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PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.701v1 | CC-BY 4.0 Open Access | rec: 16 Dec 2014, publ: 16 Dec 2014

Illustrating a free, open-source method for quantifying locomotor performance with sprinting Aegean wall lizards

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Abstract: Locomotion is an important characteristic of many animals' natural history. With the increasing availability of high-speed video cameras, videography is a powerful tool for analyzing fast or subtle motions with unprecedented resolution. However, the programs currently available for analyzing these videos are either dauntingly time intensive or prohibitively expensive. We have developed a free, open-source video analysis program, SAVRA, that enables the quick capture of scaled position data. Here we demonstrate its use with an analysis of several videos of the Aegean wall lizard (*Podarcis erhardii*). We hope making this program freely available will facilitate the analysis of video data across taxa, not just in laboratory settings but also in natural contexts.

Keywords: functional biology, locomotion, sprint speed, analytical tools, lizard, *Podarcis erhardii*

Introduction:

Whole-organism performance metrics provide valuable information on the natural history and evolution of animals; vertebrates and invertebrates (Huey, 1980; Reidy, Kerr & Nelson, 2000; Vanhooydonck, Herrel & Irschick, 2006; Hawlena et al., 2010). Locomotion performance in particular is critical for foraging and escape behaviors, and integrates a suite of associated morphological and physiological traits (Bauwens et al., 1995; Ji, Du & Sun, 1996; Herrel et al., 2008). While measuring these traits is often relatively easy, selection acts on the organism's performance as a whole (Lewontin 2000), and so methods facilitating the study of these performance traits are especially useful.

Among lizards, locomotion is directly related to escape behavior, habitat domain, and hunting mode (Huey et al., 1984; 1989; Irschick & Losos, 1998). The study of these metrics have enabled valuable insights within an evolutionary framework, across species and contexts (Huey, 1982; Bauwens et al., 1995). Several techniques have been used for these measurements. Often, photovoltaic light cells are arrayed along a gauntlet, and precision timers record when a sprinting animal breaks those light beams (Huey et al. 1981; Miles & Smith, 1987). These techniques are well established, but often obscure useful details in, for example, acceleration (Bergmann & Irschick, 2006).

In recent years, high-speed videography has enabled more detailed analyses of fast or subtle motions. While a number of software tools are available for the analysis of these videos, we believe they have not been used to full potential because they are either dauntingly labor-intensive or prohibitively expensive for most researchers.

ImageJ (National Institute of Health, Bethesda, MD, USA) is a free static-image analysis tool. While it was originally designed for microscopy measurements, it has been used for locomotion analyses through a laborious process of either calculating scaled positions frame-by-frame, or simply counting the number of frames elapsed during a movement between known "point a" and "point b." While this method has proven effective (Hawlena et al., 2010; Les et al., 2014), it is exceedingly inefficient for large sample sizes or long recordings. An additional open source program, DLTdv3 (Hedrick, 2008), has been developed to track positions of control points (e.g., beads glued to an animal) in three-dimensional space, utilizing multiple synchronized video cameras. While, with appropriate equipment, this software is immensely powerful, it requires laboratory conditions and sophisticated hardware. Several kinematics programs have also been developed for analysis of human gait or sport performance. These tools have been effectively used for non-human locomotion analysis – e.g., Eagle Eye Pro Viewer: (Logan, Cox & Calsbeek, 2014); Peak Performance MOTUS: (Vanhooydonck et al., 2006; Herrel et al., 2008) – yet these programs are prohibitively expensive (> $2,000) for many researchers.

Here, we present an open-source HTML/JavaScript program that will enable a researcher to quickly and easily analyze the frame-by-frame position of a moving subject, export those coordinates, and analyze them to determine a suite of locomotor metrics. This flexible solution is applicable to a host of locomotion questions, but we have illustrated the technique for calculating maximum spring speed using a series of videos taken of the Aegean wall lizard (*Podarcis erhardii*) in the Greek Islands.

Methods:

Wall lizard sprint speed

Podarcis erhardii is a medium-sized (snout-to-vent length 49-78 mm) lizard that is widely distributed south of the Balkans (Valakos et al. 2008). It prefers vertically-structured habitats adjacent to open patches but can be found in a wide variety of ecotypes throughout the region (Valakos et al. 2008; Roca et al. 2009). Sprint speed has never been calculated for *P. erhardii,* though several other Mediterranean *Podarcis* species have published maximum sprint speeds (e.g., [van Damme et al., 1989; Bauwens et al., 1995]).

We demonstrate sprint speed measurements with SAVRA using five *P. erhardii* adult males that were captured on the island of Naxos in the Greek Cyclades Island Cluster during the summer of 2014. The lizards were brought to a laboratory on the island and were housed in large terraria (100 cm x 45 cm x 45 cm). All lizards were given access to food (*Tenebrio* mealworms) and water *ad libitum*, and were allowed to thermoregulate along a temperature gradient created by a suspended lamp (air temperature between 45 C and 25 C).

One-by-one, the temperature of the lizards was taken (Miller & Webber T6000 cloacal thermometer), and each individual was placed in the experimental sprint speed track – a 2.5 m long, 50 cm wide cage with a sandy substrate mirroring the natural substrate of this population. The lizards were induced to run the length of this cage by loud clapping and a closely-following (never contacting) stick wielded by a research assistant. Meanwhile, a video camera (Sony HDRPJ260V) was placed on a 2 m tripod, directly over the running path. The video camera had a field of view covering 2 m of

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track, and was kept stationary for all trials of the experiment. Each lizard run was scored "good" or "bad" based upon whether the lizard ran for at least 0.5 m at seemingly maximum capacity (Losos, Creer & Schulte, 2002), and all trials were conducted during the peak activity time of this species: between 09:00 and 16:00. All procedures involving lizards were approved under Yale IACUC protocol 2013-11548 and by the Greek Ministry of Environment, Energy, and Climate Change (Permit 111665/1669).

SAVRA: the Simple Acceleration and Velocity Recording Application

SAVRA facilitates frame-by-frame analyses of performance video. The program's capabilities – describing scaled positions of an animal or structure through time – make it broadly applicable to multiple uses, not just locomotion. The program first prompts the user to choose a locally-stored video, assign an optional identifier, specify the frame rate and resolution of the video, and select the number of frames to be advanced after each click (Fig 1a). Advancing one frame at a time is recommended to maximize data resolution.

Once the initial settings are entered, the video loads and the user is then required to specify the scale of the video with two clicks. This is most easily done with a measuring tape permanently affixed in the field of view. The user can then select two points that reflect 1, 5, or 10 cm in the field of view (customizable). This scale will be displayed as video pixels, and users can repeat this process several times to check for consistency in estimate. Once the scale is set, the user should advance the video using arrow keys until the subject appears in the field of view. At this point, using the crosshair cursor, the user should mark the position of a key point on the subject (e.g., the tip of a lizard's snout). Every time a point is selected, the video will advance the selected increment (e.g., 1 frame), and an X,Y coordinate will appear in the "Details" dialogue box. The user should continue clicking that point through the remainder of the video until the subject is out of the frame of interest. Once the points are delineated, the user should select all of the scaled and unscaled coordinates in the dialogue box, copy, and then paste those comma-delimited data into their data organization program of choice (e.g., Microsoft Excel, Numbers, etc.). Note that pixel scaling works independently of both viewport scaling (pinch to zoom) and browser font size scaling.

SAVRA is capable of editing all common video file formats (.mov, .mp4, .vid, .MTS etc.). We have written the program in web-standard HTML, CSS, and JavaScript (jQuery 2.0/videoJS 4.11.0), enabling it to be used on any desktop web browser on Mac or PC platforms, with or without connection to the Internet. We have tested the software on video files exceeding 2GB in size with no noticeable performance loss (MacBook Pro 2.8 GHz Intel i5, 8 GB Ram 1600 MHz DDR3). SAVRA is open source and freely available on the software database GitHub (https://github.com/bkazez/savra). We welcome comments on and additions to the code.

Analysis of 2D coordinates for calculating locomotion variables

In order to analyze and interpret position data, a spline or smoothing function should be used to reduce displacement variability and enable numerical differentiation for calculating maximum velocities (the first derivative) and maximum accelerations (the second derivative; Walker, 1998). Many options exist for these calculations, often specialized for different fields or applications. In a review of a suite of these smoothing functions, Walker (1998) found that the mean square error (MSE) quintic spline or the zero phase shift Butterworth filter performed most robustly for calculating velocities from position data.

Quintic splines can be calculated from scaled position data using the SSR package in R (R development core team, 2014) or the SPAPI function in MatLab (MATLAB 8.0, MathWorks, Inc., Natick, MA, USA). Additionally, a visual basic (VBA) Microsoft Excel add-in for calculating fourth-order, zero phase-shift Butterworth low-pass filters is freely available from Dr. Van Wassenbergh at the University of Antwerp (https://www.uantwerpen.be/en/staff/sam-vanwassenbergh/my-website/excel-vba-tools/ accessed 10-Dec, 2014). All three techniques may be used to calculate maximum velocity and acceleration data from the position output of SAVRA.

Detailed instructions on the use of the VBA tool are available in a help document on the website above. For the purposes of illustrating SAVRA's use, we will briefly describe the use of the SPAPI function in MatLab. First, users should import data into MatLab using (for Excel data files) the xlsread() function, and then assign the time and position data arrays to corresponding time, X position, and Y position variables. The quintic spline function, spapi(), will be fit to the X position and Y position data independently, and so should be parameterized with knots equal to 6 (degree of the spline plus one), and the previously assigned variables for time, and either X position or Y position data. Taking the first derivative (using the fnder() function) of this spline fit will yield the instantaneous velocity in the X or Y direction, and a second derivative, again using the fnder() function, will yield the acceleration in that direction. These instantaneous velocity or acceleration vectors can then be combined into a two-dimensional velocity or acceleration using Pythagorean theorem (i.e. total velocity =

$[(\text{velocity X})^2 + (\text{velocity Y})^2]^{1/2}$). The maximum velocity or acceleration for that individual's run can then be calculated using the max() function.

Results:

We found that these male *P. erhardii*, at an average body temperature of 30.1 ± 1.5 C, achieved an average maximum sprint speed of 1.78 m/s (Fig. 2b). Two lizards achieved instantaneous speeds of 2.16 m/s, while the slowest lizard only achieved a maximum of 1.36 m/s.

Discussion:

Using SAVRA to track the position of these five *P. erhardii* lizards, we found an average maximum sprint speed of 1.78 m/s with a maximum of 2.16 m/s and a minimum of 1.36 m/s. While sprint speed has never been calculated for *P. erhardii*, these results fall within the published average sprint speed of two closely related lizards: *P. muralis* 1.44 m/s ± 0.07 and *P. lilfordi* 1.77 m/s ± 0.12 at approximately this body temperature (Bauwens et al., 1995). Due to this low sample size, these results should not be thought of as representative of the species. Instead we aim to illustrate the use of SAVRA for sprint speed analysis, and that the results were comparable to other studies.

SAVRA's contribution:

With the increasing availability of consumer-grade high-speed video cameras, more detailed analysis of locomotor function and performance is possible than previous sprint speed track methods (Huey et al. 1981; Miles & Smith, 1987). Videography also enables measurements over natural substrates and is frequently more convenient in the field, provided scale references can be taken. However, analysis of high-speed video is difficult, currently necessitating either time-intensive frame-by-frame export and analysis, or expensive software programs often exceeding the budget of many students and researchers. SAVRA streamlines the frame-by-frame analysis of video and provides scaled position data that can be used to calculate locomotion metrics. With its implicit scaled coordinate system, SAVRA may also be used for calculating not just speed but also angles and paths. We hope that making this code open source will enable other scientists to access and use it, increasing the number of analyses conducted on locomotion across taxa and conditions.

Acknowledgements:

The authors would first like to extend their sincere thanks to B. Redding for his help with the MatLab analysis. We would additionally like to thank M. Lambert for helpful comments on a draft of this manuscript. We finally thank our collaborators at the University of Athens, particularly P. Pafilis, for ongoing logistical and research support.

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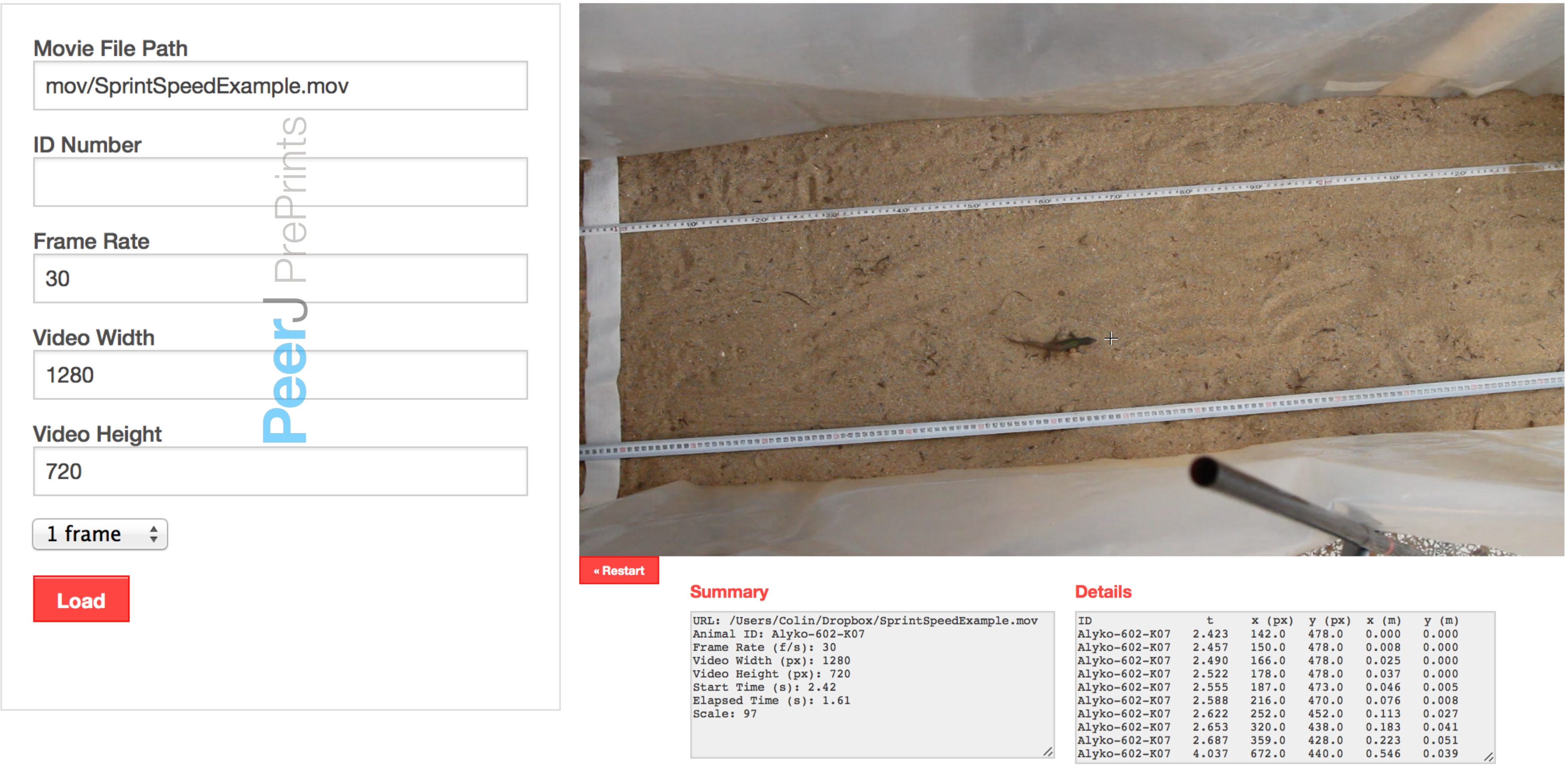
Figure 1 (on next page)

Screenshot of the SAVRA workflow

Figure 1: A screenshot of SAVRA's loading screen (a) and analysis view (b).

a) b)



Movie File Path
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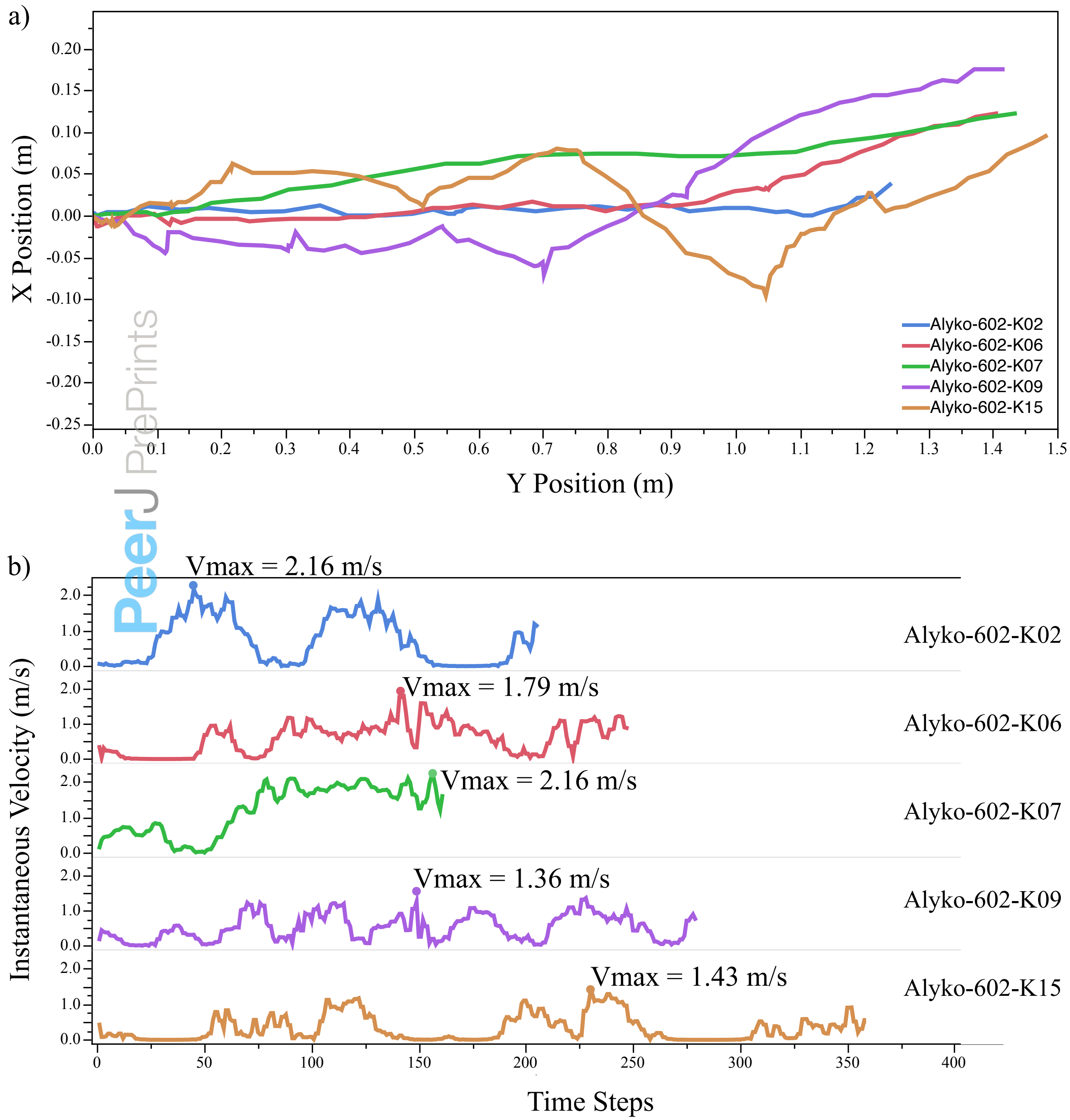
ID Number
Frame Rate
30
Video Width
1280
Video Height
720
1 frame
Load
« Restart
Summary
URL: /Users/Colin/Dropbox/SprintSpeedExample.mov
Animal ID: Alyko-602-K07
Frame Rate (f/s): 30
Video Width (px): 1280
Video Height (px): 720
Start Time (s): 2.42
Elapsed Time (s): 1.61
Scale: 97
Details
ID t x (px) y (px) x (m) y (m)
Alyko-602-K07 2.423 142.0 478.0 0.000 0.000
Alyko-602-K07 2.457 150.0 478.0 0.008 0.000
Alyko-602-K07 2.490 166.0 478.0 0.025 0.000
Alyko-602-K07 2.522 178.0 478.0 0.037 0.000
Alyko-602-K07 2.555 187.0 473.0 0.046 0.005
Alyko-602-K07 2.588 216.0 470.0 0.076 0.008
Alyko-602-K07 2.622 252.0 452.0 0.113 0.027
Alyko-602-K07 2.653 320.0 438.0 0.183 0.041
Alyko-602-K07 2.687 359.0 428.0 0.223 0.051
Alyko-602-K07 4.037 672.0 440.0 0.546 0.039

Figure 2(on next page)

Position and velocity data output from SAVRA

Figure 2. Position data (a) for the paths of five lizards after videos of their runs were processed using SAVRA. These position data were fitted with a mean square error quintic spline, and the instantaneous velocity was calculated throughout the duration of the run (b). From these velocities, a maximum was calculated, and is labeled. Visualization plots were created in JMP 10.0.0 (© 2012, SAS Institute Inc.).



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