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The Biomechanics of Tiger Salamander (Ambystoma tigrinum) Underwater Walking on Smooth and Rough Substrates

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Abstract

In nature, salamanders adapted to terrestrial and aquatic environments as well as varying substrate conditions in their habitats. Discovering more about the effects of substrate types on the kinematic measurements of underwater walking can offer us with more information regarding biological principles and limb evolution. A previous study conducted on the same salamander subjects led us to believe that a rough substrate such as gravel will provide the salamander with better traction and grip to thrust forward for walking underwater compared to a smooth substrate. Therefore, a rougher substrate is expected to have resulting kinematics that will differ significantly from underwater walking on a smooth surface. This project studied the biomechanics of underwater walking of the model organism, Tiger Salamander (*Ambystoma tigrinum*). The goal of the experiment was to analyze and collect data on the general locomotion and kinematic measurements while the salamander walked while submerged underwater. In order to obtain the information, two synchronized GoPros were used to film high speed videos of the strides of the salamanders at a lateral and dorsal viewpoint. Their unrestricted, voluntary strides were captured on both smooth and rough surfaces that provide a different expanse of traction. After filming was completed, each video was trimmed and compressed using VirtualDub. Then, the MATLAB digitizing module DLTdv5 was used to track multiple anatomical points among each salamander's body. For each frame the x and y coordinate of five landmark anatomical features were tracked for both the horizontal and vertical video. The y coordinate of the dorsal perspective provided the estimated "z" coordinate for a more accurate analysis of any existing angled strides. Based on the 2-D trends of the digitized points, a custom MATLAB code was produced in order to extract information about their overall gait cycle and therefore the individual strides. This research will help us better piece together the evolutionary changes from underwater to terrestrial locomotion and understand how these fundamental biomechanical principles apply to other organisms. The average salamander stride duration, duty factor, stride length, stride velocity, minimum foot velocity, maximum foot velocity, foot slip distance, minimum potential kinetic and potential energy, and maximum potential and kinetic energy were used as kinetic markers to compare the effects of substrate type on the kinematics of underwater walking. After statistical analysis it was determined that there were significant differences existing between two salamanders on gravel and sand substrate for the following variables: minimum kinetic energy, average velocity, average velocity in body lengths, stride duration, and stride length in body lengths. For the very first time the change in potential and kinetic energy throughout a stride of a salamander while underwater walking was able to be calculated. This current study presented statistical evidence which confirmed that the kinematics of underwater walking of a salamander differs to some degree between smooth and rough substrates.

Introduction

The salamander is an ideal organism to obtain underwater walking data from for they are identified as amphibians that are an outgroup clade to amniotes. Salamanders and early tetrapods share a similar gross anatomy and body form, however the morphology of the salamander has not changed for an estimated 150 million years (Gao and Shubin, 2001). Salamanders are the sole group of extant amphibians that have similar patterns of locomotion to other previously studied tetrapods (Liem et al., 2001). Their body plan is evolutionarily and phylogenetically significant due to their remotely unspecialized body plan, which is the reason why many studies on primitive terrestrial vertebrates utilize salamanders as their primary model. In addition to their morphological traits, salamanders also have unique characteristics regarding their locomotion and gait cycle. According to a previous study, on average a salamander was determined to have at least three or four total feet contacting the substrate beneath them for 59.4% through the duration of a single stride or gait cycle (Ashley-Ross, 1994a). Newts, which are members of a distinct family of smaller extant salamanders are another favorable model organism used in underwater locomotion and kinematic research. Locomotion studies using newts helps further explain the typical movement and kinematics of salamanders due to the large expanse of similarities of their genetics and morphology. A previous study specifically on California newts were found to have a distinctly comparable gait cycle and locomotive patterns to salamanders. On average, the newt test subjects were found to have walked with a duty factor of 77% (Ashley-Ross et al., 2009). An additional study using virtual simulations of salamanders were able to corroborate these findings with a calculated duty factor of 76% during a typical walking gait cycle. Both studies also supported that the average time that all 4 legs of the salamander or newt was in contact with the substrate during a gait cycle had a duty factor of 20% (Harischandra, Nalin et al. 2010). These previously established values will allow the data calculated from this experiment to be checked for digitizing errors, code errors, and any other discrepancy or factors that might alter the data. What these other studies lack however is the effect that buoyancy and an aquatic environment has on the locomotive patterns and the estimated changes in potential and kinetic energy for a salamander. Improvement in this particular biological field and examination of underwater walking of salamanders and similar organisms will clarify these relatively unstudied and unpublished characteristics and kinematics. The goal of this experiment was to continue the research of former University of Akron honors student Sophie Cressman and her advisor Dr. Henry Astley. Cressman's study with the same subjects were analyzed using only the horizontal perspective. This process could have led to erroneous kinematics due to the lack of detection of angular or out of plane locomotion within the field of recorded movement. The lack of the dorsal perspective also prevented the calculation of potential and kinetic energy of the salamander's gait. With the use of a lateral and dorsal camera the kinematic variables previously tested can be validated or countered. In addition, the changes in potential and kinetic energy of the salamanders while underwater walking can be quantified and compared between substrates for the very first time in the field of biomechanics.

Methods and Materials

Animal Subjects

Eight Barred Tiger Salamanders (*Ambystoma tigrinum*) were purchased from Exotic Pets LV for the purpose of this study. The salamanders were maintained under identical conditions in separate terrarium containers and fed isopods once a week. The mean snout-to-hip lengths (StH) of the salamanders was 9.03 cm with a standard deviation (SD) of .262 cm. The mean weights of the salamander were 23.8 g with a standard deviation of 2.24 g. Due to water quality issues and health ailments six of the eight salamanders passed away before all of their trials for both substrates were completed and their underwater weights could be obtained. Salamander Big 5 and Salamander Big 3 were used as test subjects as shown below:

Figure 1: Salamander Big 5

Figure 2: Salamander Big 3

Video Recording

The locomotion of each salamander was recorded using two synchronized GoPro Hero 8 HD cameras with a GoPro smart remote clicker. Each GoPro was set to 60 frames per second and at a resolution of 1440. A 20-gallon long fish tank filled $1/4^{th}$ with water treated with dechlorinator was used as the setting of the captured video trials of underwater walking. One camera was positioned laterally on a leveled tripod in front of a 20-gallon long fish tank to capture a side view of the salamander's underwater walking. The other camera was placed above the tank over its center with GoPro attachments to capture a top-down dorsal view. The camera was level and perpendicular to the substrate surface. The tank bottom was covered with small sized gravel for rough substrate trials and a homemade sandpaper insert composed of sand glued to a piece of cardboard was used for smooth substrate trials. The tank was filled roughly halfway with dechlorinated water. Before the remote clicker was pressed an eraser board and a marker was used to indicate the salamander number, trial number, and type of substrate that was being used. After the clicker was pressed the board was held in front of both go pros individually and a green laser beam was flashed into the center of the tank in order to later on synchronize the beginning of the trial for both cameras. The salamander was placed in the tank and was encouraged in walk in a straight line for at least three continuous strides. A diagonal walking pattern was accepted to be a proper trial as long as the salamander did not touch the glass sides of the tank. However, if at any point during a gait cycle where the salamander paused, swam, or stopped between a stride then that segment of the trial was not chosen to become edited and digitized for the data pool. A stride was considered starting when the salamander had their front foot at a minimum distance from their hip and ended when the foot swung back to the initial minimum distance after they moved forward. Seven trials were conducted for two salamanders for each type of substrate. The total number of trials for each salamander was 14 which lead to an accumulation of 56 total captured videos including the dorsal and lateral viewpoints. Of these trials and based on which strides fit the criteria, 6 trials on each substrate was chosen along with the corresponding side and top cameras. This led to a total of 48 videos being completely digitized. Each salamander was not out of its container and refrigerator for more than two hours since they live comfortably between a 50-65 degree environment. The tank of water was removed, replenished, and treated with dechlorinator each week of testing.

Video Processing and Stride Collection

The GoPro Hero 8 60 fps videos were first edited by searching through each large trial video file and finding the frame lengths where at least 3 strides of the salamander was documented within the proper criteria. The .MOV files were then converted to .avi files for use in MATLAB. Video files were then trimmed to the exact frame range of movement, filtered, and then compressed with the Xvid codec using VirtualDub 1.10.4 software. An excel spreadsheet was used to organize each salamander trial, the viewpoint, and the substrate used. The laser pointer beam was used as an indicator to synchronize the top and side viewpoint frame length. The frame number after the laser disappeared was documented for the top and side view. The numerical difference between the top and side view was found and the lengths of the videos were adjusted accordingly in order to have each trimmed viewpoint for each particular trial begin at the same time.

Digitization of Locomotion

In the field of biomechanics, 2D digitizing refers to the process of tracking specific anatomical points on an image of an organism or object throughout every individual frame within a video to obtain x and y coordinates of that point on a computer software. This tedious but rewarding method of digitization is what allows the quantification of that point's motion. In this experiment, a MATLAB graphical interface module called DLTdv5 was used to digitize the salamander underwater walking videos and thus create a kinematic model of their motion. However, the previous experiment conducted on the underwater walking biomechanics of salamanders only one horizontal lateral camera perspective was used to collect the x and y coordinates of the salamander's motion. In this experiment using both the lateral and dorsal viewpoints allows for data that accounts for the side to side and up and down motion of the salamander. For every frame of the edited trial videos the x and y coordinates for five landmark anatomical features were tracked. For the dorsal (top) and lateral (side) perspective the following points were digitized: the front foot carpus depending on whichever one was facing the laterally placed camera, the center of mass or the middle of the salamanders back, the top or tip of the snout, the pelvic girdle or the hip facing the lateral camera, and the area above the pectoral girdle. These anatomical points can be seen in Figure 3 below.

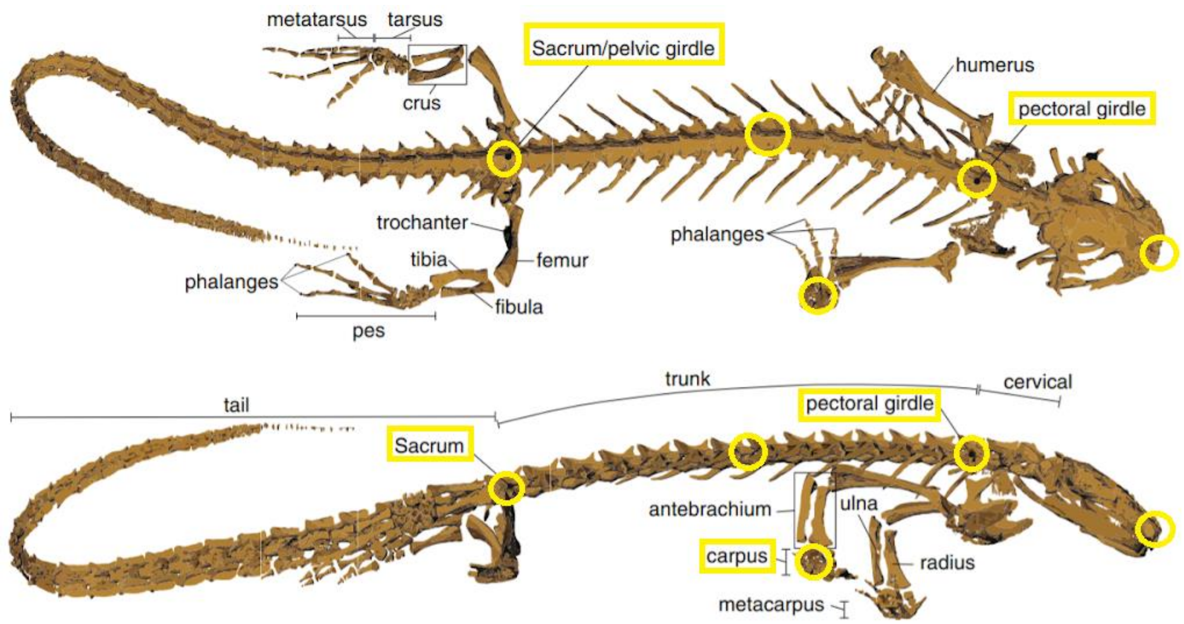


Figure 3: Basic Anatomy and Chosen Anatomical Landmarks of a Salamander from the Dorsal and Lateral Perspectives (Karakasiliotis et al., 2013)

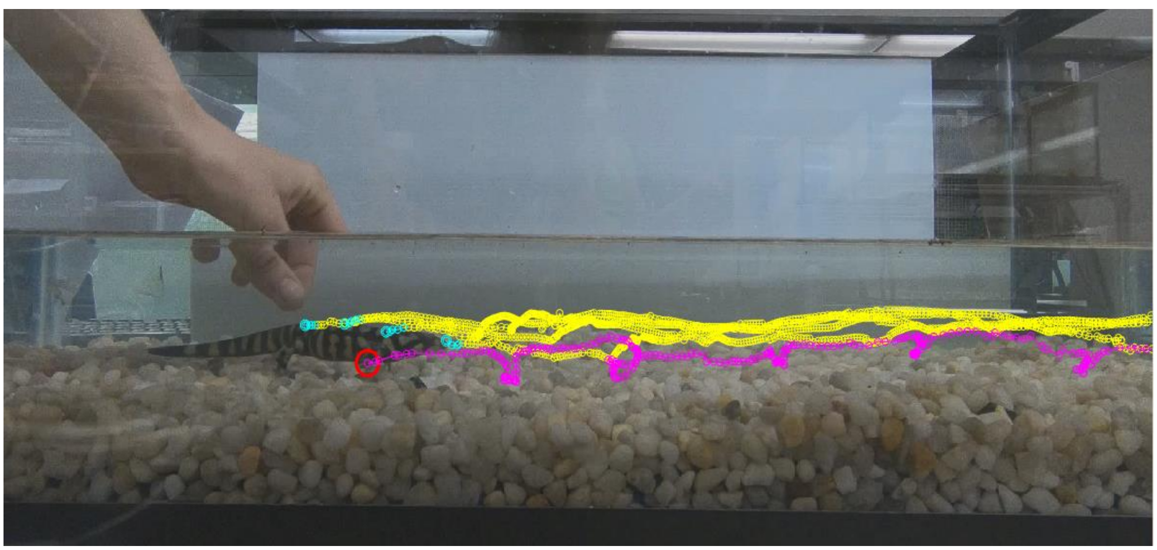


Figure 4: This screenshot shows the 2-D tracks generated by the MATLAB DLTdv5 digitizing tool after Salamander 3 completed its 7th gravel substrate trial for the five tracked anatomical points for the side or lateral view camera. The highlighted purple segment shows the 2-D tracked anatomical point number 1 being the front foot of the salamander. The x and y coordinate data were saved and stored in an excel spreadsheet for later quantification.

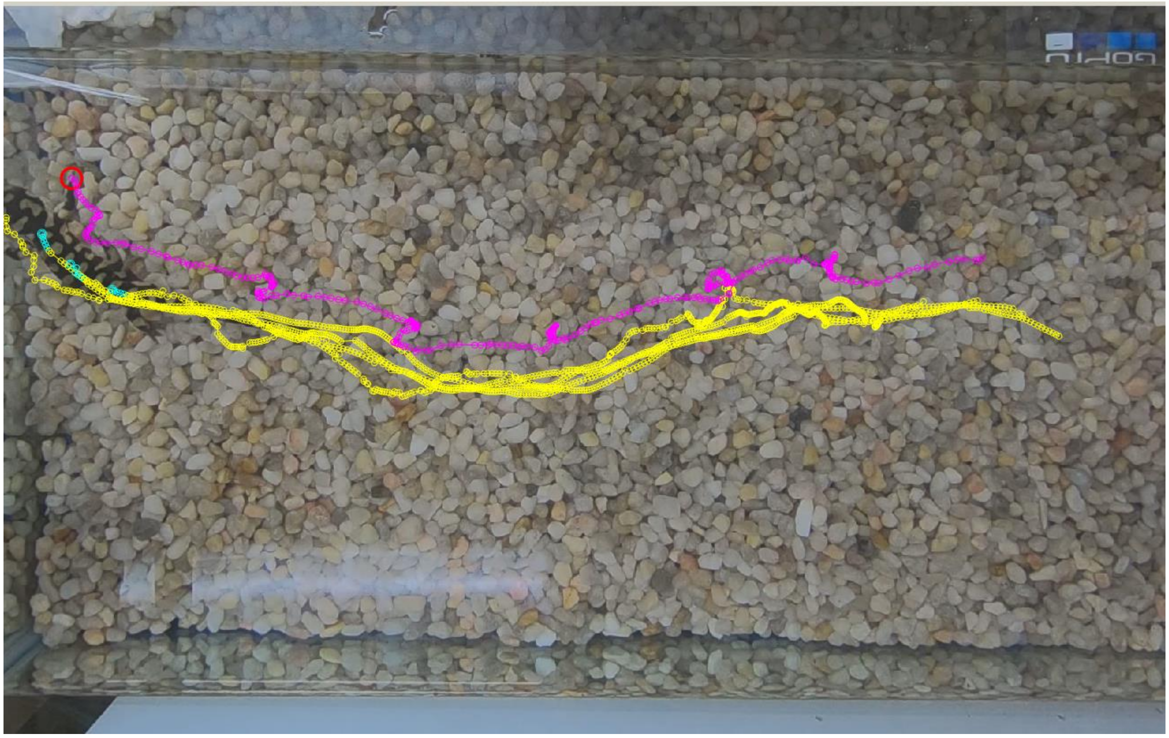


Figure 5: This screenshot shows the 2-D tracks generated by the MATLAB DLTdv5 digitizing tool after Salamander 3 completed its 7th trial for the five tracked anatomical points for the top or dorsal view camera. The highlighted purple segment shows the 2-D tracked anatomical point number 1 being the front foot of the salamander. The x and y coordinate data were saved and stored in an excel spreadsheet for later quantification.

MATLAB Analysis

Scaling and Normalizing the Dorsal and Lateral Camera Data

In the previous study of these salamanders the lateral perspective was only recorded whereas in this experiment both the lateral and dorsal perspectives were recorded and analyzed for better accuracy of locomotion of each tracked point in a more 3-D like model. Seven trials were conducted for the typical gravel substrate and the homemade smooth sandpaper cardboard insert. 6 of the trial videos for each substrate which met the appropriate criteria were chosen after the digitizing process. The DLTdv5 digitizing tool hosted within MATLAB organizes all the data collected into 4 excel spreadsheet files for each salamander, substrate, trial number, and camera perspective. For example, the very first digitized video was Salamander 5 or Big 5, gravel substrate, trial 1, and side camera viewpoint. The most important excel file that is created as a .csv file with the suffix. xypts. This file provides the x and y coordinates of each anatomical point that was assisted with the auto track function for each frame of the edited and digitized compressed video. The X and Y coordinates of the dorsal and lateral cameras will be distorted due to imperfect synchronization, human error, and the fact that the viewpoints will have different length scales therefore different pixels. However, since the viewpoints do provide a pure lateral and a pure dorsal x, y coordinate system it is possible to line up the coordinates starting at 0,0 and figure out the length and pixel ratios between the top and side viewpoints using geometric transformation. Before the ratio between the two data files was determined each file was checked to make sure there were no existing NaN or unentered values which could cause in error in MATLAB or lead to inaccurate data due to the absence in data. For the sake of control and convenience the side viewpoint X, Y coordinates were chosen to be the definitive or base coordinates in comparison to the top, dorsal viewpoint x, y coordinates. The length scale is adjusted using geometric transformation in order to find the zoom ratio between the two cameras and normalize the associated data. A final graph created within the code produces a visual representation of how close the newly adjusted data lined up with one another using two lines representing the top and side X and Y coordinate values as shown in Figure 6 below. If the lines were directly on top of each other replicating the same pattern this indicated that the data was in synch and the length constants were the same. However, if there was distortion or non-overlapping positions this indicated that there was human error digitizing the points throughout the video frames or human error when the frames of the side and top cameras were synchronized.

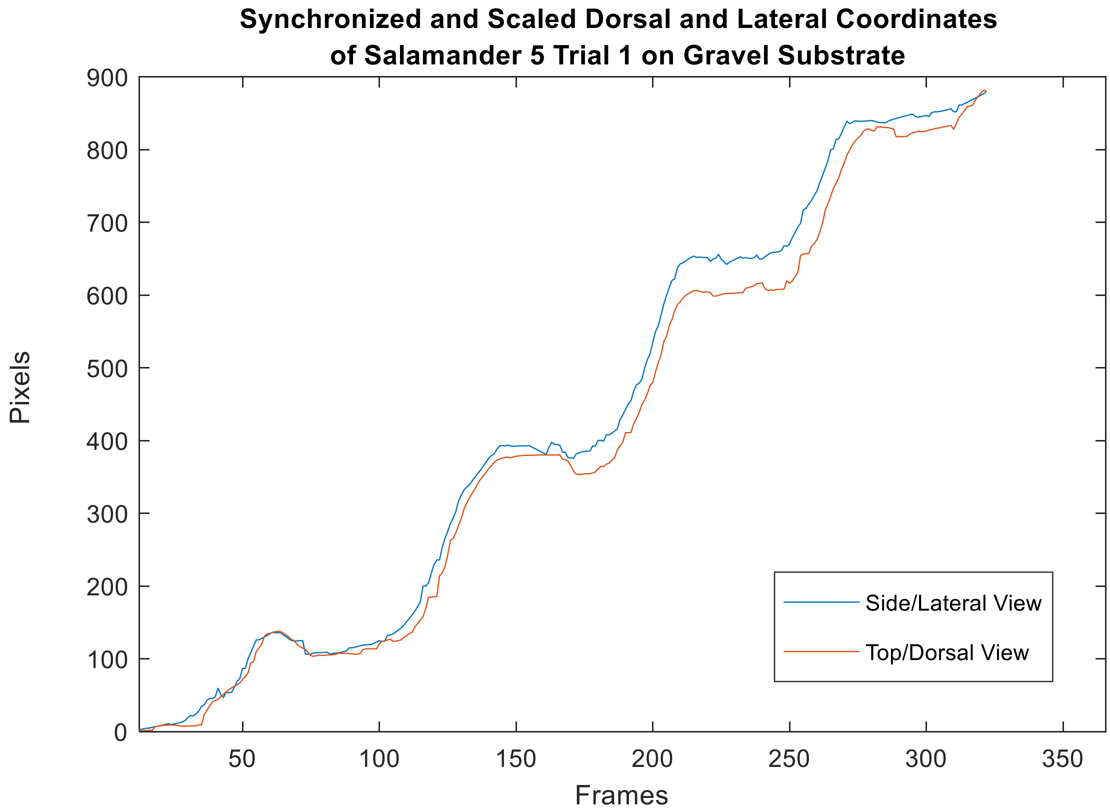


Figure 6: The raw, scaled, and geometrically transformed Lateral and Dorsal view data for the first gravel trial of salamander 1 is displayed. This shows how aligned the side and top view data are aligned in the coordinated plane and how much jitter/noise exists.

Splining Initial Scaled Dorsal and Lateral Camera Data

In this experiment it was apparent that a spline function would be necessary to smooth or reduce noise and distortion within the data. MATLAB has an add-on module known as the Curve Fitting Toolbox which enables a live script to read and analyze cubic spline functions such as the csaps argument. The csaps function essentially takes given data points recorded at the same site and creates an output value of their weighted averages for the x and y coordinates. Within the designed code it was also compulsory to designate a p value or smoothing coefficient to figure out which data points do not coincide with the norm or trend of the data or if a data point is too extreme. According to MATLAB when the smoothing coefficient is 0, the smoothing spline uses a least-squares straight line fit whereas when the coefficient is 1 the data is manipulated by a "natural" or variational cubic spline interpolant. Any coefficient above 1 produces the undesirable effect of roughening the data set rather than smoothing it. A p-value that did not undersmooth or oversmooth the data is what we were searching for (MATLAB 2019). Smooth enough to rid of unwanted extremes and potential digitizing errors but not too smooth where the consistent trend of the data is heavily altered or removed. The same smoothing coefficient was applied to both the side and top camera view excel spread sheet data. Initially a couple of different coefficient values were inputted into the constructed MATLAB code to test and plot the results. The newly splined and smoothed dorsal and lateral data was then graphed once again together as seen in Figure 7 and Figure 8 compared to the original, raw graph as seen in Figure 6. The graph with the smoothing coefficient that best reduced the jitter and noise within the data, found the basic pattern or trend within the data, and retained the original pattern or trend preexisting in the data was chosen. This chosen smoothed data saved in the form of another excel spreadsheet therefore would be used in future calculations such as stride length, duty factor, foot velocity etc. The coefficient chosen to be applied to every data set and both substrates in this experiment was p=0.999995. Unfortunately, as seen in Figure 5 and Figure 7 the lateral and dorsal camera digitized data was not perfectly overlapped throughout every frame. This could have been caused by camera positioning, micro-lag between the two videos, and human digitizing error while using the MATLAB DLTdv5 module. Therefore, the kinematic measurements will be more accurate due to two body perspectives but will also be contaminated by a small amount of error due to the slight separation of the lateral and dorsal x and y coordinates.

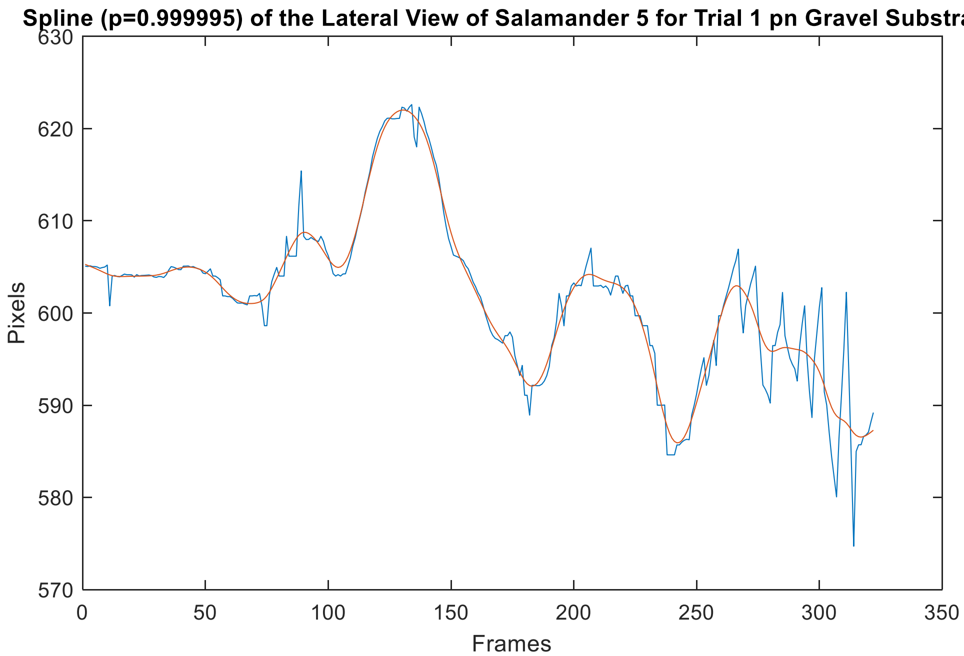


Figure 7: The raw lateral view data versus the newly smoothed data is displayed using a spline function in MATLAB to reduce jitter and noise using the p-value 0.999995 for the first side gravel trial of salamander 5.

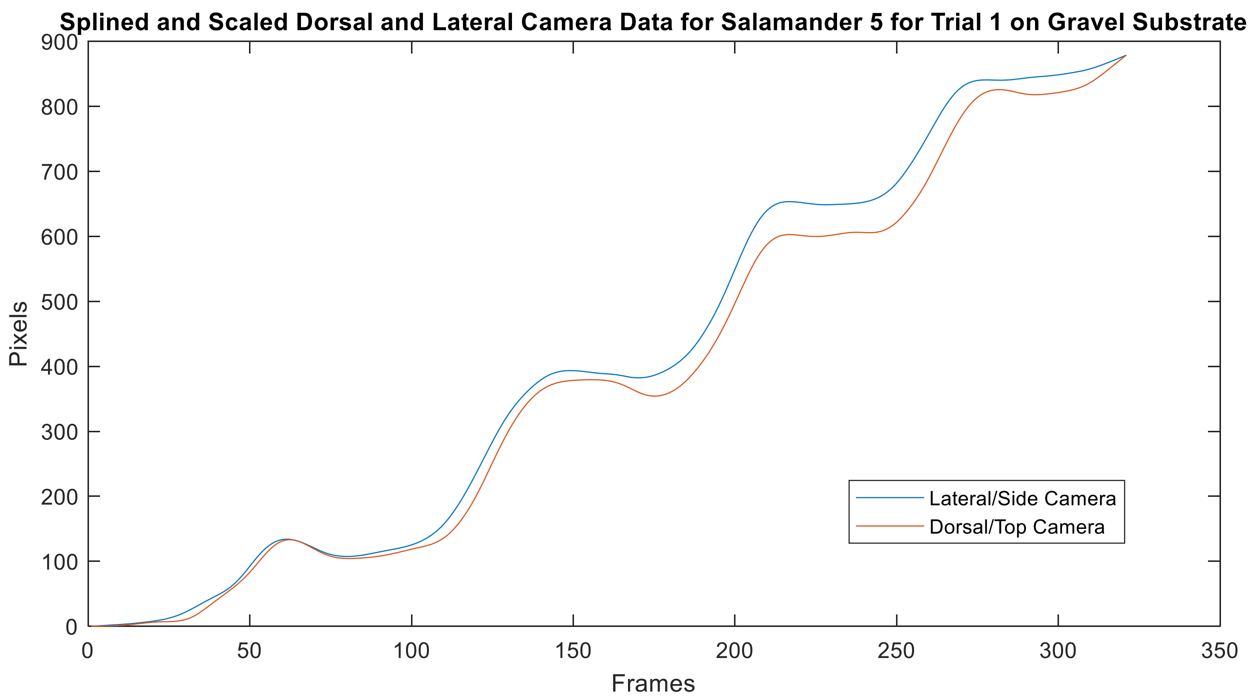


Figure 8: The final step for reducing the noise or roughness present in the lateral and dorsal data using the MATLAB splining function before statistical analysis. The chosen p value to be applied to every data set was p=0.999995.

Definitions of Distinctive Variables

- ‘Stride length’ is defined as the meters of displacement of a salamander’s foot between the time when the front foot is at a minimum distance from the hip to the next point at which the foot is at a minimum distance.
- ‘Stride velocity’ measured in meters per second is defined as the found stride length divided by the approximate stride duration measured in seconds.
- ‘Swing phase’ measured in seconds is defined as the time period within a stride where the salamander raises its front foot and marks the end of stride. In practical application within the experiment the swing phase could also be explained to start when we see that the front foot is positioned at the minimum distance from the pelvis. The swing phase therefore ends when the front foot of the salamander is positioned at the maximum distance from the pelvis (Ashley-Ross and Bechtel, 2004).
- ‘Stance phase’ measured in seconds is defined as the period of time in which during the stride over the front foot of the salamander is in direct contact with the gravel or smooth sandy sandpaper substrate. In the practical application within this experiment the stance phase starts when we see that the front foot of the salamander is at a maximum distance from the pelvis. The stance phase therefore ends when the front foot of the salamander reaches a minimum distance from the pelvis (Ashley-Ross and Bechtel, 2004).
- ‘Duty factor’ is defined as the percentage of the stride in which the front foot of the salamander is in the stance phase. This duty factor percentage can be calculated using the equation: $\frac{Stance\ Phase\ Duration(\max front\ to \max back)\ (s)}{Stride\ Duration\ (s)}$. A smaller or shorter duty factor indicates that there is less time that the salamander’s foot is in contact with the substrate which means that more force would be required in order to propel its body forward less contacts leads to more force required.
- ‘Foot slip’ which is measured in meters is defined as the distance in which the foot of the salamander facing the camera skids on the substrate during one stride. If the salamander traveled in the positive direction within a video trial it essentially traveled from the left side to the right side of the fish tank. In this case the foot slip was determined by integrating the front foot velocity curve from the exact frame where the velocity of the front foot of the salamander switched from a positive to a negative value during a stride alongside with the frame when the velocity of the foot switched back towards to the positive direction during a stride. Vice versa, when the salamander traveled from the right to the left side of the tank in the negative direction the foot slip was determined by integrating the foot velocity curve from the frame when the foot velocity of the salamander went from a negative to a positive value during a stride alongside the frame when the foot velocity switched back to negative within a stride (Cressman 2018).
- This new added variable of kinetic energy to the study of salamander locomotion allows an estimation of the amount of work that the salamander applies in both vertical and horizontal direction in order to accelerate. Generally speaking, the salamander will maintain kinetic energy unless there is a change in its speed or velocity. The kinetic energy formula is designated as $KE = (0.5)(m)(v^2)$, where the variable m is mass, v is velocity, and KE is kinetic energy (Mucha, Mathison, & Sas 2019). However, due to the salamander subject walking submerged underwater there will be discrepancies due to added mass effect and the cofounding variable of buoyancy.

- The second new variable added to this experimental study was potential energy. The purpose of calculating the potential energy of the salamander is to determine how much gravitational pull is exerted onto the salamander as well as the depth of which the salamander falls. When the salamander vertically rises in the water filled fish tank its potential energy will increase therefore its amount of stored energy increases. The potential energy formula is denoted as $PE = (m)(h)(g)$ where the variable m is mass, h is height, and g is the gravitational field strength (9.81) (Mucha, Mathison, & Sas 2019). However, due to the salamander subject walking submerged underwater there will be discrepancies due to added mass effect and the cofounding variable of buoyancy. Buoyancy was accounted for by multiplying potential energy by the ratio between the mass of the salamander on land and the weight of the salamander underwater.

Unit Conversions from Pixels to Meter and Frames to Seconds

Each kinematic variable calculated using the five digitized anatomical points on the salamander was changed to pixels per meters. The ratio utilized to calculate the final measurement was:

$$\frac{\text{StH of the salamander (pixel)}}{\text{StH of the salamander (meter)}}$$

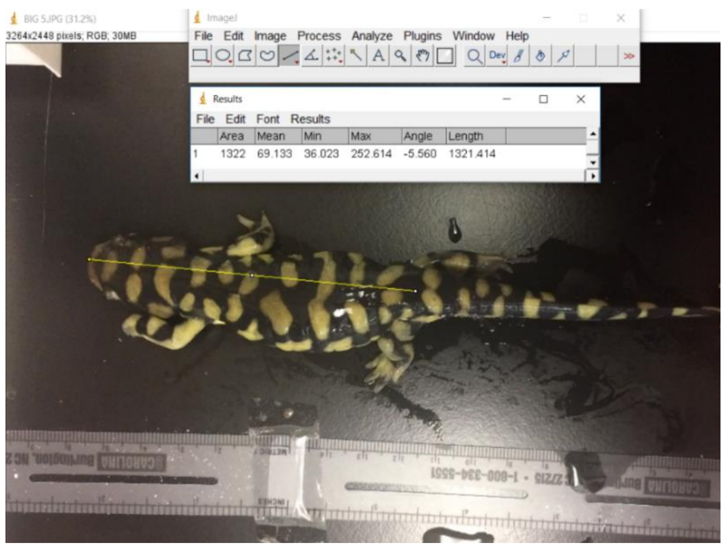


Figure 9: This screenshot shows the process of finding the pixel length of Salamander Big 5 from snout to hip using the Image J computer software, a ruler, and a dorsal picture.

The StH of the salamanders was found in meters using ImageJ software and picture of the dorsal perspective of the salamander next to a ruler in order to measure the StH in pixels. The line tool within the Image J program was able to determine the number of pixels in 10 cm (.01m). 0.1 m converts to a total length of 378 pixels. Salamander 5 was measured from the top of the snout to the base of the hip was 8.7 cm (0.087m) with a ruler and the Image J software calculated the approximate pixel length to be 1321.414 pixels. The entire photo length as shown below is 3264 pixels whereas 10cm along the ruler in the photo is 1450.424 pixels. The quotient

.911 pixels multiplied by 10 was the video adjusted size of the salamander which was equivalent to 9.11 cm. This adjusted length was inputted into MATLAB in order to convert pixels to meters and then to body length for more accurate comparisons of stride length. For Salamander 3 the Image J calculation of 10 cm among a ruler was 1279.716 pixels and the salamander was measured as 1405.675 pixels. In real-time the salamander was measured as 9.372 cm from snout to hip. The dimensions of the picture of Salamander 3 was 2448 pixels multiplied by 3264 pixels. The measurement from snout to hip in pixels was determined to be 1.09 pixels which after being multiplied by 10 would be 10.98cm.

MATLAB Plots

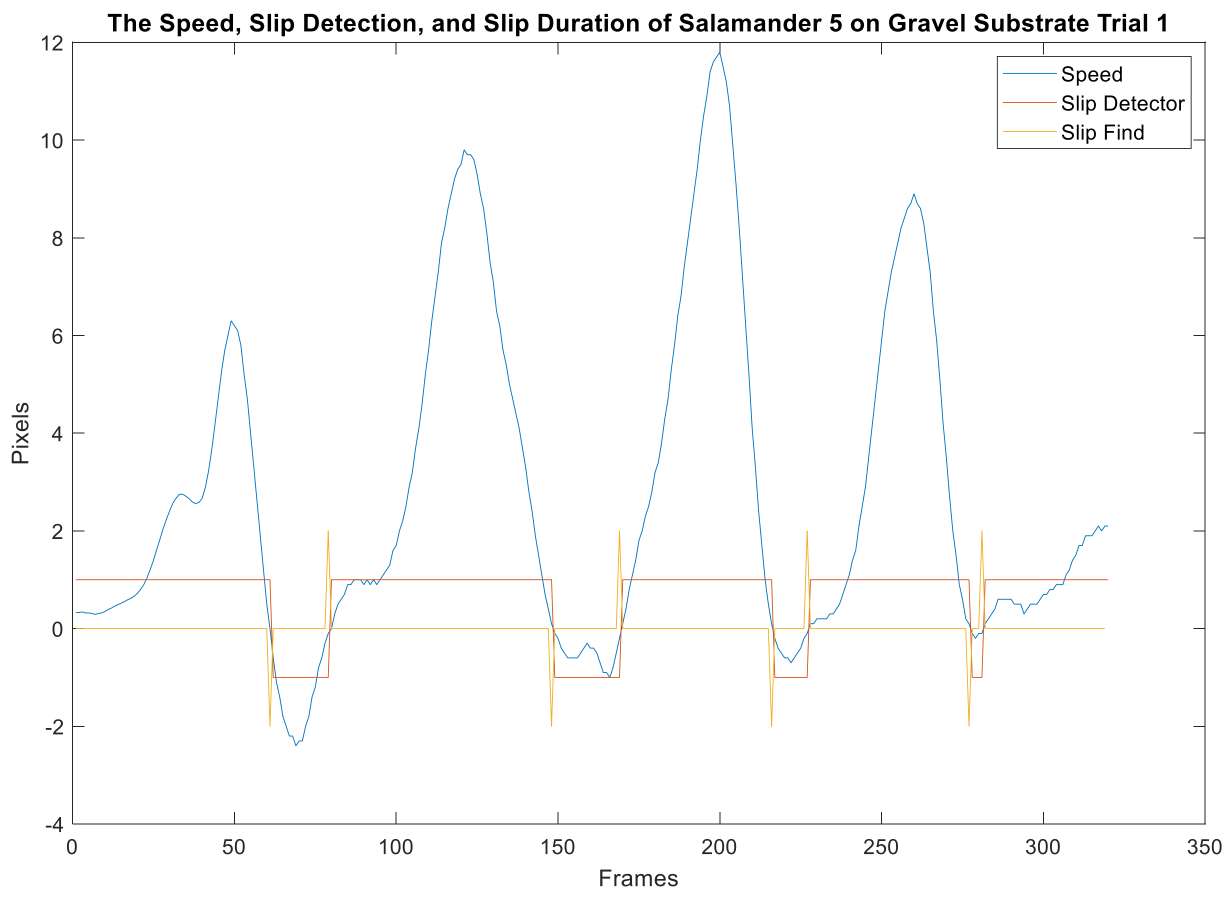


Figure 10: This is the overlay of three different MATLAB variables: "frontfootxspeed", "slipdetector", and "slipfind" in order to illustrate the gait cycle and velocity in pixels per frame of salamander 5 on gravel substrate for trial 1. Information used to construct this graph within MATLAB code was then then utilized to find the average velocity, slip duration, and slip distance of each stride length. This graph was plotted in MATLAB from the written script for all trials and both substrates.

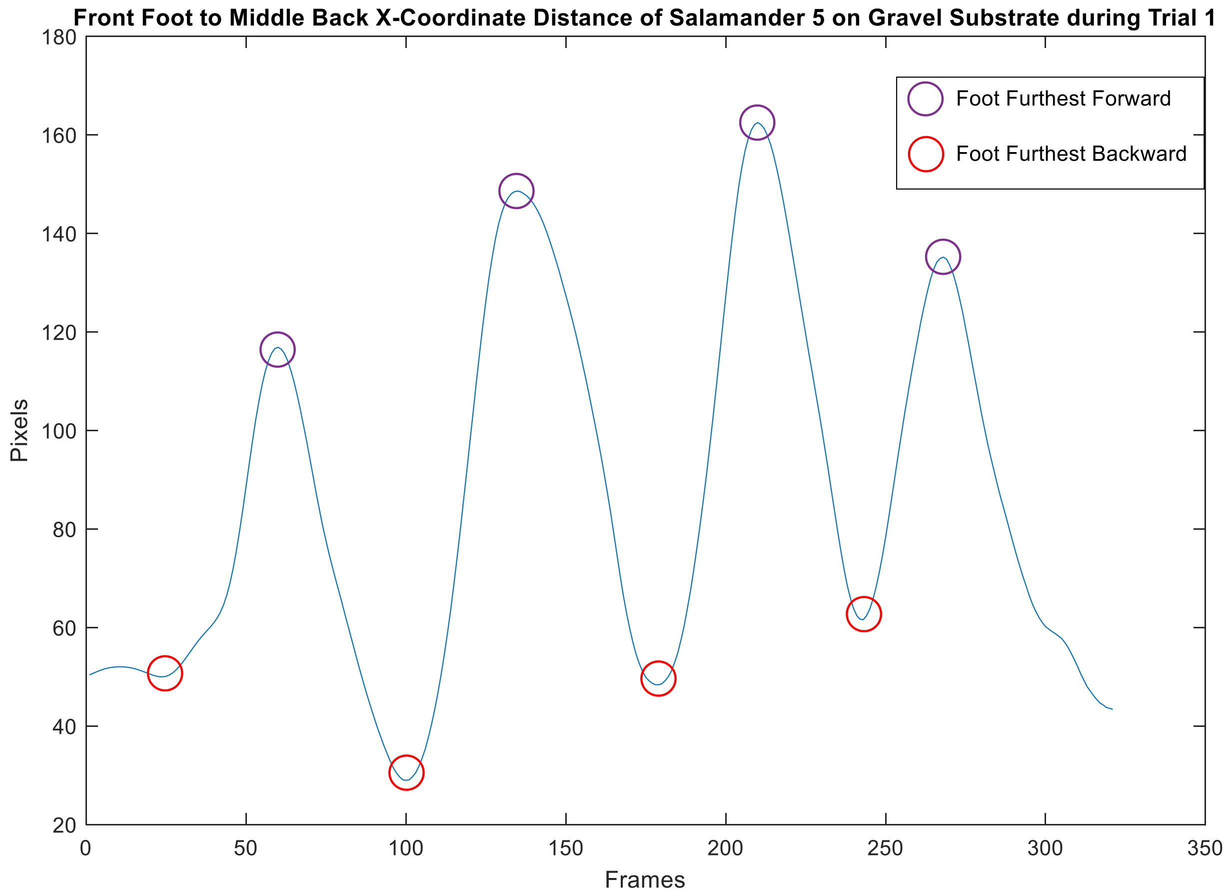


Figure 11: This portrays the front foot to middle back x-coordinate only distance for Salamander 5 on gravel substrate during the first trial. The "findpeaks" function in MATLAB extrapolated the maximum and minimums points from the original, splined excel spread sheet. The maximum points represent the frame and point in time during the video where the salamander's front foot is at its most- forward position during a stride whereas, the minimum points represent the front foot at the most-furthest back position. This graph was plotted in MATLAB from the written script for all trials and both substrates.

Kinetic and Potential Energy

In this experiment within the MATLAB code constructed, the terrestrial mass and the underwater weight of the salamander was used in calculations. From these measurements in addition to maximum velocity, minimum velocity, maximum height, and minimum height of the salamander during a stride the following calculations were made for every stride: maximum kinetic energy, minimum kinetic energy, and maximum potential energy. The minimum potential energy for every stride was set to 0. The land mass of Salamander 5 was 0.0268kg (26.8g) and the underwater weight was determined to be 0.91g (0.00091 kg). Salamander 3 has a land mass of 0.0232kg and an underwater weight of 0.0007kg. Additionally, for each trial the overall change in kinetic and potential energy was estimated using the total amount of strides within the video file and data sets. For kinetic energy change over time the equation was:

$$KEchange = (0.5)(Salamander\ Land\ Mass)(Instantaneous\ Velocity^2)$$

Potential energy change was initially calculated using:

$$PEchange = \frac{(Salamander\ Land\ Mass)(9.81)(Minimum\ Y\ Coordinate\ Middle\ Back(Y\ Coordinates\ Middle\ Back)}{pixeltometerratio}$$

The final change in potential energy was found by taking the value of PEchange and multiplying it by the ratio between the terrestrial mass and the underwater weight of the salamanders as seen in the equation below:

Salamander 5:

$$\frac{0.00091\ kg(Salamander\ 5\ Underwater\ Weight)}{0.0268\ kg\ (Salamander\ 5\ Land\ Mass)} X100\% = 3.395\%$$

$$PEchangefinal = \ (PEchange)(0.03395)$$

Salamander 3:

$$\frac{0.0007\ kg(Salamander\ Underwater\ Weight)}{0.0232\ kg\ (Salamander\ Land\ Mass)} X100\% = 3.017\%$$

$$PEchangefinal = \ (PEchange)(0.03017)$$

These resulting changes in potential and kinetic energy for each frame over the course of each trial were calculated and plotted in MATLAB and can be seen in Figure 12 and 13. See the **Appendix** section for the complete MATLAB code which was scripted for this experiment.

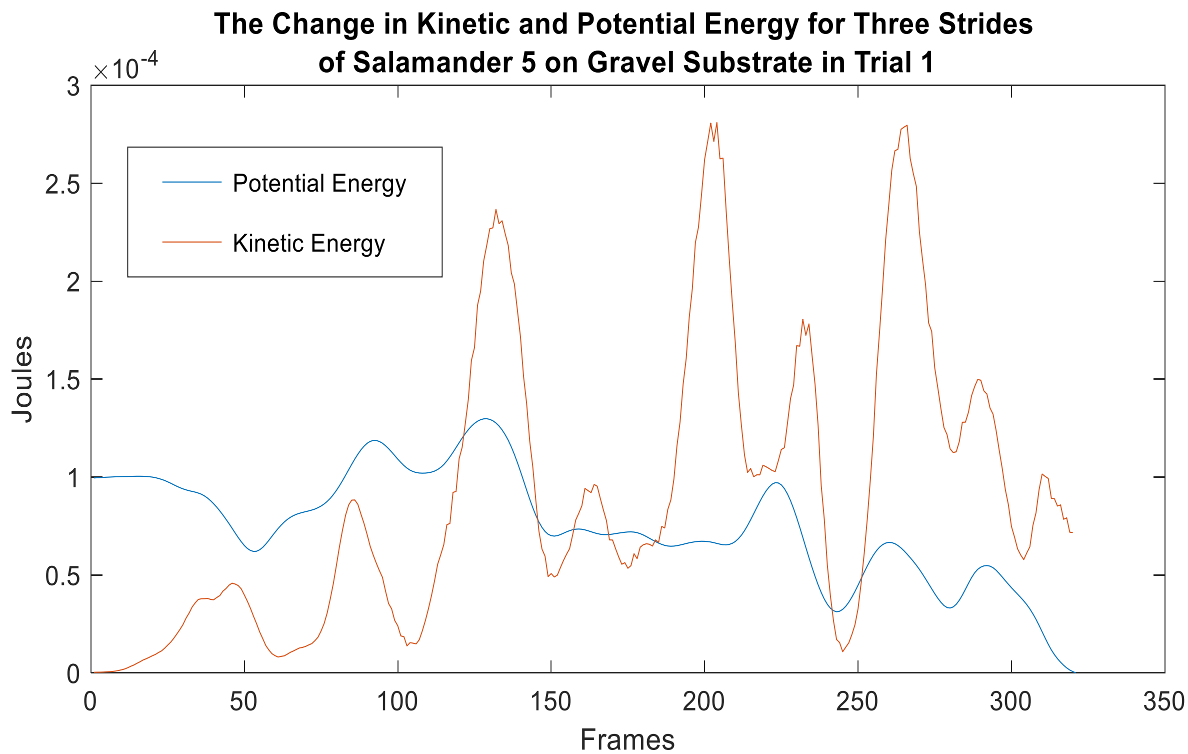


Figure 12: These oscillations show the change in kinetic and potential energy throughout the strides of Salamander 5 on gravel substrate during trial 1.

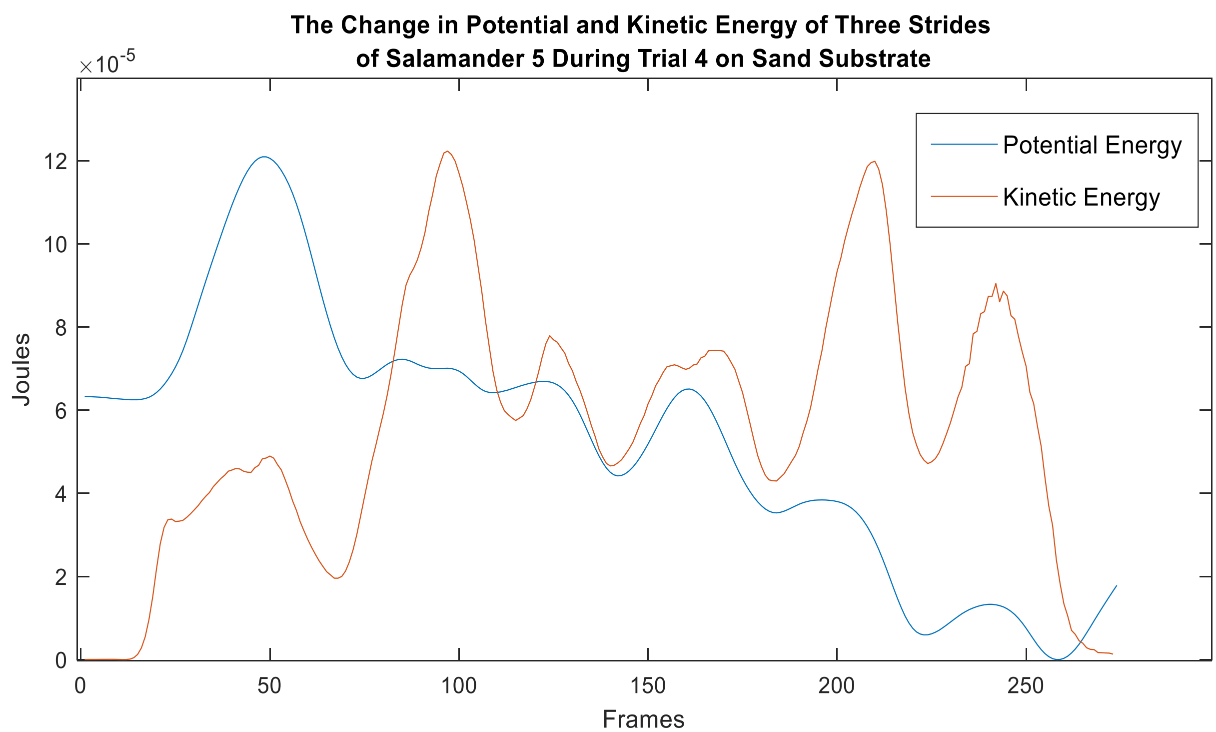


Figure 13: These oscillations show the change in kinetic and potential energy throughout the strides of Salamander 5 on the sandpaper substrate during trial 4.

Statistical Analysis and Results

Using the constructed code in MATLAB the sandpaper and gravel substrates were compared using stride duration, stride lengths, duty factor, slip lengths, average front foot velocity, potential energy, and kinetic energy for each individual stride. Using a transposed excel file saved from MATLAB holding all of the calculated variables, a statistical analysis was conducted after putting every excel file's data into the same compendium. This final excel spreadsheet containing the data from both Salamander 5 and Salamander 3 was then pooled together into JMP Pro 14 for statistical analysis using T-tests to test for any significant difference between the gravel and sand substrates.

Results from multiple T-tests indicated that there were significant differences existing between the salamanders on gravel and sand substrates for the following variables: minimum kinetic energy, average velocity, average velocity in body lengths, stride duration, and stride length in body lengths. Out of the 13 variables inputted as a Y variable or response factor in JMP these 5 kinematic variables were determined to be statistically different between gravel and sand substrates as their p-values were less than 0.05. Slip length in pixels, meters, and body lengths were not found to be statistically different between the gravel and sand substrate. Stride length in pixels and meters were also found to not be statistically different when comparing the salamanders on gravel and smooth substrates. Additionally, the maximum kinetic energy and the maximum kinetic energy of the salamanders were not statistically different between the sand and gravel substrates. The statistically insignificant kinematic variables and their resulting data can be found within the **Appendix** section at the end of this report.

Statistically Significant Variables:

Stride Duration and Stride Length in Body Lengths

As shown in Figure 14, the mean stride duration on gravel substrate was 1.084 seconds whereas the mean on the sandpaper substrate was 0.97 seconds. Figure 16 displays a T-test conducted for stride duration between the two substrates which calculated a p-value of 0.0092. This indicated that there was a statistical difference between the stride durations of the salamanders on gravel and sandpaper substrates. According to Figure 15 the mean stride length measured in salamander body lengths for the gravel substrate was $3.321e^{-4}$ while the sandpaper substrate had a mean of $3.804e^{-4}$. As shown in Figure 17 the p-value was determined to be 0.0485 which supported that there was statistical difference between the stride length in body lengths on the gravel and sandpaper substrates.

Substrate									
Gravel					Sand				
Stride Duration					Stride Duration				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
56.38365	1.0843009615385	0.2122503353668	0.66667	1.7667	52.38335	0.970062037037	0.2308805548606	0.61667	1.6667

Figure 14: Summary statistics of stride duration in seconds for gravel and sandpaper substrates.

	Substrate									
	Gravel					Sand				
	Stride Length BL					Stride Length BL				
	Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
	1.73e-2	3.321e-4	0.000129534	2.21e-5	7.50e-4	2.05e-2	3.804e-4	0.00011884	1.37e-4	7.89e-4

Figure 15: Summary statistics of stride length in body lengths for gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	-0.11424	t Ratio	-2.6535
Std Err Dif	0.04305	DF	103.7809
Upper CL Dif	-0.02886	Prob > |t|	0.0092*
Lower CL Dif	-0.19962	Prob > t	0.9954
Confidence	0.95	Prob < t	0.0046*

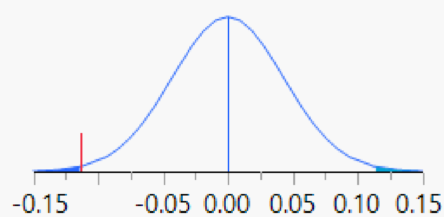


Figure 16: T-test results of stride duration in seconds. The p-value was 0.0092 which supported that there was a statistical difference between stride durations on the gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	0.000048	t Ratio	1.996589
Std Err Dif	0.000024	DF	102.4274
Upper CL Dif	0.000096	Prob > |t|	0.0485*
Lower CL Dif	3.189e-7	Prob > t	0.0243*
Confidence	0.95	Prob < t	0.9757

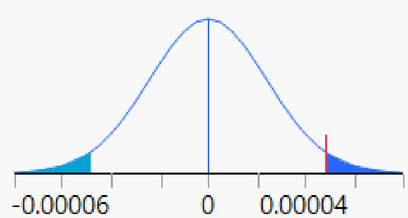
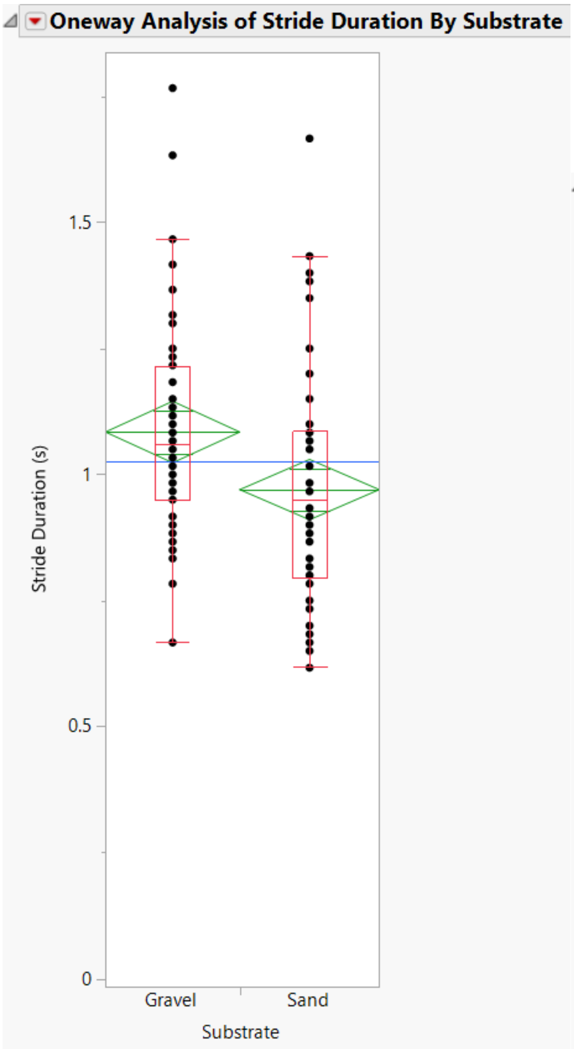


Figure 17: T-Test results of stride length in body lengths. The p-value was 0.0485 which supported that there was statistical difference between stride length in body lengths on the gravel and sandpaper substrates.



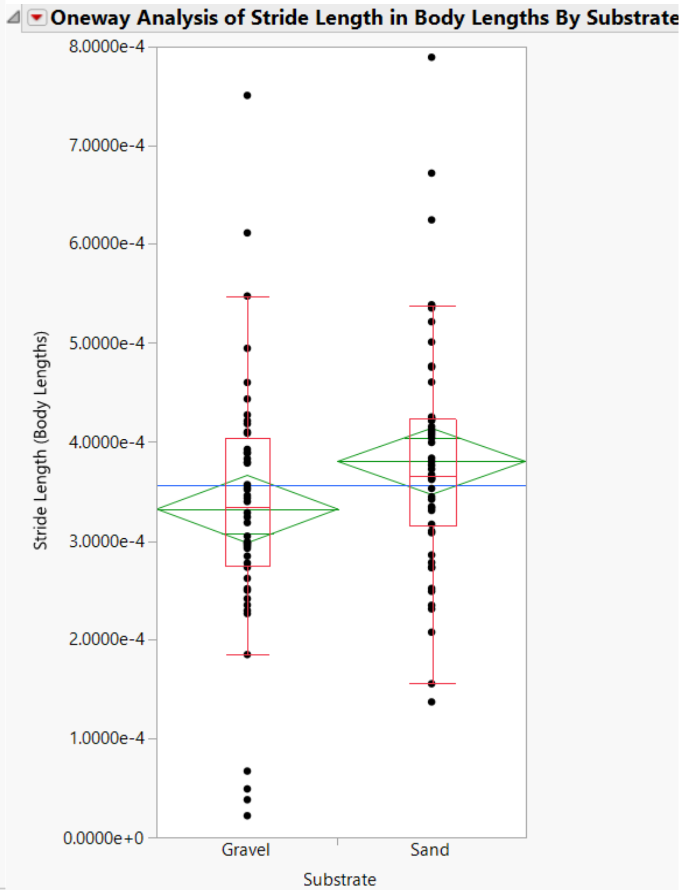


Figure 18 (left): Scatterplot of stride duration (s) displayed with box-plots and mean diamonds comparing the two substrates. The salamanders' strides on average were longer on the gravel substrate compared to the sandpaper.

Figure 19 (right): Scatterplot of stride lengths (body lengths) displayed with box-plots and mean diamonds comparing the two substrates. The salamanders on average had longer body lengths during their stride on the sandpaper substrate compared to gravel.

Front Foot Velocity and Front Foot Velocity in Body Lengths

The kinematic variables front foot velocity (m/s) and front foot velocity (body lengths) were also found to be statistically different. In Figure 20 the mean velocity (m/s) of the front foot of the salamanders was 0.08439 m/s on the gravel substrate while the mean on the sandpaper substrate was 0.1034 m/s. As shown in Figure 22 the p-value was 0.0012 which supported that there was statistical difference between the average front foot velocity (m/s) on the gravel and sandpaper substrates. For front foot velocity measured in body lengths the mean of the salamanders on gravel substrate was $3.360e^{-4}$ body lengths while the mean of the salamanders on the sandpaper substrate was $4.112e^{-4}$ as listed in Figure 21. Additionally, a T-test in Figure 23 was conducted and found that the p-value between the two substrates was 0.0030 which indicated that there was a significant difference between the two means.

Substrate									
Gravel					Sand				
Velocity (m/s)					Velocity (m/s)				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
4.388660367	0.08439731475	0.023092641745	0.044253	0.15103	5.584946	0.1034249259259	0.0346371421426	0.048961	0.21458

Figure 20: Summary statistics of front foot velocity (m/s) for gravel and sandpaper substrates.

Substrate									
Gravel					Sand				
Velocity BL					Velocity BL				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
1.75e-2	3.360e-4	0.000092191	1.86e-4	6.00e-4	2.22e-2	4.112e-4	0.000154597	1.93e-4	8.16e-4

Figure 21: Summary statistics of velocity measured in body lengths for gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	0.019028	t Ratio	3.33908
Std Err Dif	0.005698	DF	92.69598
Upper CL Dif	0.030344	Prob > |t|	0.0012*
Lower CL Dif	0.007711	Prob > t	0.0006*
Confidence	0.95	Prob < t	0.9994

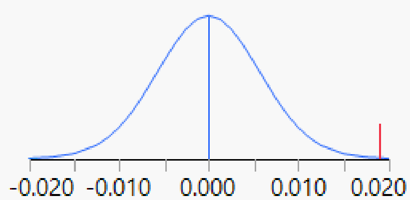


Figure 22: T-Test results of average front foot velocity (m/s). The p-value was 0.0012 which supported that there was statistical difference between the average front foot velocity (m/s) on the gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	0.000075	t Ratio	3.056346
Std Err Dif	0.000025	DF	87.03728
Upper CL Dif	0.000124	Prob > |t|	0.0030*
Lower CL Dif	0.000026	Prob > t	0.0015*
Confidence	0.95	Prob < t	0.9985

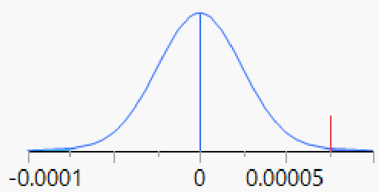
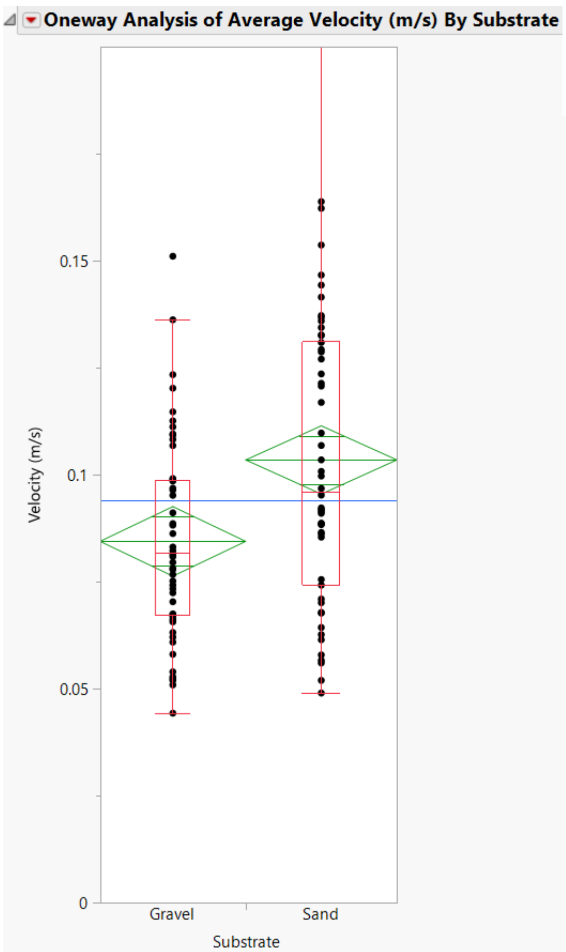


Figure 23: T-Test results of the average front foot velocity in body lengths. The p-value was 0.0030 which supported that there was statistical difference between stride length in body lengths on the gravel and sandpaper substrates.



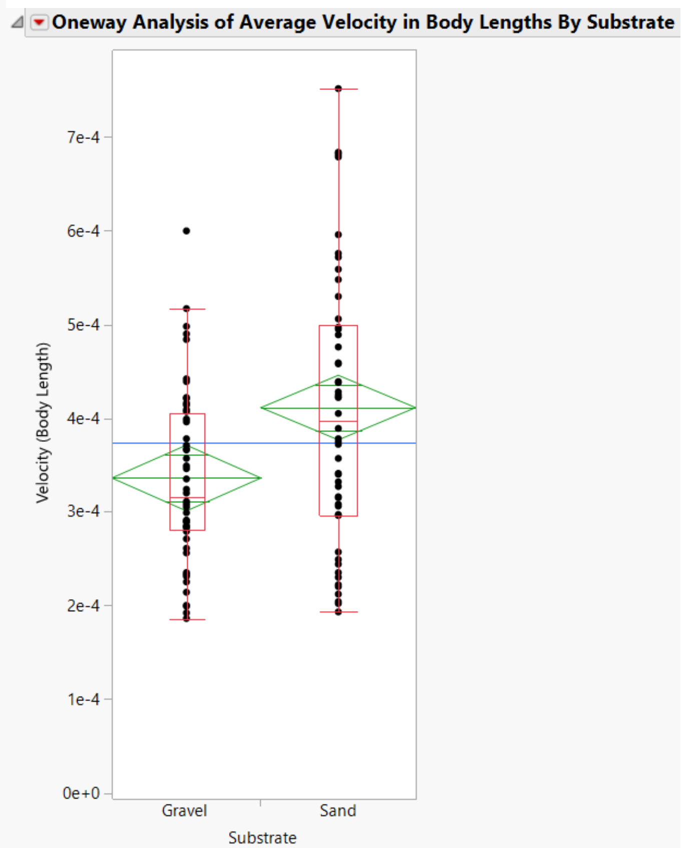


Figure 24 (left): Scatterplot of front foot velocity (m/s) displayed with box-plots and mean diamonds comparing the two substrates. The salamanders on average had a faster front foot velocity (m/s) when they were walking on the sandpaper substrate.

Figure 25 (right): Scatterplot of front foot velocity (body lengths) displayed with box-plots and mean diamonds comparing the two substrates. The salamanders on average had a faster front foot velocity (body lengths) when they were walking on the sandpaper substrate.

Minimum Kinetic Energy

The minimum kinetic energy measured in joules throughout each stride of the salamanders were calculated and averaged. Figure 26 displays that the mean kinetic energy minimum for the salamanders on gravel substrate was $4.049e^{-5}$ joules while the mean on the sandpaper substrate was $9.508e^{-5}$. The minimum kinetic energy mean for the salamanders on the sandpaper substrate was doubled during their strides compared to the gravel substrate. In Figure 27 displaying t-test results shows that the p-value between the two substrates was less than 0.0001 indicating a large statistical difference between the minimum kinetic energy for the sandpaper and gravel substrates.

Substrate									
Gravel					Sand				
Kemin					Kemin				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
2.11e-3	4.049e-5	3.087138e-5	2.55e-6	1.17e-4	5.13e-3	9.508e-5	0.000069494	1.41e-6	3.91e-4

Figure 26: Summary statistics of the minimum kinetic energy during a stride on the gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	0.000055	t Ratio	5.257977
Std Err Dif	0.000010	DF	73.73068
Upper CL Dif	0.000075	Prob > |t|	<.0001*
Lower CL Dif	0.000034	Prob > t	<.0001*
Confidence	0.95	Prob < t	1.0000

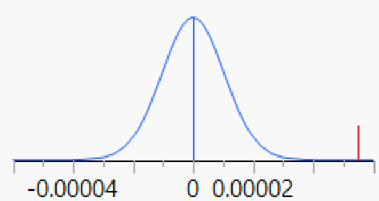


Figure 27: T-Test results of the minimum kinetic energy during a stride. The p-value was 0.0001 indicating statistical difference between the minimum kinetic energy of a stride on the gravel and sandpaper substrate.

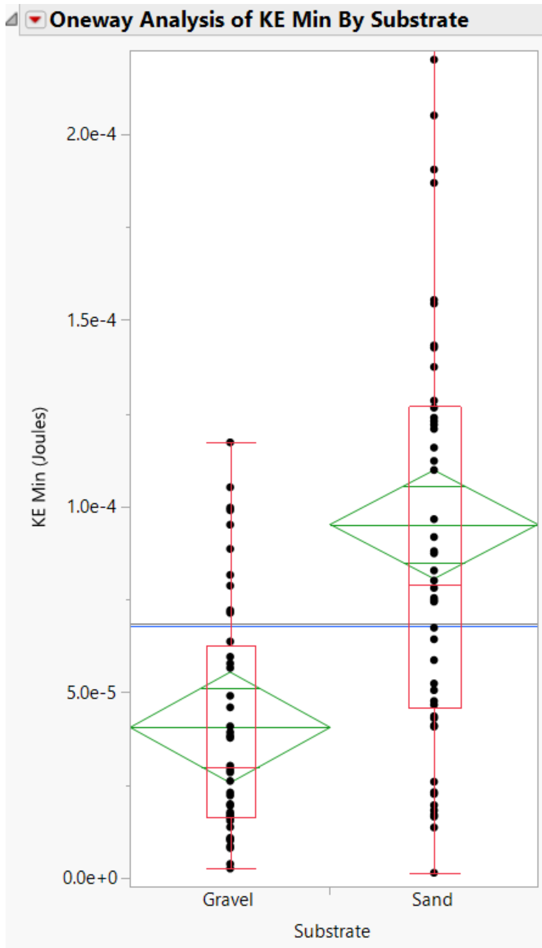


Figure 28: Scatterplot of the minimum kinetic energy in joules displayed with box-plots and mean diamonds comparing the two substrates. The salamanders on average had a lower kinetic energy minimum when they were walking on the gravel substrate.

Discussion

One of the biggest issues with the previously recorded sequences of salamanders underwater walking was that only one camera and visual perspective was analyzed. Even if the salamander appeared to walk continuously and perfectly straight to the human eye the analysis of data proved to be distorted and unreliable. The problem with digitizing only the lateral perspective was that the data could not describe the salamander's complete range of motion when the salamander's locomotion was angled and moved in left or right direction. In addition, many of the collected and digitized video trials did not all meet the proper criteria for statistical analysis due to the lack of three or more total and continuous strides. Due to this unsuitable data the , "average stride length, stride duration, stance duration, swing duration, duty factor, stride velocity, slip distance, percentage into the stride maximum foot velocity occurred, and percentage into the stride minimum foot velocity occurred were calculated from a variable number of analyzed strides for each salamander on each substrate type" (Cressman 2018). Based on these analyzed strides, “JMP analysis of averaged stride duration, stance duration, and swing duration values for each salamander on each substrate condition revealed that all durations are significantly greater for walking on gravel versus glass substrate” (Cressman 2018). It was proposed that the gravel substrate provided more foot grip which allowed for more propulsion therefore causing a longer stride duration. In addition, there appeared to be no significant difference for any other calculated kinematic variable between the two substrates. However, there were expectations that there would be a present significant difference between the calculated kinematic variables of stride velocity and foot slip between the rough gravel and smooth glass bottom substrate. Therefore, it was generally concluded that, “the kinematics of underwater salamander walking differ to some degree when the salamander walks on smooth or rough substrate” (Cressman 2018). The very purpose of this current experiment was to continue and compare results with the previous study conducted last year.

Stride duration in this experiment was statistically different between the two substrates with the sandpaper substrate having on average a greater stride duration. However, in the previous study conducted by Cressman, stride duration was indeed statistically different with the smoother plexiglass substrate compared to gravel. This result is contradictory to prior research that only involved the use of one camera. Unlike the previous experiment’s results, this experiment acquired more variables that proved to have statistical significance between the two tested substrates. The average velocity in meters per seconds, average velocity in body lengths, and the stride length in body lengths of the salamanders were found to be statistically different for the gravel and sandpaper substrate. In the previous experiment there were no statistical differences found between these variables. In addition, with the use of two cameras in this project we were finally able to calculate the minimum and maximum potential and kinetic energy for each stride collected from the trial videos of the two salamanders. This enabled the salamanders change in kinetic and potential energies to be graphically represented for each trial on both sandpaper and gravel substrate. After interpretation of the results and re-evaluating the expectations from these variables, the sandpaper substrate perhaps was not the best substrate to test the salamanders on. Perhaps using the bottom smooth plexiglass surface of the tank or actual sand could have been used instead. The gravel and sandpaper substrate might provide the salamanders with a similar level of traction and grip, which would explain why there is no statistical difference between any of the three foot slip variables. The next step in this experiment would be to purchase more force sensors and create a runway for a salamander to step upon each force sensor in order to record exactly how much force a salamander uses to propel its body via its feet with contact to its substrate while underwater. Therefore, we would be able to gather more information such as energy expenditure and a more accurate measurement in the change in both kinetic and potential energy while the salamander is underwater walking.

Conclusion

Salamander underwater walking although studied using various techniques and having applied information utilizing other viable and similar model organisms, has yet to be completely pieced together. The goal of this study was to further advance the previous endeavor to quantify the kinematics extracted from five anatomical points on a salamander using a lateral and dorsal video camera viewpoint. This set-up enabled the improved MATLAB code to take into account vertical, horizonal, and diagonal movement of the salamander's strides. Improvement of the frictionless and tractable substrate from having the salamander walk on the smooth bottom plexiglass surface of the fish tank to the homemade cardboard sandpaper-like "sand" substrate allowed for more accurate digitization. Also, the addition of the fifth point tracking the area above the pectoral girdle on the salamander provided a better understanding and corroboration to other previous studies that also digitized this anatomical point and extracted new kinematic measurements. This experiment was successful in finding five statistically different kinematic variables between the gravel and sandpaper substrate for the two tested salamanders. However, there were contradictory results when compared to the previous experiment such as for the variable, stride duration. Of these five variables, the new measurement minimum kinetic energy was found to be statistically different between the gravel and sandpaper substrates. This variable along with maximum kinetic energy and maximum potential energy allowed MATLAB to calculate and plot the change in kinetic and potential energy of the salamanders during their trials. This information on potential and kinetic energy of a salamander while underwater walking is relatively new and not yet published in the field of biology and biomechanics. Overall, it was statistically warranted to state that the kinematics of underwater walking for salamanders differ to some degree between smooth and rough substrates.

Acknowledgements

This experiment could not have been conducted without the lab of and the vast biomechanical knowledge of organisms from Dr. Henry Astley. I would like to thank Sophie Cressman for providing technical help in filming the salamanders and allowing me to continue her research. I also thank Dr. Henry Astley, Dr. Janna Andronowski, and Dr. James Holda who provided critical comments and constructive criticism on the rough and final draft of this report.

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Appendix

Statistically Insignificant Variables:

Stride Length in Pixels and Stride Length in Meters

Substrate									
Gravel					Sand				
Stride Length (pixels)					Stride Length (pixels)				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
11909.7068634	229.03282429615	57.384521025788	128.62	432.58	13267.57	245.69574074074	76.49898405052	147.27	603.61

Figure 29: Summary statistics of the stride length in pixels of the gravel and sandpaper substrates.

Substrate									
Gravel					Sand				
Stride Length (m)					Stride Length (m)				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
4.647005684	0.0893654939231	0.0238241353874	0.051853	0.18879	5.191304	0.0961352592593	0.0279707426478	0.043739	0.211

Figure 30: Summary statistics of the stride length in meters for the gravel and sandpaper substrates.

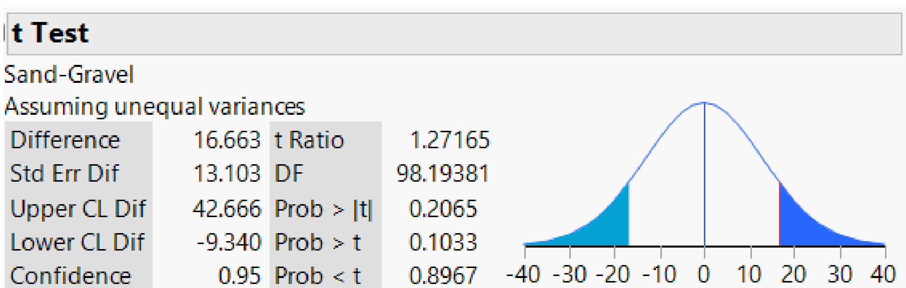


Figure 31: T-Test results of the stride length in pixels. The p-value was .2065 which indicated that there was no statistical difference between the stride length (pixels) of the salamanders on the gravel and sandpaper substrates.

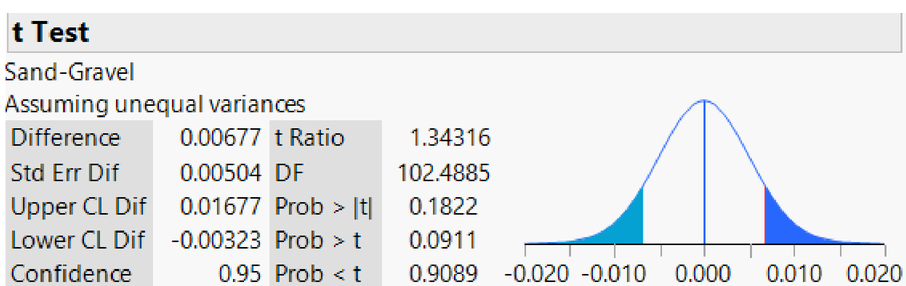
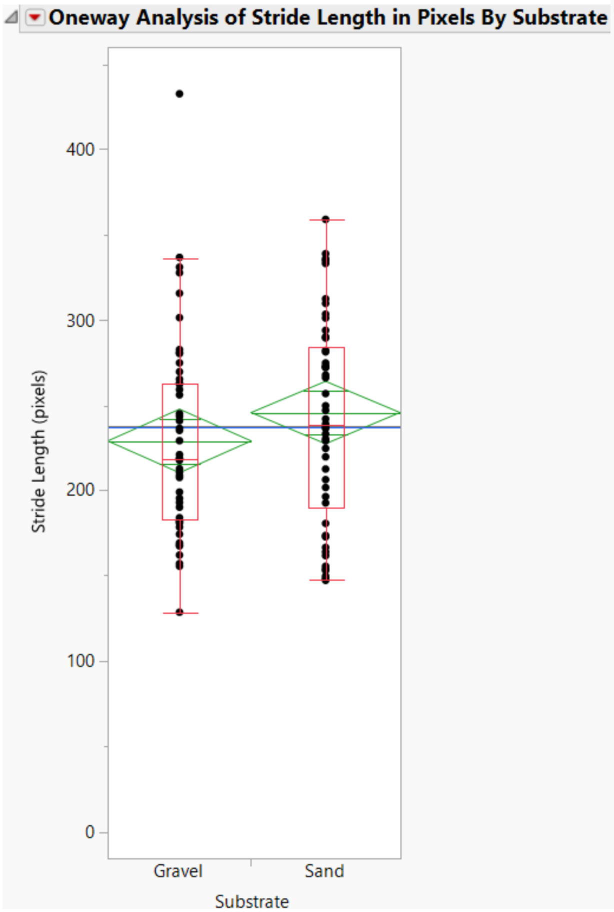


Figure 32: T-Test results of the stride length in meters. The p-value was .1822 which indicated that there was no statistical difference between the stride length (meters) of the salamanders on the gravel and sandpaper substrates.



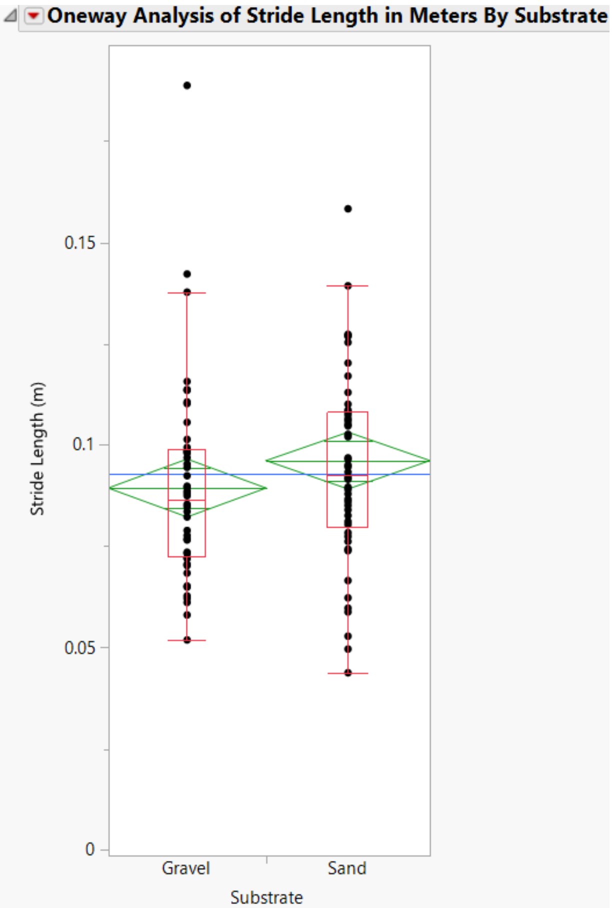


Figure 33 (left): Scatterplot of the stride length in pixels displayed with box-plots and mean diamonds comparing the two substrates. The salamanders on average had a longer stride length (pixels) on the sandpaper substrate but was not statistically different from gravel.

Figure 34 (right): Scatterplot of the stride length in meters displayed with box-plots and mean diamonds comparing the two substrates. The salamanders on average had a longer stride length (meters) on the sandpaper substrate but was not statistically different from gravel.

Duty Factor and Front Foot Slip in Pixels

Substrate									
Gravel					Sand				
Duty Factor					Duty Factor				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
26.701241228	0.5134854082308	0.0665967382398	0.39683	0.69231	27.53001	0.509815	0.0980681214095	0.24324	0.79545

Figure 35: Summary statistics of duty factor (%) for gravel and sandpaper substrates.

	Substrate									
	Gravel					Sand				
	Slip (pixels)					Slip (pixels)				
	Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
	6305.89666954	121.267243645	122.90890934744	0.60382	360.27	7304.0989	135.26109074074	134.37915759449	0.4704	419.5

Figure 36: Summary statistics of slip in pixels for gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	-0.00367	t Ratio	-0.22616
Std Err Dif	0.01623	DF	93.60796
Upper CL Dif	0.02856	Prob > |t|	0.8216
Lower CL Dif	-0.03590	Prob > t	0.5892
Confidence	0.95	Prob < t	0.4108

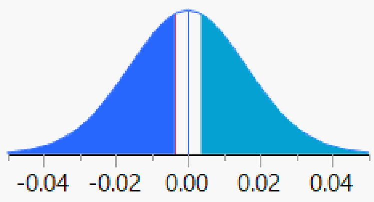


Figure 37: T-Test results for the percentage of duty factor. The p-value was 0.8216 which indicated no statistical difference in the duty factors between the sandpaper and gravel substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	13.994	t Ratio	0.559792
Std Err Dif	24.998	DF	103.73
Upper CL Dif	63.568	Prob > |t|	0.5768
Lower CL Dif	-35.580	Prob > t	0.2884
Confidence	0.95	Prob < t	0.7116

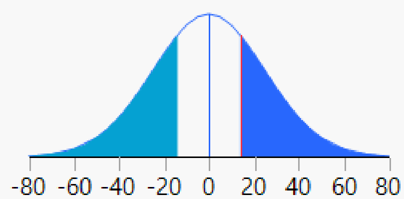
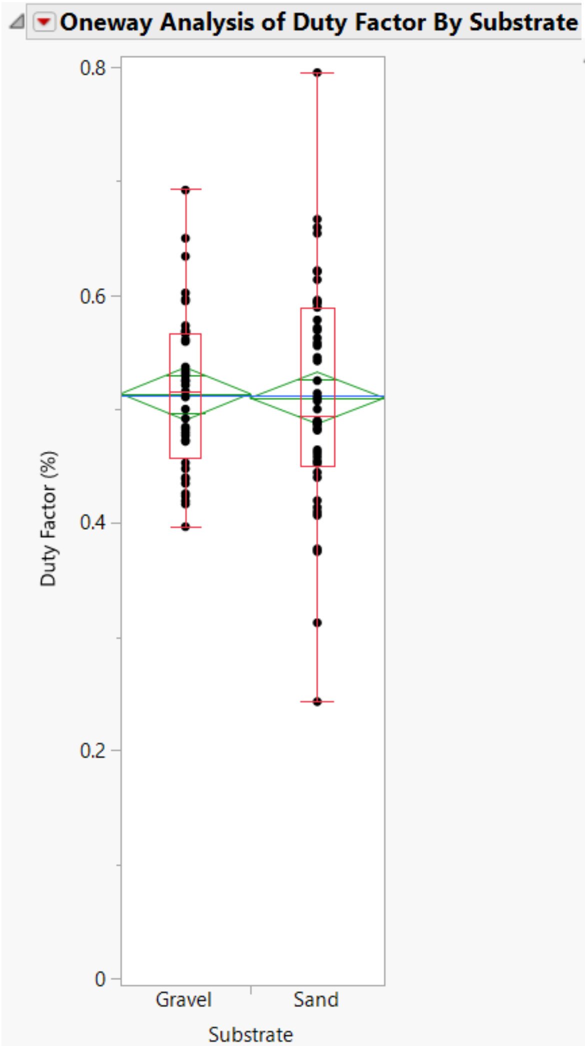


Figure 38: The T-Test result for the foot slip in pixels. The p-value was 0.5768 was indicated that there was no statistical difference in foot slip distance (pixels) between the gravel and sandpaper substrates.



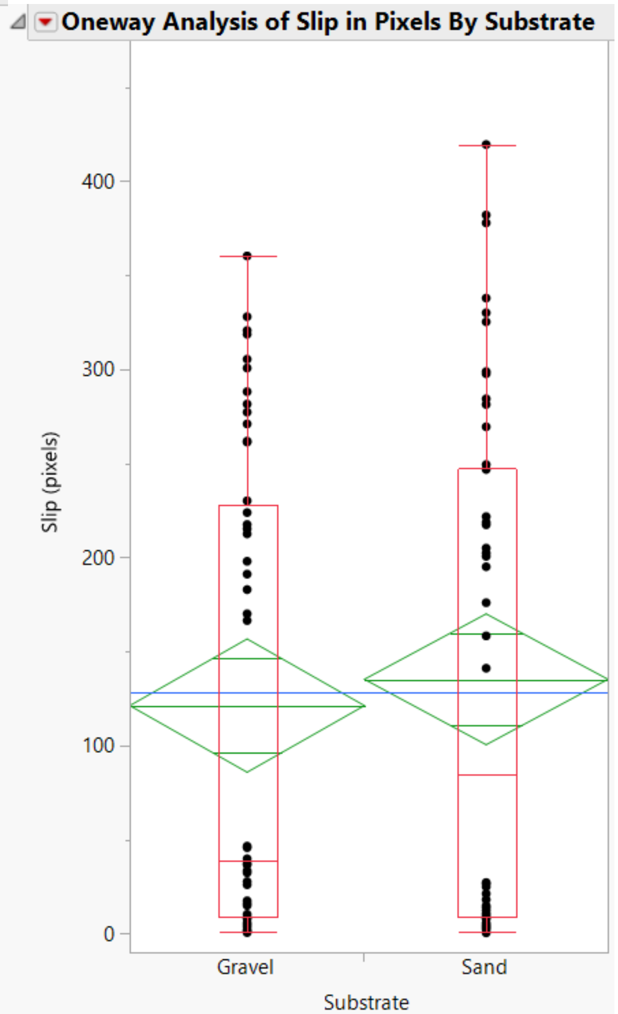


Figure 39 (left): Scatterplot of the duty factor percentage displayed with box-plots and mean diamonds comparing the two substrates. There was no statistical difference between the duty factor of the sandpaper and gravel substrate.

Figure 40 (right): Scatterplot of the foot slip distance in pixels displayed with box-plots and mean diamonds comparing the two substrates. The salamanders on average had longer slip (pixels) on the sandpaper substrate but was not statistically different from gravel.

Slip in Meters and Slip in Body Lengths

	Substrate									
	Gravel					Sand				
	Slip (meters)					Slip (meters)				
	Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
	2.426718586	0.0466676651154	0.0474457614685	0.00024342	0.15723	3.08216158	0.0570770662963	0.0564376246659	0.00018824	0.16285

Figure 41: Summary statistics of the front slip distance in meters for the gravel and sandpaper substrates.

	Substrate									
	Gravel					Sand				
	Slip BL					Slip BL				
	Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
	9.57e-3	1.840e-4	0.000188107	1.08e-6	6.25e-4	1.25e-2	2.313e-4	0.000236393	8.27e-7	8.11e-4

Figure 42: Summary statistics of foot slip in body lengths for the gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	0.01041	t Ratio	1.029294
Std Err Dif	0.01011	DF	102.1588
Upper CL Dif	0.03047	Prob > |t|	0.3058
Lower CL Dif	-0.00965	Prob > t	0.1529
Confidence	0.95	Prob < t	0.8471

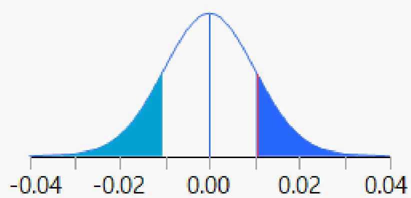


Figure 43: T-Test results of front foot slip in meters. The p-value was 0.3058 which indicated no statistical difference in foot slip (meters) between the gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	4.738e-5	t Ratio	1.144007
Std Err Dif	4.142e-5	DF	100.4716
Upper CL Dif	0.00013	Prob > |t|	0.2553
Lower CL Dif	-3.48e-5	Prob > t	0.1277
Confidence	0.95	Prob < t	0.8723

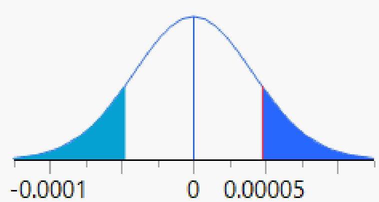
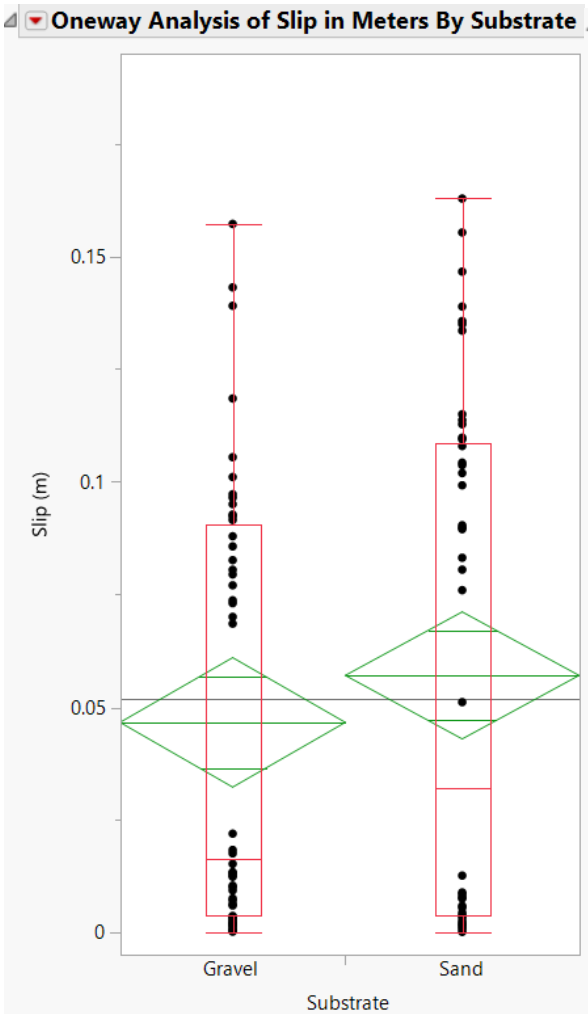


Figure 44: T-Test results of the front foot slip in body lengths. The p-value was 0.2553 which indicated no statistical difference between slip (body lengths) for the gravel and sandpaper substrates.



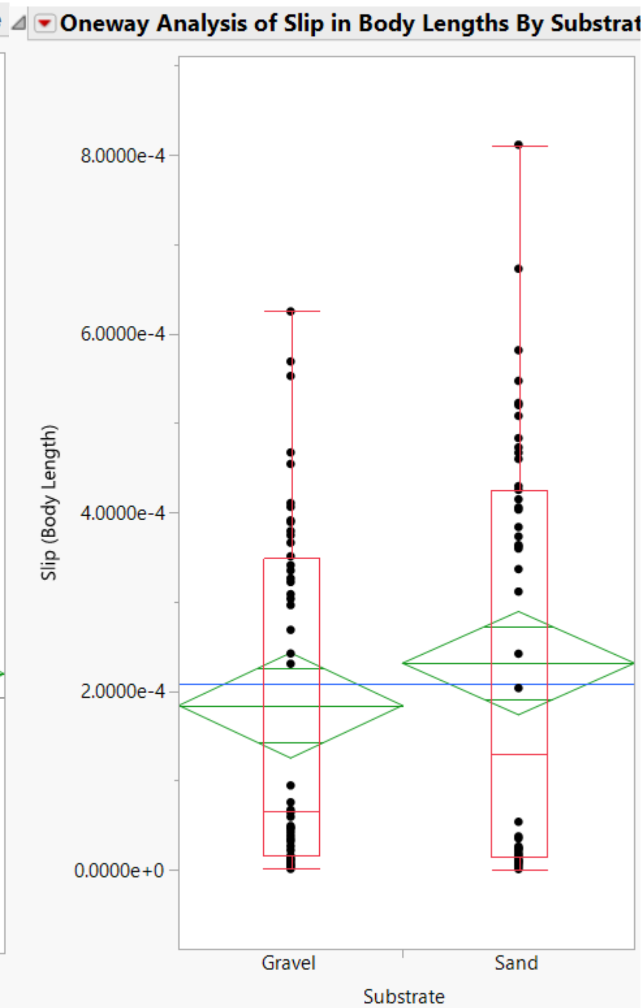


Figure 45 (left): Scatterplot of the front foot slip distance in meters displayed with box-plots and mean diamonds comparing the two substrates. On average, the salamanders had more slip on the sandpaper substrate, however there was no statistical difference between the gravel substrate.

Figure 46 (right): Scatterplot of front foot slip distance in body lengths displayed with box-plots and mean diamonds comparing the two substrates. On average, the salamanders had more slip (body lengths) on the sandpaper substrate, however there was no statistical difference between the gravel substrate.

Maximum Potential and Kinetic Energy

Substrate									
Gravel					Sand				
Pemax					Pemax				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
0.081445215	0.0015662541346	0.0007578736515	0.00040333	0.0042195	0.07292271	0.0013504205556	0.0006073376842	0.00041462	0.0026503

Figure 47: Summary statistics of the maximum potential energy in joules during a stride for the gravel and sandpaper substrates.

Substrate									
Gravel					Sand				
Kemax					Kemax				
Sum	Mean	Std Dev	Min	Max	Sum	Mean	Std Dev	Min	Max
1.07e-2	2.059e-4	0.000085609	5.91e-5	5.33e-4	1.25e-2	2.307e-4	0.000138091	6.30e-5	7.68e-4

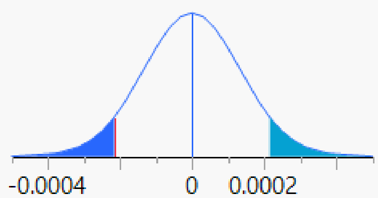
Figure 48: Summary statistics of the maximum kinetic energy in joules during a stride for the gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	-0.00022	t Ratio	-1.61428
Std Err Dif	0.00013	DF	97.64754
Upper CL Dif	4.951e-5	Prob > |t|	0.1097
Lower CL Dif	-0.00048	Prob > t	0.9452
Confidence	0.95	Prob < t	0.0548



4

Figure 49: T-Test results of the maximum potential energy during a stride in joules. The p-value was 0.1097 which indicated no statistical significance for the maximum potential energy (joules) during a stride between the gravel and sandpaper substrates.

t Test

Sand-Gravel

Assuming unequal variances

Difference	2.477e-5	t Ratio	1.11431
Std Err Dif	2.223e-5	DF	89.01372
Upper CL Dif	0.000069	Prob > |t|	0.2681
Lower CL Dif	-0.00002	Prob > t	0.1341
Confidence	0.95	Prob < t	0.8659

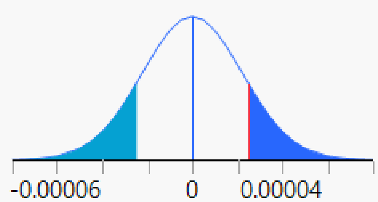


Figure 50: T-Test results of the maximum kinetic energy in joules during a stride. The p-value was 0.2681 which indicated that there was no statistical significance in the maximum kinetic energy (joules) between the gravel and sandpaper substrates during a stride.

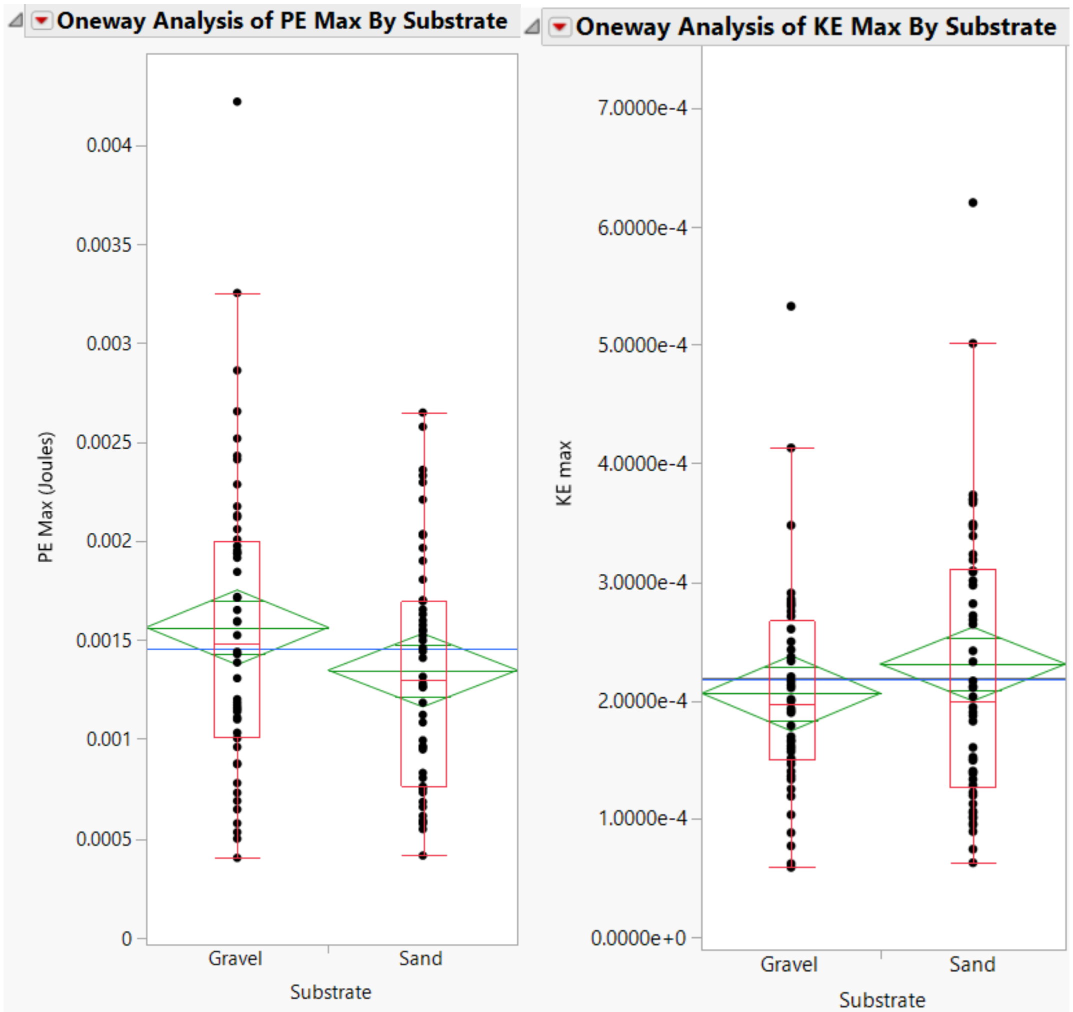


Figure 51 (left): Scatterplot of the maximum potential energy during a stride in joules displayed with box-plots and mean diamonds comparing the two substrates. On average, the salamanders had a larger potential energy maximum (joules) on the gravel substrate during a stride, however there was no statistical difference between the sandpaper substrate.

Figure 52 (right): Scatterplot of the maximum kinetic energy in joules during a stride displayed with box-plots and mean diamonds comparing the two substrates. On average, the salamanders had a larger kinetic energy maximum on the sandpaper substrate, however there was no statistical difference between the gravel substrate.

Salamander MATLAB Script
Dr. Henry Astley and Megan Lee

```
clear all

uiopen('C:\Users\Owner\Desktop\Honors Project Code and Figures\Salamander 5 Excel Sheets\Gravel Smooth\032019_Sal5_gravel_side_vid1__smoothed.csv',1)
uiopen('C:\Users\Owner\Desktop\Honors Project Code and Figures\Salamander 5 Excel Sheets\Gravel Smooth\032019_Sal5_gravel_top_vid1_smoothed.csv',1)
FileSide= '032019_Sal5_gravel_side_vid1__smoothed.csv';
FileTop= '032019_Sal5_gravel_top_vid1_smoothed.csv';
date=str2double(FileSide(1:6));

a=strfind(FileSide,'Sal');
salnumber=str2double(FileSide(a+3));

b=strfind(FileSide,'NoSub');
if isempty(b)==1
  substrate='gravel';
else
  substrate='sand';
end
date=str2double(FileTop(1:6));

a=strfind(FileTop,'Sal');
salnumber=str2double(FileTop(a+3));

b=strfind(FileTop,'NoSub');
if isempty(b)==1
  substrate='gravel';
else
  substrate='sand';
end

v=strfind(FileTop,'vid');
d=strfind(FileTop,'xy');
vidnum=str2double(FileTop(v+3:d-1));

salamanderside= csvread(FileSide,1,0);
salamandertop= csvread(FileTop,1,0);

%X/Y OFFS SIDE
xoff1=salamanderside(1,1);
yoff1= salamanderside(1,2);
salamanderside(:,1)= salamanderside(:,1)-xoff1;
salamanderside(:,2)= salamanderside(:,2)-yoff1;
```

```
%xoff1=salamanderside(1,3);
%yoff1= salamanderside(1,4);
salamanderside(:,3)= salamanderside(:,3)-xoff1;
salamanderside(:,4)= salamanderside(:,4)-yoff1;

%xoff1=salamanderside(1,5);
%yoff1= salamanderside(1,6);
salamanderside(:,5)= salamanderside(:,5)-xoff1;
salamanderside(:,6)= salamanderside(:,6)-yoff1;

%xoff1=salamanderside(1,7);
%yoff1= salamanderside(1,8);
salamanderside(:,7)= salamanderside(:,7)-xoff1;
salamanderside(:,8)= salamanderside(:,8)-yoff1;

%xoff1=salamanderside(1,9);
%yoff1= salamanderside(1,10);
salamanderside(:,9)= salamanderside(:,9)-xoff1;
salamanderside(:,10)= salamanderside(:,10)-yoff1;

%(over and over, always with xoff1 and yoff1)
%(repeat with NEW xoff2 yoff2 values for 2nd video)
%X/Y OFFS TOP
xoff2=salamandertop(1,1);
yoff2= salamandertop(1,2);
salamandertop(:,1)= salamandertop(:,1)-xoff2;
salamandertop(:,2)= salamandertop(:,2)-yoff2;

%xoff2=salamandertop(1,3);
%yoff2= salamandertop(1,4);
salamandertop(:,3)= salamandertop(:,3)-xoff2;
salamandertop(:,4)= salamandertop(:,4)-yoff2;

%xoff2=salamandertop(1,5);
%yoff2= salamandertop(1,6);
salamandertop(:,5)= salamandertop(:,5)-xoff2;
salamandertop(:,6)= salamandertop(:,6)-yoff2;

%xoff2=salamandertop(1,7);
%yoff2= salamandertop(1,8);
salamandertop(:,7)= salamandertop(:,7)-xoff2;
salamandertop(:,8)= salamandertop(:,8)-yoff2;

%xoff2=salamandertop(1,9);
%yoff2= salamandertop(1,10);
```

```
salamandertop(:,9)= salamandertop(:,9)-xoff2;
salamandertop(:,10)= salamandertop(:,10)-yoff2;
Scale1=salamanderside(end,1)-salamanderside(1,1);
Scale2=salamandertop(end,1)-salamandertop(1,1);

if Scale1<0
    salamanderside(:,1)=-1*salamanderside(:,1);
    salamanderside(:,3)=-1*salamanderside(:,3);
    salamanderside(:,5)=-1*salamanderside(:,5);
    salamanderside(:,7)=-1*salamanderside(:,7);
    salamanderside(:,9)=-1*salamanderside(:,9);
end
if Scale2<0
    salamandertop(:,1)=-1*salamandertop(:,1);
    salamandertop(:,3)=-1*salamandertop(:,3);
    salamandertop(:,5)=-1*salamandertop(:,5);
    salamandertop(:,7)=-1*salamandertop(:,7);
    salamandertop(:,9)=-1*salamandertop(:,9);
end

videoratio=abs(Scale2/Scale1);
salamandertop(:,:)=salamandertop(:,:)/videoratio;

%plot to check
figure(1)
plot(salamanderside(:,1));hold on;plot(salamandertop(:,1));hold off;

%FRONT FOOT
frontfoot=salamanderside(:,1:2);
frontfoot(:,3)=salamandertop(:,2);


frontfootxval= frontfoot(:,1);
frontfootxspeed=diff(frontfootxval);
slipdetector=frontfootxspeed./abs(frontfootxspeed);
slipdetector(isnan(slipdetector))=1;
slipfind=diff(slipdetector);

figure(2)
plot(frontfootxspeed);hold on;plot(slipdetector);hold on;plot(slipfind)

[pts,slipends,w,p]=findpeaks(slipfind);
[pts,slipstarts,w,p]=findpeaks(slipfind*-1);

frontfoottobackXdist=frontfootxval-salamanderside(:,3);
[pts,locs,w,p]=findpeaks(frontfoottobackXdist);
```

```
pts(p<20)=[];
locs(p<20)=[];
w(p<20)=[];
p(p<20)=[];
[pts2,locs2,w2,p2]=findpeaks(frontfoottobackXdist*-1);
pts2(p2<20)=[];
locs2(p2<20)=[];
w2(p2<20)=[];
p2(p2<20)=[];
footfurthestforwardsframes=locs;
footfurthestbackwardsframes=locs2;

numberofstrides=size(locs,1)-1;

figure(3)
plot(frontfoottobackXdist)

%THE BACK
back=salamanderside(:,3:4);
back(:,3)=salamandertop(:,4)
backxval=back(:,1)

%Foot and Back Points
FootX=frontfoot(:,1);
FootY=frontfoot(:,2);
FootZ=frontfoot(:,3);
BackX=back(:,1);
BackY=back(:,2);
BackZ=back(:,3);

%THE HIP
hip=salamanderside(:,7:8);
hip(:,3)=salamandertop(:,8);
HipX=hip(:,1);
HipY=hip(:,2);
HipZ=hip(:,3);

%THE SNOUT
snout=salamanderside(:,5:6);
snout(:,3)=salamandertop(:,6)
SnoutX=snout(:,1);
SnoutY=snout(:,2);
SnoutZ=snout(:,3);


%Conversion to Meters
```

```
bodylengthframes=((HipX-SnoutX).^2+(HipY-SnoutY).^2+(HipZ-SnoutZ).^2).^0.5;
bodylength=mean(bodylengthframes)
if salnumber==5
    mlength=.0911;

 end
pixeltometerratio=bodylength/mlength;
bodylength=bodylength.*pixeltometerratio;

if locs2(1)< locs(1)
   locs2(1)=[]
end

instantaneousvel=(((diff(BackX).^2+diff(BackY).^2+diff(BackZ).^2).^0.5)*60)/(pixeltometerrat
io);

for i = 1:numberofstrides
   strideduration(i)=(locs(i+1)-locs(i))/60;

   stancephase(i)=(footfurthestbackwardsframes(i)-footfurthestforwardsframes(i))/60;

   dutyfactor(i)=stancephase(i)/strideduration(i);

   stridelength(i)=sqrt(((BackX(footfurthestforwardsframes(i+1))-
BackX(footfurthestforwardsframes(i)))^2)+((BackY(footfurthestforwardsframes(i+1))-
BackY(footfurthestforwardsframes(i)))^2)+((BackZ(footfurthestforwardsframes(i+1))-
BackZ(footfurthestforwardsframes(i)))^2));

   stridelengthmeters(i)=stridelength(i)/pixeltometerratio

   stridelengthBL(i)=(stridelength(i))/(bodylength)

   slip(i)=sqrt(((FootX(slipstarts(i))-FootX(slipends(i)))^2)+((FootY(slipstarts(i))-
FootY(slipends(i)))^2)+((FootZ(slipstarts(i))-FootZ(slipends(i)))^2));

   slipmeters(i)=(slip(i))/(pixeltometerratio)

   slipBL(i)=(slip(i))/(bodylength)

   avevelocity(i)=stridelengthmeters(i)/strideduration(i);

   avevelocityBL(i)=stridelengthBL(i)/strideduration(i);

   maxvelocity(i)=max(instantaneousvel(locs(i)+1:locs(i+1)+1));
   minvelocity(i)=min(instantaneousvel(locs(i)+1:locs(i+1)+1));
   maxheight(i)=max(BackY(locs(i)+1:locs(i+1)+1))/(pixeltometerratio);
```

```
    minheight(i)=min(BackY(locs(i)+1:locs(i+1)+1))/(pixeltometerratio);

    if substrate=='gravel'
        sublist(i)=1;
    else
        sublist(i)=0;
    end

    salmass=0.0268; %kg
    %Underwater weight=.91g (0.00091 kg)
    KEmax(i)=(.5)*(salmass)*(maxvelocity(i)^2);
    KEmin(i)=(.5)*(salmass)*(minvelocity(i)^2);
    PEmax(i)=(salmass)*(maxheight(i))*(9.81)-(salmass)*(minheight(i))*(9.81);
    PEmin(i)=0;


end
KEchange=(.5)*(salmass)*(instantaneousvel.^2);
PEchange=(salmass)*9.81*(BackY-min(BackY))/(pixeltometerratio);
%insert waterweight?? block wood down frictionless ramp. friction=losing
%potential energyX ratio between land and water weight
%KE only chnages momomentum
figure(4)
plot(PEchange*0.03395); hold on; plot(KEchange);
%3.39% is the ratio between land and water weight

newFileSide = strrep(FileSide,'.csv','.mat');
save(newFileSide);



DataTable=horzcat(sublist',strideduration',dutyfactor',stridelength',stridelengthmeters',stridelengt
hBL',slip',slipmeters',slipBL',avevelocity',KEmax',KEmin',PEmax',PEmin');

dtname=strrep(FileSide,'.csv','_DATA.csv');
csvwrite(dtname,DataTable);
```