

SHORT COMMUNICATION

Skittering locomotion in cricket frogs: a form of porpoising

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ABSTRACT

Multiple species of frogs in the *Ranidae* family have been observed to ‘skitter’ across the water surface, but little is understood about the biomechanical or physical mechanisms that underlie this behavior. All documented descriptions are anecdotal, asserting simply that the frogs can cross the water surface without sinking. To study this form of interfacial locomotion, we recorded high-speed video of the northern cricket frog *Acris crepitans* and quantified its kinematics. We also compared its semi-aquatic behavior with the frogs’ terrestrial locomotion. Contrary to expectations based on anecdotal knowledge, we found that cricket frogs do not maintain an above-surface position throughout the locomotor cycle. Instead, the frogs are completely submerged during both the launching and landing phase of a jump cycle, similar to porpoising in other animals. It is possible that leg-retraction time constrains these frogs from performing true surface-only locomotion.

KEY WORDS: Water-surface locomotion, Biomechanics, Jumping, Amphibians

INTRODUCTION

Frogs and toads (anurans) exhibit many forms of terrestrial and aquatic locomotion, including hopping and jumping (Cox and Gillis, 2015), bounding (Reilly et al., 2015), walking (Reynaga et al., 2018), running (Ahn et al., 2004) and swimming (Gal and Blake, 1988). Frogs have also been reported to perform an additional form of locomotion known as ‘skittering’, a hopping-like behavior involving the air–water interface. Skittering has been described in the natural history literature as ‘jumping on the water surface without sinking’ (Gans, 1976), which suggests that the animal’s body remains above the water surface. If so, water hopping in skittering may be governed by similar interfacial dynamics to those in other animals such as the water-running basilisk lizards (Glasheen and McMahon, 1996a,b; Hsieh, 2003; Hsieh and Lauder, 2004) and grebes (Clifton et al., 2015).

However, although 11 species of frogs have been noted to skitter (Annandale, 1918; Blair, 1950; Chabanaud, 1949; Cunningham, 1964; Duellman and de Sá, 1988; Dunn, 1928; Flower, 1896; Herrmann and Edwards, 2006; Inger, 1966; Romer, 1951), all previous accounts stem from anecdotal observations. For such fast-moving animals, answering questions involving locomotor kinematics requires high-speed imaging or some other fast

time-resolved technique. Recent observations using high-speed video, for example, have revealed that geckos slap the water’s surface like a water-running basilisk lizard, but do not produce enough force to lift the body completely above the surface, and thus ‘skim’ on the water surface (Nirody et al., 2018). These geckos show that interfacial locomotion in atypical animals is possible even with a partially submerged body.

Do frogs also submerge their bodies when they skitter? Geckos maintain the posterior portion of the body underwater during water-running (Nirody et al., 2018), and frogs are capable of jumping from the water surface while partially submerged (Nauwelaerts et al., 2004; Wilkinson, 2014), suggesting that skittering need not exclusively be an above-surface form of locomotion. To test the hypothesis that skittering in frogs is a solely above-surface phenomenon, we examined the interfacial locomotion of the cricket frog *Acris crepitans*, which has been previously noted to skitter (Blair, 1950; Hudson, 1952; Milstead, 1972; Wright and Wright, 1933). Using high-speed videography, we also compared skittering performance with terrestrial hopping in this species.

MATERIALS AND METHODS

Animals

A total of 15 male cricket frogs (*Acris crepitans* Baird 1854) were collected from a swamp in Merchants Millpond State Park (Gatesville, NC, USA) in June 2013. Only males were collected in an effort to minimize the effect on the pond’s natural breeding population. Frogs were kept individually in 35.6×27.9×15.9 cm (L×W×H) containers [Sterilite® deep clip box (1965), Sterilite Corporation, Townsend, MA, USA] lined with coconut fiber or reptile eco carpet (Zoo Med Laboratories, Inc., San Luis Obispo, CA, USA) and fed crickets *ad libitum*. All experiments were approved by Virginia Tech’s Institutional Animal Care and Use Committee (13-014, 15-228). Animal collection was approved by the Virginia Department of Game and Inland Fisheries (permit #047841), the North Carolina Division of Wildlife Management (permit #13-SC00789), and Merchants Millpond State Park (research permit #R13-34). See [Supplementary Materials and Methods](#) for morphometrics of frogs used in the analysis.

Aquatic kinematic data collection

Recordings of locomotion were conducted in a 20 gallon glass tank (~76 l, 76.2×30.5×30.5 cm) with approximately 20 cm of water treated with a dechlorinator (API Stress Coat+, Mars Inc., McLean, VA, USA). Floating platforms, consisting of fake lily pads or Styrofoam slabs, were provided at both ends of the tank. The tank was illuminated from the top and side with two tungsten lights diffused through a softbox (Everlight, JTL Corp, Miranda, CA, USA). To begin a trial, frogs were released by hand into the tank at one end and then (if necessary) encouraged to move from one end of the tank to the other by gentle prodding with an acrylic rod or with the experimenter’s fingers. Resulting sequences of interfacial locomotion were recorded with a front-facing high-speed camera (Fastec TS3, Fastec Imaging, San Diego, CA, USA), which was

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positioned orthogonally to the long end of the tank. Video was recorded at 250 or 500 frames s^{-1} . The frogs were encouraged to repeatedly perform interfacial locomotion until they swam to the bottom of the tank or feigned death, a behavior also noted in the wild (McCallum, 1999). A total of 45 experimental trials were recorded from 5 frogs.

As an additional qualitative check in the wild, 4 frogs were captured at the pond and released immediately, while filming their resulting escape behavior in the water (GoPro Hero4 Black, GoPro, Santa Cruz, CA, USA). These trials were not included in data analyses but are provided as a supplementary video (Movie 2).

Data analyses

For all aquatic jump sequences, the duration of time that the frog spent above, below and intersecting the water surface was determined using frame-by-frame analysis and a custom Python script. Each sequence contained up to four consecutive jumps, but not all occurred in the camera's field of view. The start of a jump cycle's aerial phase was defined as the frame immediately after the animal broke the surface of the water. Of the 45 recorded sequences, 30 contained at least one full jump cycle, which were used for estimating the ratio of underwater to above-water timing and then pared down for further duration analyses.

In a subset of these sequences in which the frog's trajectory was generally orthogonal to the camera ($n_s=15$ sequences, encompassing $n_j=31$ jumps), we analyzed the kinematics in more detail. Some of these sequences contained partial jumps, with the frog jumping into or out of the field of view. This approach resulted in not all kinematic metrics having the same n_j – if only the end of a jump was on camera, for example, that partial jump could be used to measure recovery phase timing, but not the metrics in the start of the jump. Prior to analysis, images were corrected for uneven refraction and magnification caused by density differences between air and water using a custom Python script (see Fig. S1). The scale of the aerial portion of the body was adjusted so that the body was fully aligned to the underwater portion; this scale value was used to correct all frames in the corresponding trial.

To track the approximate center of mass and body angle of the frog, we used a custom Python script that allowed a user to identify the frog's trunk in each video frame using the watershed algorithm of OpenCV (3.4.1-dev; <https://opencv.org>) (Bradski, 2000) (see Fig. S2). The body angle (relative to the waterline) and (X, Y) position of the center of the body were calculated from the image moments of the resulting segmentation contour. The time-varying body angle and position data were smoothed independently using a fourth order, zero phase shift Butterworth filter applied twice, with the cutoff frequency determined using residual analysis to balance the signal-to-noise ratio (Winter, 2009).

To determine when the frog's limbs were either retracting (moving towards the body) or extending (moving away from the body), each frame in a video was compared with the following frame to score the state of the leg. Only legs on one side of the body (those closest to the camera) were analyzed.

To determine instantaneous velocity, we calculated the second-order central difference of the smoothed position data using `numpy.gradient` from SciPy (<https://scipy.org/>; Virtanen et al., 2020).

Terrestrial experiments

For terrestrial experiments, frogs were placed in 20 gallon glass tank (~ 76 l, $76.2 \times 30.5 \times 30.5$ cm) lined with black foam mats. The tank

was front-lit with two LED light banks (Model id5000-V1, Ikan International, Houston, TX, USA). To begin a trial, frogs were paced by hand onto the foam substrate on one side of the tank at one end and then encouraged to move from one end of the tank to the other by gentle prodding with an acrylic rod or with the experimenter's finger. Frogs had to be encouraged to jump every time, including multiple jumps in the same sequence. Resulting sequences were recorded with a front-facing high-speed camera (Photron APX-RS, Photron USA, Inc., San Diego, CA, USA) at 500 frames s^{-1} , which was positioned orthogonally to the front of the tank. Frogs were able to jump 2–3 times in the tank before reaching the other side, after which the frog would be turned around and encouraged to jump some more. A total of 67 jumps from 29 sequences for 3 frogs were recorded, from which $n_s=26$ sequences, encompassing $n_j=59$ jumps, were used for analysis. Only generally orthogonal jumps were included, excluding jumps to or from the side tank wall.

To provide comparisons with the skittering trials, propulsion time, aerial time, wait time and recovery time were determined from manual frame counts in visual inspection of the videos. Of these sequences, jumps in which the frog jumped mostly orthogonal to the camera had their jump distance and maximum jump height measured in ImageJ (Schneider et al., 2012).

Statistical methods

Terrestrial and aquatic locomotion were compared using a mixed model in JMP statistical software (SAS, Cary, NC, USA). The 'Fit Model' platform was used to estimate model parameters and determine statistically significant differences between kinematic variables. Restricted maximum likelihood (REML) estimation was used with bounded variance components. Experiment type (skittering vs terrestrial) was fit as a fixed effect while frog ID, sequence, and jump number (in the sequence) were specified as random nested effects. The jump range, height, recovery time (time for hindlimbs to retract completely) and wait time (the time between landing and the beginning of hindlimb retraction) were analysed. More details about statistical methods can be found in the supplemental material and on figshare (doi:10.6084/m9.figshare.20720935).

RESULTS AND DISCUSSION

Interfacial locomotion in *A. crepitans* consists of rapid, consecutive jumps that originate underwater, in which the frog's limbs push against the water to generate thrust, producing cycles with both underwater and above-water components. We defined each jump cycle as consisting of four phases: takeoff, aerial, re-entry and recovery (Fig. 1; see Movie 1). Takeoff begins with the frog completely submerged (mean \pm s.d. depth 0.86 ± 0.2 cm, $n_s=15$, $n_j=25$) as the animal initiates hindlimb extension, and ends when the frog's hindlimbs are fully extended, and thus force production from the hindlimbs ends. The duration of takeoff averaged 77.5 ± 3.4 ms ($n_s=15$, $n_j=26$), with the frog being at 20.0 ± 5.7 deg ($n_s=15$, $n_j=26$) at water exit, resulting in a maximum velocity of 112.5 ± 1.8 cm s^{-1} ($n_s=15$, $n_j=24$). During the aerial phase, which lasts until the frog re-contacts the water (duration 159 ± 52.3 ms, $n_s=15$, $n_j=13$), the frog follows a mostly ballistic trajectory and the hindlimbs remain fully extended (Fig. 1). While in the air, body angle tends to decrease toward more horizontal, and the frog's forelimbs move from being held against the body to extended anteriorly (Fig. 1). The re-entry phase or wait time (duration 110.8 ± 41.3 ms, $n_s=15$, $n_j=16$) begins as the frog first contacts the water with its hindlimbs (when the tracked center body point hits the

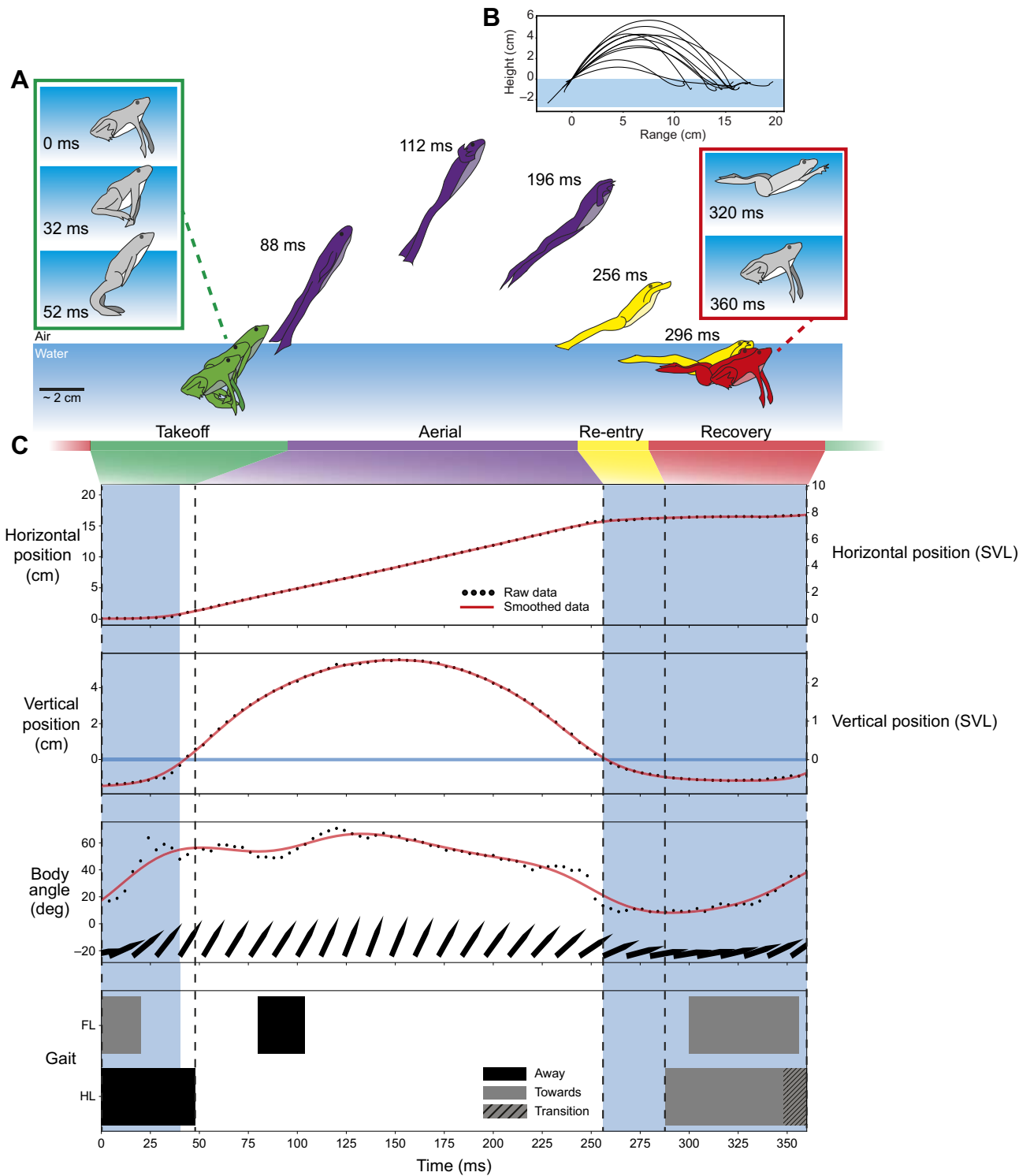


Fig. 1. Kinematics of interfacial locomotion in the cricket frog *Acris crepitans*. Colors correspond to takeoff (green), aerial (purple), re-entry (yellow) and recovery (red) phases. Data in A and C are from a single trial; data from other trials are provided in the supplementary information (Supplementary Materials and Methods, 'Figure collection S3', see figshare doi:10.6084/m9.figshare.20720935). (A) Representation of postural changes in a frog (mass 1.09 g; snout–vent length, SVL, 21.4 mm) during one full cycle of interfacial locomotion, shown in side view. The inset boxes show details obscured from view during phases with little translation. (B) Height versus range for the 11 complete jumps in the dataset. (C) Kinematics of one representative cycle versus time. Data represent the position of the center of the frog's body, determined by the watershed method (see Fig. S2). The background is blue whenever the frog's body center is under the water surface. Phase transitions between take-off, aerial, re-entry and recovery are marked by vertical dashed lines. Body angle was calculated relative to the horizontal water surface; the black icons below the trace provide a visual representation of the angle for select times. In the gait diagram, bars represent times when the forelimbs (FL) or hindlimbs (HL) were moving away from (extension) or toward (retraction) the body. The transition region indicates when the hindlimbs were switching from retraction to extension, and as such the state was hard to manually grade.

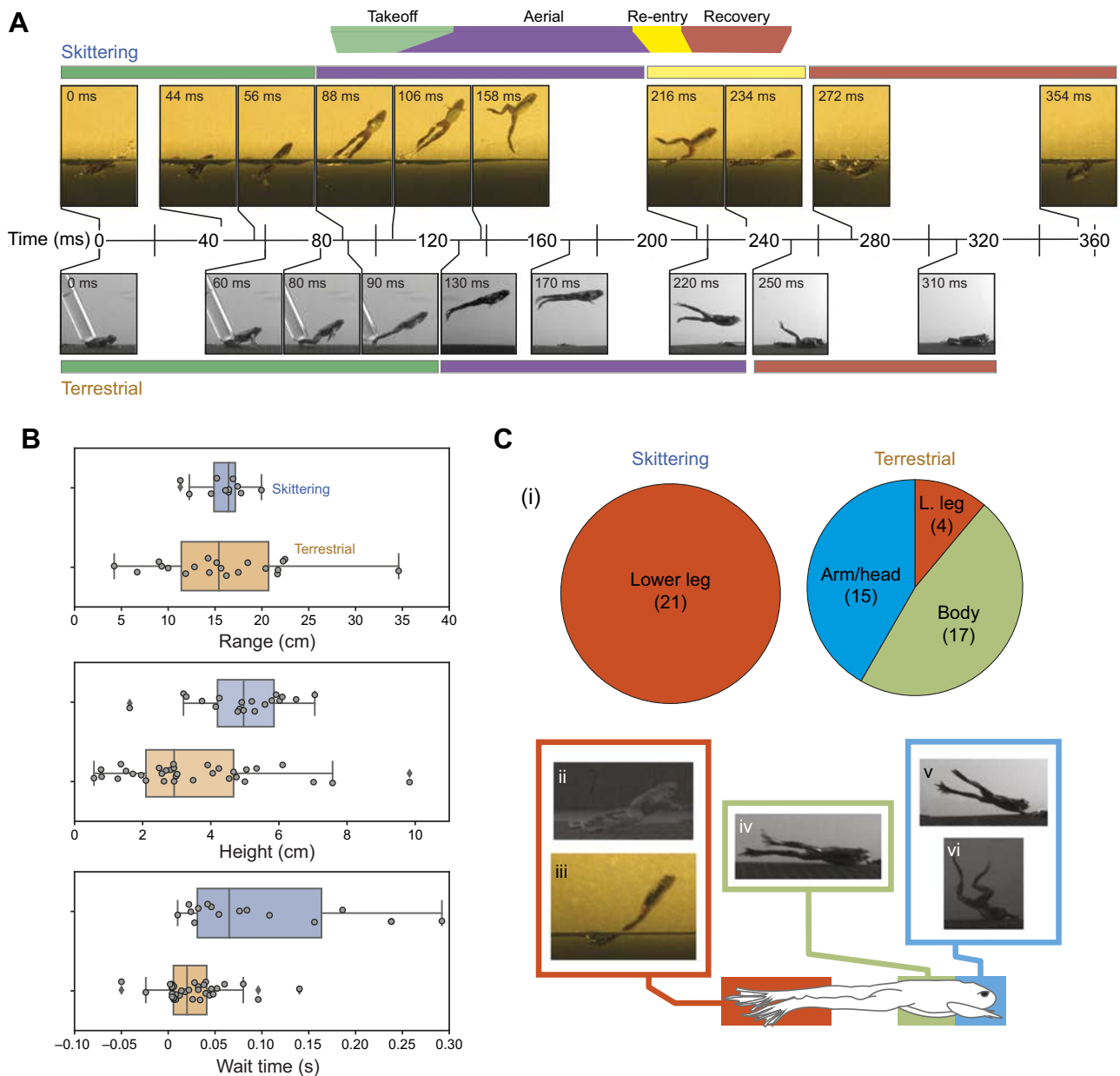


Fig. 2. Terrestrial versus skittering locomotion in *A. crepitans*. (A) Comparison of interfacial (skittering; top) and terrestrial (bottom) locomotion in the same frog (mass 1.55 g, SVL 22.3 mm). (B) Comparison between interfacial jumps and terrestrial jumps for range (skittering $n_s=15$ sequences, $n_j=11$ jumps; terrestrial $n_s=16$, $n_j=20$), height above water (skittering $n_s=15$, $n_j=22$; terrestrial $n_s=22$, $n_j=33$), and for wait time, the time between water/land contact and the beginning of recovery (hindlimb retraction) (skittering $n_s=15$, $n_j=16$; terrestrial $n_s=24$, $n_j=36$). In terrestrial trials, negative wait time values represent when the frog began retracting its limbs before touching the ground. Box plots show median, upper and lower quartiles and $1.5\times$ the interquartile range. Only wait time was significantly different between terrestrial and skittering locomotion, as determined using mixed models in JMP (SAS, Cary, NC, USA) with type as a fixed effect and frog ID, sequence and jump as completely nested random effects. (C) Comparison of landing position of the frog (skittering $n_s=15$, $n_j=21$; terrestrial $n_s=24$, $n_j=36$). The pie charts (i) indicate the number of jumps in which the frog contacted the substrate with each body part (ii–vi). Owing to the resolution of the image, it was difficult to distinguish between arm landing (v) and face planting (vi), so they were combined into one category.

water surface, the frog has a body angle of 18.1 ± 2.4 deg, $n_s=15$, $n_j=18$) and ends once the frog is fully submerged. The hindlimbs are the first body part to re-enter the water, followed by the trunk and head (Fig. 2A,C). As the frog sinks below the water surface, the hindlimbs continue to remain nearly fully extended, with the animal holding this posture, and the body translation comes to a complete stop (see Supplementary Materials and Methods, ‘Figure collection S3’, see figshare doi:10.6084/m9.figshare.20720935). After re-entry comes the recovery phase (duration 77.5 ± 3.4 ms, $n_s=15$, $n_j=21$), where the frog retracts both

its hindlimbs into a fully flexed configuration and its forelimbs into an adducted position against the trunk (Fig. 1). Overall, the frog spends 54.2% (148 ± 34.7 ms, $n_s=30$) of the cycle underwater and 45.8% (125 ± 29.4 ms, $n_s=30$) in the air, with a total displacement of 16.2 ± 0.3 cm ($n_s=15$, $n_j=11$) horizontally and 3.7 ± 2.0 cm ($n_s=15$, $n_j=19$) vertically. During the experiment in the tank, frogs jumped up to 3 times in a row without additional encouragement. In the natural pond using a similar handling protocol, freshly caught frogs exhibited up to 8 consecutive jumps (see Movie 2).

The kinematics of interfacial locomotion are similar to hopping on land. Using mixed models in JMP, the height ($P=0.0734$) and range ($P=0.9843$) were determined to not be significantly different between water and land hopping (see Fig. 2B). Recovery time was determined to be significantly different among water- and land-hopping frogs ($P=0.0175$), with a mean difference of -0.039 s for water–terrestrial and a 95% confidence interval of $(-0.070$ s, -0.008 s), indicating terrestrial jumps incur slightly longer preparation time. Wait time (the time between body impact on land/water and beginning of recovery; see Fig. 2B) was also determined to be statistically significant ($P=0.0006$), with a mean difference of 0.072 s for water–terrestrial and a 95% confidence interval of $(0.034$ s, 0.111 s). During interfacial skittering, frogs always hit the water with their lower legs, with a consistent body angle (s.d., ~ 2 deg); by contrast, terrestrially hopping frogs landed in multiple configurations including landing on their belly and head, and using their forelimbs to attempt to control landing. Additionally, during interfacial skittering, frogs would usually jump multiple times in a row with no additional encouragement; in terrestrial trials, frogs required encouragement for each jump.

Based on undocumented filming, Gans (1976) described skittering in the frog *Rana cyanophlyctis* (now *Euphlyctis cyanophlyctis*) as a locomotor behavior that takes place exclusively on and above the water's surface. However, our high-speed videos reveal that the skittering frog *A. crepitans* employs a different form of locomotion, characterized by two unexpected features: (1) separate phases in which the frog is completely in the air, partially submerged or completely submerged; (2) a momentary but distinct pause between locomotor cycles as the animal comes to a stop below the water before initiating the subsequent propulsive jump. These results demonstrate that Gans' (1976) conception of skittering does not apply to all skittering frogs. How widespread this conclusion is among the 11 identified species of skittering frogs remains unknown. This discovery corroborates the power of modern techniques to help clarify and sometimes counter canonical knowledge derived from natural history observations.

Other animals such as dolphins (Lusseau, 2006), birds (Blake and Smith, 1988), seals (Williams and Kooyman, 1985), otters (Packard and Ribic, 1982) and fish (Hsieh, 2010) also locomote above and below the water's surface using a combination of jumping and aerial ballistics known as porpoising. When porpoising, an animal swims to build speed, leaps out of the water, splashes down, coasts inertially and finally accelerates using muscle power to launch from water again, all in continuous motion. These animals are momentum jumpers, using the energy from the previous jump to aid their next leap out of the water (Chang, 2018). Cricket frogs appear to use a form of porpoising, with one key distinction: cricket frogs come to a stop following each leap from the water and thus do not harness any energy from the previous jump (see figure collection S3 at doi:10.6084/m9.figshare.20720935.v1). Their lack of momentum travel likely results from their small body mass in relation to their relatively high surface area and thus their high drag.

Cricket frogs are capable of propelling themselves from a completely submerged position to several centimeters above the water from a standstill. The question remains, why do they not remain in the air, jumping on the water surface itself? Our kinematic data comparing aquatic and terrestrial sequences suggest that limb retraction speed provides a biomechanical constraint. In order to jump on the water surface without sinking, an animal must have its limbs prepared for another jump before it reaches the water surface again. Essentially, a frog must be able to use a bounding form of locomotion in order to skitter on the water

surface. However, in both terrestrial and porpoising locomotion in *A. crepitans*, the frog reaches the landing surface ~ 50 ms before it begins to retract its hindlimbs. After contacting the land or water, it takes an additional 750 ms to fully retract the limbs to prepare for another jump. This retraction speed may reflect a phylogenetic constraint (Reilly et al., 2016), suggesting that a barrier to jumping or running on water for all animals may not be force production, but speed.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: T.W., G.B.G., J.J.S.; Methodology: T.W.; Software: T.W.; Validation: J.V.M.; Formal analysis: T.W., J.V.M.; Investigation: T.W., J.J.S.; Resources: J.J.S.; Data curation: T.W.; Writing – original draft: T.W., G.B.G., J.J.S.; Writing – review & editing: T.W., G.B.G., J.V.M., J.J.S.; Visualization: T.W.; Supervision: G.B.G., J.J.S.; Project administration: J.J.S.; Funding acquisition: J.J.S.

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Data availability

Additional supplemental material is available on figshare (doi:10.6084/m9.figshare.20720935.v1) and GitHub (https://github.com/TheSochaLab/Skittering.locomotion.in.cricket.frogs).

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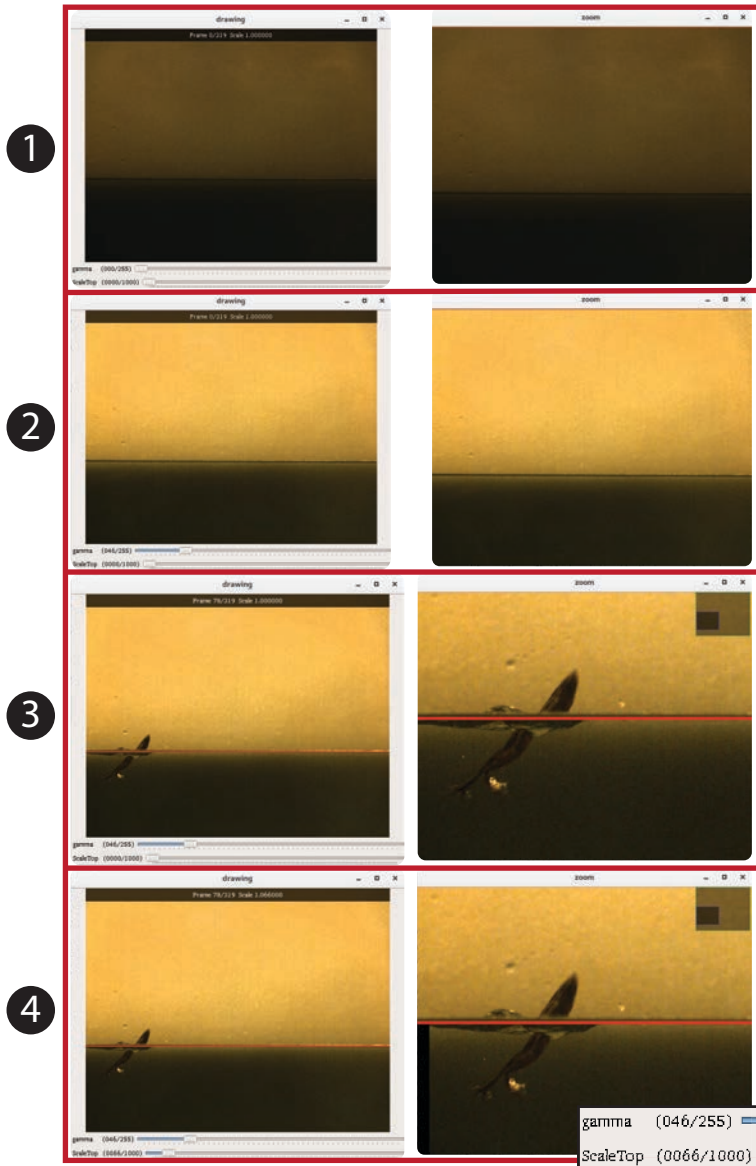
Supplementary Materials and Methods

Morphometrics

Data from 5 frogs was used in determining the gross amount of time spent above and below the water surface for all skittering trials. However, only 3 of these frogs had sequences which were perpendicular to the camera view, and could therefore be used in the remaining kinematics analyses. These frogs were all male and consisted of: ID AC01, SVL 20.1 mm, mass 1.25 g; ID AC03, 22.4 mm, 1.09 g; ID AC04, 22.3 mm, 1.55g. The additional two frogs used for the analysis of how much time was spent above and below water were AC02, 1.13 g and AC08, 3.06 g.

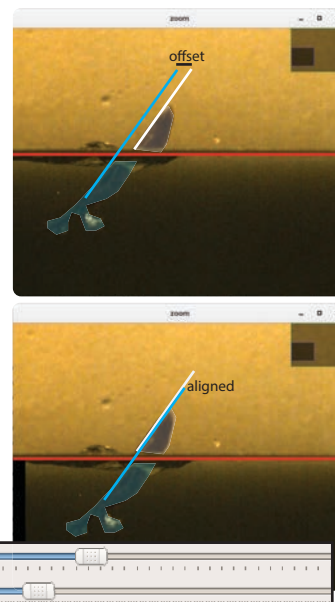
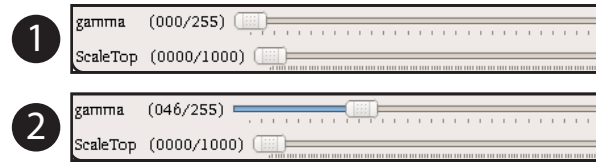
A table of this information can be found in Table S1 on figshare (doi:10.6084/m9.figshare.20720935).

Because the optical properties of air and water differ, the scale of the frog under and above water in the experimental images are quite different. This needed to be corrected to more accurately determine kinematics. This program, running once per video analyzed, resizes different parts of the image in each frame to correct this scale difference



On initial run of the program, the user is presented with two screens. On the left is the original image, and on the right is the edited image. Using the arrow keys, the user can scroll through the video. Below the image on the left screen are the controls the user can use to change the brightness of the image and the eventual scale increase of the air portion compared to the bottom.

Below shows the change of gamma used to brighten the image between (1) and (2)



After image brightness is corrected, the user left clicks on the left image to determine the air-water interface. The user can also zoom into each frame to help visualize the offset between the frog's body above and below water.

When increasing the ScaleTop slider, the program rescales the above water portion of the image. The user does this until the frog's body above and below water are aligned. The user can also scroll through the video to see ensure that the scale works for all frames of the video.

RESULT

When the user is happy with the increased scale of the above water portion, the program will go through the entire video and resave the images with the scale modification performed. The heights and width of the image are modified according to the following formula:

$$\begin{aligned} {}^s h_a &= \text{round}(h_a \times \text{scale}) \\ {}^s h_w &= \text{round}(h_w \times \text{scale}) \\ {}^s w_a &= w_a \\ {}^s w_w &= w_w \end{aligned}$$

Where $\text{scale} = \text{ScaleTop}/1000 + 1$
The below water portion of the frame is then centered with respect to the rescaled above water portion

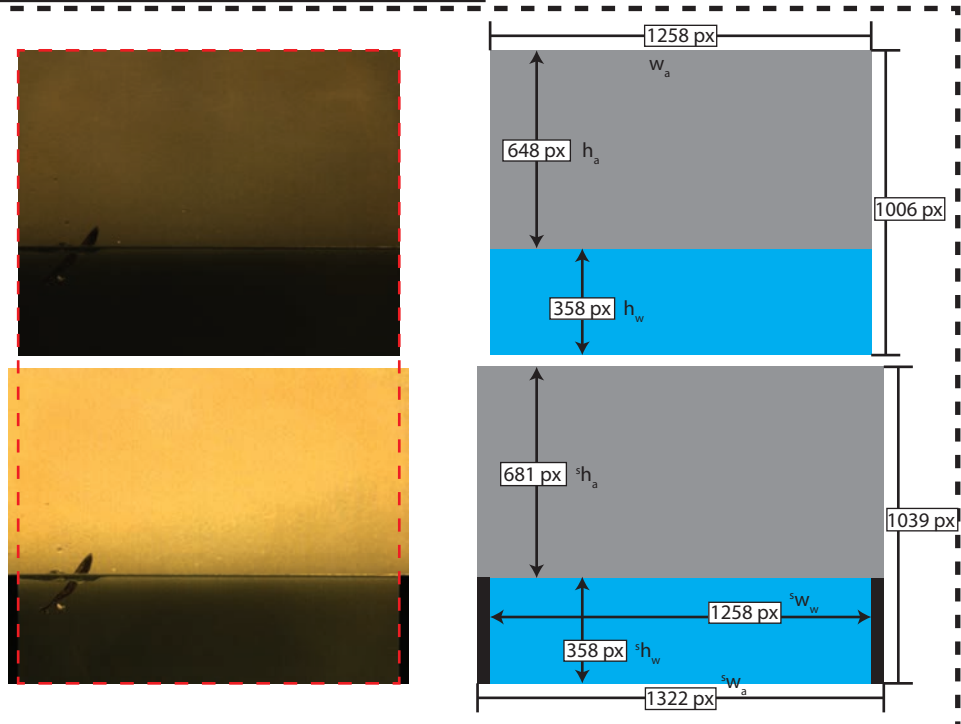
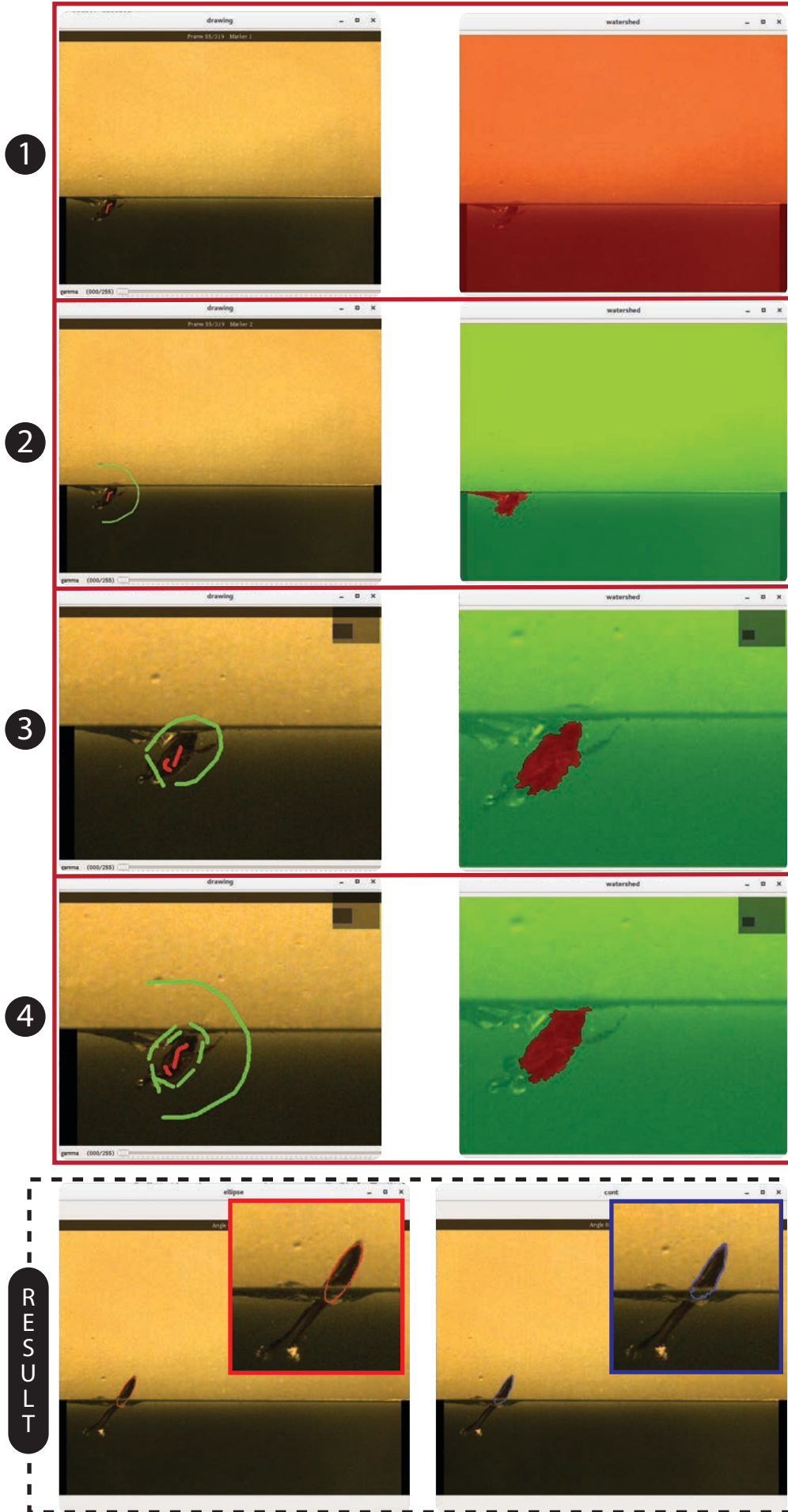


Fig. S1. Water/air scale adjustment and frog body alignment

For each frame of every analyzed sequence, a supervised watershed segmentation algorithm was performed to get a segmentation of only the frog's body (not counting arms and legs) for kinematic analysis. Below shows screenshots of the custom python code run on each image frame



The user chose two colors with which to seed the watershed algorithm. These are chosen with numbers on a keyboard. The user then draws on the left image with the mouse. The program uses the marked locations as seeds in the color watershed algorithm, and shows the resulting segmentation on the right.

The user then iteratively marked the desired foreground and background of the frame by adding more markers of each color to the seeds for the watershed algorithm.

This program also allows zooming on the image for more precise marker placement to get a better resultant segmentation

After several iterations of adding more markers on the left image, and the user is happy with the resulting segmentation shown on the right, the user presses a key to finalize the segmentation.

The marker locations and final segmentation mask are then saved with a reference to the video and frame number, and a contour is calculated (using OpenCV functions) for the final segmentation

The final contour and fit ellipse is shown on the frame based on the finished watershed segmentation.

From this final contour, the image moments are calculated to yield the centroid and orientation of the frog's body.

Fig. S2. Watershed segmentation of the frog body

Figure Collection S3: Detailed kinematics for all aquatic sequences

Data for all 15 sequences in which the frog jumped perpendicular to the camera is provided on figshare (doi:10.6084/m9.figshare.20720935).

The title of the graphs gives the frog ID and the sequence number of the trial. The blue background of the graph indicates that the center of the frog's body is underwater.

For these figures, smoothed data (red line) were calculated using a fourth order, zero-phase shift Butterworth filter applied twice, with the cutoff frequency determined using residual analysis to balance the signal-to-noise ratio [1]. Body angle is calculated relative to horizontal; the black icons below the trace provide a visual representation of the angle for select times. In the gait diagram, bars represent times when the forelimb (FL) or hindlimb (HL) are moving away from (extension) or toward (retraction) the body. The transition region indicates when the hindlimbs were switching from retraction to extension. A black dot-dashed line is overlaid on the gait, velocity, and acceleration graphs at the time hindlimb extension ends. These lines help to make comparisons of end-of-propulsion to the velocity and acceleration profiles easier to analyze.

Instantaneous velocity was calculated by taking the second-order central difference of the smoothed position data using `numpy.gradient` from SciPy [2,3]. A dotted, black horizontal line at 0 cm/sec velocity and 0 cm/sec² acceleration is provided to more easily determine when the frog is at rest, and when maximum velocity occurs. Additionally, a horizontal black line is provided on the vertical and total acceleration plots at the value of gravitational acceleration.

Statistical Methods Description

All tables in this section can be found on the figshare collection (doi:10.6084/m9.figshare.20720935).

The 'Fit Model' platform from JMP statistical software was used to estimate model parameters and determine statistically significant differences. Restricted maximum likelihood (REML) estimation was used with bounded variance components. Experiment type (skittering vs terrestrial) was fit as a fixed effect (fixed effects are used to compare specific levels of an independent variable). In this context, fixed effects test the null hypothesis of whether all group means are equal or there at least one group mean is different from the others.

Individual frog ID and jump sequence (frog) were specified as random nested effects. The effect of jump(sequence, frog) functions as the residual. Random effects are used to indicate that frog, sequence

with frog, and jump with sequence and frog are sources of variation. However, comparing specific frogs, sequences, or jumps are of little value as these same animals and occurrences will not be observed again. Instead, this approach acknowledges that these frogs, sequences, and jumps are sampled from a larger population. Random effects test the null hypothesis of whether or not a variance is different from zero. Nesting accounts for the hierarchical nature of the model (i.e., jumps are within sequences for each frog). One would expect jump sequences within a frog to be more related than jump sequences across frogs. Similarly, one would expect jumps within a sequence to be more related than jumps across sequences. The time order of jumps was not considered in this analysis. Details of the analysis are shown in the form of p-values, means, differences in means, and their associated confidence intervals in subsequent sections.

Results and Interpretation

p-values

The table of p-values summarizes the statistically significant effect of type for each response can be found in Table S-2 on figshare (doi:10.6084/m9.figshare.20720935).

Group means for type

The group mean, standard error, and 95% confidence interval for each response and each level (water and terrestrial) are shown for the fixed effect of Type in Table S3 on figshare (doi:10.6084/m9.figshare.20720935).

Differences in Group Means for type

In Table S4, the differences of the means for each level of the fixed effect (water versus terrestrial) are shown along with their associated confidence intervals. Consistent with the statistical significance shown through the p-values, the 95% confidence intervals with second duration prep (i.e., recovery time) and second duration wait (re-entry) do not include zero. The average difference in water versus terrestrial jumps for SecDurationPrep is -0.039 s, indicating that terrestrial jumps have a longer preparation time when compared to water jumps. The 95% confidence interval indicates that a range of likely values for the true mean difference in SecDurationPrep for (water-terrestrial) is (-0.070 s, -0.008 s). The average difference in water versus terrestrial jumps for SecDurationWait is 0.0724 s, indicating that water jumps have a longer wait time (or re-entry time) when compared to terrestrial jumps. The 95% confidence interval indicates that a range of likely values for the true mean difference in SecDurationWait for (water-terrestrial) is (0.034 s, 0.111 s). Note the error term for the confidence intervals is estimated from the variance components in the mixed mode and is a combination of frog to frog, sequence within frog, and jump within sequence and frog variability.

Variance Components

In models, variance components are also of interest to the researcher. In this case, the mixed model estimates the frog-to-frog variability, sequence-to-sequence variability, and jump-to-jump variability. For all responses analyzed in the study, jump-to-jump variability within sequence and frog (modeled as the residual) accounts for the majority of the total variability, with the exception of propulsion time. These majorities range from 62%-96% as shown in Table S5. Propulsion time yields a variance component for jump within sequence and frog of 43%. Sequence-to-sequence variability within frog is the next largest source of variability in AboveWaterActual Height in cm, secDurationPrep, and secDurationWait. It is the largest source of variability for secDurationPrep. It is negligible for the Range responses. Frog-to-frog variability is the smallest for all responses.

Limitations and Generalizability of Statistical Results

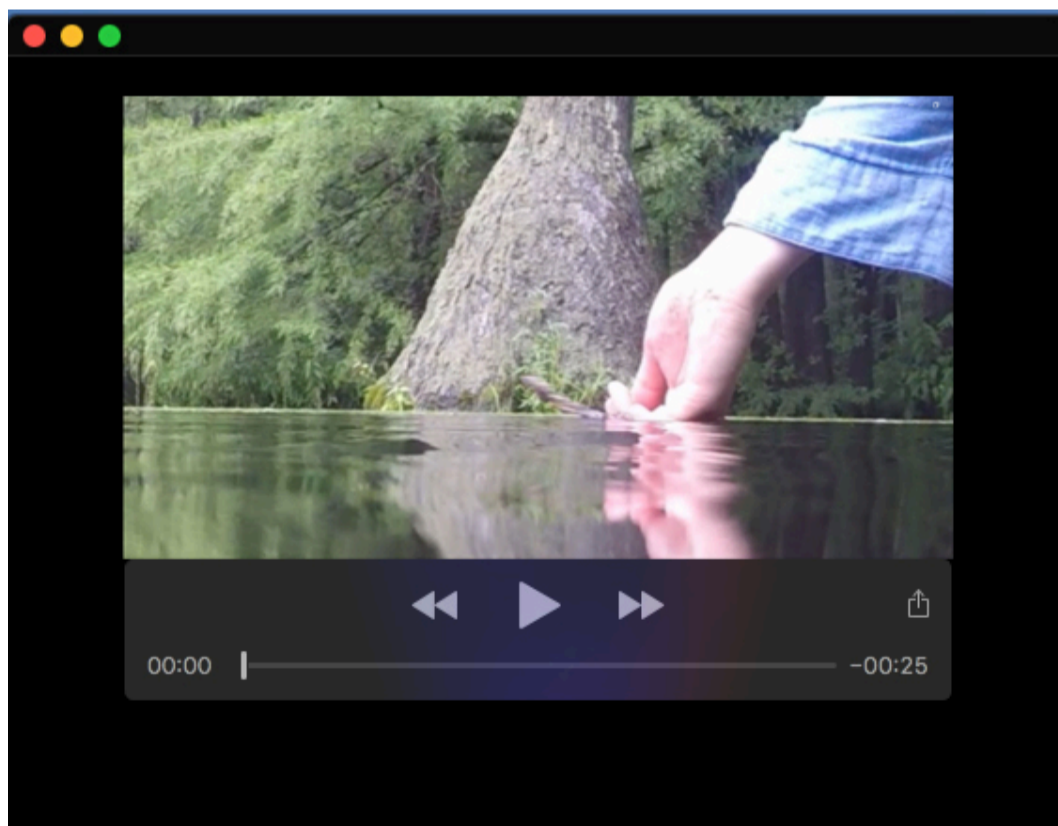
As stated in the body of the study paper, there are many sources of limitations for the statistical results. Results should be generalized with caution and corroborated with future expanded studies. Limitations include the limited number of frogs, the varied number of sequences and jumps within sequence per frog, and the fact that only two of the four frogs in the study participated in both types of experiments. In addition, only three of the four frogs completed each type of jumping. The small sample sizes make the study more susceptible to the effects of outliers in terms of individual jumps, sequences, and frogs. Variation in the camera set-up also contributes to the limitations of the study. Future experiments should be carried out with more subjects and, as much as possible, subjects participating across the experimental conditions in a balanced way. Furthermore, experimental setups should be as consistent as biologically possible to ensure the statistical analysis is measuring the effect of type versus confounding factors. The variance components in this study could be used to estimate the number of subjects required in a power analysis. However, due to the low number of subjects, a scenario planning approach is recommended which would account for increases in the variance components observed in this study.

Supplemental references

- [1] Winter DA. 2009 *Biomechanics and Motor Control of Human Movement*. 4th edn. Wiley.
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- [3] Virtanen P *et al.* 2020 SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nat. Methods* **17**, 261–272. (doi:10.1038/s41592-019-0686-2)



Movie 1. An example of skittering locomotion in the frog *Acris crepitans* in an experimental trial. The sequence is shown first at normal speed and then at 5% speed.



Movie 2. An example of skittering locomotion in the frog *Acris crepitans* released shortly after capture. The specimen was captured at Merchants Millpond State Park (Gates County, NC, USA), and the skittering bout was filmed within two hours of capture. Duckweed was pushed away from the filming area to prevent interaction. Filmed on a GoPro Hero4 Black. The sequence is shown first at normal speed and then at 10% speed (zoomed in 2X, and roughly motion tracked). In the sequence, the frog jumped 7 times.