their thought on unequal force production by the pectoral fins is plausible, however, I have not observed movement of the pectoral fins that would suggest they are unequally generating propulsive force to alter the lateral trajectory of the fish. Overall, I believe that the body parts primarily responsible for prone jump propulsive force production are the tail and pectoral fins. Force plate measurements of prone jumping is required to accurately determine force contribution and direction of the tail and paired fins as it is difficult to determine from kinematics alone.

The relationship between body position, food position and jump trajectory.

One of the goals of this study was to determine how body and tail placement influenced jump performance. Specifically, questions were asked about how tail placement relative to the body's center of mass changed relative to food position (hypothesis 1) and altered jump trajectory (hypothesis 5). It seems from this study that fish plant their tail close to their CoM regardless of vertical food angle if their target is far away and adjust the position of their planted tail relative to the vertical food angle if their target is close (Figure 13). The distance of their planted tail relative to their center of mass for low distance jumps predicted the initial vertical jump trajectory of the fish (Figure 18B), indicating that the planting position of the tail has an impact on the direction the fish travels. However, given that the fish do not adjust tail position relative to their CoM when the food is far away suggests they can produce different jump trajectories without adjusting tail planting position and that they may be maximizing tail coiling to increase force production when the food is far away. Based on these findings, it appears fish can produce a wide range of vertical jump trajectories if their tail is positioned close to their center of mass but can only produce a small range of jump trajectories if their tail is planted far away from their center of mass. In other words, the further the tail is planted away from the center of mass, the lower the resulting vertical trajectory and the range of possible jump trajectories. However, this brings up the question, why do they not always plant their tail close to their center of mass if it gives them a wider range of jump trajectories?

Minimizing tail bending can save energy from a muscle activation perspective and save time spent bending their tail. Therefore, the fish may balance maximal force production/jump range and the need to conserve energy/minimize time when deciding on the degree of tail bending.

This study also confirms the prediction that fish plant their tails contralaterally to the prey position (Figure 14). This makes sense as the forces produced by the tail have both a vertical and horizontal component, the tail pushing the fish's center of mass upward and forwards but also laterally away from the side the tail is planted on. Indeed, tail planting side predicted the initial lateral trajectory of the fish, such that fish with tails planted on the left would generally jump to the right while fish with tails planted on the right would jump to the left (Figure 19). Interestingly, there was no pattern in which side a tail will coil when the food is placed directly in front of the fish (around 0 degrees; Figure 14). Fish planted their tails on either side of the body in this case suggesting that the species as a whole does not have a preferential bias in tail side placement.

Another goal of this study was to determine whether the pectoral fins resist unwanted lateral movement and rolling (Hypothesis 2). Inverse to the prediction, the pectoral fin on the opposite side of the planted tail (contralateral fin) was planted closer to the pelvic girdle than the fin on the same side as the planted tail (ipsilateral fin) (Figure 17). This does not rule out the possibility that the contralateral fin acts as a support since the contralateral fin is still planted on the ground, but it does indicate that the fish do not need to extend their contralateral fin more laterally than the ipsilateral fin for support. The fish could be placing the ipsilateral fin more laterally to make room for tail planting since the caudal fin was observed occasionally just slipping past the posterior edge of the ipsilateral fin before planting.

Fish may use their pectoral fins to adjust the height of their CoM relative to the vertical food angle (hypothesis 3), and the height of the CoM may an impact on their vertical jump trajectory (hypothesis 5). From the present study, it is difficult to answer whether the pectoral fins are raising or lowering the CoM relative to the vertical food angle since the height of the CoM changes when the fish pitches its head towards the food (Figure 16). Therefore, it is difficult to dissociate the effect of head pitch on CoM height. On the other hand, the height of the CoM did relate to vertical jump trajectory (Figure 18A). The effect CoM height has on vertical jump trajectory could be due to the impact of CoM height on force direction or could be due to the confounding effect of the fish pitching their heads towards the food. Future studies should look at the actual direction of the forces produced by the tail relative to the fish's CoM.

We asked whether the mudskippers orient their heads towards the target of their prone jump to better perceive the food's position (Hypothesis 4). Indeed, there was a strong positive linear relationship between head pitch and the vertical food angle that supported this hypothesis (Figure 15). Work on a similar species of mudskipper, *Periopthalmus modestus,* suggests they pitch their heads towards their target to orient the food within the region of their field of view with higher visual acuity(Takiyama et al., 2016). This could likely be the case for *Periopthalmus argentilineatus* as well, but research into the structure of their eyes is required. Also, given the mobility of their eyes and their ability to jump and catch prey with considerable precision, it has been presumed that mudskippers have binocular vision (Takiyama et al., 2016). Therefore, orienting of the head could also be used to position the food within the region of their eye with binocular vision since this would allow them to better perceive the distance of their target. Head pitching would also displace their center of mass relative to their planted tail since as the head pitches upwards the mass of its head moves upwards and backwards. Displacing their center of mass via head pitching would impact the direction of the ground reaction forces produced by their tail relative to their center of mass, therefore, likely impacting jump trajectory. Upwards pitching of the head would also direct the mouth towards the food, minimizing the need to reorient the mouth relative to the food after take-off. Thus, head pitching may provide multiple performance advantages.

Based on this study's findings, mudskippers do adjust certain aspects of their initial body position depending on the food's position or their tail colling direction.-The strongest relationships were found between head pitch and tail coiling side relative to food position, indicating that these aspects of body positioning might be more critical to the control of jump performance. Such that, if the fish did not coil its tail to the opposite side of its body relative to the food and the food was sufficiently off-centre from the fish it would likely miss the food. Also, if the fish did not perceive the food's position by orienting its head towards the food, it would not be able to properly adjust its jump kinematics and its accuracy would likely suffer. The other aspects of body position relative to food position or tail coiling side (tail to CoM distance, pectoral fin distance and the height of the CoM) had weaker relationships and therefore appear less critical in controlling jump performance. Many reasons could explain why these aspects do not have strong relationships: They could simply not have a great impact on jump performance, the fish could adjust for variation in their position during the propulsive and aerial phase of the jump, or they affect aspects of the jump performance that were not considered. For example, the CoM height and tail to CoM distance may have had weaker relationships with food position because the fish rapidly displaces the center of mass relative to the tail during the initial moments of the propulsive phase. Therefore, the initial position of the tail and CoM would only impact the jump direction during the initial moments of the propulsive phase. On the other hand, pectoral fin distance relative to tail coiling side may have had a weaker relationship because it does not have a large impact on jump performance or because it is the passive result of the tail coiling towards one side and does not impact jump performance.

The relationship between max velocity, food position and acceleration

The sixth hypothesis was that mudskippers adjust their maximum jump speed and acceleration relative to the distance the food is from the fish. This study found a positive linear slope between maximum jump speed and food distance, the steepness of said slope increases with vertical food angle (Figure 20A). The interaction indicated that there was a synergistic effect between vertical food angle and food distance on maximum jump speed. The effect of vertical food angle on jump speed was not predicted but indicates that fish may have a wide performance envelope where they are able to increase performance even against gravity to reach their prey. It also suggests that the jumping elicited in this project did not approach their maximum performance. Fish also demonstrated that when they increase their maximum velocity, they did so in the vertical direction rather than the horizontal (Figure 20B and 20C). Increasing horizontal force production increases the force the tail pushes with along the surface of the substrate, if that force exceeds the static friction force of the ground the tail will slip. Vertical forces on the other hand are normal to the ground and therefore they do not slip. By increasing the vertical velocity component while maintaining some component of the horizontal velocity, simple ballistics will allow for greater horizontal distances with reduced chance of slip.

Although a weaker relationship, fish also increased their average acceleration as food distance and vertical food angle increased (Figure 21). The weak relationship indicates that the fish may also control slip by changing the duration of acceleration to maximum velocity. Minimizing instantaneous forces to stay below the slip coefficient of the surface and avoid slipping. Overall, it appears the fish do control jump performance by adjusting the speed of their jumps and by adjusting their average acceleration rate and duration.

Course correction

The seventh hypothesis was that mudskippers change body conformation in the air to course correct for initial ballistic trajectories that would not reach the food. At first glance, data suggests that the initial trajectory of the fish were highly accurate (Figure 23C), and the fish did not need to course correct since the mean predicted accuracy was not significantly different from the mean fish accuracy (Figure 23B and 23D). However, of the trials analyzed (N = 29), the majority had very minor observed changes in body conformation and were generally short. This dataset, therefore, has selected for successful jumps that may not have required course correction and so did not capture the possible variation in aerial correction that may exist across successful and unsuccessful jumps. I include the minimum distance between the predicted trajectory of the fish and the food to illustrate how close the predicted trajectory of the fish got to the food when allowed to continue until ground contact (Figure 23E). Overall, the fish I observed generally did not visibly course correct and this was likely due to the short distance between the fish and the food and ballistic trajectories that would direct them towards the food. The analysis of course correction would be better suited to a subset of filmed trials with obvious changes in body conformation. It would also be prudent to select jumps of approximately the same aerial food distance or duration since increasing either of these variables would likely increase the distance of the predicted accuracy compared to shorter duration or distance trials.

Evolution of prone jumping

When mudskippers crutch on deformable inclined substrates such as sandy slopes, they occasionally use their tail to provide extra propulsion and to resist slipping (Naylor & Kawano, 2022). Comparisons can be made between prone jumps and the crutching behaviour in which the mudskipper uses its tail for extra propulsion. During both behaviours, mudskippers curl then plant their caudal fin on the substrates and extend their tails into the substrate to propel themselves forward over their pectoral fins. Based on the commonalities between both movements, I hypothesize that the prone jumping movement may have evolved from the crutching movement in which the mudskipper uses its tail to provide additional propulsive force. To transition from the modified crutching behaviour to a prone jumping behaviour, mudskippers would have had to increase the proportion of force generated by the tail and change other aspects of the crutching behaviour such as planting their paired fins during tail coiling. The non-aerial prone jumps observed could be an intermediate behaviour that evolved before aerial prone jumping behaviour since non-aerial prone jumps are produced for shorter distance targets that require less force and they do not require the calculation of the aerial jump trajectory. More detailed comparisons between the kinematics of modified crutching using the tail and prone jumps are required to support my hypothesis.

Limitations

One of the main limitations of this study stems from individual variation in the behaviour of animals. Some fish were very bold and fed readily in the filming setup, others required a great deal of coaxing. As a result, although I tried to get a wide distribution of food angles and distances for each fish, it was not always possible. Fish were coaxed to jump by moving the food towards the animal so acquiring long distance or high vertical jumps was difficult. In addition, there was a lack of high distance low vertical food angle jumps, since when the food was held low to the ground the fish would often crutch or prone jump towards the food before performing the final prey-capture prone jump.

Conclusion

Mudskippers can adjust the trajectory of their prey-capture prone jumps depending on the position of their prey. The present study suggests that mudskippers do this in part by adjusting their initial body position before jumping relative to the prey. There were significant relationships between all the observed aspects of body position (tail position, pectoral fin position, head pitch, CoM height) and food position or tail coiling side. The more critical body positioning aspects were pitching of the head towards the food to perceive the food position, and adjusting tail coiling direction relative to left/right position of the food to adjust lateral jump trajectory. During the propulsive phase of the jump, the fish increased their maximum speed as both vertical food distance and vertical food angle increased. Vertical food angle and food distance had a synergistic effect on maximum jump speed. The fish primarily increased the maximum speed of their jumps by increasing the vertical velocity component of their jump relative to the food distance and vertical food angle. They also increased the rate and duration of their average acceleration relative to the food's position. Finally, it had been observed that mudskippers occasionally changed body conformation during the aerial phase. It was hypothesized that these movements were used to course correct their jump trajectory. The jumps analyzed did not indicate that the fish needed to course correct. This conclusion was likely influenced by selection bias for shorter and better jumps. While these are only initial steps in understanding how mudskippers control jump trajectory, further research into this topic will not only provide a better understanding of the biomechanics of prone jumping but could also provide insight into the evolution and control of terrestrial locomotion.

Chapter 3: Future directions

In this chapter, I will talk about the future directions of this research and methodology. The goal of my research was to explain how mudskippers control their prone jump trajectory. I did so by measuring how mudskippers position and move their bodies for different target positions during prey-capture prone jumps. While these may be great initial steps in determining the mechanism behind the control of prone jump trajectory, there is still much to be discovered on the control of prone jump trajectory with the existing data and in future studies.

For example, in my existing data, I observed that the mudskippers rapidly lift their head upwards during the initial moments of the propulsive phase. This pattern of upwards lifting of the head/anterior body after the start of the propulsive phase would rapidly change the orientation and position of the fish (ie. change the position of the center of mass) relative to the force-generating tail and thus would likely impact the trajectory of the fish. While this may not seem like it is critical to jump performance, this exemplifies that there is a pattern of body movement during the propulsive phase that could explain how the fish adjust their jump trajectory and that has not yet been quantified in this analysis. Also, with the existing data there is still much more that could be analyzed to understand what factors affect jump performance. For example, since I did not analyze trials with slipping, it would be prudent to look back at the trials where slipping occurred to determine if slipping impacted jump trajectory and the kinematics of the jump. It would also be prudent to compare jump performance between failed and successful jumps, regardless of tail slipping. Below I discuss the future directions in this research.

Force measurement (kinetics)

The direction the mudskipper moves during the aerial phase of a prone jump is ultimately decided by the magnitude, direction and timing of the ground reaction forces and torque produced by the mudskipper. The measurement of prone jump kinetics (how forces relate to movement) could reveal more about the control of prone jump trajectory, as is difficult to explain with kinematics alone. For example, I suggest that most of the propulsive force of prone jumps is generated by the extension of the tail into the ground and not the pectoral or pelvic fins. This suggestion is motivated by my observations of how the fish accelerate after the paired fins (pectoral and pelvic fins) leave the ground and that the fish move in the direction opposite of their extending tail. However, it was unclear from my observations and kinematic analysis whether the pectoral fins or pelvic fins contribute propulsive force to the jump and whether those forces have a meaningful impact on the jump trajectory since they only remain in contact with the ground briefly during the propulsive phase of the jump and do not appear to push into the ground. From kinematic data alone, I cannot determine the forces produced by the axial and appendicular anatomy and how these forces relate to the direction of the prone jump. Therefore, I believe a logical next step in the analysis of prone jump trajectory control would be to have the fish jump on force plates and measure the forces produced by the axial (tail) and appendicular anatomy (paired fins) during the prone jump. It would be best to have separate force measurements for the forces produced by the appendicular and the axial anatomy during prone jumping. The coaxing technique I employed by suspending food in tweezers in front of the fish and waiting for the fish to approach the food could be used to position the fish over these force sensors before the prone jump. Also, suspending the food in different positions relative to the fish as I have done in this research and measuring how this affects force production by the fish could provide further insight into how the fish controls jump trajectory.

In my analysis of how the mudskippers changed prone jump velocity for different food positions, I found that the fish were primarily adjusting the vertical velocity of their jumps compared to the horizontal velocity. I hypothesized that the fish do not alter their horizontal velocity as much compared to their vertical velocity since the tail is incapable of increasing horizontally directed force without slipping. Measuring the forces produced by the fish's tail during prone jumps where the fish slipped and jumps where the fish did not slip could be used to determine the threshold force that results in slipping and whether slipping affects the forces produced during the prone jump. Overall, understanding how the magnitude, direction, and pattern of force production, and how the relative force contribution of the appendages and axial skeleton relates to jump trajectory could be very beneficial to the understanding of prone jump trajectory control. Especially if the force measurements are used in conjunction with kinematic data on the position and movement of the fish's axial and appendicular anatomy.

Biomimetic mudskipper robot

Animals have evolved adaptations (e.g. cognitive processes, morphology and neural control systems) over millions of years that allow them to locomote effectively in their given environment. For this reason, they are often used as inspiration for building better robots that are capable of locomoting effectively (Gao et al., 2019). For example, researchers have already created a mudskipper-inspired amphibious robot that can swim in water and crutch on land, demonstrating how bio-inspired design can improve movement efficiency and maneuverability (Lin et al., 2023). Biomimetic robots are also commonly used in the study of vertebrate locomotion to validate and expand current understandings of the factors that affect locomotion (e.g. cognition, neural control circuits, morphology, etc.) (Ijspeert, 2008; McInroe et al., 2016; Ramdya & Ijspeert, 2023). For example, they are often used in the study of the neural control of vertebrate locomotion since they allow researchers to validate hypothesized neural circuits that control locomotion (Ijspeert, 2008; Ramdya & Ijspeert, 2023). Another example is the biomimetic crutching mudskipper robot that was used to model how tail use during crutching can improve robustness to suboptimal kinematics and substrate conditions (McInroe et al., 2016).

Biomimetic jumping mudskipper robots would be excellent for validating how specific changes in prone jump kinematics influence jump trajectory. A mudskipper robot capable of prone jumping with variable tail to center of mass distance could be used to validate my hypothesis on how adjusting the planting position of the tail relative to the center of mass affects jump trajectory. A benefit of using robots to address specific questions like this is that they can be systematically programmed to attempt a multitude of jumps that can explore function and performance across a landscape. Robots capable of prone jumping could also have real world applications such as search and rescue due to their ability to navigate complex environments by jumping over obstacles.

One limitation of biorobotic approaches to understanding animal locomotion is the necessary simplification that robots are in comparison with real biological systems. For example, technology limits our ability to sense and produce power in small robotic systems. This is likely a reason for the current lack of robotic jumping mudskippers.

Aerial movement analysis

In Chapter 2 I analyzed whether mudskippers course correct during the aerial phase of their prone jumps. The analysis of course correction was largely inconclusive since most of the analyzed prone jumps did not include obvious changes in body conformation. Therefore, an obvious next step would be to capture trials where the fish course correct in the air. This may be difficult since the course correcting behaviour appears to happen the most when the mudskipper slips or produces bad jump trajectories that are not directed at the target. However, I have also observed that the mudskippers reorient themselves midair before touching the ground, via spinning and bending of their tail and movement of their pectoral fins. As a result of this midair movement, the fish rarely ever lands on its back and protects its eyes from ground contact. Future studies aiming to understand how they orient themselves midair might be able to elicit this midair orienting behaviour more readily than the course-correction behaviour since it appears to always happen before the fish touches the ground. Future researchers may even be able to drop mudskippers upside down or from their sides over water to elicit this behaviour.

Comparative analyses

Comparative analyses involving changing environmental properties are commonly used to study amphibious fish locomotion (McInroe et al., 2016; C. M. Pace & Gibb, 2009; Standen et al., 2016). This is because many factors that affect locomotion such as the forces generated by their movement and the strategies the fish use to move on land are impacted by their physical environment. For example, mudskippers modulate their crutching gait by using their tail and by changing the way they use their tail and fins when walking on different substrates (solid mud, semi-solid mud and dry sand) and substrate inclinations (0°, 10°, and 20°) (Naylor & Kawano, 2022). Another amphibious fish, *Polypterus senegalus,* has also been found to change movement gaits between axial-based terrestrial locomotion for complex substrates and axial-appendage based for flatter substrates (Standen et al., 2016).

A comparative analysis of mudskippers producing prey-capture prone jumps in different environments could help us better understand the mechanics of prone jumps. For example, I hypothesize that tail slipping has a negative impact on jump performance (reduces jump distance and makes jumps less accurate). By comparing jumps performed on substrates with varying coefficients of friction (slipperiness) I could use slip location (fins vs tail) and ultimate jump performance to evaluate which parts of the fish are essential during force production.

Another form of comparative analysis could be between mudskippers with different morphologies. These types of comparisons are commonly made between species with different morphologies or between control fish and fish that have been surgically modified in a way that affects their performance (Hidayat et al., 2022; Quigley et al., 2022; Takiyama et al., 2016; Tran et al., 2022, 2023; Wicaksono et al., 2016; Wilga & Lauder, 1999). A comparative study that could help better understand the function of the pectoral and pelvic fins in the control of prone jump trajectory would be to compare jump performance between mudskippers with modified pectoral or pelvic fin anatomy and mudskippers without modified anatomy. I had performed prey-capture prone jumping trials of mudskippers with amputated pectoral fins to determine whether the pectoral fins were required for prone jumping trajectory control (see appendix). However, due to time constraints and issues eliciting prey-capture prone jump behaviours from fish with amputated pectoral fin rays before they regenerated, I did not include this research in the present study. Please see the appendix for more information on the amputated trials. Another comparative study that could test presence of binocular vision and its usefulness for prey-capture prone jumping would be to compare the jumping ability of mudskippers with two eyes and mudskippers missing an eye. Interestingly, a population of *Periopthalmus argentilineatus* living in polluted Mtoni mangroves of Tanzania have a high occurrence (3.3% of observed mudskippers) of unilateral anophthalmia (missing left eye) and would be ideal fish to compare with two-eyed mudskippers (Kruitwagen et al., 2006).

Conclusion

My research is the first of its kind on the control of prone jump trajectory and is ultimately an incremental step in the understanding of the mechanics and control of prone jumps. While I have addressed how the mudskippers adjust certain kinematic aspects (body positioning, max velocity and acceleration) of their prone jumps to control jump trajectory, there is still more that could be learned. In this chapter, I have outlined a few future directions (further research into kinematics, force measurement, robotics, aerial movement analysis and comparative analysis) for this research and briefly talked about the applications of this research to robotics. The present work and future work on the control of prone jumping will contribute to the understanding of the biomechanics of prone jumping and the strategies and adaptions of amphibious fish that allow them to locomote effectively in terrestrial environments.

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Appendix

Ray amputation of the pectoral fins

To better understand the contribution of the pectoral fins during prey-capture prone jumps I elicited and filmed prey-capture prone jumps from mudskippers with amputated pectoral fins. The left and right pectoral fins of all five fish included in the non-amputated analysis were amputated where the radials meet the rays (Table A1). These trials were not analysed due to time constraints and issues such as eliciting enough prey-capture prone jumps from all the fish and regrowth of the fins during the filming period. The total number of trials filmed can be found below in Table A1.

Table A1 | Total trials filmed of mudskippers post pectoral fin amputation and their recorded length at the end of the post amputation trials.

Fish ID	Trials analysed	Trials recorded Worms	Trials recorded Crickets	Length (cm)
1	0	22	0	5.4
2	0	21	0	5.4
3	0	0	10	5.3
4	0	0	0	9.1[a b]
5	0	0	0	5.4[ad]
Total	0	43	10	

[a] Fish 4 and 5 did not complete prey-capture prone jump behaviours during the filming period and showed no interest in approaching the crickets or blood worms.

[b] 36 Escape response prone jumps were elicited from fish 4 in a final attempt to collect data on the contribution of the pectoral fin from the fish.

Summary of data collected.

Table A2| All trials collected from the mudskippers and the number of good trials observed.

Good trials are when the fish caught the food, did not slip and was visible in both camera views.

Year	Month	Day	FishID	length	Weight	Trials filmed	Good trials	# of cameras	FPS	Calibration object	Comments
2022	March	18	F1	5.2cm	1.36g	7	5	2	500	wand	
2022	March	22	F2	5.2cm	1.14g	6	5	2	500	wand	
2022	March	23	F3			1	0	2	500	wand	
2022	March	24	F3			10	5	2	500	wand	
2022	March	29	F3			7	6	2	500	wand	
2022	March	30	F3	5.2cm	0.97g	12	8	2	500	wand	
2022	August	09	F2			3	2	2	1000	wand and cube	
2022	August	16	F2			5	3	2	1000	wand and cube	
2022	August	17	F2	5 cm	1.09g	7	7	2	1000	wand and cube	
2022	August	20	F1			2	2	2	1000	wand and cube	
2022	August	21	F1			4	3	2	1000	wand and cube	
2022	August	22	F1	5.2cm	1.16g	16	9	2	1000	wand and cube	
2022	August	25	F4			4	4	2	1000	wand and cube	
2022	August	26	F4			10	9	2	1000	wand and cube	
2022	August	27	F4	8.4cm	5.48g	21	20	2	1000	wand and cube	
2022	September	06	F5			14	13	2	1000	wand and cube	
2022	September	07	F5	4.95cm	1.1g	6	6	2	1000	wand and cube	
▼ Post Amputation trials ▼											
2022	November	15	F4	9cm	7.59g						Right/left amputation
2022	November	15	F2	5.1cm	1.13g						Right/left amputation
2022	November	15	F5	5.4.cm	1.15g						Right amputation
2022	November	15	F3	5.2.cm	1.27g						Right amputation
2022	November	15	F1	5.4cm	1.17g						Right amputation
2023	February	14	F4	9.1cm	missing						Right/left amputation
2023	February	21	F4			23	0	2	1000	wand	
2023	February	22	F4	9.1cm	7.65g	13	0	2	1000	wand and cube	
2023	February	22	F3	5.3cm	1.08g						Right/left amputation
2023	February	24	F3			3	0	2	1000	wand	
2023	February	25	F3			3	0	2	1000	wand	

2023	February	26	F3				0			wand	
2023	February	27	F3			9	0	2	1000	wand	
2023	March	01	F3			8	0	2	1000	wand	
2023	March	02	F3			21	3	2	1000	wand	
2023	March	03	F3			5	1	2	1000	wand	
2023	March	04	F3			9	1	2	1000	wand	
2023	March	05	F3			11	1	2	1000	wand	
2023	March	06	F3			9	2	2	1000	wand	
2023	March	06	F4	8.6cm	7.13g						Fish euthanized
2023	March	07	F1	5.4cm	1.3g						Right/left amputation
2023	March	07	F3	5.3cm	1.1g	7	2	2	1000	wand	
2023	March	10	F1			1	0	2	1000	wand	
2023	March	14	F1			3	0	2/1	1000	wand	missing ventral videos
2023	March	16	F1			3	0	2	1000	wand	
2023	March	18	F1			1	0	2	1000	wand	
2023	March	19	F1			15	3	2	1000	wand	
2023	March	20	F1			34	13	2	1000	wand	
2023	March	21	F1	missing	missing	16	6	2	1000	wand	
2023	March	21	F5	5.4cm	1.35g						Right/left amputation
2023	March	22	F5			2	0	2	1000	wand	
2023	March	28	F1	5.4cm	1.17g						Euthanized
2023	March	28	F3	5.3cm	1.12g						Euthanized
2023	April	4	F5			1	0	2	1000	wand	
2023	April	12	F5	5.4cm	1.17g						Last day in filming tank
2023	April	18	F5	5.4cm	1.25g						Euthanized
2023	April	18	F2			10	5	2	1000	wand	
2023	April	19	F2			19	8	2	1000	wand	
2023	April	21	F2	5.1cm	1.12g						Right/left amputation
2023	April	21	F2			2	0	1	1000	wand	Missing lateral videos
2023	April	24	F2			13	8	2	1000	wand	
2023	May	3	F2	5.4cm	1.04g						Last day in filming tank
2023	May	9	F2	5.1cm	1.12g						Euthanized

Model statistics

Table A3 | Summary of final models' statistics. Parameter estimates ($\beta_{0\text{-}3}$) are listed in the order they appear in the model specification. Asterisks (*) are placed next to significant P-values.

Model type[a]	**Model specification**	**$\beta_{intercept}$ (P-value) [$CI_{95\%, lower}$, $CI_{95\%, upper}$]**	**β_1 (P-value) [$CI_{95\%, lower}$, $CI_{95\%, upper}$]**	**β_2 (P-value) [$CI_{95\%, lower}$, $CI_{95\%, upper}$]**	**β_3 (P-value) [$CI_{95\%, lower}$, $CI_{95\%, upper}$]**	**$R^2_{LMM(m)}$ $R^2_{LMM(c)}$**
LMM	***a***. _TPd ~ VFA + d + VFA*d + (1|FishID)_	0.352 (2e-16)* [0.3, 0.407]	-3.95e-2 (3.11e-8)* [-4.05, -0.181]	0.294 (4.82e-6)* [-0.405, -0.181]	5.11e-2 (9.42e-5)* [2.75e-3, 7.34e-3]	0.559 0.701
LMM	**b.** Distance ~ _Fin side + (1|FishID) + (1|FishID:Trial)_[d]	0.155 (1.98e-6)* [0.144, 0.167]	1.52e-2 (8.04e-6)* [9.04e-3, 2.13e-2]			0.11 0.465
LMM	**c.** _Ph~ Hp + (1|FishID)_	1.87e-2 (3.72e-5)* [1.11e-2, 2.64e-2]	4.26e-4 (1.78e-6)* [2.7e-4, 5.85e-4]			0.293 0.463
LMM	**d**. _Hp~ VFA + d + (1|FishID)_	13.9 (1.32e-5)* [8.92, 19]	0.549 (2e-16)* [0.48, 0.619]	7.61 (5.48e-2)* [0.104, 15.2]		0.788 0.85
LMM	**e**. _Vertical trajectory angle ~ TPd + Ph + (1|FishID)_	36.2 (1.68e-4)* [19.6, 52.8]	-138 (5.72e-6)* [-189, -84.8]	633 (2.18e-4)* [329, 939]		0.432 0.705
LMM	**f.** _Lateral trajectory angle ~ Tail plant side + (1 |FishID)_	2.45 (0.261) [-1.46, 6.76]	-9.86 (1.18e-5)* [-13.9, -5.19]			0.283 0.389
LMM	**g.** _Max speed ~ VFA + d + VFA*d + (1|FishID)_	0.5 (1.24e-3)* [0.216, 0.784]	-1.22e-4 (0.97) [-6.43, 6.04]	6.76e-2 (0.825) [-0.528, 0.66]	1.48e-2 (2.39e-2)* [2.64e-3, 2.74e-2]	0.657 0.728
LMM	**h**. _Vertical max speed ~ VFA + d + VFA*d + (1|FishID)_	8.94e-2 (0.567) [-0.211, 0.386]	1.23e-2 (0.711) [-5.19e-3, 7.66e-3]	-0.323 (0.308) [-0.929, 0.289]	2.5e-2 (3.7e-4)* [1.23e-2, 3.77e-2]	0.776 0.851
LMM	**i.** _Horizontal max speed ~ VFA + d + VFA*d + (1|FishID)_	0.241 (7.54e-2) [-1.28e-2, 0.503]	6.08e-3 (4.14e-2)* [3.73e-3, 1.17e-2]	0.962 (1.01e-3)* [0.416, 1.49]	-1.7 (4.84e-3)* [-2.8e-2, -5.63e-3]	0.169 0.331
LMM	**j.** _Acceleration to max speed ~ VFA + d + (1|FishID)_	4.51 (3.61e-2)* [0.431, 8.58]	0.203 (4.66e-7)* [0.134, 0.273]	11.1 (3.01e-2)* [4.15, 18.1]		0.514 NA[c]
LMM	**k.** _Distance ~ Cat1 + (1 |FishID) + (1|FishID:Trial)_[d]	0.255 (2e-16)* [3.43e-2, 4.94e-2]	-2.6e-2 (2.03e-2)* [-4.74e-2, -4.69e-3]			0.091 NA[c]
LMM	***l.*** _Distance ~ Cat2 + (1 |FishID) + (1|FishID:Trial)_[d]	0.239 (2.81e-2)* [0.213, 0.257]	-8.21e-3 (0.196) [-2.05e-2, 4.14e-3]			0.008 0.755
MELR	**m**. Tail plant side ~ _Lateral food angle_ + (1|_FishID_)	-0.833 (0.4) [-3.44, 1.58]	-0.393 (8.16e-4)* [-0.705, -0.215]			0.823 0.898

	Model ID	**Variable 1**	**Variable 2**	**$t_{\alpha=0.05}$**	**df**	**P-value**	**[$CI_{95\%}$]**
T-test	**n.**	Non-aerial food distance	Aerial food distance	-3.00	4	0.0399*	[-0.382,-1.48e-2]
T-test	**o.**	Non-aerial vertical food angle	Aerial vertical food angle	0.828	4	0.454	[-11.7,21.7]

[a] LMM = Linear mixed effects model, MELR = Mixed effects logistic regression

[b] *VFA* = Vertical food angle at the end of the preparatory phase, *d* = distance between the mouth and the food at the end of the preparatory phase, *TPd* = horizontal distance between the tail locus and pelvic girdle at the end of the preparatory phase, *Ph* = height of the pelvic girdle at the end of the preparatory phase, *Hp* = head pitch, Fin side = ipsilateral or contralateral, Tail plant side = left or right, Trajectory = predicted or real.

[C] The conditional coefficient of determination could not be calculated due to the lack of variance explained by FishID.

[d] Trial is a nested random effect within *FishID*. This pairs fin distance measurements from the same trial.

Table A4 | Correlation of fixed effects included in the final models.

Fixed effects	Degrees of freedom	Pearson correlation coefficient
Food distance (*d*) ~ Vertical food angle	58	0.355
Pelvic girdle (CoM) height ~ Tail-pelvic distance	58	-0.207

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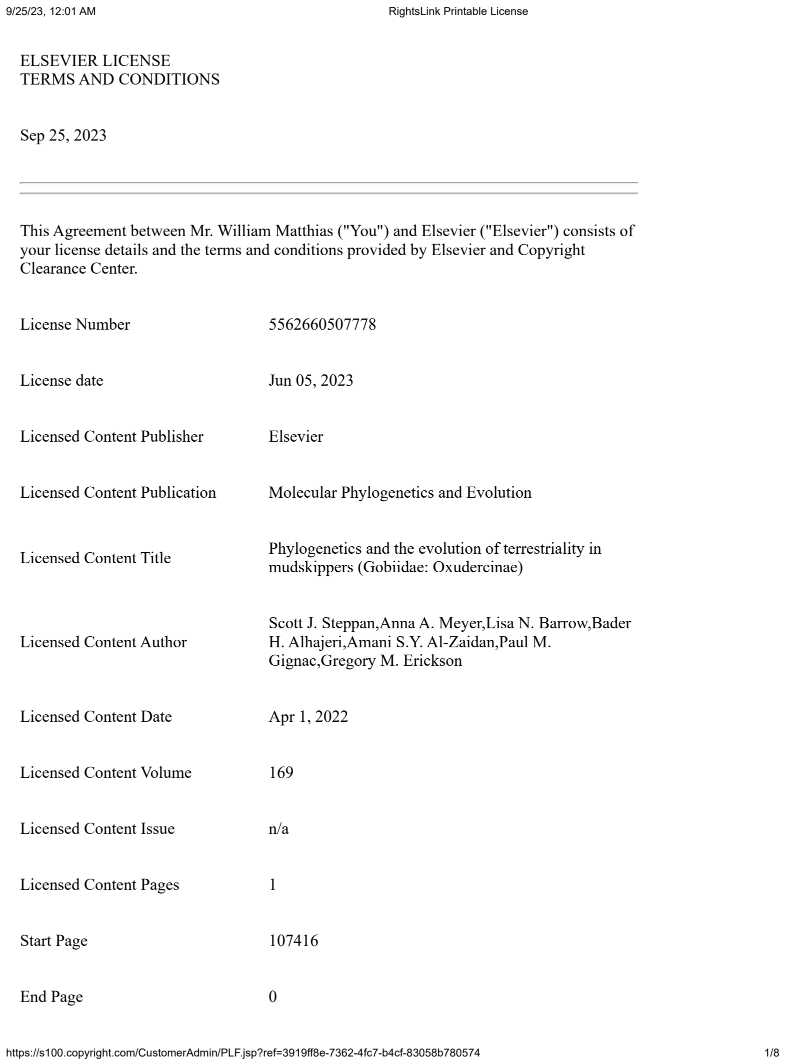

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