A Comparison of Feeding and Jumping Behaviors in the Northern Leopard Frog,

Lithobates pipiens

by Candice Johnson, Master of Arts in Teaching

A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of
Master of Science
in the field of Biological Sciences

Advisory Committee:

Richard Essner, Chair

David Jennings

Daniel Welch

Graduate School
Southern Illinois University Edwardsville
December 2019

ProQuest Number: 27545866

ProQuest.

ProQuest 27545866

ABSTRACT

A Comparison of Feeding and Jumping Behaviors in the Northern Leopard Frog,

Lithobates pipiens

by

Candice Johnson

Chairperson: Dr. Richard Essner

Anuran jumping is an explosive movement that requires rapid, synchronous hind limb extension; however, the ancestral tetrapod bauplan is structured around asynchronous locomotion (i.e., lateral undulation). This raises the question as to the origin of synchronous locomotor behavior. It has been hypothesized that synchronous movement progressively developed from the motor training of a riparian feeding behavior. Lunge feeding has been proposed as a possible precursor to jumping in anurans, since it too involves rapid, synchronous hindlimb extension and is the ancestral feeding condition for both frogs and salamanders. In order to elucidate the relationship between jumping and feeding, we used high-speed video to quantify kinematics during jumping and feeding behaviors in the Northern Leopard Frog, *Lithobates pipiens*.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

Chapter

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Ancestral/Fossil Forms

Anurans are comprised of thousands of species who use various modes of locomotion. Most species exhibit synchronous locomotor behaviors. These behaviors are characterized by both forelimbs, along with both hind limbs moving in the same direction at the same time. Alternately, asynchronous locomotion is exhibited when forelimbs, or hind limbs move in opposite directions from each other when moving. It has been hypothesized that synchronous movement progressively developed from the motor training of a riparian feeding behavior. Lunge feeding has been proposed as a possible precursor to jumping in anurans, since it too involves rapid, synchronous hind limb extension and is the ancestral feeding condition for both frogs and salamanders. The morphology of anuran ancestral forms gives insight into the evolution of frog anatomy and in turn their behaviors.

Triadobatrachus massinoti, an early Triassic fossil found in Madagascar, is considered the oldest known anuran-like ancestor. It had an anuran-like skull but exhibited an elongated vertebral column relative to modern frogs. There were at least 26 vertebrae, possibly consisting of 15 presacral, 1 sacral, and 10 caudal vertebrae (Ascarrunz et al., 2016). The 15th vertebra is considered to be a sacral vertebra. This bone was short and did not contribute to a joint (Rage and Rocek, 1989). Modern frogs have shorter vertebral columns, consisting of a maximum of nine presacral vertebrae. The sacrum contributes to a urosacral joint and is connected to an elongated pelvic girdle. While modern adult frogs do not have tails, *Triadobatrachus* had 10

caudal bones. This could potentially be due to the specimen being a post-metamorphic juvenile that did not undergo complete resorption of its caudal vertebrae, but the life stage of the specimen is not certain (Ascarrunz et al., 2016). It appears that evolutionary changes leading to the anuran bauplan occurred first in the skull. This is because the *Triadobatrachus* cranial skeleton is more advanced, being more anuran-like than the postcranial skeleton. With its long body, short pelvic girdle, and tail, *Triadobatrachus* was not specialized for jumping as seen in modern frogs, contributing to the assumption that saltatorial locomotion must have evolved later (Rage and Rocek, 1986).

Prosalirus bitis is the earliest known anuran and illustrates that many distinctive anatomical features of modern frogs were established by the Early Jurassic (Shubin and Jenkins, 1995). Hind limb and tarsal elongation, radio-ulnar and tibiofibular fusion, and truncal shortening are exhibited. These characteristics all contribute to saltatory locomotion. The characteristic that is unique to frog saltatory locomotion is the "very flexible" iliosacral joint that is representative of jumping frogs (Green, 1931). The flexion-extension of the back provided by this joint is a critical component of anuran saltation (Emerson, 1982). The mobility of this joint allows the trunk to extend and in turn align with the propulsive force of the hind limbs during the preliminary phase of the launch. Although some have proposed that jumping has aquatic origins, the anatomy of *Prosalirus* suggests that terrestrial saltation is the primitive locomotion of anurans (Jenkins and Shubin, 1998).

Neural Control

The conversion of sensory signals into motor commands plays an essential role when generating behaviors (Fotowat et al., 2011). As the mechanics of frog movement evolved, the

nervous system developed along with it. Frogs receive visual stimuli from their environment through the ganglion cells of the retina, which then transport their projections to the superficial layers of the tectum in the brain. Prey capture and escape jumping rely heavily on the proper functioning of these cells (Gabriel et al., 1998). Information received through sensory input is transferred by neural networks that eventually cause motoneurons to fire at appropriate times. Various behaviors result from sensory information being transferred through different neural pathways. Startle responses in tadpoles, for instance, are initiated through the monosynaptic connections between Mauthner cells of the brainstem and the spinal motoneurons; whereas, swimming in adult frogs is mediated by the central pattern generators (CPG) which originate from the spinal cord (Stehouwer, 1992). CPGs are responsible for rhythmic locomotor behaviors such as swimming, flying, or walking (Wolfe et al., 2009). Once sensory input is received, organisms produce tightly coupled, stereotyped patterns used for most frequent modes of locomotion such as jumping in anurans.

Evolution of Jumping

Anuran jumping is an explosive movement that requires rapid and synchronous hindlimb extension (Peters et al., 1996); however, the ancestral tetrapod bauplan is designed around asynchronous locomotion (i.e., lateral undulation). While other members of Class Amphibia have asynchronous locomotion, such as the salamanders, anurans move synchronously when jumping. Frogs that display more primitive anuran characteristics, such as *Ascaphus truei,* jump synchronously, but use a more energetically inefficient, asynchronous gait when swimming (Abourachid and Green, 1999). Most anurans within the Suborder Neobatrachia, such as the Northern Leopard frog, *Lithobates pipiens*, exhibit synchronous, repeated, discrete hops when

active. The locomotion of toads, a member of the Superfamily Hyloidea of anurans, demonstrates an alternate way of moving that is less common and even more advanced by displaying a mammalian, synchronous bounding gait (Reilly et al, 2015). They are the only non-mammalian tetrapod known to do so. The progression of synchronous locomotion among various anuran species raises the question as to the origin of synchronous locomotor behavior in frogs.

Early amphibians exhibit aquatic specializations where hind and forelimbs were about equal in size. Equivalent limb proportions contributed to all limbs serving equally in support, steering, and propulsion (Peters et al., 1996). Early anurans show trends that alter this bauplan, such as shortening of the trunk and lengthening of hind limbs (Reilly et al., 2015). This would be advantageous in bipedal swimming as well as efficient terrestrial movement such as hopping if periodically leaving the water. The biomechanics of hopping and swimming both correlate strongly with their morphology (Peters et al., 1996). Asynchronous swimming, demonstrated by *Leiopelma hochstetteri* and *Ascaphus truei,* shows that trot swimming is an independent locomotor trait that derived separately from hopping (Abourachid and Green, 1999). The behavioral or morphological similarities between synchronous swimming and jumping might indicate that they share the same evolutionary origin.

Many frogs have restricted home ranges and exhibit limited movement outside of acquiring food, mates, or protection (Wells, 2010). The riparian hypothesis of origin for jumping suggests that frog-like ancestors might leave the water to feed, rest, or bask along the banks of bodies of water. Synchronous movement may have progressively developed from the motor training of a riparian feeding behavior such as catching prey along the banks of water (Gans and Parsons, 1966). Motor training occurs when a motor task is continually repeated and the muscles strengthen and perform without conscious effort. Behaviors can go through rapid development in

their initial stages due to high predator-induced selective pressures. Feeding terrestrially, while potentially advantageous for acquiring food, would cause the pre-frog to be more susceptible to terrestrial predators. The developed synchronous movement would then be beneficial in providing the ability to escape jump into the water. Therefor jumping may have adapted from feeding and become an exaptation for escape.

Feeding in Amphibians

Understanding amphibian feeding is essential to understanding the transition from aquatic to terrestrial behaviors that are found in basal lineages of all tetrapods (Lauder and Reilly, 1994). The transition to terrestrial life involved principally carnivorous species. Among carnivorous amphibian species today, there are two main methods of prey capture: one that reduces the mass of the part of the predator that comes in contact with the prey to more easily and inconspicuously reach its prey, and the second that attracts or baits the prey to the vicinity of the predator through methods like emitting attractive chemicals. Amphibians typically exhibit the first prey capture behavior. An example of this is through a well-developed projectile tongue, which typically occurs rapidly and reaches as far as, or beyond, the mandible. The soft tissue on the tongue's tip contains a mass of mucous glands that form around the prey upon impact and allow the tongue to bring prey back into the mouth cavity as it retracts (Vree and Gans, 1994). Many believe the hyoid is solely responsible for tongue projection, but muscle activity plays a major role as well (Gans and Gorniak, 1982).

There are basic feeding behaviors characteristic of the three orders of amphibians. Though many amphibians feed through tongue projection, there are a variety of feeding modes among the various life stages and species that include: jaw prehension, suction feeding, filter

feeding, and lunge feeding (Schwenk, 2000). While several feeding styles exist, most share a common developmental transition from aquatic larval suction-based feeding to a terrestrial adult tongue and jaw-based feeding behavior. In the case of many of the aquatic adult feeders, modifications of terrestrial feeding, such as tongue protraction and jaw prehension mechanisms, are still used (Deban, 2001).

Feeding in Salamanders

The Order Caudata, or salamanders, have both aquatic and terrestrial feeders. Aquatic feeding salamanders use a suction feeding mechanism to capture prey. Many species who begin as suction feeding aquatic larvae, eating microorganisms, transition to terrestrial feeding adults. Lauder and Reilly (1994), identified two key behaviors in the biomechanics of terrestrial salamander feeding which include the initial strike of prey and the transport of prey to the esophagus. Salamanders are known to use the same kinematic patterns no matter the feeding conditions. Adult salamanders have thick, short tongues that are flipped forward to catch prey. Their tongues are internally supported by the hyobranchial bones, which are used in coordination with the skull for prey capture (Lauder and Reilly, 1994). The tongue morphology varies greatly with regard to projection distance, muscle attachment, and hyobranchial folding (Schwenk, 2000). Tongue protraction, or tongue protrusion, is an imprecise movement that is triggered by vision (Deban, 2001). Salamanders capture their prey by tongue, which is extended from the mouth and returns to the buccal cavity with the prey attached. This is not accomplished through "tongue projection", which is a term reserved for mechanisms in which the tongue is fired from the mouth ballistically and reaches the prey on its own (Wake and Deban, 2000). The typical strike of a salamander uses a full body lunge to reach prey, having the tongue just barely leave its

mouth for prey contact. The full body lunge is a synchronous movement, involving both sides of the body. Lunging increases the strike distance, as well as the strike force (Wake and Deban, 2000). After the initial strike, prey is transported from the mouth to the esophagus through repeated cycles of jaw and hyoid motions (Lauder and Reilly, 1994). The transport of prey in aquatic species has not yet been studied in detail.

Feeding in Caecilians

The Order Gymnophiona, or caecilians, are mostly fossorial with several aquatic and semiaquatic species, with many having an aquatic larval stage. From the little that is known about caecilian diets, they are assumed to be carnivores (O'Reilly, 2000). Caecilian larvae use suction feeding in a similar manner to that of salamanders, but differ from them by locating prey through a combination of electrical, chemical, and tactile cues (Deban, 2001). Their eyes are likely not capable of sight, but their retina is capable of light reception (Himstedt, 1995). Their sense of hearing is not well developed either, so caecilians depend on other senses for prey recognition. They use olfaction and electroreceptors located on their heads to detect prey (Himstedt and Fritzsch, 1990). Caecilians have large tongues, and unlike salamanders and frogs, they do not flip them forward, but instead capture prey through jaw prehension (O'Reilly, 2000). Relative to frogs and salamanders, the movements of adult caecilians during feeding behavior is extremely slow and is modified by sensory information gathered during the strike (O'Reilly, 1994).

Feeding in Frogs

The Order Anura, or frogs, possess feeding mechanisms extensively diverse in functional and morphological configurations. Unlike salamanders, frogs have highly modified strike kinematics based on variables such as prey size and capture success (Lauder and Reilly, 1994). Many frog larvae are herbivores that use filter and suction feeding initiated by chemical and mechanical cues (Deban, 2001). Following metamorphosis, most species are carnivorous, eating primarily invertebrates, with prey capture triggered by sight (Nishikawa, 2000). Although there are a variety of feeding methods among frogs depending on the size and type of prey, most species feed terrestrially, using a forward, full body lunge movement in combination with the use of a short, thick tongue to capture prey. The lunge method in anurans is more precise than that of salamander and caecilians. Lunge feeding has been found to be the feeding method of the tailed frog *Ascaphus,* which is a basal taxon exhibiting a suite of ancestral morphological and behavioral features, supporting the conclusion that lunge feeding is the ancestral feeding behavior for frogs (Nishikawa and Cannatella, 1991).

While salamander feeding is primarily hyoid-based in both aquatic and terrestrial species and used in prey strike as well as transport, the hyoid appears to play a relatively insignificant role in tongue projection in frogs (Lauder and Reilly, 1994). Unlike salamanders, the anuran tongue consists of muscles and connective tissue only, not allowing the extent of tongue protrusion or projection mechanisms seen in salamanders (Nishikawa, 2000). Frogs have evolved anatomical and behavioral feeding adaptations that make them an exceptional clade of amphibians. Frogs are among the only orders that jump to increase their foraging radius, allowing access to more prey without having to anatomically extend a body part such as tongue projection in salamanders (Gans and Parsons, 1966).

Objectives and Hypothesis

The goal of this paper is to determine whether feeding behavior in the Northern Leopard Frog, *Lithobates pipiens,* share the same basic kinematic patterns as jumping behavior. In order to test the hypothesis that jumping gradually evolved from a preexisting feeding movement, I conducted a comparative study of lunge feeding and escape jumping behaviors in *Lithobates pipiens*. I hypothesize that lunge feeding is a precursor to jumping, and predict that the kinematic data will support this through similar joint angles and timing features.

CHAPTER II

METHODOLOGY

<u>Design</u>

Lithobates pipiens was filmed performing lunge feeding and jumping behaviors on a

nonslip surface in an arena. The Northern Leopard frog was chosen as the model species for the

study because it is a standard laboratory frog that will not be discouraged by the presence of

arena equipment and frequently engages in both lunge feeding and jumping behaviors. Frogs

were filmed using a Redlake Motionscope M3 high-speed video camera at a rate of 250 frames

per second. The camera was positioned vertically above a mirror, which was situated at a 45°

angle from the base of the arena. Placing the frog at the base of the mirror allowed the camera to

record two views of each trial which were used to calculate 3D kinematic data (Figure 1). Small,

white, flat beads were used as markers and glued onto 13 limb joints and other bony landmarks

including: the snout, occiput, urosacrum, urostyle, hip, iliosacrum, shoulder, elbow, wrist, finger,

knee, ankle, and foot (Figure 2). The points were digitized for each frame of the event and

transformed to three-dimensional coordinates with MaxTRAQ 3D (Innovision Systems Inc.).

Five *Lithobates pipiens* individuals were used, and approximately twenty trials – ten

trials of feeding and ten trials of jumping were recorded per individual. The five best trials from

each behavior were selected for analysis. The criteria for the best feeding trials chosen included

that the prey item remain near the designated mark on the arena floor, as well as the frog lunging

forward towards the prey and parallel to the mirror. The criteria for best jumping trials included

that the frog jump parallel with the mirror and stay within the arena boundaries. The trials that had the most parallel movements to the mirror and both prey and frog specimen began nearest the designated starting marks were chosen for analysis. The frogs were coaxed to lunge feed by placing mealworms approximately 10 cm from the snout. Escape jumping was encouraged by tapping directly behind the rear of the individual for each trial.
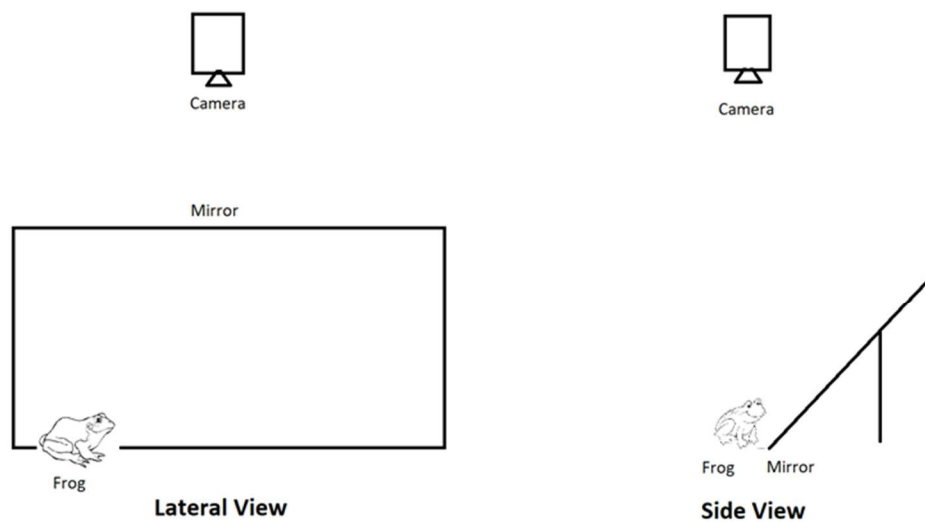

Figure 1: *Design of Experimental Set-up for Jumping and Feeding Arena* for *Lithobates pipiens*. The mirror was set at a 45° angle, and a single camera was positioned vertically.

Figure 2: *Photograph of Frog with Landmarks.* Photo of *Lithobates pipiens* with markers that include: 1) snout, 2) occiput, 3) urosacrum, 4) urostyle, 5) hip, 6) iliosacrum, 7) shoulder, 8) elbow, 9) wrist, 10) finger, 11) knee, 12) ankle, and 13) foot.

Data Analyses

Kinematic variables between lunge feeding and jumping behaviors were statistically compared using a Hotelling's Paired-Sample T^2 test in NCSS 2007. An alpha of 0.05 was used. A Monte Carlo randomization test was run to project a larger sample data set of 10,000 samples. Kinematic variables included performance and angular variables, as well as timing variables. The performance and angular variables include: takeoff velocity, jump and lunge distance, duration, takeoff angle, as well as the minimum, maximum, and excursion data for each of the following angles: urosacral (snout, urosacrum, urostyle), elbow (shoulder, elbow, wrist), wrist (elbow, wrist, finger), hip (iliosacrum, hip, knee), knee (hip, knee, ankle), and ankle (knee, ankle, foot). The takeoff angle was calculated using the x, y, and z coordinates of the snout, foot, and the horizontal at the toe's last contact with the ground. The horizontal coordinates were estimated by

taking the foot's x, y, and z coordinates and projecting a point in the y direction. Takeoff velocity was estimated as the average of five frames centered at the frame that the toe left the ground (Marsh and Alder, 1994). The timing variables include time to hands up and time to peak angle for all angles. Kinematic events included beginning of movement (frame before movement), front limb off (first frame front limb leaves ground), and maximum extension (last frame body fully extended; Figure 3).

Mean kinematic profiles of angles were constructed by filtering data with a fourth order Butterworth lowpass filter with a cutoff frequency of 10 Hz and normalizing to percent duration with Biomechanics Toolbar version 1.02. Resulting data were entered into SigmaPlot 11.0 to graph angular difference between behaviors.
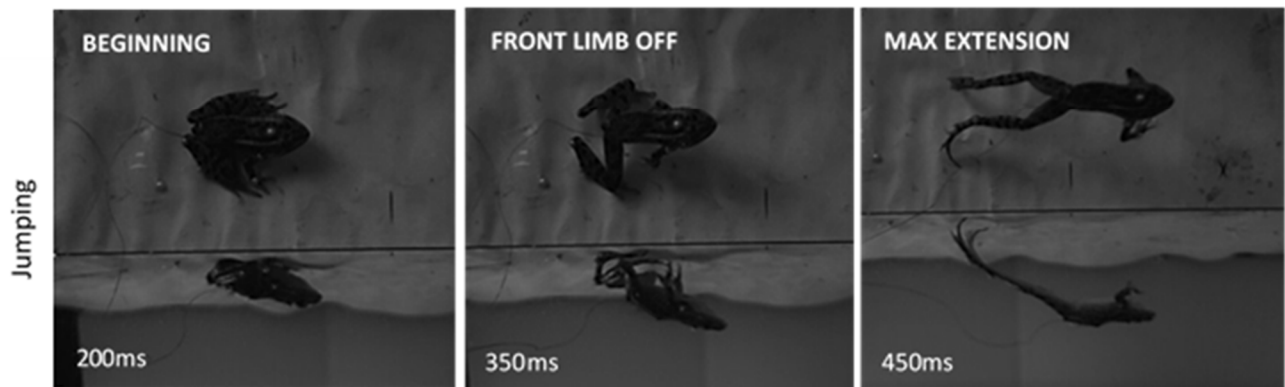
A
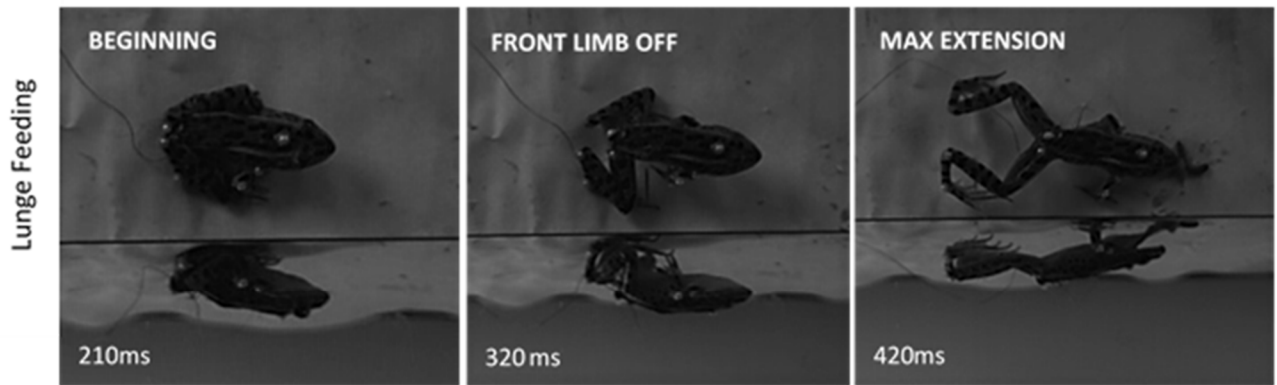
B

Figure 3: *Key Kinematic Events* in *Lithobates pipiens*. A) lunge feeding, B) jumping. Beginning (frame before movement), front limb off (first frame limb leaves ground), and maximum extension (last frame body fully extended).

CHAPTER III

RESULTS

Kinematic Profiles

During lunge feeding and escape jumping in frogs, the locomotor cycle begins with the

frog in a crouched position with all limbs bent at minimum angles near the torso. The frog moves

in a synchronous saltatory motion that begins in a similar pattern for both behaviors. Hind limb

extension begins at the beginning of movement (Kamel et al., 1996). The ankle angle starts at a

minimum angle of $47.1 \pm 3.2°$ and slightly increases during the lunge to its maximum ($74.1 \pm$

$4.0°$). The ankle angle increases from the minimum angle ($41.6 \pm 4.2°$) to the maximum angle

($156.0 \pm 2.6°$) much more rapidly during a jump, where the frog pushes further off and away

from the ground. The pace of the increase in the ankle angles slows as the frog reaches maximum

extension for all angles (Figure 4). The knee angle starts at a minimum angle of $13.1 \pm 3.1°$ and

increases at a steady pace during the lunge and finally slows to the maximum angle ($72.8 \pm$

$6.0°$). During jumping, the frog goes from the minimum knee angle ($16.0 \pm 1.1°$) to the

maximum ($144.1 \pm 2.7°$) in a similar movement pattern as in the lunge, just at a higher rate

(Figure 5). The final hind limb joint, the hip angle, begins at the minimum angle ($64.9 \pm 2.8°$)

and gradually increases until maximum extension for lunging ($119.0 \pm 4.3°$). When jumping, the

hip angle follows a similar pattern of movement occurring at a higher maximum ($152.2 \pm 1.9°$)

and in turn a greater excursion ($84.3 \pm 3.2°$; Figure 6).

At the beginning of movement, the pelvic joints bend towards the ground, decreasing the

urosacral angle and then extending rapidly for both behaviors at the moment the forelimbs leave

the ground. This angle plateaus at maximum extension while in the air during jumping (163.7±

1.4°), but continues to increase and straighten out once prey is caught in feeding (162.1± 2.0°;

Figure 7).

Unlike the hind limbs, forelimbs do not begin movement with joint extension. The wrist

angle decreases as the frog leans forward and then begins to increase as it extends to use the

hands to push off the ground in a forward motion. Immediately after maximum extension of the

wrist in the lunge (155.0± 3.3°), the frog begins to lean back on its hands that have returned to

the ground and then sits even deeper into the starting position. This causes the angle to be

smaller than at the start (86.7± 3.2°). For the jump, the hand begins to bend back to the starting

position at maximum extension (170.0± 1.1°), while still off the ground, causing the wrist angle

to remain larger than at the starting position. Aside from the absence of the ground at maximum

extension of the wrist, the movement pattern is the same for both behaviors, with higher

maximum wrist angles for jumping (Figure 8). The elbow angle gradually increases at the start of

motion and then decreases again in air as it reaches maximum extension for jumping (136.5±

3.1°). With lunging, the elbow angle increases at the start of movement (112.0 ± 4.5°) and then

decreases again, similar to the movement in jumping. The primary difference is that as prey is

being caught during lunging, the frog's head often falls forward towards the ground causing the

elbow angle to increase slightly as the head rises upon maximum extension as it prepares to

return to start (Figure 9).

Comparing similar angles demonstrates that both lunge feeding and jumping movements

involve the extension of the knee, hip, wrist, and elbow angles with them being more extreme in

jumping, indicating that greater extension and velocity is exhibited during jumping. The ankle is

only minimally used in lunging, while it is a major component in jumping. Lunging seems to be

powered primarily by the knee and hip, whereas jumping is powered by the knee, hip, and ankle (Peters et al., 1996). The urosacral angle is unique when comparing the two behaviors. It is the only angle that is used equally, or possibly even more during lunging than in jumping. If used more during feeding, the flexion and extension happening may aid in precise targeting during prey capture. As previously mentioned, frogs slightly alter the way they feed depending on prey variables to increase the likelihood of prey capture. The only joint that indicates modulated movement during feeding is the urosacral joint.

ANKLE ANGLE

Figure 4: *Mean Kinematic Profile for Ankle Angle* from start of movement to maximum extension for lunge feeding and jumping behaviors. (N=5 individuals, five trials each, twenty-five trials total.) Percent duration is zero to 100 illustrated on the X axis with angle in degrees illustrated on the Y axis. Lunge feeding (black circle) and jumping (white circle). Error bars represent the Standard Error of 4.76% of the total duration.

KNEE ANGLE

Figure 5: *Mean Kinematic Profile for Knee Angle* from start of movement to maximum extension for lunge feeding and jumping behaviors. (N=5 individuals, five trials each, twenty-five trials total.) Percent duration is zero to 100 illustrated on the X axis with angle in degrees illustrated on the Y axis. Lunge feeding (black circle) and jumping (white circle). Error bars represent the Standard Error of 4.76% of the total duration.
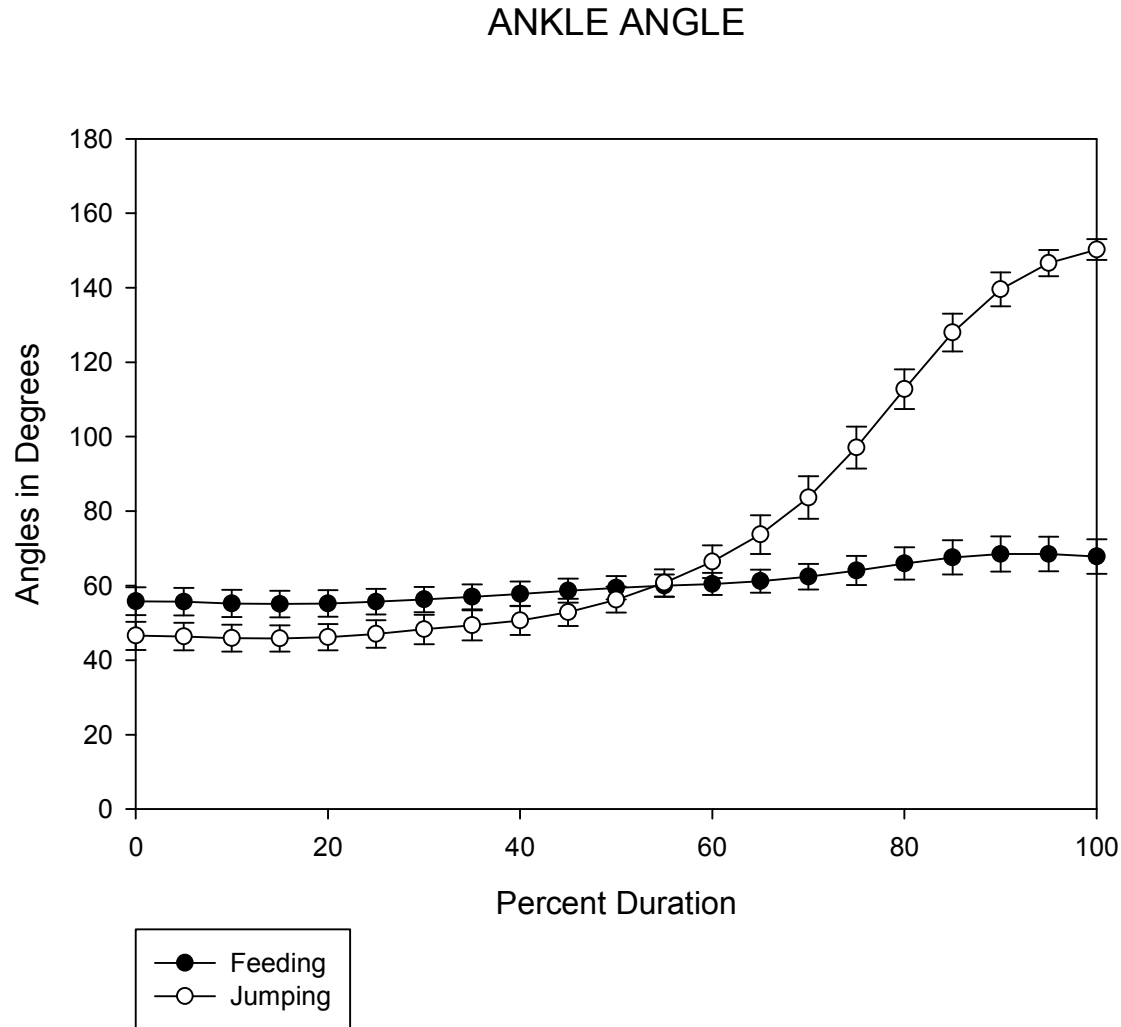
HIP ANGLE

Figure 6: *Mean Kinematic Profile for Hip Angle* from start of movement to maximum extension for lunge feeding and jumping behaviors. (N=5 individuals, five trials each, twenty-five trials total.) Percent duration is zero to 100 illustrated on the X axis with angle in degrees illustrated on the Y axis. Lunge feeding (black circle) and jumping (white circle). Error bars represent the Standard Error of 4.76% of the total duration.

UROSACRAL ANGLE
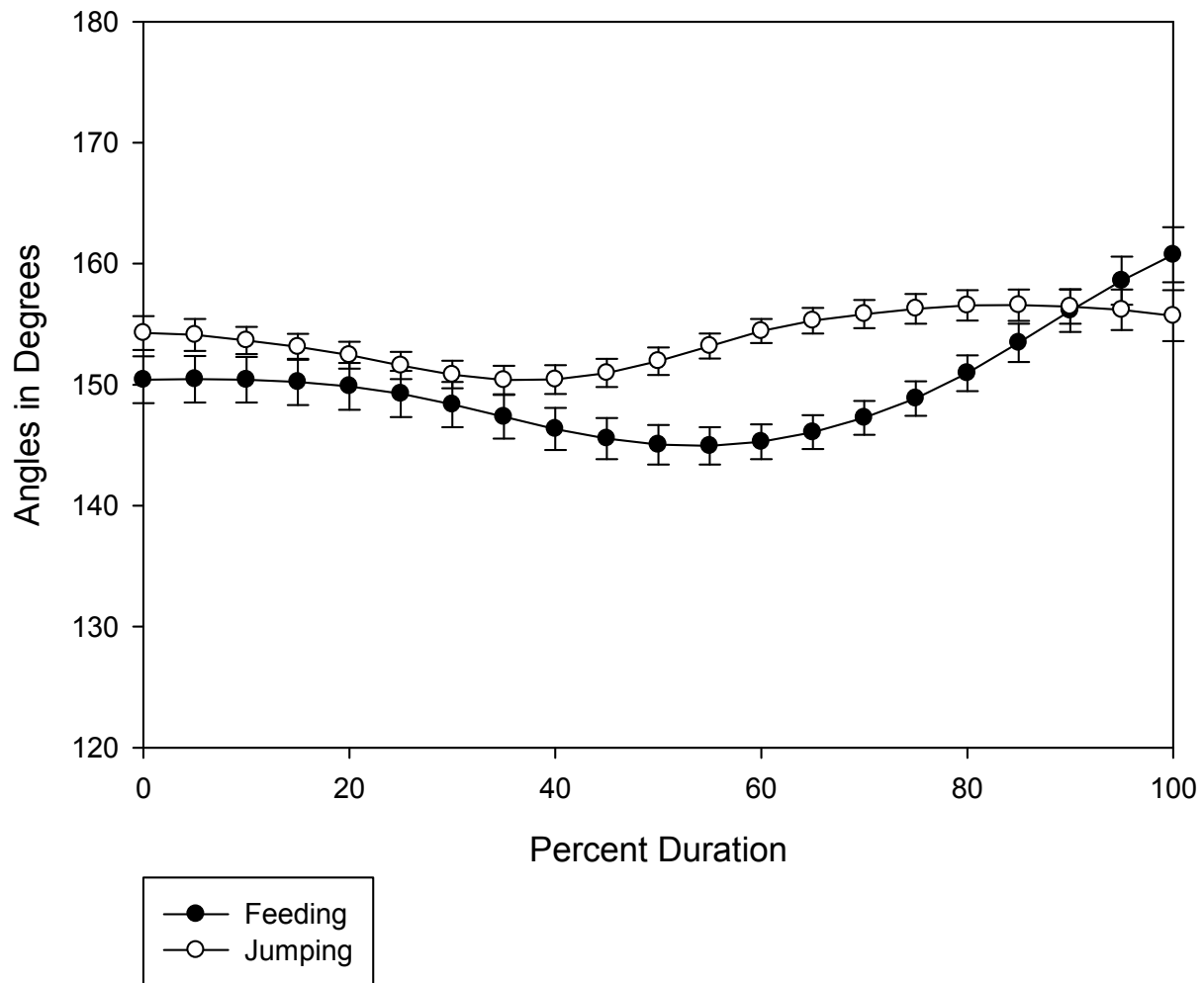
Figure 7: *Mean Kinematic Profile for Urosacral Angle* from start of movement to maximum extension for lunge feeding and jumping behaviors. (N=5 individuals, five trials each, twenty-five trials total.) Percent duration is zero to 100 illustrated on the X axis with angle in degrees illustrated on the Y axis. Lunge feeding (black circle) and jumping (white circle). Error bars represent the Standard Error of 4.76% of the total duration.
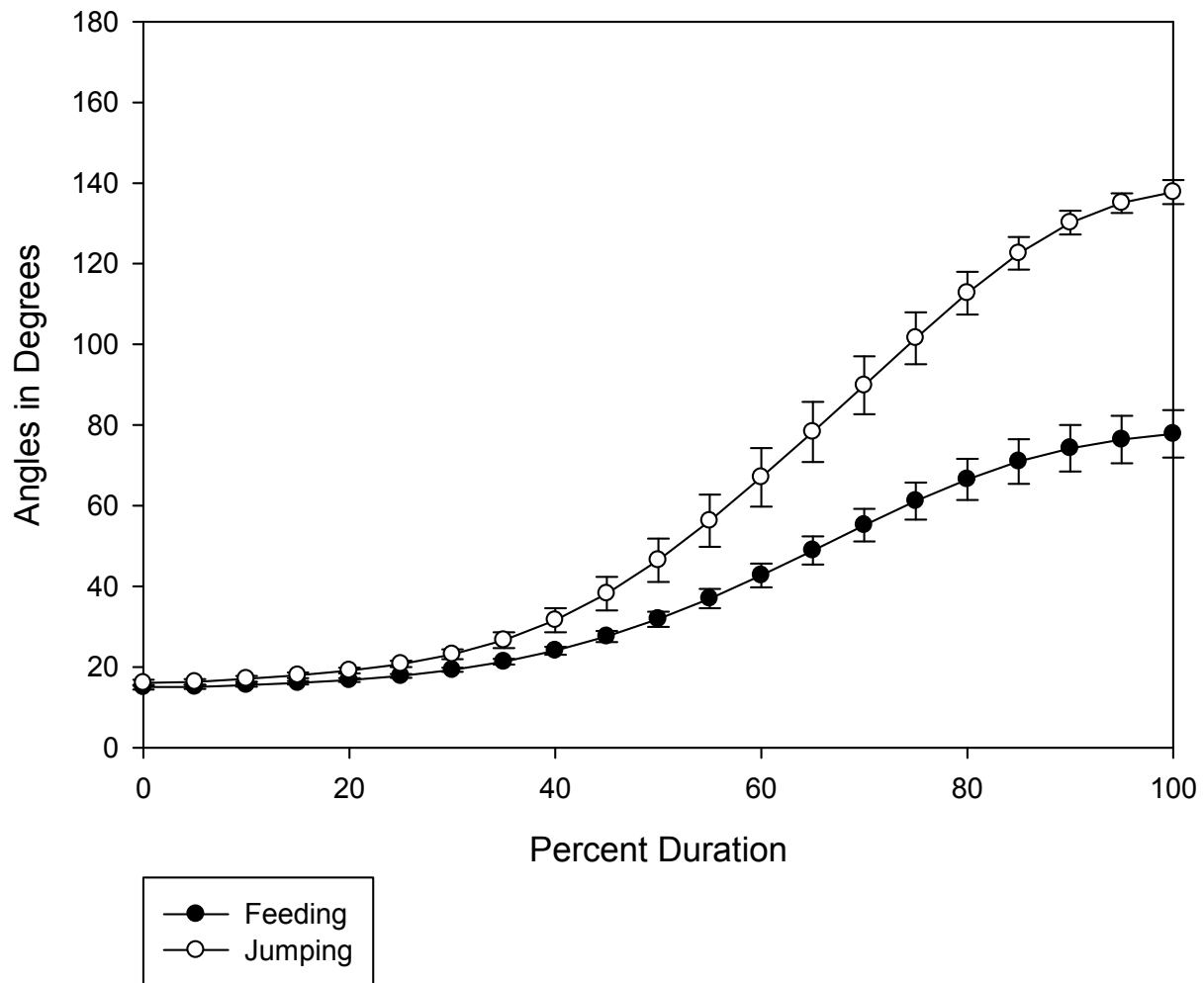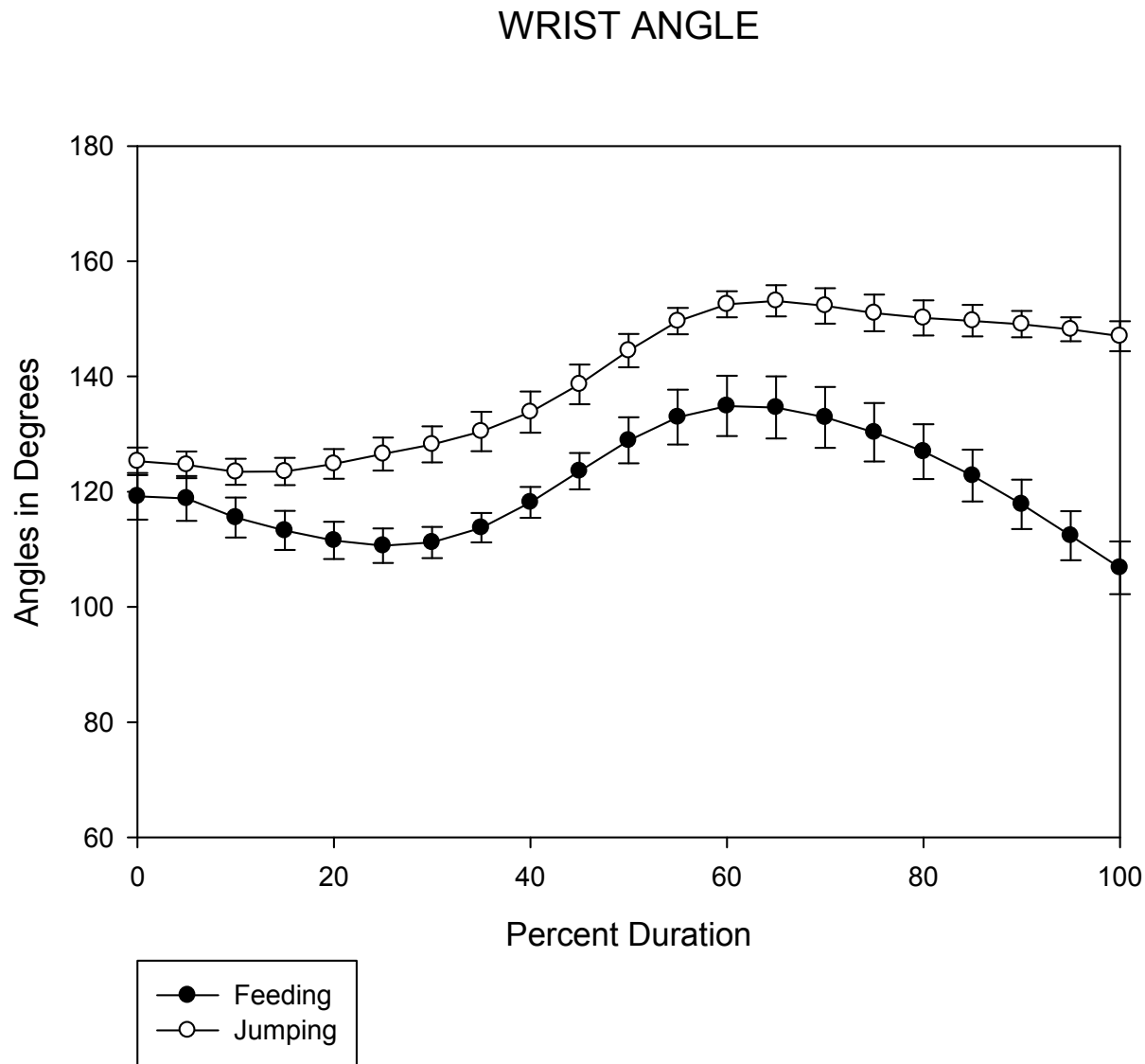
WRIST ANGLE

Figure 8: *Mean Kinematic Profile for Wrist Angle* from start of movement to maximum extension for lunge feeding and jumping behaviors. (N=5 individuals, five trials each, twenty-five trials total.) Percent duration is zero to 100 illustrated on the X axis with angle in degrees illustrated on the Y axis. Lunge feeding (black circle) and jumping (white circle). Error bars represent the Standard Error of 4.76% of the total duration.
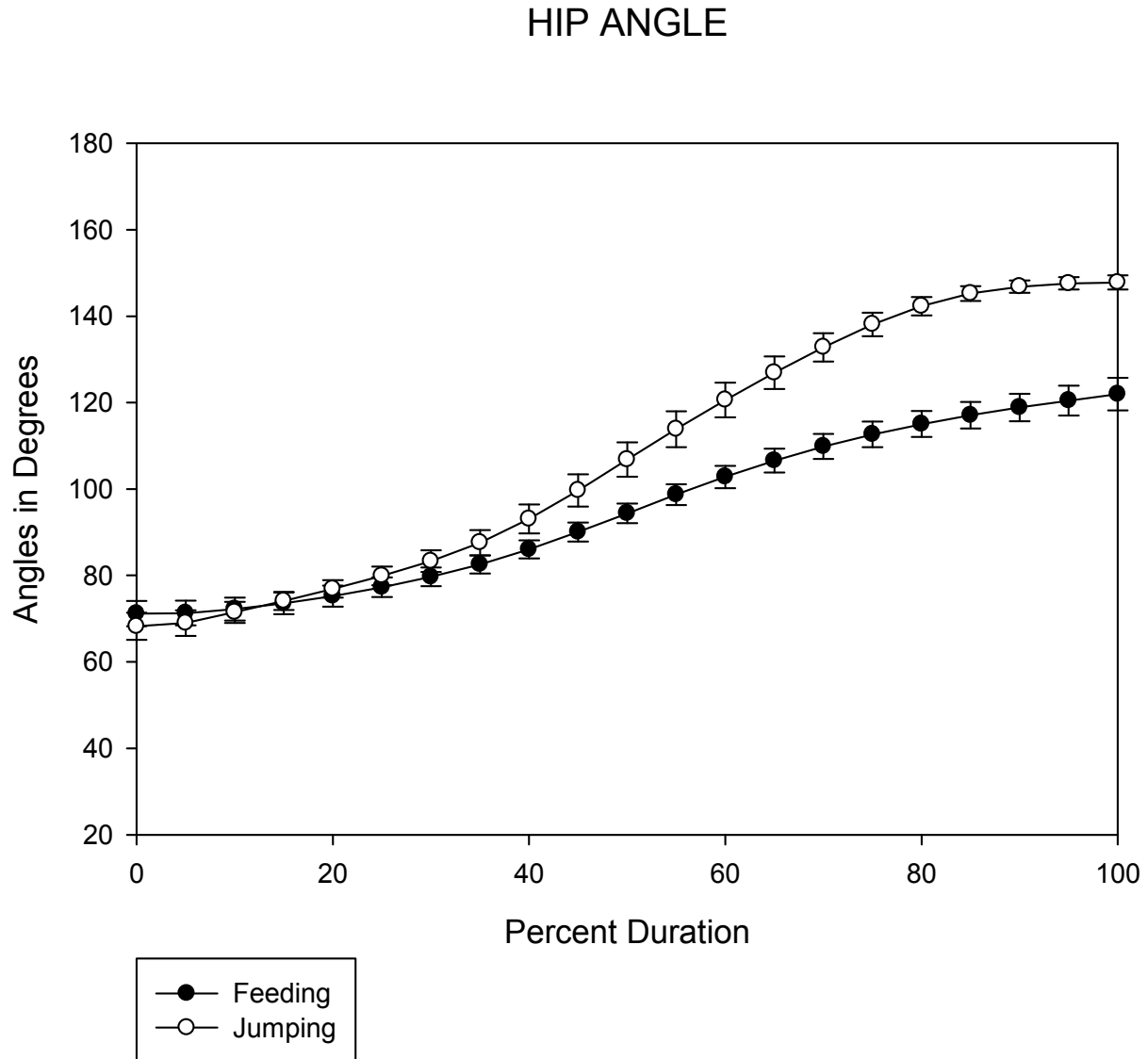
ELBOW ANGLE

Figure 9: *Mean Kinematic Profile for Elbow Angle* from start of movement to maximum extension for lunge feeding and jumping behaviors. (N=5 individuals, five trials each, twenty-five trials total.) Percent duration is zero to 100 illustrated on the X axis with angle in degrees illustrated on the Y axis. Lunge feeding (black circle) and jumping (white circle). Error bars represent the Standard Error of 4.76% of the total duration.
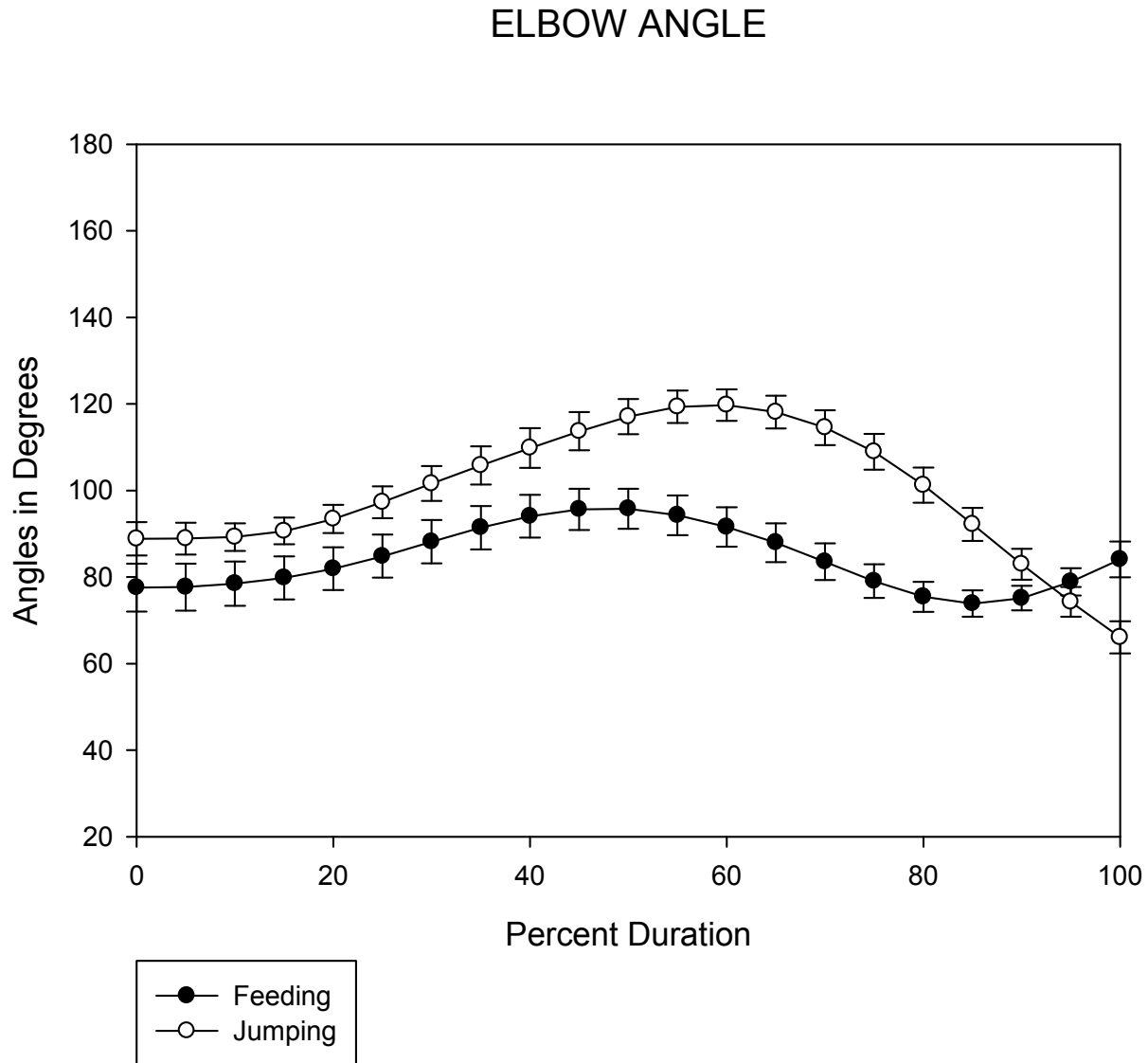
Kinematic Variables

A Hotelling's T^2 test was run with angular, performance, and timing variables collected from the trials (Table 1). Takeoff velocity was significantly different between the two behaviors, with frogs moving faster during jumping (3.1± 0.3m/s) than they do during feeding (1.0 ± 6.6m/s).

The excursion angles represent the difference between the minimum and maximum values for each angle. Variation between the excursions of the two behaviors was significant for all except the wrist. The wrist excursion angle changes more rapidly than any other angle, but the movement maintains a similar pattern for both behaviors. Shortly after the beginning of movement, the wrist angle decreases in preparation for the launch. The wrist angle decreases more prior to lunge feeding (68.3 ± 4.3°) than prior to lifting off of the ground during jumping (62.6 ± 2.9°).

Takeoff angle was significantly different between jumping (30.4 ± 1.3°) and feeding (-13.3 ± 2.7°). The takeoff angle for jumping is considerably larger than that of the takeoff angle of feeding. This is due to the snout being positioned higher than the ankle at takeoff during jumping, but lower than the ankle during lunging, causing a negative angle for all lunge takeoff angles. For most of the behaviors, the minimum angle is the same as that at the beginning of movement except for the wrist and the knee. As discussed previously, the wrist angle exhibits flexion during feeding immediately after the beginning of movement.

The comparisons between all maximum angles were significantly different except for the urosacral angle. During jumping, the majority of angles reach full extension, while in feeding many did not. The urosacral angle seems to be an exception in terms of timing. The time to peak urosacral angle did not differ significantly between the two behaviors, while it did for most other

angles (Table 1). While the other joints are reaching maximum extension during jumping, the urosacrum has a wider range of movement and reaches maximum extension during feeding. This may be an indication that the urosacrum is being used meaningfully during feeding. The frog might be moving the urosacral joint to precisely target prey. A multivariate regression was done with prey distance as the independent variable and takeoff angle, takeoff velocity, urosacral, hip, knee, ankle, elbow, and wrist maximum angles as dependent variables. This regression indicated that the only significant variable when prey distance is changed was the maximum urosacral angle (Table 2).

Table 1: *Hotelling's Paired-Sample T^2 Test Report of Angular/Performance, and Timing Variables.* Significant p-values are italicized.

	Jumping		Feeding		
	Mean	SD	Mean	SD	P
Angular and Performance Variables					
Takeoff Velocity (ms)	*3.1*	*± 0.3*	*1.0*	*± 6.6*	*0.0001*
Distance (mm)	*601.0*	*± 56.4*	*104.7*	*± 7.7*	*0.0001*
Duration (ms)	*225.3*	*± 8.3*	*193.3*	*± 5.2*	*0.0039*
Urosacral Excursion Angle (degrees)	*20.4*	*± 1.2*	*24.9*	*± 2.0*	*0.0453*
Elbow Excursion Angle (degrees)	*72.5*	*± 4.3*	*59.4*	*± 3.9*	*0.0395*
Wrist Excursion Angle (degrees)	62.6	± 2.9	68.3	± 4.3	0.1891
Knee Excursion Angle (degrees)	*128.1*	*± 3.0*	*60.0*	*± 6.3*	*0.0001*
Ankle Excursion Angle (degrees)	*114.5*	*± 5.2*	*27.0*	*± 2.6*	*0.0001*
Hip Excursion Angle (degrees)	*84.3*	*± 3.2*	*54.1*	*± 3.9*	*0.0001*
Takeoff Angle (degrees)	*30.4*	*± 1.3*	*-13.3*	*± 2.7*	*0.0001*
Urosacral Minimum Angle (degrees)	143.3	± 1.0	137.2	± 3.1	0.0716
Elbow Minimum Angle (degrees)	63.0	± 3.4	53.7	± 2.4	0.0563
Wrist Minimum Angle (degrees)	*107.3*	*± 2.8*	*86.7*	*± 3.2*	*0.0001*
Knee Minimum Angle (degrees)	*16.0*	*± 1.1*	*13.1*	*± 0.6*	*0.0280*
Ankle Minimum Angle (degrees)	41.6	± 4.2	47.1	± 3.2	0.3281
Hip Minimum Angle (degrees)	67.9	± 2.8	64.9	± 2.8	0.4691

Variable					
Urosacral Maximum Angle (degrees)	163.7	± 1.4	162.1	± 2.0	0.5624
Elbow Maximum Angle (degrees)	136.5	± 3.1	112.0	± 4.5	0.0012
Wrist Maximum Angle (degrees)	170.0	± 1.1	155.0	± 3.3	0.0001
Knee Maximum Angle (degrees)	144.1	± 2.7	72.8	± 6.0	0.0001
Ankle Maximum Angle (degrees)	156.0	± 2.6	74.1	± 4.0	0.0001
Hip Maximum Angle (degrees)	152.2	± 1.9	119.0	± 4.3	0.0001
Timing Variables					
Time to Hands Up (ms)	114.8	± 6.5	84.7	± 10.1	0.0051
Time to Peak Urosacral Angle (ms)	169.0	± 15.6	162.3	± 13.0	0.7457
Time to Peak Elbow Angle (ms)	132.7	± 8.6	2252.	± 2134.2	0.8111
Time to Peak Wrist Angle (ms)	180	± 10.1	93.4	± 11.7	0.0001
Time to Peak Knee Angle (ms)	214.6	± 10.0	187.1	± 5.2	0.0208
Time to Peak Ankle Angle (ms)	220.4	± 8.8	154.3	± 12.5	0.0001
Time to Peak Hip Angle (ms)	210.1	± 10.0	179.7	± 6.9	0.0195

Table 2: *Multivariate Regression Report of Prey Distance* in all feeding trials, N=25, verses takeoff velocity, takeoff angle, and all maximum angles. Significant p-values are italicized.

Variable	t-value	P
Takeoff Velocity	1.2	0.2592
Takeoff Angle	2.0	0.0612
Urosacral Maximum Angle	*2.9*	*0.0074*
Elbow Maximum Angle	0.3	0.7116
Wrist Maximum Angle	-0.4	0.7021
Knee Maximum Angle	1.4	0.1621
Ankle Maximum Angle	1.1	0.2691
Hip Maximum Angle	1.7	0.0938

CHAPTER IV

DISCUSSION

The hypothesis that lunge feeding is a precursor to jumping was supported by the kinematic data of the joint angles and timing features. The data reinforced that feeding and jumping are essentially the same behaviors using the same parts of the body, with jumping being more extreme with more work being done by the frog. The two behaviors are similar in the overall movement they go through, but the timing and extension is different. Completing a jump takes more time and covers more distance on average then completing a lunge. Also, during a jump the angles reach maximum extension, where most angles in a lunge do not fully extend. An escape jump is an attempt for a frog to get as far away as possible in one discrete hop, while the purpose of a lunge is to reach a nearby prey item to capture. Where jumping is powered mostly by the knee, hip, and ankle, lunging is powered by the knee and hip. Ostry et al. (1991) found that the hip and knee are synchronous movements in lunging. These joints begin movement at the same time, but studies show that the movement amplitude is greatest for the knee and least in the hip (Ostry et al., 1991). In jumping, the joint extensors in the knee, hip and ankle activate synchronously (Kamel et al., 1996), but joint movement occurs in a proximal-to-distal sequence (Astley and Roberts, 2014). Many animals use elastic energy storage to move faster and more powerfully than muscle alone is capable of. For elastic energy to be pre-loaded at the ankle, it requires movement at the hip or knee to precede it. While jumping, previous extension of more proximal joints could increase ground reaction force (GRF) before ankle extension and cause higher ankle muscle forces and storage of elastic energy (Astley and Roberts, 2014). In jumping, the data supports that the hip extends first, followed by the knee, and then the ankle. The data supports the involvement of the hip and knee joints in lunging, but the onset timing along with

other variables need to be explored further. Other possible variables include the knee's minimum angle, whose kinematic data was notably different between the two behaviors. This is peculiar because most other angles had comparable minimum angles being that those were also the angles at the starting position, which appeared the same for all behaviors in the kinematic profiles. The ankle, knee, and hip all begin movement close to the same time during locomotion, and each joint begins at the same respective angle, no matter the behavior. The unique knee data, as well as the timing of all joint movement, could be explained by the energy storage mechanism described by Astley and Roberts (2014), or possibly the process of motor unit recruitment for each behavior. Motor unit recruitment of the muscles involved in joint movement can be explored further through electromyography (EMG). Electromyography is an important tool that is used to link behavioral and morphological characteristics in many studies (Kamel et al., 1996).

The angle that has a unique kinematic profile compared to the other angles is the urosacral angle. This proves to be the one angle that goes through greater excursion angles during feeding than in jumping. The excursion angles represent the difference between minimum and maximum angles. This difference is typically greater in jumping because the joints are more fully extended leading to greater maximum angles. To explore feeding behavior further, a multivariate regression for prey distance was taken. Prey was initially placed approximately 10 cm from the snout of the frog for all trials; however, natural movement of the prey and/or the frog resulted in variation in prey distance among trials. We examined variation in prey strike distance with multivariate regression. The results indicated that the only significant variable was maximum urosacral angle, indicating that frogs primarily use the urosacral joint to modulate the prey strike (Table 2). This would appear to offer novel insight into the evolution of the urosacral joint. The presumed anuran ancestral form exhibited in *Triadobatrachus massinoti*, does not

possess a movable urosacral joint, but by the Early Jurassic, frogs such as *Prosalirus bitis* did have a moveable urosacral joint. Since *Prosalirus* was not considered a prolific jumper (Herrel et al., 2016), this may indicate that a moveable urosacral joint was a preadaptation that initially evolved for feeding. It then likely became secondarily used as a way of further increasing foraging radius in the vertical direction, as well as facilitating jumping by enabling an upward rotation of the anterior trunk during jumping. This would allow for the center-of-mass to be better aligned with the forces generated by hind limb extension. Jumping allowed a further increase in foraging radius—quickly closing the gap between the frog and distant prey, as well as allowing for rapid escape behavior. Confirmation of this hypothesis requires more detailed analysis with electromyography to determine if motor patterns between feeding and jumping are similar. However, the generally similar movement patterns between the two behaviors is consistent with the Gans and Parsons (1966) hypothesis that jumping is an evolutionary modification of lunge feeding behavior.

Anuran jumping is often thought to have evolved simultaneously with synchronous swimming. Most frogs are saltatory, with specific locomotor habits varying slightly among species. Morphologically archaic species, such as *Leiopelma hochstetteri* and *Ascaphus truei*, jump synchronously (moving both sets of limbs simultaneously) while on land, but use alternate leg movements when swimming. While trot swimming evolved in ancestral frog species, derived frogs developed a more energy efficient, synchronous gait for both jumping and swimming (Abourachid and Green, 1999). Many derived frog species, such as *Lithobates pipiens*, are excellent jumpers and swimmers which use simultaneous leg movements to swim and hop. Several morphological structures, including consolidated vertebrae, loss of tail, enhanced pelvis, and lengthened hind limbs correlate with, and even demand, simultaneous motions of hind limbs

during both jumping and swimming (Peters et al., 1996; Abourachid and Green, 1999). According to the riparian hypothesis of origin, anuran ancestors that were originally aquatic, may have left the water to feed, rest, or bask along the banks of bodies of water. Synchronous movement may have progressively developed from the motor training of a riparian feeding behavior like catching prey along the banks of water (Gans and Parsons, 1966). It is likely that, separate from the primative asynchronous swimming, both synchronous jumping and synchronous swimming later evolved from lunge feeding.

Future Work

Future research will involve a detailed electromyographic study of lunge feeding and jumping, as well as a more detailed analysis of the effects of prey distance on kinematics and motor patterns of lunge feeding. Examining kinematics based upon varying prey distance, especially in the vertical direction, may give additional insight on the role of the urosacrum in targeting prey. Moreover, a detailed study of muscle function via electromyography may provide insight into the role of the trunk, caudopelvis, and limb musculature in modulating prey strike. The overall kinematic data that was collected could potentially be supported by additional EMG data. This would show the exact process of motor unit recruitment that contributes to the movement of muscles in the feeding and jumping behaviors of *Lithobates pipiens*. The notable kinematic data found for the knee and urosacrum can be further explored using EMG to explain their unique kinematic results. The minimum angle being significantly different between both behaviors for the knee is unexplained thus far, as well as the exact reason why the urosacral maximum angle is the only maximum angle of no significant difference between the two

behaviors. Individual muscles that contribute to the movement of the knee and the urosacral joint could be quantified for motor patterns and given further insight to explain their results.

Lunge feeding is ancestral in both salamanders and frogs, and yet each order approaches the behavior differently. While frogs lunge straight forward in a precise way (Nishikawa and Cannatella, 1991), salamanders lunge to the side, allowing for a less accurate prey capture. The differences in technique may be due to the anatomical differences among the trunks of frogs and salamanders. Salamanders have between ten and sixty mostly uniform vertebrae, including atlas and caudal vertebrae. Frogs have only six to nine pre-sacral vertebrae, and one or two sacral vertebrae, with the caudal vertebrae being fused into a rod-shaped skeletal element called the urostyle (O'Reilly et al., 2000). Even though frogs and salamanders share a common amphibian ancestor, each order has morphological differences, receive environmental feeding cues differently, and therefore possess varying behavioral characteristics that accompany lunge feeding. These differences would be worth researching in a future study comparing the lunge feeding behaviors among anurans and caudatans. A detailed kinematic and EMG study would provide insight into the muscles responsible for the variation in lunge feeding behaviors. EMG would be focused on trunk muscles such as the muscles from the vertebral column and pelvic girdle, including: the coccygeoiliacus, coccygeosacralis, dorsalis trunci, interspinalis, iliolumbaris, intertransversarii, and longissimus dorsi. During a comparative lunge feeding study, additional evidence would be gathered to further test the hypothesis of O'Reilly et al. (2000) that muscle function is likely to be conserved during evolution. The vertebral column has clear structural differences among frogs and salamanders and exploring the muscle function that accompanies those structures during lunge feeding would be a valuable contribution to our understanding of Lissamphibian evolution.

REFERENCES

Abourachid, A., & Green, D. M. (1999). Origins of the frog-kick? Alternate-leg swimming in primitive frogs, families Leiopelmatidae and Ascaphidae. *Journal of Herpetology*, 657-663.

Ascarrunz, E., Rage, J. C., Legreneur, P., & Laurin, M. (2016). Triadobatrachus massinoti, the earliest known lissamphibian (Vertebrata: Tetrapoda) re-examined by μCT scan, and the evolution of trunk length in batrachians. *Contributions to Zoology*, *85*(2), 201-234.

Astley, H. C., & Roberts, T. J. (2014). The mechanics of elastic loading and recoil in anuran jumping. *Journal of Experimental Biology*, *217*(24), 4372-4378.

De Vree, F., & Gans, C. (1994). Feeding in tetrapods. In *Biomechanics of Feeding in Vertebrates* (pp. 93-118). Springer, Berlin, Heidelberg.

Essner, R. L., Suffian, D. J., Bishop, P. J., & Reilly, S. M. (2010). Landing in basal frogs: evidence of saltational patterns in the evolution of anuran locomotion. Naturwissenschaften, 97(10), 935-939.

Fotowat, H., Harrison, R. R., & Gabbiani, F. (2011). Multiplexing of motor information in the discharge of a collision detecting neuron during escape behaviors. *Neuron*, *69*(1), 147-158.

Gabriel, R., Völgyi, B., & Pollak, E. (1998). Calretinin-immunoreactive elements in the retina and optic tectum of the frog, Rana esculenta. *Brain research*, *782*(1-2), 53-62.

Gans, C., & Gorniak, G. C. (1982). Functional morphology of lingual protrusion in marine toads (Bufo marinus). American Journal of Anatomy, 163(3), 195-222.

Gans, C., & Parsons, T. S. (1966). On the origin of the jumping mechanism in frogs. Evolution, 92-99.

Green, T. L. (1931, December). 57. On the Pelvis of the Anura: a Study in Adaptation and Recapitulation. In *Proceedings of the Zoological Society of London* (Vol. 101, No. 4, pp. 1259-1290). Oxford, UK: Blackwell Publishing Ltd.

Herrel, A., & Measey, G. J. (2012). Feeding underground: kinematics of feeding in caecilians. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *317*(9), 533-539.

Herrel, A., Moureaux, C., Laurin, M., Daghfous, G., Crandell, K., Tolley, K. A., ... & Boistel, R. (2016). Frog origins: inferences based on ancestral reconstructions of locomotor performance and anatomy. *Fossil Imprint*, *72*(1-2), 108-116.

Himstedt, W. (1995). Structure and function of the eyes in the caecilian *Ichthyophis-kohtaoensis* (Amphibia, Gymnophiona). *Zoology-Analysis of Complex Systems*, *99*(1), 81-94.

Himstedt, W., & Fritzsch, B. (1990). Behavioural evidence for electroreception in larvae of the caecilian Ichthyophis kohtaoensis (Amphibia, Gymnophiona). *Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere*, *94*(4), 486-492.

Jenkins Jr, F. A., & Shubin, N. H. (1998). Prosalirus bitis and the anuran caudopelvic mechanism. *Journal of Vertebrate Paleontology*, *18*(3), 495-510.

Kamel, L. T., Peters, S. E., & Bashor, D. P. (1996). Hopping and swimming in the leopard frog Rana pipiens: II. A comparison of muscle activities. *Journal of Morphology, 230*(1), 17-31.

Lauder, G. V., & Reilly, S. M. (1994). Amphibian feeding behavior: comparative biomechanics and evolution. In *Biomechanics of feeding in vertebrates* (pp. 163-195). Springer, Berlin, Heidelberg.

Marsh, R. L., & John-Alder, H. B. (1994). Jumping performance of hylid frogs measured with high-speed cine film. Journal of Experimental Biology, 188(1), 131-141.

Nishikawa, K. C. (2000). Feeding in frogs. *Feeding: Form, Function and Evolution in Tetrapod Vertebrates (ed. K. Schwenk)*, 117-147.

Nishikawa, K. C. (1999). Neuromuscular control of prey capture in frogs. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 354(1385), 941-954.

Nishikawa, K. C., & Cannatella, D. C. (1991). Kinematics of prey capture in the tailed frog Ascaphus truei (Anura: Ascaphidae). Zoological journal of the Linnean Society, 103(3), 289-307.

O'Reilly, J. C. (1994). *Comparative studies on the evolution of prey capture behavior in amphibians* (Doctoral dissertation, Northern Arizona University).

O'Reilly, J. C. (2000). Feeding in caecilians. *Feeding: form, function and evolution in tetrapod vertebrates*, 149-166.

Ostry, D. J., Feldman, A. G., & Flanagan, J. R. (1991). Kinematics and control of frog hindlimb movements. *Journal of Neurophysiology*, *65*(3), 547-562.

Peters, S. E., Kamel, L. T., & Bashor, D. P. (1996). Hopping and swimming in the leopard frog, Rana pipiens: I. Step cycles and kinematics. *Journal of Morphology*, *230*(1), 1-16.

Rage, J. C., & Rocek, Z. B. Y. N. Ë. K. (1989). Redescription of Triadobatrachus massinoti (Piveteau, 1936) an anuran amphibian from the early Triassic. Palaeontographica A, 206(1-3), 1-16.

Rage, J. C., & Rocek, Z. (1986). Triadobatrachus revisited. Studies in herpetology. Charles University Press, Prague, 255-258.

Reilly, S. M., Montuelle, S. J., Schmidt, A., Krause, C., Naylor, E., Jorgensen, M. E., & Essner Jr, R.L. (2016). Pelvic function in anuran jumping: Interspecific differences in the kinematics and motor control of the iliosacral articulation during take☐off and landing. Journal of morphology, 277(12), 1539-1558.

Reilly, S. M., Montuelle, S. J., Schmidt, A., Naylor, E., Jorgensen, M. E., Halsey, L. G., & Essner, R. L. (2015). Conquering the world in leaps and bounds: hopping locomotion in toads is actually bounding. Functional Ecology, 29(10), 1308-1316.

Schwenk, K. (Ed.). (2000). Feeding: form, function and evolution in tetrapod vertebrates. Elsevier.

Shubin, N. H., & Jenkins Jr, F. A. (1995). An early Jurassic jumping frog. *Nature*, *377*(6544), 49.

Stehouwer, D. J. (1992). Development of anuran locomotion: ethological and neurophysiological considerations. *Journal of neurobiology*, *23*(10), 1467-1485.

Wells, K. D. (2010). The ecology and behavior of amphibians. University of Chicago Press.

Wolf, E., Soffe, S. R., & Roberts, A. (2009). Longitudinal neuronal organization and coordination in a simple vertebrate: a continuous, semi-quantitative computer model of the central pattern generator for swimming in young frog tadpoles. *Journal of computational neuroscience*, *27*(2), 291-308.