

**A DAY IN THE LIFE OF A TAPEWORM: INVESTIGATING THE LIFE CYCLE OF  
AND POSSIBLE INTERMEDIATE HOSTS FOR *DISTOICHOMETRA BUFONIS* IN  
WOODHOUSE'S TOAD, *BUFO WOODHOUSII***

Kristin K. Brotan, Kristina J. Hubbard, Tyler M. Humphrey, Jake A. McNair, Benjamin G. Powers, Rachel E. Rudolph, and Mackenzie R. Waltke

School of Biological Sciences, University of Nebraska—Lincoln, Lincoln, Nebraska 68588,  
U.S.A. e-mail: [mwaltke1@bigred.unl.edu](mailto:mwaltke1@bigred.unl.edu).

**ABSTRACT:** Cestode life cycles in general are rarely studied and present a wide variety of questions about the evolution of parasites. The cestode *Distoichometra bufonis*, Dickey (1921) has been reported in *Bufo woodhousii* Girard, 1854, but the intermediate host in this lifecycle has never been identified. We examined the stomach contents and feces of these toads to determine possible intermediate hosts. In addition, two species of ants were presented with gravid proglottids to determine if they were ingesting proglottids and possibly serving as an intermediate host. Results suggested that beetles and ants were most likely the intermediate host based on their frequency in the stomachs of infected toads and their visitation to the feces. Some gravid proglottids were eaten when placed near ant hills adding further evidence that an invertebrate, probably ants, is indeed the intermediate host.

Woodhouse's or the Rocky Mountain toad, *B. woodhousii*, is known to be the definitive host for the cestode *D. bufonis*. Very little is known about the life cycle of this particular tapeworm and the intermediate host is completely unknown. It is known that gravid proglottids are released in the feces of the toad where they must be ingested by an intermediate host. This host is then eaten by the toad and the tapeworm can mature and reproduce. Mackiewicz (1988) has provided a basis for the understanding of some cestode life cycles. These life cycles are very

fragile and involve a great deal of co-evolution between the parasite and host, however some plasticity in intermediate hosts has been observed. Life cycles are also susceptible to a variety of external pressures including prey habitat and behavior, environmental conditions, population dynamics, and the reproductive potential of the parasite. Hardin and Janovy (1988) explored the interactions between *B. woodhousii* and *D. bufonis* showing that interruption of the host population has a limited effect on the parasite population. This study also attempted some experimental infections in several beetle families, but was unable to produce any infections.

Other studies of intermediate hosts in cestodes have shown that several orders of invertebrates are capable of ingesting proglottids and transmitting the worms. Fritz (1985) showed that oribatid mites and ants were capable of transmitting the cestode *Moniezia expansa*, while Smirnova and Kontrimavichus (1977) demonstrated that collembolans transmit *Paranoplocephala* sp. Evans et. al. (1998) have shown that Tenebrionid beetles serve as the intermediate host for *Hymenholepis diminuta*. Based upon these findings, it is reasonable to suggest that *D. bufonis* is also using an invertebrate as its intermediate host.

Transmission from the intermediate host to the definitive host is influenced by the behavior of the toad host. Predation by the toads must also be considered when suggesting possible intermediate hosts. According to a study done by Beck and Ewert (1979), the common toad, *Bufo bufo*, responds to not only the shape of the prey stimulus, but also its direction of motion. The behavior of the toads, as predators, is affected by the behavior of their prey. Studies by Norris (2004) have shown that toad vision is most acute in the lateral direction. Therefore, a stimulus moving sideways across a toad's field of vision will gather the toad's initial attention, otherwise known as the orienting behavior. Regardless of what happens after the initial stimulus action, the toad will follow the rest of its hunting response to completion. Thus, toads might not

be influenced by the prey item itself, but the prey's movement. Possible intermediate hosts must be among these prey.

This study explored the life cycle of *D. bufonis* in order to identify possible intermediate hosts with 3 main goals: (1.) to determine what the toads, especially infected ones, are eating, (2.) to determine what invertebrates are visiting the feces and may become infected with proglottids, and (3.) to examine if it is possible to experimentally infect any of these invertebrates with proglottids.

## **MATERIALS AND METHODS**

### **Toad Collection**

*B. woodhousii* specimens were collected at Beckius Pond (41.12529, -101.37276) near Ogallala, NE, U.S.A. Twenty-eight toads were collected during the night from Beckius Pond on July 23, 2006. A second collection was taken during the day at Beckius Pond on July 27, 2006 where twenty-eight toads were gathered. Toads were placed in refrigeration until dissection.

### **Stomach Contents**

Fifty-six *B. woodhousii* were anesthetized before dissection. Prior to the ventral incision, snout to vent length and gape measurements were recorded. The stomach, small intestine, and large intestine were removed, and the stomach was separated from the rest of the gastrointestinal tract for further examination. Each stomach was opened and emptied into a numbered vial with 70% ethanol for content preservation. Upon completion of all the dissections, the partially digested matter was placed into a Petri dish and examined under a dissecting microscope. Specimens were identified and classified into several broad categories consisting of Ants, Other Hymenoptera, Coleoptera, Hemiptera, Homoptera, Diptera, Orthoptera, Odonata, Chelicerata,

Isopoda, Caterpillars, and Toads. These classifications included larval stages and all identifiable body parts. Insects were identified using common order characteristics as described in *A Field Guide to Insects: America North of Mexico* (1970). Each intact individual was measured and recorded. If recognizable but not intact, the specimen was recorded in the classification data, but no measurements were taken.

### **Intestinal Tract**

Upon dissection, the intestinal tract was separated and subsequently examined for the presence of the tapeworm *D. bufonis*. If the toad was infected, the tapeworm was removed from the intestine and stored in a vial containing water. The abundance of tapeworms was recorded and in order to conduct the feeding tests later, gravid proglottids were separated from the posterior end of the tapeworm and placed in plastic Petri dishes each with a single drop of water.

After collection of the data, statistical analysis was conducted using Microsoft Excel investigating prevalence, mean abundance, and intensity. Correlation values were also examined between snout-vent length and gape, as well as snout-vent length and abundance of tapeworms. Next, a t-test was used to check the significance of the correlation between the abundance of worms versus the size of the toad. Using Hardin and Janovy (1988), the toads were split into two groups according to size: those larger than 65mm and those smaller than 65mm. Another t-test was used to examine the significance of the correlation between the abundance of worms compared to the sex of the toad.

### **Fecal Matter Collection and Investigation**

Fecal matter was collected at Cedar Point Biological Station near Ogallala, NE (41.21517, -101.52220) over a period of two days. Each fecal pellet was collected, measured for length and width, and examined for the presence of proglottids. Fall traps were set at the location

of each fecal matter. Fall traps consisted of small vials placed in holes flush with the surrounding ground. They were filled with 70% ethanol and the fecal pellets were placed over the top.

Control traps were set in the same manner, using twigs of a similar size placed over the top of the vials. When the feces were small enough to fall through the trap, a wire mesh screen was placed over the vial opening and the feces placed on top of the screen. These traps were checked after 12 and 24 hours. The types of invertebrates were catalogued using a dissecting microscope according to order. Collections from these traps were also made at 6:00 am to determine which invertebrates were visiting the traps at night and at 8:30 pm to determine which were visiting during the day. Larger fall traps were set using plastic cups. One trap had tapeworms, one feces smeared on the trap, one a fecal pellet wrapped in tapeworms, and one a fecal pellet alone. These traps were checked after 12 hours and the invertebrates catalogued in the same manner as above.

### **Fecal Matter Temperature**

Ground and feces surface temperatures were measured using a Raytek® STPro™ noncontact thermometer. Temperatures of all the fecal matter were taken at 1:30 pm and again at 3 hour intervals over a 12 hour period (6:00am-6:00pm). With the fall traps, temperatures of each fecal pellet and the ground temperature next to it were measured. In addition, 3 pellets were placed in grass and 3 pellets were placed in sand and measured over the same intervals.

### **Experimental Infections**

The gravid proglottids collected during the dissection were removed from the plastic Petri dishes and placed on plastic cover-slips. Half of the slides contained only the dry proglottids, while the remaining half contained both the dry proglottids and a drop of honey to attract the ants. Nine ant hills were selected, and one slide of each type was placed next to the entrance of the ant hill. The first trial was conducted during the night, and another trial was repeated the next

day. The slides were then collected after a twelve hour time interval and examined for the absence of the gravid proglottids. The data was recorded, and using a contingency table, the  $\chi^2$  value was calculated to determine whether there was difference between the number of proglottids eaten during the day compared to those eaten at night.

## RESULTS

### Toad Collections

There were only slight differences in the average sizes of the toads collected during the day versus those collected at night. The day average was 44.2 mm while the night was 45.4 mm. The prevalence, mean abundance, and intensity of the parasitism of *D. bufonis* in *B. woodhousii* are listed in Table I with size distribution listed in Figure 1. Also, a graphical representation of the correlation between snout-vent length and gape width is shown in Figure 2. For further exploration, a prevalence distribution is listed in Figure 3 with the toad sizes divided in ten millimeter increments. After statistical analysis, the correlation between snout-vent length and gape resulted in a value of 0.94, and the correlation between snout-vent length and abundance of tapeworms resulted in a value of 0.53. For statistical purposes, this relationship is shown in Figure 4, where the abundance of tapeworms versus snout-vent length of each of the sexes of toads.

Using a t-test, the relationship between snout-vent length and abundance of tapeworms proved to be significant ( $p < 0.05$ ). Another t-test was performed on the relationship between the sex of *B. woodhousii* and abundance of tapeworms. However, this was not significant ( $p > 0.05$ ).

## Stomach Contents

The average prey size during the day was 4.1 mm and the night was 5.5 mm. Diet analysis of *B. woodhousii* indicated that diurnally active toads predominantly consumed insects of the order Diptera with 90.5% prevalence (Figure 5). The remainder of their diet consisted primarily of Ants, other Hymenoptera and Coleoptera. The diet of nocturnally active toads varied considerably for each individual. Ants comprised 56.7%, although the remainder of the toads' diets were largely made up of other Hymenoptera, Diptera, and Coleoptera (Figure 6).

A  $\chi^2$  analysis was used to compare the different categories of organisms found in the day and night toads. As demonstrated by Figure 7, there was a difference between the percentage of ants present in the toads collected during the day compared to the percentage of those found in the toads collected at night. There were more ants found at night, with 56.7% abundance and the day value of only 4%. There were also differences found between the day and night consumption of Coleoptera (day: 1.7% and night: 15.3%) and Diptera (day: 90.5% and night: 17.1%). However, the other Hymenoptera category showed no difference. The toads were separated into size classes by 10 mm increments, starting with 20-29 mm through 70-79 mm. There was a strong correlation of 0.95 between the average size of the toad and the average size of the organisms consumed. Overall, as the size of *B. woodhousii* increased, the size of prey increased as well (Figure 8). This is also the case when diurnal (Figure 9) and nocturnal (Figure 10) toads are compared separately.

## **Fecal Matter Collection and Investigation**

A total of nine fecal pellets was observed. The feces ranged in length from 21 mm-80 mm, with an average length of 36.33 mm. The feces averaged 10.22 mm in width with a range of 8 mm-16 mm. There were no proglottids found on any of the feces collected.

Several groups of invertebrates were discovered in the fall traps including ants, beetles, mites, spiders, flies, wasps, and springtails. Ants were the most prevalent in all fall traps, comprising almost 60% of all the invertebrates found (Figure 11). Very few spiders or wasps were found. There was no difference found between the small fall traps with fecal matter and the control traps (Figure 12). Again, with almost 50% in both traps, ants were the most prevalent. There was also no difference in the prevalence of any of the invertebrates found in small traps with screen or ones without screens. Ants were the most common (Figure 13). Beetles, mites, and springtails all showed differences in the number collected from traps during the day with those collected from traps during the night (Figure 14). Springtails and beetles were found more often at night, while mites were more common during the day.

In the large fall traps with tapeworms compared to those with feces, statistical analysis showed a difference in the number of ants, mites, and springtails. Ants and mites were more common in traps with tapeworms, while springtails were less common in these traps (Figure 15). In traps with fecal pellets wrapped in tapeworms and fecal pellets alone, only ants showed a difference in the number caught (Figure 16). More ants were caught in traps with only fecal pellets.

## **Fecal Matter Temperature**

A minimum temperature of 20.6°C and a maximum of 73.8°C were measured for the surface of the fecal pellets. A wide fluctuation in the surface temperature was observed



throughout the day, with the highest average temperature for the day (43.66°C) found around 3:00 pm (Figure 17). Temperatures were also compared between fecal matter on two different substrates, sand and grass. Again, there was a temperature fluctuation throughout the day with the highest temperatures around 3:00 pm. The surface temperatures of the feces on sand were higher than those of the feces on grass (Figure 18). The highest average temperature for the sand feces was 42.33°C, while the highest average temperature on grass was 38.13°C. Temperatures were lowest around 6:00 am.

### **Experimental Infections**

The results from the two feeding trials are summarized in Table II. Some ant hills lack appropriate data because the slides were lost, due to wind. To interpret this data, a contingency table was constructed, which resulted in a  $\chi^2$  value of 1.77 with one degree of freedom. There was not a difference between the number of gravid proglottids eaten during the day compared to the number eaten at night.

### **DISCUSSION**

The average sizes of the toads collected during the day and night were similar. This suggests that the difference in diet is not due to size, but rather to the time of day at which toads are feeding. Different types of prey may be more prevalent at different times of the day and may account for the varied stomach contents. Although many of the smaller toads had a high number of organisms in their stomach, there was relatively little diversity when looking at each toad individually. The larger toads had more diversity, including a few instances of large toads eating smaller toads. Previous studies have remarked on toad predation response to visual stimulus and size rather than the type of prey. *B. woodhousii* may simply be looking for food that is

proportional to its gape, rather than for certain organisms. Larger toads may simply ingest a wider diversity than smaller toads, because they can capture large and small prey. These observations suggest that the tapeworm life cycle may be limited by the less diverse diet of the smaller toads. *D. bufonis* would need to infect both large and small toads to increase its survival, so the intermediate host is most likely found in smaller toads.

Ants and beetles were two of the most commonly ingested invertebrates found within the stomach contents. Both of these invertebrate groups fit within the desirable gape size for almost all toads. Ants and beetles are also common within the *B. woodhousii* habitat and are easily accessible as they are frequently found along the ground rather than in the air. In total, 94% of the toads that were infected were found to have consumed either beetles (69%) or ants (25%). This suggests that if *D. bufonis* is indeed using either beetles or ants as an intermediate host the chances of transmission to the definitive host are high.

There are several different groups of invertebrates, including beetles and ants that visit toad feces and could function as a possible intermediate host for *D. bufonis*. Based on the frequency of these visitations as well as other accounts, along with ants and beetles, springtails and mites appear to be the most likely contenders for the intermediate host. However, neither mites nor springtails were found in abundance in the stomachs of the toads. It is possible that these smaller organisms are absent or unidentifiable because they are digested more quickly. However, based on our findings the most likely intermediate hosts remain ants and beetles.

Despite the visitation of possible hosts, transmission is not easily accomplished. The location of a trap as well as the time of day appears to influence which invertebrate is visiting said trap. In addition, the temperature of the feces varies widely and may also influence the behavior of the invertebrates. All these factors present numerous environmental stresses upon the

proglottids, which may be influencing transmission. For example, it is possible that the proglottids must be transmitted while the feces are still fresh to prevent desiccation during the day and ensure survival.

From statistical data gathered, there was no difference between the number of gravid proglottids eaten during the day versus the number of gravid proglottids eaten at night. This suggests that ants are just as likely to eat proglottids during the day as at night. This is also supported by the data from the fall traps that saw no difference in the number of ants visiting the feces during the day and night. Based on all of the data gathered in this experiment ants continue to be a likely intermediate host. However, further study must be conducted using a number of other invertebrate hosts, including beetles, as so little is known about this particular tapeworm and its life cycle.

## **ACKNOWLEDGMENTS**

The researchers would like to thank Rob Anderson and the Cedar Point Biological Station for the use of the lab and access to research material. We would especially like to express our appreciation to Dr. Matt Bolek for the research ideas and the constant enthusiasm that made our class exciting and unforgettable. We would also like to thank the toads for sacrificing their bodies for science and the Cedar Point lunch ladies for keeping us well nourished. JAM would like to thank Ben Powers and Kristin Brotan for the rides to the white gate and beach where many of our hypotheses and research methods were thought up and contemplated. We would like to acknowledge Ryan Murphy, the creator of Nip/Tuck, for providing us with motivation on Mondays and constant break entertainment. This research was supported by the hard earned

money the students paid for tuition to the University of Nebraska Lincoln. A special thanks to Dr. John Janovy, Jr. for providing partial funding.

## LITERATURE CITED

- Beck, A and J. P. Ewert. 1979. Prey selection by toads (*Bufo bufo* L.) in response to configurational stimuli moved in the visual field z,y-coordinates. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **129**: 207-209.
- Evans, W. S., A. Wong, M. Hardy, R. W. Currie, and D. Vanderwel. 1998. Evidence that the factor used by the tapeworm, *Hymenolepis diminuta*, to direct the foraging of its intermediate host, *Tribolium confusum*, is a volatile attractant. *Journal of Parasitology* **84**: 1098-1101.
- Fritz, G. N. 1985. A consideration of alternative intermediate hosts for *Moniezia expansa* (Cestoda: Anoplocephalidae). *Proceedings of the Helminthological Society of Washington* **52**: 51-53.
- Hardin, E. L. and J. Janovy Jr. 1988. Population dynamics of *Distoichometra bufonis* (Cestoda: Nematotaeniidae) in *Bufo woodhousii*. *Journal of Parasitology* **74**: 360- 365.
- Mackiewicz, J. S. 1988. Cestode transmission patterns. *Journal of Parasitology* **74**: 60-71.
- Norris, S. 2004. Common toad (*Bufo bufo*) prey catching behavior. Retrieved August 3, 2006, from <http://instruct1.cit.cornell.edu/courses/bionb424/students/ssn3/behavior.html>.
- Smirnova, L. V. and V. L. Kontrimavichus. 1977. Collembola--intermediate hosts of cestodes of Muridae in Chukotka. *Dokl, Akad. Nauk. S. S. S. R.* **236**: 771-772. (In Russian.)

Table I. Prevalence, mean abundance, and intensity of *Distoichometra bufonis* in *Bufo woodhousii*.

	<b>Male</b>	<b>Female</b>	<b>2nd yr.</b>	<b>3rd yr.</b>	<b>Total</b>
<b>Prevalence</b>	0.25	0.44	0.24	0.83	0.3
<b>Mean Abundance</b>	1.05	2	0.98	4.17	1.32
<b>Intensity</b>	4.2	4.57	4.08	5	4.35

Table II. Results from feeding trials with gravid proglottids placed near the entrance of ant hills.

<b>Ant Hill No.</b>	<b>Day</b>	<b>Night</b>
	<b>Eaten</b>	<b>Eaten</b>
<b>1</b>	Yes	No
<b>1-honey</b>	Yes	No
<b>2</b>	Yes	Yes
<b>2-honey</b>	No	Yes
<b>3</b>	No	No
<b>3-honey</b>	No	No
<b>4</b>	Yes	No
<b>4-honey</b>	Yes	Yes
<b>5</b>	Yes	Yes
<b>5-honey</b>	Yes	Yes
<b>6</b>	Yes	No
<b>6-honey</b>	No	No
<b>7</b>	Yes	No
<b>7-honey</b>	Yes	Lost
<b>8</b>	Yes	Yes
<b>8-honey</b>	No	No
<b>9</b>	Yes	Yes
<b>9-honey</b>	Yes	Lost

**FIGURE LEGENDS**

FIGURE 1. a. The correlation between snout-vent length versus abundance of each sex of *B. woodhousii*. b. Linear regression results including  $r^2$  and slope.

FIGURE 2. A positive correlation of snout-vent length with gape width.

FIGURE 3. Prevalence of *D. bufonis* in *B. woodhousii* separated into sizes of 10mm increments.

FIGURE 4. Size distribution of sampled *B. woodhousii* divided 10mm size increment.

FIGURE 5. Distribution of stomach contents as a percent of the total organisms recovered from diurnal toads.

FIGURE 6. Distribution of stomach contents as a percent of the total organisms recovered from nocturnal toads.

FIGURE 7. Comparison of the percentage of invertebrates present in the stomach for diurnal and nocturnal toads.

FIGURE 8. Toad size class versus prey size for total number of toads collected, showing a strong correlation (.95) between toad size and prey size.

FIGURE 9. Toad size class versus prey size for toads collected during the day showing a slight correlation (.56) between toad size and prey size.

FIGURE 10. Toad size class versus prey size for toads collected at night showing a strong correlation (.89) between toad and prey sizes.

FIGURE 11. The total number of invertebrates found in the all of the fall traps. Invertebrates were collected over a three day period and classified into large groups for identification purposes.

FIGURE 12. Prevalence of invertebrates found in the small experimental and control fall traps, showing that there was no difference between the traps with or without feces.

FIGURE 13. Prevalence of invertebrates found in small fall traps with and without screens.

There was no difference for any of the invertebrates found, showing that the presence of a screen to hold the feces does not prevent visitation or trapping.

FIGURE 14. Comparison of invertebrates found in traps during the day and night. There was a difference between beetle ( $\chi^2 = 5.42$ ), mite ( $\chi^2 = 27.83$ ), and springtail ( $\chi^2 = 29.75$ ) visitation.

FIGURE 15. Comparison between the large trap with tapeworms and the large trap with feces.

Ants ( $\chi^2 = 43.30$ ), mites ( $\chi^2 = 28.05$ ), and springtails ( $\chi^2 = 93.81$ ) all showed a difference in their prevalence in the traps.

FIGURE 16. Comparison between large traps with feces wrapped in tapeworms and feces alone.

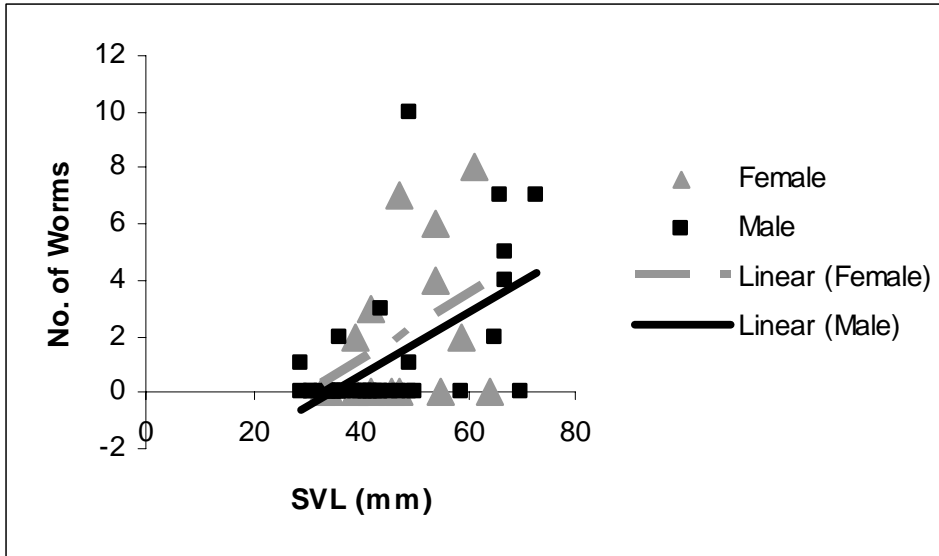
Ants alone showed a differential preference for feces alone ( $\chi^2 = 6.24$ ).

FIGURE 17. Average temperature fluctuation for feces surface temperature over a 12 hour period (6:00am-6:00pm). Highest temperatures were reached around 3:00pm and lowest temperatures around 6:00am.

FIGURE 18. Comparison of the average surface temperature of feces found on grass and sand.

Temperatures were taken over the same 12 hour period.





a.

	Slope	y-intercept	$r^2$
<b>Male</b>	0.1108	-3.8045	0.3301
<b>Female</b>	0.1208	-3.7026	0.1731

b.

