

AN ABSTRACT OF THE THESIS OF

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Title: The Effects of Body Temperature on the Transit Rate and Hopping Endurance of *Anaxyrus woodhousii*.

Abstract approved:

Anaxyrus woodhousii occupies an extensive range of habitats in the United States. However, as an ectotherm, body temperature plays a critical role in digestive physiology and behavior of *A. woodhousii*. Therefore, I was interested in investigating whether different body temperatures can affect transit rate and hopping endurance in *A. woodhousii*. I designed two experiments to investigate the effects of different temperatures on transit rate and endurance of hopping behavior. First, I used 20 field-collected individuals to test the digestive rate at four different temperatures: 16, 21, 26 and 31 °C. I kept all the toads individually in clear shoe boxes with a water bowl and plastic retreat, which allowed them to access water and hide themselves. In my experiment, I put all the toads in a walk-in chamber or acclimation chambers, which were set to a specific temperature and a 12-hour photoperiod starting at 0800 CDT. Before I started the transit rate experiment, all the toads were maintained at room temperature and fed with crickets every day for four weeks. During the experiment of transit rates, I acclimated all four groups of toads at a constant temperature and fed them crickets which had a different colored plastic bead glued to them for seven days. All the toads went through four temperatures but in different orders of temperature. I checked for feces three

times per day and over 12 hours a day. Once I found the bead in the feces, the bead color marked how long it stayed in the digestive tract of *A. woodhousii*. In this experiment, I found there was no significant difference in transit rate of *A. woodhousii* over the four temperatures I tested. In a separate experiment, I tested for effects of different temperatures (10, 15 and 20 °C) on endurance of hopping behavior of eight *A. woodhousii* individuals. I acclimated all the toads for four days before starting each trial, and I tested each toad 3 times at each temperature to determine the length of time it could hop. In this experiment, all toads hopped on a small treadmill with a speed of 0.074 m/s. Hopping endurance at 20 °C was significantly longer compared to both 10 and 15 °C. Over the temperature range frequently encountered by toads, transit rate was unaffected, but hopping endurance was diminished at low temperatures.

Key words: *Anaxyrus woodhousii*, temperature, transit rate, hopping endurance

The Effects of Body Temperature on the Transit Rate and Endurance of
Hopping Behavior of *Anaxyrus woodhousii*

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PREFACE

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INTRODUCTION

The relationship among environmental temperature, organismal development, physiology and behavioral thermoregulation in ectotherms has been of interest to biologists for a long period (Angilletta et al., 2002). For ectotherms, body temperature (T_b) has a dominant role in many aspects of behavior and physiology (Huey and Stevenson, 1979; Huey, 1982), including locomotion (McConnell and Richards, 1955; Bennett, 1980; Hirano and Rome, 1984; Weinstein, 1998; Ojanguren and Brana, 2000), immune function (Mondal and Rai, 2001), sensory input (Stevenson et al., 1985; Werner, 1976), foraging ability (Greenwald, 1974; Ayers and Shine, 1997), and rates of feeding and growth (Warren and Davis, 1967; Dutton et al., 1975; Kingsolver and Woods, 1997). Previous evidence reveals that T_b is one of the most important environmentally related factors governing the physiological performance and behavior of ectotherms (Angilletta et al., 2002). The most obvious example is that the juveniles of ectotherms show a highly correlated relationship between growth rate and temperature (Gotthard, 2001; Lee and Roh, 2010).

Because the T_b of ectotherms are highly correlated with environmental temperature, it may be expected to select micro-habitats in the environment to maintain an optimal T_b (Carey, 1978). Acute exposure to a diverse range of environmental temperatures often reveals that performance of ectotherms is maximized at an optimal temperature and minimized at the ends of tolerable T_b (Angilletta et al., 2002). Limits exist to the ability of ectotherms to function at extreme temperatures; the critical thermal limits are the minimum (CT_{min}) and maximum (CT_{max}) T_b s where death occurs if the

animal is not removed from that ambient temperature (Gvoždík, and Castilla, 2001; Lutterschmidt and Hutchison, 1997). The optimal temperature is typically near the CT_{max} (Huey and Bennett, 1987). At this T_b , ectotherms show best physiological performance, but temperatures above this narrow range could cause damage to ectotherms or even lead to death (Lutterschmidt and Hutchison, 1997).

The importance of T_b on physiological performance suggests that ectotherms employ multiple strategies to respond to different thermal conditions. In most situations, ectotherms might use behavior, physiology, or both to regulate their T_b s within a range narrower than the range of environmental temperatures (Cowles and Bogert, 1944; Casey, 1981; Avery, 1982; and Hutchison and Dupre, 1992). Behavioral thermoregulation allows rapid heat gain or loss to the surrounding environment or the maintenance of a stable T_b . Ectotherms select appropriate microhabitats and can use physiological mechanisms to some extent to maintain a T_b within a tolerable range (Dunham et al., 1989). Due to unstable environmental temperature, precise thermoregulation might be difficult or even impossible in some conditions, or it might not be beneficial in some environments because of the cost associated with behavior (Huey and Slatkin, 1976). Behavioral thermoregulation does not help ectotherms maintain optimal T_b at extreme ambient temperatures. Therefore, the physiological performances of ectotherms are still sensitive to environmental temperature changes (Logan et al., 2014).

As an ectotherm, *A. woodhousii* occupies an extensive range of habitats in the United States, occurring throughout the Great Plains, and from Canada to Mexico (Sullivan 1989). With such a wide distribution, *A. woodhousii* uses a wide range of habitats including open woodlands, grassland, and the riparian valleys of larger streams

and rivers (Sievert and Sievert, 2011). Its extensive range suggests that *A. woodhousii* has an ability to deal with temperature changes throughout the year and at different locations. Like most amphibians, T_b is highly correlated to environmental temperature, and physiological performance and function are highly influenced by T_b . As a toad, environmental temperature affects its behavior, metabolism, immune system and development (Raffel et al. 2006; Whitford, 1973). Therefore, toads must be able to tolerate a range of T_b s. *A. woodhousii* deals with environmental fluctuations by short-term behavioral adjustments or long-term changes in thermal sensitivity through acclimation or adaptation (Huey and Bennett, 1987; Gvozdk and Castilla, 2001). However, the question is whether *A. woodhousii* can deal with a big range of temperature and maintain the same level of physiological performance. Previous studies threw doubt on the ability of this toad to maintain a constant performance. For instance, *A. woodhousii* exhibits greater mean velocity and mean jump length at warmer acclimation temperatures compared with cold temperatures (Londos and Brooks, 1988). In addition, *A. woodhousii* shows increasing oxygen consumption while being maintained at temperatures from 5 to 25 °C (Maher, 1967), and an increase in T_b (20 to 35 °C) can alter the pattern of postprandial metabolic rate in some toads (Secor and Faulkner, 2002). Wei and Hou (2008) found that ingested energy in *Anaxyrus bankorensis* is significantly affected by temperature and generally increased with increased temperature. These studies indicate the physiological performance of *A. woodhousii* is highly sensitive to temperature.

The energy ingested from food is essential for animals to survive, grow, develop, and reproduce (Randall et al. 2001). Therefore, animals need to maximize their energy

input from food. Because ectotherms do not have the ability to keep a constant T_b , they must deal with daily fluctuations in T_b . T_b is a major factor that influences the rate at which complex molecules found in food are digested (McNab, 2002). Most ectotherms regulate their metabolic rate by increasing or decreasing T_b and thereby control the speed of digestion and frequency of needing to consume food (Du et al., 2000; Wang et al., 2003). Even though digestion in ectotherms is dependent on T_b , effects of T_b on nutrient up take by the gut are conflicting (Beaupre et al., 1993). In the case of cornsnakes, Sievert et al. (2005) showed T_b had no effect on transit rate. However, some studies showed ectotherms have different thermal optima for digestive functions (Dorcas et al., 1997). These studies suggest the effect of T_b on digestion and transit rate may vary among species, which may be related to the diversity of habitats used by different species. Field observations and experimental laboratory studies show that digestive tract anatomy and function of many species including ectotherms are flexible and can change in response to variation in environmental conditions (Piersma and Lindstrom, 1997; Starck, 1999; McWilliams and Karasov, 2001). Since *A. woodhousii* exhibits a wide range of distribution, I was interested in investigating effects of T_b on transit rate in these toads.

Thermal and exercise physiology are not independent in anurans. For instance, jumping or calling, is relatively easy to quantify is directly related to temperature and important in fitness (Navas et al., 2008). Several studies reveal the influence of temperature on jumping and the physiology of leg skeletal muscle (Rand, 1952; Hirano and Rome, 1984; Lutz and Rome, 1994; Marsh, 1994; Navas et al., 1999; Wilson, 2001). There are two different types of locomotion among anurans. Most frogs maximize jump length as an escape behavior (Gans and Parsons, 1966), while toads have traded their

jump length for endurance and a strategy of repeated hopping (Reilly et al., 2015). Locomotion is essential in food hunting, migrating to a new habitat, and escaping predators. Even though toads have shorter legs and jump length than most frogs, toads have greater endurance, muscle efficiency and aerobic scope (Jorgensen and Reilly, 2013; Zug 1978; Bennett and Licht, 1973; Zug 1985; Gibbs and Chapman, 1974; Josephson, 1975; Walton and Abderson, 1988). Locomotion of toads is an aerobic exercise, which allows them to hop for a long period (Walton and Anderson, 1988). Factors that can influence locomotion have been measured, such as oxygen consumption (VO₂), temperature, metabolic rate, and muscle performance (Walton and Anderson, 1988; Londos and Brooks, 1988; Anderson et al., 1991). Londos and Brooks (1988) showed *A. woodhousii* had mean velocity, jump frequency, and jump length that varied with T_b, but they did not demonstrate if T_b could affect endurance of hopping. Therefore, I was interested whether temperature can alter the hopping endurance of *A. woodhousii* when toads need to keep hopping to escape from a predator.

In this research, I measured the speed of food passage and maximal endurance of hopping in *A. woodhousii* at different temperatures. The primary goals of this study were to investigate two questions: (1) Does T_b affect transit rates of *A. woodhousii* (2) Does T_b affect the endurance of hopping activity in *A. woodhousii*? My hypotheses were (1) there is no significant difference in transit rate of *A. woodhousii* over the four test temperatures and (2) there is a significant difference in hopping endurance in *A. woodhousii* over the three test temperatures.

MATERIALS AND METHODS

I collected 20 adult *A. woodhousii* (14 female and 6 male) in Lyon County, Kingman County and Coffee County, Kansas, in May 2017. The toads were habituated to captivity for several weeks to ensure they would reliably eat crickets. The toads were initially 14.5 to 90.8 g body mass. Throughout the experiment, all the toads were housed in a 33 x 20 x 13 cm ventilated plastic container with a water bowl and PVC retreat, which allowed them to access water and hide. The containers and water bowls were cleaned every week or more often if needed.

Experiment 1: TRANSIT TIMES

To test the transit times of food through the digestive tract, I assigned toads to one of four groups ($n = 5$ per group) that only differed in the order of the four treatment temperatures of 16, 21, 26 and 31 °C (see Appendix A for more details). Each chamber was maintained under the same 12 h light: 12 h dark photoperiod with the photophase starting at 0800 hr CDT. All toads were tested between May and September 2018.

Prior to testing, each toad in its container acclimated seven days before the first of four feeding experiments at each different temperature. During this time, toads were fed at 1200 hr CDT each day. Small toads were given two adult crickets (*Gryllodes sigillatus*), and large toads were given three adult crickets. To measure the transit rate of *A. woodhousii* for seven consecutive days, I glued a colored plastic bead on the back of each cricket and used those crickets to feed toads. The glue I used was Elmer's Washable No Run School Glue, which is nontoxic. Moreover, the plastic bead was only 0.1 cm diameter and each toad was given one bead of a different color every day. In this

experiment, I always followed a specific order of colored beads to make sure that I knew how long a bead stayed in the digestive tract of a toad (Appendix B). All the toads were given one cricket with a bead and one or two crickets without beads depending on the toad's size. I monitored all the toads until I found all the beads in their feces. I fed toads at 1200 CDT for seven days and followed by checking for feces three times per day at 0800, 0100, and 2000 hr CDT. I recorded the transit rate in days in this experiment.

EXPERIMENT 2: HOPPING ENDURANCE

The hopping endurance was measured in eight adult *A. woodhousii* caught in Lyon County, Kingman County and Coffee County, Kansas. The toads in this experiment had a range of body mass from 20 to 45 g. All the toads were tested at 10, 15, and 20 °C between July and November 2019. I put the toads into a walk-in chamber at the first of three testing temperatures and allowed them to acclimate at this temperature for seven days prior to the hopping experiments. A treadmill was constructed from a converted belt sander with an endless belt, a speed controller to control the speed of the belt and wood baffle to prevent toads from jumping out. The treadmill was housed in the walk-in chamber and the speed of treadmill was set to 0.074 m/s (0.266 km/h). Prior to the test, I started the treadmill to make sure the speed remained constant. I put a toad on the middle part of the moving treadmill and started the timer. During the whole process, the toads hopped without any artificial prodding. When the toads stopped hopping, the timer was stopped. I tested all the toads three times at each temperature and recorded the time each one hopped on the treadmill in seconds. Toads were fed crickets and/or mealworm larvae (*Tenebrio molitor*) every day. On the test day, toads were fed 30 to 60 minutes before testing every day. This was done because toads performed better if fed before testing

rather than after. Preliminary trials showed that toads often did not perform unless they had eaten.

STATISTICAL ANALYSES:

All statistical analyses were completed using SPSS version 24.0 (IBM) Statistical Package for the Social Sciences and ProStat. One-Sample Kolmogorov-Smirnov tests and QQ plots tested data for normality and Levene's test tested homogeneity of variance. The results from the One-Sample Kolmogorov-Smirnov test indicated transit rate data and hopping endurance data of *A. woodhousii* were not normally distributed. I log transformed data and excluded data from toad #7 because it was a significant outlier (Appendix. E). After transformation, all groups of data were normally distributed ($P_{16}=0.200$; $P_{21}=0.200$; $P_{26}=0.200$; $P_{31}=0.200$). QQ plots also indicated that transformed data were normally distributed. In the hopping endurance experiment, I excluded toad #12 because it did not hop at 10 and 15 °C. The Kolmogorov-Smirnov test showed hopping endurance of *A. woodhousii* was normally distributed with Lilliefors Significance Correction after excluding toad #12 ($P_{10}=0.073$; $P_{15}=0.199$; $P_{20}=0.200$).

In the transit rate experiment, I averaged the time that it took the seven beads to be expelled in the feces of each toad at each temperature. I used One-way ANOVA with repeated measures (ProStat Program) to check for differences in transit rate among test temperatures. This was followed by Student-Newman-Keuls (SNK) test to determine where the difference occurred.

In the hopping endurance experiment, each toad was tested three times at each temperature. I used the maximum time of hopping endurance from three trials as the time

of hopping endurance for each toad at each temperature. I used One-way ANOVA with repeated measures (ProStat Program) to check for differences in endurance of hopping activity among test temperatures. This was followed by Student-Newman-Keuls (SNK) test to determine where the difference occurred.

RESULTS

The transit rate of beads did not differ among *A. woodhousii* across the four acclimation and test temperatures (df= 3, P= 0.8471; Figure 1). Despite being acclimated and tested at a constant temperature the range from 16 to 31 °C did not alter transit rate. In this experiment, I found 159 feces from 17 toads and 28% of feces contained one bead, 24% of feces contained two different colored beads and 25% of feces contained three beads (Table 1).

Hopping endurance of *A. woodhousii* was significantly different over the three test temperatures (df= 2, P= 0.0005; Figure 2). Moreover, Student-Newman-Keuls (SNK) indicated hopping endurance of *A. woodhousii* at 20 °C was significantly higher compared with that at 10 °C (P= 0.007) and 15 °C (P=0.018). Hopping endurance did not differ between 10 and 15 °C (P= 0.39).

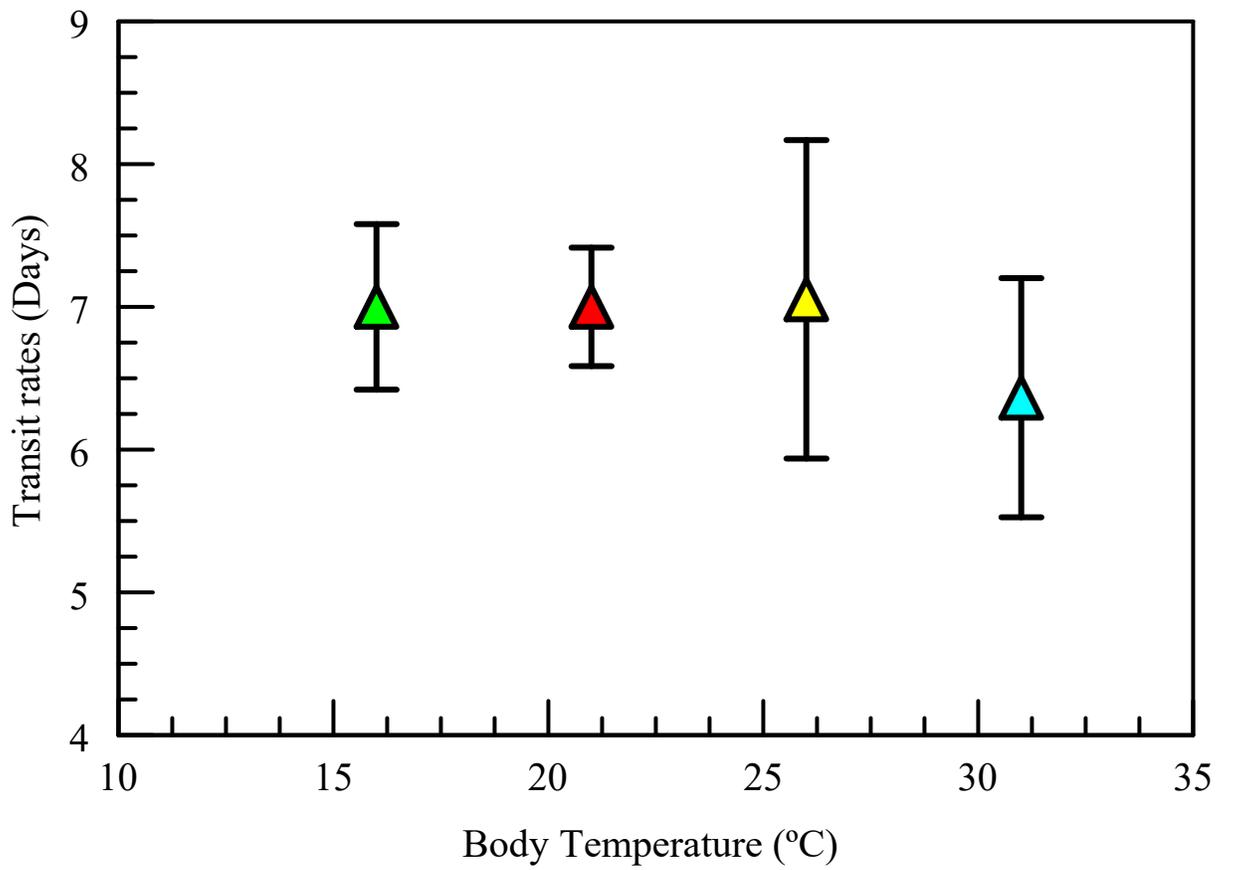


Figure 1. Transit rate ($\bar{x} \pm SE$) of *Anaxyrus woodhousii* at 16, 21, 26, and 31 °C (n= 17).

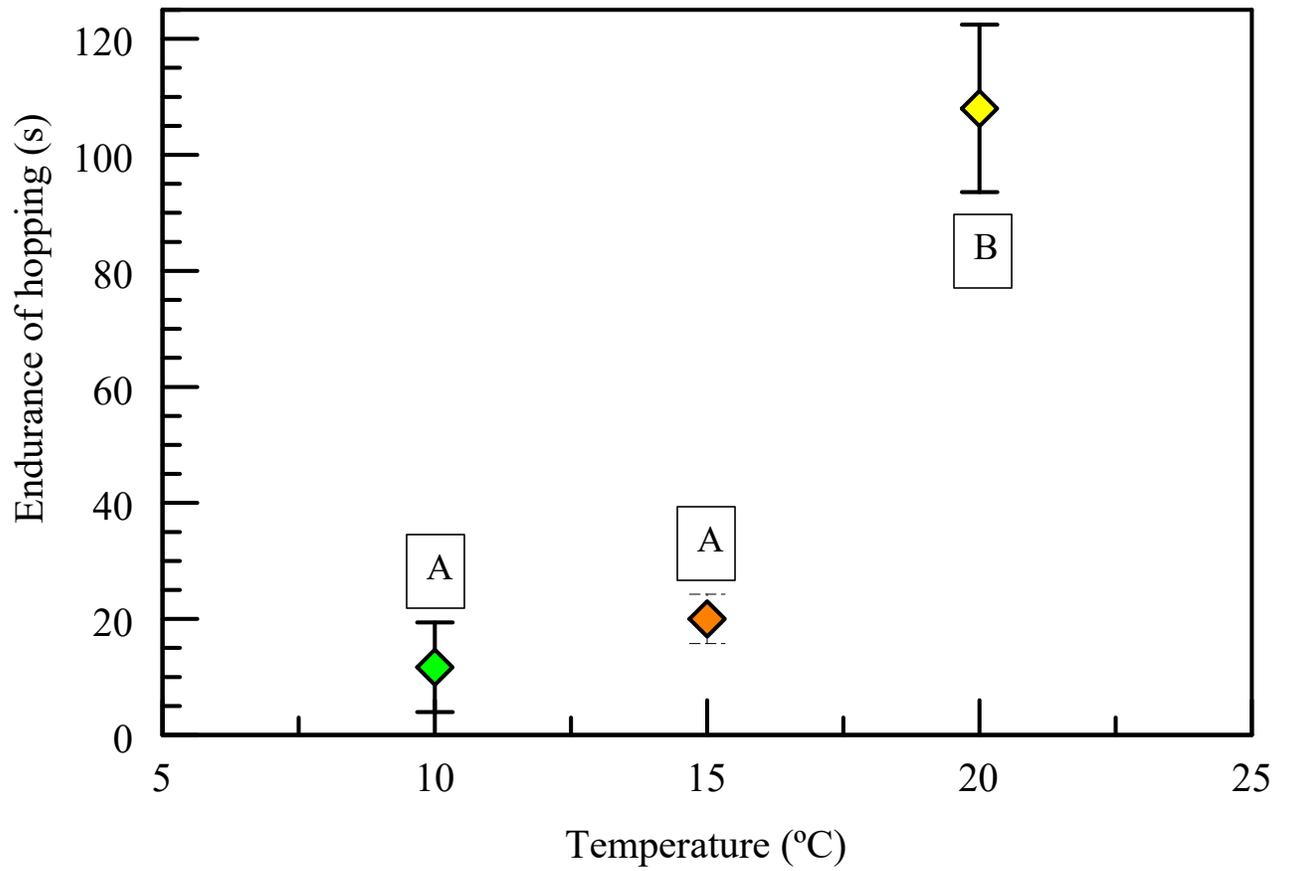


Figure 2. Hopping endurance ($\bar{x} \pm SE$) of *Anaxyrus woodhousii* at 10, 15, and 20 °C (n=7). Bars labeled with different letters are significantly different.

Table 1. The proportion of fecal samples from 17 *Anaxyrus woodhousii* (n= 159) according to numbers of beads found within them.

Number of beads in feces	Total number of feces	Proportion of total
1	44	27.67%
2	38	23.89%
3	40	25.16%
4	12	7.50%
5	11	6.90%
6	5	3.10%
7	9	5.66%

DISCUSSION

The goals of this research were to determine effects of T_b on transit rate and hopping endurance of *A. woodhousii*. I accepted my first hypothesis that the range of T_b s I tested did not significantly affect the transit rate of *A. woodhousii*. This outcome is not surprising because the testing temperatures in this experiment were close to the range that allows successful physiological performance of *A. woodhousii* (22 to 35 °C; Brattstrom, 1963). Moreover, the test temperatures I chose were based on the temperature range encountered by *A. woodhousii* in Kansas. For instance, the seasonal climate of Kansas includes summer (July to September) average monthly temperatures of 21.7 to 27.2 °C, warm spring and fall average monthly temperatures of 13.9 to 24 °C (KDWPT). *A. woodhousii* needs to be able to deal with a relatively wide range of temperatures in such a variable environment. My results indicated the food passage time of *A. woodhousii* was relatively stable (Figure 3) within T_b ranging from 16 to 31 °C. My methods allowed toads to acclimate to each temperature before measuring transit rate, therefore mimicking the acclimation that would occur in the field. The temperature I used remained constant whereas the temperature experienced by free-living toads is variable over the course of hours or days. Given that my test animals did not show differences in transit rate at constant high or low temperature suggests that transit rate in free-living toads is independent of ambient temperature within the range of 16 to 31 °C.

I observed some feces contained several beads from days that were not contiguous. This observation can be explained by the beads sitting somewhere in the digestive tract and thereby changing the order in which beads exited the digestive tract. I observed that

undigested insect parts, such as crickets' heads, showed up in toads' feces. This was especially noticeable in freshly collected toads. These insect parts often exceeded the size of the beads used in my experiment. I also observed toads did not produce feces every day when I fed them with two or three crickets every day. In addition, I also found toads have the ability to retain feces for a long period. In this experiment, Toad #7 produced a relatively huge feces that contained all seven beads 25 days after the day it was given the first bead. These data were significant outliers so I excluded them from final analysis. However, I did not observe any significant pattern for retaining feces in toads. I observed toads retain feces at T_b s of 21, 26, and 31 °C, but none of the toads retain feces at a T_b of 16 °C. Most interesting, I only observed one toad retain feces twice in this experiment, which was toad #6. This toad retained feces at 26 °C and 31 °C, but this toad was not moved directly from 26 to 31 °C. Toad #6 was in group 2, before moving to 31 °C, it was tested at 16 °C (Appendix A). Finally, my results and observation suggest the technique used in my research to determine the transit rate was a crude measure of time passage. I could not monitor where food was within the digestive tract as has been done in snakes, because the digestive tract of toads is not straight (Stevens and Hume, 1995; Sievert et al., 2005; Bontrager et al., 2006). I tested acclimation effects of temperature on transit rate. It is possible that if I had measured the effect of acute temperature on transit rate I might have found some combinations of acclimation temperature and test temperature that altered my results.

In contrast with transit rate, a T_b of 20 °C did significantly affect hopping endurance of *A. woodhousii* compared with that at 10 °C and 15 °C. Therefore, I accepted my second hypothesis in this experiment. My results are consistent with previous results

on *A. woodhousii* (Londos and Brooks, 1988). In addition, I observed better hopping performance at 20 °C than at both 10 and 20 °C. Toads struggled on the treadmill when temperatures were at 10 and 15 °C, but not at 20 °C. Most toads could not make several hops continuously, and they tried to walk on the treadmill instead of hopping at the lower temperature. My results suggested that cold acclimation had significant effects on toad's muscle performance. This outcome agrees with studies showing that cold acclimation in ectotherms affects maximum velocity and maximum power output of muscle, contractile properties, and nervous system function (Rome, 1990; Rome, 2007).

CONCLUSION

In summary, test temperatures of 16, 21, 26, and 31 °C did not significantly affect the transit rates of *A. woodhousii*. In contrast, test temperatures of 10, 15, and 20 °C did significantly affect the hopping endurance of *A. woodhousii*. The first result from this research indicated the T_b s within the test temperature range had no significant effect on food going through the toad's digestive tract, which showed *A. woodhousii* can digest at different T_b s and showed the digestive system had thermal flexibility. The transit rate remains stable over a relatively wide range of T_b s. Moreover, I observed toads retained their feces at three test temperatures in this research but did not observe any temperature related pattern of retaining feces. I also found the technique of this research provided a crude but non-invasive way to measure transit time. The second result from this research indicated that T_b had a significant effect on hopping endurance of *A. woodhousii*. The maximum hopping endurance at 20 °C was significantly higher than that at 10 °C and 15 °C. I also observed toads had better performance at 20 °C compared to 10 °C and 15 °C, and most toads struggled at the lower T_b s. I found the same result of hopping activity compared with Londos and Brooks (1988). Finally, I found maintaining toads at 31 °C continually can be harmful to toad's normal physiological performance. I observed 2 toads died at this temperature in this experiment.

Overall, one result of my research suggests that *A. woodhousii* can subdue prey, digest food and produce feces at 16, 21, 26, and 31 °C, which is in keeping with the temperature of wild toads in the natural habitat (Brown et al., 2007). For instance, the average monthly temperature of Kansas is ranging from 13.9 to 27.2 °C during the time

this toad is active each year (KDWPT). However, another result of my research suggests that muscle performance of *A. woodhousii* was temperature dependent. Therefore, temperature in this species has a larger impact on muscle performance than digestion.

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Appendices

Appendix A: The random order of different temperatures used in testing.

	Group 1	Group 2	Group 3	Group 4
Temperature 1	16 °C	21 °C	26 °C	31 °C
Temperature 2	21 °C	31 °C	21 °C	26 °C
Temperature 3	26 °C	16 °C	31 °C	16 °C
Temperature 4	31 °C	26 °C	16 °C	21 °C

Appendix B: The order of different colored beads given to the toads.

Days	1	2	3	4	5	6	7
Color	White	Black	Blue	Yellow	Orange	Dark blue	Green

Appendix C: The print-out from the one-Sample Kolmogorov-Smirnov test for digestive rates of *Anaxyrus woodhousii* over different temperatures: 16, 21, 26, and 31 °C.

One-Sample Kolmogorov-Smirnov Test

		Log16	Log21	Log26	Log31
N		17	17	17	17
Normal Parameters ^{a,b}	Mean	.7224	.7259	.7765	.7459
	Std. Deviation	.17519	.14037	.24594	.23338
Most Extreme Differences	Absolute	.168	.116	.158	.113
	Positive	.140	.070	.158	.113
	Negative	-.168	-.116	-.150	-.094
Test Statistic		.168	.116	.158	.113
Asymp. Sig. (2-tailed)		.200 ^{c,d}	.200 ^{c,d}	.200 ^{c,d}	.200 ^{c,d}

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

Appendix D: The print-out from the one-Sample Kolmogorov-Smirnov test for endurance of hopping activity of *Anaxyrus woodhousii* over different temperatures: 10, 15, and 20 °C.

One-Sample Kolmogorov-Smirnov Test

		VAR00014	VAR00015	VAR00016
N		7	7	7
Normal Parameters ^{a,b}	Mean	24.6667	40.3333	86.2857
	Std. Deviation	20.47039	11.27271	38.20434
Most Extreme Differences	Absolute	.292	.252	.212
	Positive	.292	.186	.212
	Negative	-.203	-.252	-.154
Test Statistic		.292	.252	.212
Asymp. Sig. (2-tailed)		.073 ^c	.199 ^c	.200 ^{c,d}

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

Appendix E: The average time beads stayed in the digestive tract of *Anaxyrus woodhousii* at 16, 21, 26, and 31 °C (Days).

*. The toads died during the experiment.

**.. Showed this toad was excluded in this research.

Red color indicates this data was excluded. Blue color indicates retaining feces.

Toad number	Temperature (°C)			
	16	21	26	31
1	7	7	13.4	4.5
2	8.6	4.4	6.4	2.71
3	4.42	4	3.3	1.83
4	2.5	4	6.2	13
5	4.2	4.5	18	7
6	3.5	8.5	13	11
7**	2.5	25	9	5
8	6.5	6	5	6.6
9*	5.4	2.833	5	N/S
10	6.5	7	4.7	5
11	5.4	2.83	4.6	3.5
12	4	5	4.16	6.67
13	3.6	5	5.5	7.42
14	4	8.57	14.25	4
15	3.6	7.2	7	14
16	6.6	3.1	2.8	4.25
17	6.5	5.4	3.25	5
18	8	6.3	3.83	4.2
19	12	6	4.5	7.5
20*	6.25	N/S	N/S	N/S

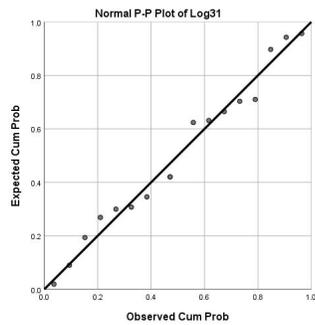
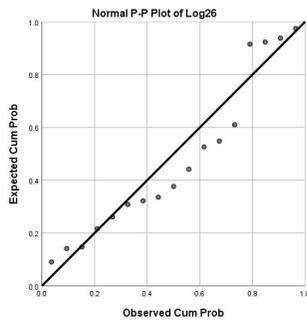
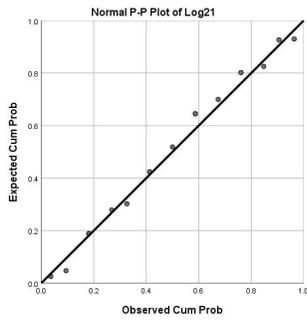
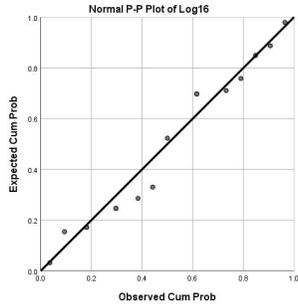
Appendix F: The average time of endurance of hopping activity of *Anaxyrus woodhousii* over 10, 15, and 20 °C (Given in seconds).

*. Indicated toad did not hop at this situation.

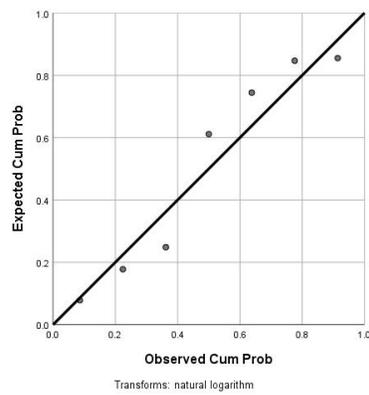
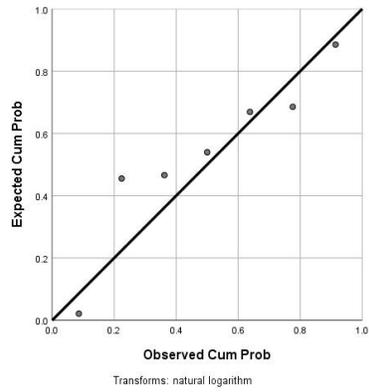
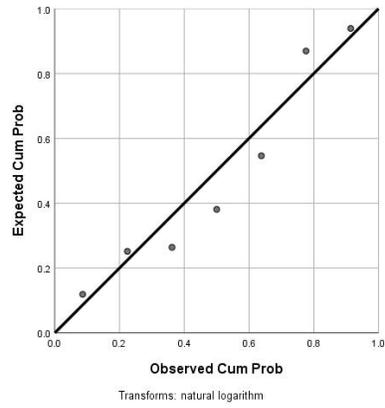
** . Showed this toad was excluded in this research.

Toad number	Temperature (°C)		
	10	15	20
1	11.7	20	108.00
3	7.7	45.33	129.00
6	61.7	44.7	131.3
11	11.3	37.3	39.3
12**	0*	0*	18.7
13	20.7	40.00	56.3
15	15.00	37.7	50.00
18	44.7	57.3	90.00

Appendix G. The result of QQ plots of transformed transit rate of *Anaxyrus woodhousii* at 16, 21, 26, and 31°C.



Appendix H. The result of QQ plots of hopping endurance of *Anaxyrus woodhousii* at 10, 15, and 20°C.



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The Effects of Body Temperature on the Transit Rate and Endurance of Hopping Behavior of
Anaxyrus woodhousii

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