To Eat or Not to Eat? Behavioral Responses to Novelty and Aposematic Signals

by

Sarah A. Candler, B.S.

A Thesis

In

BIOLOGY

Submitted to the Graduate Faculty of Texas Tech University in
Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCES

Approved

Dr. Ximena E. Bernal, Ph.D.
Co-chair of Committee

Dr. Nancy E. McIntyre, Ph.D.
Co-chair of Committee

Dr. Krista A. McCoy, Ph.D.

Dr. Mark Sheridan, Ph.D.
Dean of the Graduate School

August, 2014
ACKNOWLEDGEMENTS

I would like to thank my family, friends, and mentors for their continued help and support throughout the course of my Masters of Science degree. First, I would like to thank my advisor, Dr. Ximena E. Bernal, for her guidance. I truly appreciate her trust and support, both of which have helped me grow not only as a scientist, but also as a person. I would also like to thank the members of my committee, Dr. Nancy McIntyre and Dr. Krista McCoy. Dr. McIntyre for helpful comments and wisdom throughout my studies. Dr. McCoy for her advice in the beginning stages of my project and continued interest and feedback.

In addition to my committee, many of my fellow graduate students helped me throughout the past two years. I would like to thank the following people for providing support in a plethora of ways from reviewing manuscripts to simply listening to frustrations: Doug Perez, Lauren Eveland, Andrea Reinhardt, Emma Roberts, Morgan Cocke, and Sarah Mangum. Priyanka de Silva, you helped in more ways than you will ever know; I appreciate everything you have done for me and will truly miss working with you. Several undergraduate students also helped me tremendously; Katie Smith, thank you for your help conducting field work; Bianca Rendon, thank you for your comments on my manuscript, your enthusiasm in my project, and all of your help in the field.
Last, but not least, I would like to thank my family for their continued support. My parents, Jerry and Patti Candler, and siblings, Jerry and Erin Candler--thank you for believing in me, listening to me, and providing me with distractions when I needed a break. Thank you to my grandmother, Lucille Kiessling, for providing opportunities for me to have fun and for always listening to me. Finally, my fiancé, Thomas Small, has provided unbelievable support over the past two years. Thank you for everything that you do, but mostly for making me laugh and always being there for me.
# Table of Contents

Acknowledgements .................................................................................................................................. ii

Abstract .................................................................................................................................................... vi

List of Figures ........................................................................................................................................ viii

I. General Introduction .......................................................................................................................... 1

II. Differences in neophobia between cane toads from introduced and native populations ............... 4

   Abstract ................................................................................................................................................ 4

   Introduction .......................................................................................................................................... 5

   Methods ............................................................................................................................................... 8

      Study Species .................................................................................................................................. 8

      Study Areas ...................................................................................................................................... 9

      Experimental Set-up ....................................................................................................................... 10

      Novel Prey ....................................................................................................................................... 11

      Novel Object .................................................................................................................................... 12

      Statistical Analyses ......................................................................................................................... 13

   Results ................................................................................................................................................ 14

      Novel Prey ....................................................................................................................................... 14

      Novel Object .................................................................................................................................... 16

   Discussion ........................................................................................................................................... 17

   Figure Legends ..................................................................................................................................... 20

III. Neophilia in the cane toad *Rhinella marina* ................................................................................. 27

   Abstract ............................................................................................................................................. 27
IV. Behavioral responses of predators to fireflies: Do courtship signals also function as aposematic signals? .................................................... 39

Abstract .................................................................................................................. 39

Introduction ............................................................................................................. 40

Methods .................................................................................................................. 43

Cane Toads .............................................................................................................. 43

Bats .......................................................................................................................... 45

Data Analysis ......................................................................................................... 46

Results ..................................................................................................................... 47

Toad Trials .............................................................................................................. 47

Bat Trials ................................................................................................................ 47

Discussion .............................................................................................................. 51

Figure Legends ...................................................................................................... 55

V. Conclusion .......................................................................................................... 61

Bibliography .......................................................................................................... 64
ABSTRACT

Foraging behavior is an integral part of an individual’s life. Without food, organisms will not have the energy needed to find shelter, reproduce, etc.; therefore, understanding the ways in which individuals find and respond to food can provide insights into their success. This is particularly true in the case of invasive species, which must cope with novel prey items when moving into new habitats. We examined the response of cane toads (Rhinella marina) from native and introduced ranges to novel and familiar prey items. Native cane toads were less likely to consume novel prey items and thus more neophobic than introduced toads. We also tested native toads for neophilic tendencies. Toads were presented with both novel and familiar prey at once. We found that native toads do not preferentially feed on novel prey and, therefore, are not neophilic. In addition to affecting the individual’s survival, foraging behavior can also impact the prey species. The evolutionary pathway of a prey species can be altered through the foraging behavior of predators. We examined the response of three common nocturnal predators, the cane toad and two species of insectivorous bats (Myotis nigricans and Molossus molossus), to two species of Neotropical fireflies (Photurus trivattatta and Bicellonycha amoena) to determine if flashing serves as an aposematic signal to predators in addition to functioning as a courtship signal. Both species of bats rejected fireflies, but ate mealworms coated in pyrazine. Fireflies, however, did not deter cane toads. This suggests that fireflies are unpalatable to some predator species, but pyrazine is not
the main deterrent. By understanding the foraging behavior of a species insight can be gained into mechanisms for their success as well as their impact on the evolution history with and effect on prey species.
LIST OF FIGURES

2.1. Neophobia Experimental Set-up .................................................................23
2.2. Novel Prey Experiment Results ....................................................................24
2.3. Novel Object Experiment ............................................................................25
2.4. Novel Object Experiment Movement Results .............................................26
3.1. Neophilia Experimental Set-up ....................................................................38
3.2. Response to Prey .........................................................................................39
4.1. Latency to Eat ...............................................................................................58
4.2. Time Spent Chewing and Percentage of Time Spent Eating in *My. nigricans*
    and *Mo. molossus* .......................................................................................59
4.3. Number of Shakes in *My. nigricans* and *Mo. molossus* ............................60
4.4. Percent of Prey Eaten Across Trials in *My. nigricans* and *Mo. molossus* ....61
CHAPTER I

GENERAL INTRODUCTION

Finding and obtaining food for energy is a basic need for all living things. Individuals need this energy order to find shelter, locate mates, reproduce, etc. Thus, foraging behavior is crucial to an organism’s survival, and many successful foraging strategies exist (Schoener 1971). These strategies are shaped by evolutionary history, the environment, and also behavior. Recently, behavior has been increasingly integrated into the study of evolution since behavior not only shapes evolutionary pathways, but is itself shaped by evolution (Sih et al. 2010).

The following chapters examine the foraging and exploratory behavior in a known invasive species, the cane toad (Rhinella marina), and how this species responds to various types of prey, both novel and familiar. The second chapter, which is the main study of this thesis, focuses on the response of individuals to novel and familiar prey as well as novel feeding situations in populations from different habitats and ranges. Because invasive species are a major threat to native biota, understanding the behavior involved in their ability to invade multiple habitats is crucial for conservation. This study is currently in review in Behavioral Ecology and is co-authored by Dr. Ximena E. Bernal. The third chapter formatted for Behavioral Ecology explores a similar question concerning the response to novelty in cane toads, but at the opposite end of a behavioral continuum. Whereas avoidance of novelty is examined in the second chapter, the third chapter examines
exploratory interest in novelty. The purpose of this chapter is to build on the second chapter to better understand the role of behavior in invasive ability.

In response to novelty, we expect native individuals to be more hesitant to explore and consume novel prey items than invasive individuals. In addition, individuals from different habitats within a single range are expected to differ. Individuals living in a rural environment should be more hesitant to exploit novelty than those living in an urban environment. Additionally, because cane toads are highly successful as an invasive species, we expect them to opt to exploit novelty when faced with a choice between familiar and novel food.

In addition to shaping the evolution of the focal predator species, foraging behavior can also change the evolutionary trajectory of the prey species. The fourth chapter in this thesis describes the response of three common nocturnal predator species (cane toads and two insectivorous bat species: *Myotis nigricans* and *Molossus molossus*) to Neotropical fireflies. This study tests the hypothesis that fireflies are an unprofitable prey species and flashing serves not only as a courtship signal, but also as an aposematic signal to predators. Although cane toads are not expected to be deterred by fireflies or their chemical compounds, the bat species examined are expected to reject both due to more specialized diets when compared to cane toads.

Understanding the behavioral response of an invasive species to novelty will provide insight into the ability to invade new environments. Although behavior has been shown to be important in the success of introduced species (Thorsen et al. 2000; Sol et al. 2002; Martin and Fitzgerald 2005), no studies have examined it in
anurans. This study, therefore, is the first to directly examine the response of an anuran to novel prey items in both its native and invasive range as well as multiple habitats within its invasive range. In addition, understanding more about the response of multiple nocturnal predators to prey known to be noxious to other species sheds light on the evolutionary history of not only the predator species, but also the prey species.
CHAPTER II

DIFFERENCES IN NEOPHOBIA BETWEEN CANE TOADS FROM INTRODUCED AND NATIVE POPULATIONS

Abstract

For species moving into new environments, locating and using unfamiliar resources is crucial for survival. The cane toad (*Rhinella marina*) has been successful in many countries worldwide, persisting in both urban and rural landscapes. Given that animals exploiting urban habitats are confronted with novel feeding opportunities, individuals in those areas are expected to exhibit reduced neophobic tendencies compared to individuals in rural populations. Additionally, individuals persisting in introduced populations are expected to be less neophobic than individuals in native populations, taking advantage of novelty as their range expands. To investigate such predictions, we examined the response to novel prey and a novel object in native toads in Panama and introduced populations (urban and rural) in Florida. Toads were tested in an arena with novel or familiar prey and later with a novel object next to familiar prey. We found differences in response to novelty between cane toads in different ranges but not in different habitats. Most introduced individuals from both urban and rural habitats consumed novel prey with no difference in latency to eat between prey types. Few native toads, however, consumed any prey during trials, spending most of their time moving about the arena. When familiar prey was presented near a novel object, more than half of the
introduced toads ate, but no native individuals ate. This study emphasizes the importance of behavior as a mechanism used by invasive species to exploit novel resources and successfully colonize new environments.

Key Words: *Bufo marinus*, invasive, neophobic, novelty, marine toad, *Rhinella marina*

**Introduction**

Introduced species are increasingly common worldwide and recognized as threats to biodiversity and a source of economic concern (reviewed in Pimentel, Zuniga, and Morrison 2005). Although the exact reasons explaining a species’ success in novel environments are often difficult to determine or remain largely unknown, several hypotheses have been proposed about mechanisms promoting invasive ability (Lodge 1993). Release from selective pressures from predators, competitors, or diseases, for instance, increase survival within the introduced ranges of multiple taxa (e.g., Phillips, Brown, and Shine 2003; Torchin et al. 2003; Callaway and Ridenour 2004). Specific characteristics, such as recent disturbance, can make habitats more likely to be invaded by a given species (reviewed in Lodge 1993). In addition, particular life history traits including high fecundity and rapid developmental rates are considered critical characteristics that determine the outcome of an invasive species (e.g., Lodge 1993; Sakai et al. 2001; Rouget and Richardson 2003; Beard and Pitt 2005). Recently, behavioral traits have been recognized as important mechanisms for successful invasions in many taxa (e.g., birds: Sol, Lefebvre, and Timmermans 2002; mammals: Thorsen et al. 2000;

One behavioral trait receiving increased attention in species confronted with new environments is neophobia, or avoidance of novelty (Greenberg and Mettke-Hoffman 2001). Neophobia was traditionally considered one end of an approach-avoidance continuum conflict of possible responses to novelty with neophilia, exploratory interest in novelty, at the other end (Montgomery 1955). These behaviors, however, are now recognized to correspond to different motivations (Wood-Gush and Vestergaard 1993; Mettke-Hofmann et al. 2002) and personality axes (Gosling and John 1999). Neophilia and neophobia may also be linked to different genes (Powell et al. 2003; Weissstaub 2006). Because introduced species expand their range across new habitats, they are under strong selection to exploit novel resources (Sol and Lefebvre 2000). Reduced neophobia, along with neophilia, is expected to enable individuals to locate food and safe areas within their new range (Sol et al. 2011). Studies investigating neophobia in mammals and birds are consistent with such predictions. Introduced Norway rats (*Rattus norvegicus*), for instance, are less neophobic than individuals from native populations (Thorsen et al. 2000). Similarly, invading house sparrows (*Passer domesticus*) are less neophobic than individuals from resident populations (Martin and Fitzgerald 2005). Although multiple studies have examined neophobia in many species (e.g., rats, Barnett 1957; warblers, Greenberg 1983; sparrows, Greenberg 1992; convict cichlids, Brown et al. 2014), few studies directly compare native and invasive individuals of the same
species (Parker et al. 2013) or populations of individuals inhabiting different environments (Bókony et al. 2012).

Within the invasive range, different habitats are expected to impose different selective pressures. Urban and rural habitats, for example, pose varying challenges on individuals resulting in different successful strategies (Kark et al. 2007). One important reason for such differences may be availability of novel resources. Urban areas present more novelty and fewer vertebrate predators than rural areas (Faeth et al. 2005). Hence, urban individuals that frequently encounter novel food under lower predation risk are hypothesized to exhibit lower neophobia. Despite predictions that invasive and urban populations should respond better to novelty challenges, there is no agreement across the few species examined to date. Although invasive common mynas (Acridotheres tristis) in urban habitats are more exploratory than those in rural habitats (Sol et al. 2011), house sparrows from populations in differently urbanized areas exhibit no difference in latency to eat novel food (Bókony et al. 2012). In this study, we investigate neophobia in populations from different habitats in a widespread introduced species, the cane toad (Rhinella marina, formerly Bufo marinus), to investigate the role of behavior in the success of introduced species.

Cane toads are an ideal species to study behavioral changes associated with the invasion process (e.g., Child, Phillips, and Shine 2008). This species is one of the most widespread anuran species (Pitt, Vice, and Pitzler 2005) and has been introduced from its natural range in Central and South America to more than 20
countries worldwide (Lever 2001; Kraus 2009). While the history of cane toad introductions has been well-documented (reviewed in Lever 2001), little is known about introductions to areas other than Australia. The success of introduced cane toads has been attributed to many factors including their life history strategy (Freeland and Martin 1985; Van Bocxlaer et al. 2010) and ability to outcompete natives (Phillips and Shine 2004). The role of behavior in their success, however, has received little attention. Here we investigate the hypothesis that reduced neophobia promotes invasive ability allowing cane toads to better cope with novelty. We test the responses of cane toads to novel prey and a novel object in their native and introduced ranges. This species also inhabits both urban and rural habitats (Zug and Zug 1979). To examine the role of urbanization on cane toad behavior, we investigate neophobia in urban and rural populations within the introduced range. We predict that introduced individuals from urban and rural populations will be less neophobic than native individuals. Within the introduced range, urban individuals are expected to exhibit reduced neophobia compared to rural individuals since opportunities for novelty are reduced in rural environments. To our knowledge, this is the first study to investigate anuran neophobic tendencies and how they relate to invasive potential.

**Methods**

**Study Species**

Cane toads are large, terrestrial anurans native to the Neotropics. Their natural foraging behavior is well-documented (Zug and Zug 1979) and their diet has
been reported across their natural (Easteal 1981; Lever 2001) and invasive ranges (Zug, Lindgren, and Pippet 1975; González-Bernal 2011). Cane toads are generalists that consume a variety of insects in their native range, but also include small mammals and conspecifics in their invasive range (Easteal 1981; Pizzatto, Child, and Shine 2008). Anecdotal evidence suggests cane toads also exploit novel food sources such as trashcans and pet food bowls (Alexander 1964).

**Study Areas**

To investigate neophobia, juvenile cane toads were collected in populations from their introduced and native ranges. Following Zug and Zug (1979), toads under 9cm in length and with multi-coloration pattern were considered juveniles. Individuals were checked for nuptial pads to confirm they were not sexually active. Two populations in the introduced range and one population in the native range were sampled.

Twenty cane toads were collected each from an urban and rural population in their introduced range in Florida. Rural toads were collected near the Everglades Research and Education Center in Belle Glade (26°40'15"N, 80°38'17"W). This area is largely agricultural consisting mostly of sugar cane and rice fields. Urban toads were collected behind a shopping center in Wellington (26°40'57"N, 80°12'15"W). These two sampling populations are approximately 65 km apart and, therefore, are likely distinct populations (Zug, Lindgren, and Pippet 1975).

Introduced individuals were euthanized with an overdose of the anesthetic MS-222, tricaine methanesulfonate following the completion of experimental trials.
Toads were immersed in a solution of 0.50 g/L of MS-222 (0.05%) (Fellers, Drost, and Heyer 1994; Cecala, Price, and Dorcas 2007) and died without any struggle within 20-30 minutes.

In their native range, 22 cane toads were collected in Gamboa, Panama (9°7'0.46"N, 79°42'2.79"W) around the facilities of the Smithsonian Tropical Research Institute. Gamboa is a small town surrounded by mature forest of the Soberania National Park. All toads were found near buildings in town with the exception of a single individual found on a road. Following experimental trials, individuals were marked by toe-clipping to avoid retesting and released at the site of collection. The Guidelines for the Use of Live Amphibians and Reptiles in Field Research compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists’ League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR) were followed when toe-clipping toads. A maximum of two toes were clipped from each individual and no thumbs on the forefeet were clipped given their importance in amplexus.

Upon capture, all individuals from all populations were weighed using an Adam® HCB302 Highland 300g X 0.01g portable scale. Snout-vent length (SVL) was measured with an ROK 150mm digital caliper.

**Experimental Set-up**

Toads were housed individually in plastic containers (introduced: 38cm x 30.5cm x 15cm; native: 33cm x 40.5 cm x 16.5cm) covered with clear plastic. Each individual was given a blue water bowl and blue food bowl. During the first night of
captivity, each individual was given two mealworms (*Tenebrio molitor*) inside their home container. The following day, home containers were checked for presence of mealworms. Toads that did not eat overnight were given fresh mealworms. All toads ate both mealworms within three days and no toads were excluded from experiments. Toads were considered to be familiar with the mealworms after they ate both worms. Food was then withheld for three nights. Trials were run on the fourth night to mimic natural feeding behaviors in the wild (Zug and Zug 1979).

To investigate neophobia, each toad was individually placed in a test arena (91.5 cm x 61 cm x 61 cm; Fig. 1). A food bowl identical to that in the home tank was placed at one end of the arena. A red light was placed above the arena to simulate natural feeding conditions that usually occur under low light intensity (Zug and Zug 1979). A Sony HDR-UX20 camera set on night shot function was used to record trials. To eliminate subjective bias, an observer unaware of the population of the toads later scored the video recordings.

**Novel Prey**

Toads were presented with familiar prey, mealworms, or novel prey, yellow-colored waxworms (*Galleria mellonella*). Although waxworms have a broad distribution, they live exclusively in beehives (Paddock 1918), making them inaccessible to toads in the wild. Waxworms are also considered novel prey because they differ from mealworms in their general shape and locomotion pattern. Preliminary studies indicated that yellow-colored worms elicited stronger neophobic responses in toads (e.g., long latency to eat) than worms of other colors.
(e.g. pink). Although cane toad color vision has not been investigated, toads respond to blue and yellow visual components (Reuter and Virtanen 1976) and differentiate blue moving prey on grey backgrounds (Dietz 1972). The blue food bowls coupled with yellow waxworms likely increased conspicuousness of this novel prey to the toads.

At each population, half of the toads were presented with familiar prey first and the other half with novel prey first. The prey was placed in the bowl first. Then, the toad was placed into the center of the arena facing the food bowl, approximately 30cm away (Fig. 1a). Once the first prey item was eaten, the second was placed in the bowl and the toad was returned to its starting position. Videos were analyzed for time to prey detection, time spent moving, time spent along edges of the arena (15cm out from wall), escape attempts, latency to eat, and reaction to prey items, i.e. leaning in and examining prey versus striking from a distance. Latency to eat was measured as time from detection of prey to striking at or eating the prey. A toad was considered to have detected the prey when it looked toward the prey and oriented to it. Escape attempts were defined as a rapid jump or set of connected jumps toward the arena’s wall as the toad attempted to go above or beyond it.

Novel Object

To further test the hypothesis that introduced and urban individuals are less neophobic, toads were presented with familiar prey by a novel object following previous studies (e.g., Webster and Lefebvre 2001; Mettke-Hofmann, Winkler, and
Leisler 2002; Martin and Fitzgerald 2005). Feeding near unfamiliar objects is risky in many contexts and, therefore, is considered a bold behavior.

Toads were allowed to rest one night between the novel prey and novel object experiments. During this time, individuals were not handled or given food. Using the same arena as the novel prey experiments, a mealworm was placed in a food bowl directly next to a novel object (Fig. 1b). We used a blue ball (15cm) patterned with white flowers as a novel object following previous studies (Martin and Fitzgerald 2005; Lendvai, Bókony, and Chastel 2011). A blue object was chosen because, as mentioned above, toads respond to blue dummy prey on grey backgrounds (Dietz 1972). Videos were analyzed to measure latency to eat the mealworm, time to prey detection, time spent moving, time spent near the walls of the arena, time spent near the novel object (10cm out from ball), time spent touching the novel object, and escape attempts.

**Statistical Analyses**

A one-way ANOVA was performed to compare the size of the toads across populations. We used Fisher’s exact tests to compare the proportions of individuals that ate as well as the proportions of each type of prey eaten between populations. The fiducial level of significance, $\alpha$, was set at 0.025 to control for error rate in pairwise comparisons. A Mann-Whitney test was used to compare latency to eat the different prey between populations following previous neophobia studies (Greenberg 1992; Kelly and Marples 2004; Martin and Fitzgerald 2005). Finally, we performed independent analyses of variance (ANOVA) to examine how behavior
compared among populations (Zar 2010). Analyses were performed for time spent moving, in the center of the arena, near the novel object, and on the novel object. To examine whether movement in the arena was associated with trying to escape, we performed Pearson correlations between time spent moving and number of escape attempts.

**Results**

Juvenile cane toads from native and both introduced populations tested in this study were similar in size. There was no difference in the snout-vent length or weight of the toads from different populations (SVL: $F_{1,112}= 5.771, P=0.115$; Weight: $F_{1,112}=23.016, P=0.848$).

**Novel Prey**

Introduced cane toads were more likely to eat in the arena than native cane toads (Fisher’s exact test, $P= 0.021$; Fig. 2). Native individuals visually examined both prey items, but few consumed either prey. To examine whether this was due to lack of hunger, native individuals were given mealworms in their home container following testing. All individuals ate mealworms when presented in this context. Although there was no difference between urban and rural populations in the proportion of individuals that ate (Fisher’s exact test, $P=0.2$, $n_{rural}=20$, $n_{urban}=20$), both populations were significantly different than native toads (Fisher’s exact test, urban vs native: $P=0.04$; rural vs native: $P=0.00038$; $n_{native}=22$). Of the individuals that did not eat in the arena, 90.9% of native toads detected the prey while 32.5% of introduced individuals detected it. In addition, introduced toads did not
preferentially eat either prey item (Fisher’s exact test, rural: \( P=0.527, n_{\text{rural}}=11 \); urban: \( P=0.716, n_{\text{urban}}=17 \)). Neophobia is also expected to result in latency to eat. Consistent with our predictions, introduced toads showed no signs of neophobia to novel prey. Among introduced cane toads that consumed both prey items, mealworms were eaten no quicker than waxworms (Mann Whitney test, \( U=358, P=0.6; n_{\text{rural}}=11, n_{\text{urban}}=17 \); Fig. 2b). In contrast, the few native individuals that ate showed signs of neophobia, eating slower than introduced individuals (urban vs native: \( U=289, P=0.000032 \); rural vs native: \( U=671, P=0.0015 \)).

All populations differed in their movement in the arena (Fig. 2c). Individuals from the urban population spent less time in the center of the arena compared to rural individuals (\( F_{1,112}=31.395, P<0.001 \)). However, there was no difference in time spent moving between urban and rural toads (\( F_{1,45}=0.007, P=0.938 \)). The native population moved less (rural: \( F_{1,112}=109.77 \); urban: \( F_{1,112}=69.286 \)) and spent more time along the walls (rural: \( F_{1,112}=15.148 \); urban: \( F_{1,112}=10.521 \)) than the introduced populations (\( P<0.001 \) for all cases). Time spent moving was correlated to escape attempts (Pearson correlation; \( r=0.468, P=0.01 \)). Overall, the movement in the arena of toads from both introduced populations was more similar to one another (pairwise comparisons; Hotelling’s t-square=2.662, \( P=0.277 \)) than to the native population (urban vs native: Hotelling’s t-square=12.742, \( P=0.003 \); rural vs native: Hotelling’s t-square=6.998, \( P=0.036 \)).
**Novel Object**

There were differences in consumption of familiar prey by a novel object between native and introduced individuals (Fisher's exact test, $P<0.001$; Fig. 3a). While native individuals did not consume mealworms next to the novel object (0 out of 22 toads), introduced individuals did (urban: 12 out of 20 toads; rural: 10 out of 20 toads). There was, however, no difference in latency to consume mealworms next to the novel object among urban and rural toads (Mann Whitney test, $U=52$, $P=0.6277$; Fig. 3b). Of the individuals that did not eat, 32.5% of native individuals detected the prey while 9.4% of introduced individuals detected the prey.

Introduced populations differed in how individuals used their time and moved around the arena. Urban toads moved more than rural toads ($F_{1,45}=19.259$, $P<0.001$; Fig. 4a); however, rural toads spent significantly more time near the novel object ($F_{1,45}=10.309$, $P=0.002$; Fig. 4b) and in the center of the arena ($F_{1,45}=4.838$, $P=0.033$; Fig. 4a) than urban toads. Urban and rural individuals did not differ in time spent touching (on) the novel object ($F_{1,45}=0.004$, $P=0.949$; Fig. 4b).

Introduced and native populations also differed in their movement (Fig. 4). Toads from both introduced populations spent less of their time moving (urban vs native: $F_{1,45}=133.163$, $P<0.001$; rural vs native: $F_{1,45}=35.485$, $P<0.001$; Fig. 4a) and more time in the center of the arena than native individuals (urban vs native: $F_{1,45}=26.135$, $P<0.001$; rural vs native: $F_{1,45}=9.427$, $P=0.004$). Similar to the novel prey experiment, time spent moving during novel object trials was correlated to escape attempts (Pearson correlation: $r=0.427$, $P=0.021$). Individuals from native
and introduced populations did not differ in time spent on the object (urban vs native: $F_{1,45}=0.121, P=0.729$; rural vs native: $F_{1,45}=0.004, P=0.947$; Fig. 4b). Toads from native populations spent the same amount of time near the novel object as urban toads ($F_{1,45}=0.168, P=0.068$) but less time than rural toads ($F_{1,45}=14.854, P<0.001$; Fig. 4b).

**Discussion**

We found that, in a novel arena, introduced juvenile cane toads readily ate novel and familiar prey while native cane toads were less likely to eat either prey. Native individuals showed signs of increased neophobia and anxiety-like behaviors when confronted with a novel environment; they spent more time by the arena walls and were less likely to approach a novel object. These behaviors match those expected in habitats where encounters with unfamiliar resources are rare while predation risk is high (Greenberg and Mettke-Hoffmann, 2001; Sol et al. 2011). Previous studies in birds and mammals support the hypothesis that introduced individuals are less neophobic than native individuals (Thorsen et al. 2000; Sol, Lefebvre, and Timmermans 2002; Martin and Fitzgerald 2005; Sol et al. 2011). Accordingly, we found that cane toads from Florida have a higher propensity to eat novel prey than native toads from Panama. Such reduced aversion to novelty, perhaps coupled with neophilia, may have facilitated the establishment of invasive cane toad populations due to increased ability to cope with and exploit novelty.

The amount of movement and areas visited in the arena by native toads differed from introduced toads. Native toads spent most of their time by the walls,
away from the center of the arena. Equivalent behaviors are associated with high levels of anxiety in rodents (Bailey and Crawley 2009). The proportion of time moving differed between native and introduced individuals during novel prey and novel object experiments. While native toads spent less time moving than introduced toads in the novel prey experiment, they moved more than introduced toads during the novel object experiment. This change in movement, however, is due to the decreased movement and reduced escape attempts by introduced toads during the novel object experiment. Similar behavior in both experiments may reflect lack of habituation of native toads to the arena.

While not eating novel prey indicates neophobia, long latency to eat is also a sign of increased neophobia. Although cane toads from both introduced populations ate novel food and familiar food by the novel object, subtle differences in their response such as latency to eat could be expected. Contrary to expectations, we found no differences in latency to eat between urban and rural populations. It is possible that the level of novelty perceived by the toads was not enough to reveal subtle differences between introduce populations in their response to unfamiliar prey. In addition, urban toads showed more anxiety-like behaviors than rural toads, spending more time moving around the arena edges. These results contrast with previous studies (i.e., Echevarría and Vassallo 2008). In birds, the ability to exploit novel resources is recognized as an important trait favoring individuals and species in urban habitats (Kark et al. 2007; Møller 2009; Sol et al. 2011). Urban habitats provide more novelty challenges, confronting animals with human-derived
resources absent in their natural habitat. Individuals able to exploit novel resources are expected to succeed and become established (Webster and Lefebvre 2001; Liker, Bókony, and Brown 2009). Although urban birds are more likely to exploit new resources in some contexts, there are exceptions to this pattern. For example, house sparrows from habitats of varying levels of urbanization do not differ in their feeding behavior when confronted with novel food or novel feeding situations (Bókony et al. 2012). To our knowledge, no studies have examined the neophobic behavior of anuran species in urban and rural habitats. It is thus unclear whether lack of decreased neophobic behavior in urban cane toads is representative of the strategies in this taxonomic group.

In addition to habitat, experience may play a role in behavioral plasticity of neophobic responses (Kendal, Coe, and Laland 2005; Mettke-Hofmann et al. 2006; Brown et al. 2014). Differences in exploratory behavior and neophobic tendencies may be expected between juveniles and adults. For instance, juvenile chimango caracaras (Milvago chimango) are less neophobic toward novel objects than adults (Biondi, Bó, and Vassallo 2010). In contrast, adult vervet monkeys (Chlorocebus pygerythrus) are less neophobic because of a greater variety of experiences (Hauser 1988). How neophobia changes over development is likely affected by whether individuals have positive or negative experiences with novelty throughout their life. Juvenile cane toads may be more exploratory, or neophilic, and less neophobic than adults. Because cane toads disperse during the early stages of life (Zug and Zug 1979), juveniles must exploit novelty when moving across new habitats. Examining
neophobia over a broad age range in cane toads is necessary to determine the role of experience in such behaviors and whether differences between rural and urban populations are accentuated over ontogeny.

Overall, we found that introduced toads are less neophobic than native toads. In addition, bolder, potentially neophilic individuals that readily approach novel prey/objects are present in all cane toad populations studied here. Through increased exploration and decreased avoidance of novel prey, introduced cane toads are likely able to exploit novel resources. Successful establishment of introduced species relies on multiple factors (reviewed in Lodge 1993). Cane toads are thought to be successful for several reasons: they lay large numbers of eggs that develop quickly, eat a wide variety of prey, and possess toxins that release them from predation in some of their introduced ranges (Zug and Zug 1979). Recently, the role of behavior in successful invasive species has received increased attention (Sol and Lefebvre 2000; Sol, Lefebvre, and Timmermans 2002; Martin and Fitzgerald 2005; Kark et al. 2007). To our knowledge, this is the first study investigating neophobia in anurans and examining the role of such behaviors in invasive potential in this taxonomic group. In light of current worldwide population decline of anurans (Stuart et al. 2004), this work provides valuable insights of how behavior influences the fate of introduced anuran species.

**Figure Legends**

**Figure 2.1.** Diagram of experimental set-up used to investigate neophobia in cane toads. Individuals were released in the experimental arena at the middle (indicated
by the toad) and presented with novel prey (a) or a novel object by familiar prey (b). The arena was divided into different areas to quantify the behavior of the toads:

edge (dark gray area), center (white area), and, in the novel object experiments, near the object (striped area).

**Figure 2.2.** Behavior of cane toads in response to novel and familiar prey in individuals from native (black bars) and introduced populations (rural: light grey bars; urban: dark grey bars). a) Proportion of individuals from each population that ate prey items; b) latency to eat prey items (Mean±SE of individuals that chose to eat prey); and, c) proportion of time during each trial toads from each population spent moving and in the center of the arena. *=P<0.05; **=P<0.001; ***=P<0.0001.

**Figure 2.3.** Behavior of cane toads in response to familiar prey by a novel object in individuals from native (black bars) and introduced populations (rural: light grey bars; urban: dark grey bars). a) Proportion of individuals from each population that ate familiar prey when presented next to a novel object; and (b) latency to eat mealworms when presented next to a novel object (Mean±SE).

**Figure 2.4.** Behavior of cane toads in response to a novel object by familiar prey in individuals from native (black bars) and introduced populations (rural: light grey bars; urban: dark grey bars). (a) Proportion of time toads from each population spent moving and in the center of the arena during novel object trials; and (b)
proportion of time toads from each population spent near and on the novel object.

Mean ±SE are shown. * = P < 0.05; ** = P < 0.001; *** = P < 0.0001.
Figure 2.1. Neophobia Experimental Set-up
Figure 2.2. Novel Prey Experiment Results
Figure 2.3. Novel Object Experiment
Figure 2.4. Novel Object Experiment Movement Results
CHAPTER III

NEOPHILIA IN THE CANE TOAD *Rhinella marina*

Abstract

Anthropogenic-driven environmental change can occur rapidly and is increasingly common worldwide. Because of this, species are often forced to cope with novel situations and resources. Although there are several possible mechanisms for coping with such rapid change, dispersing to more suitable habitat or evolutionary responses are often not viable options. Individuals within a species may, however, possess behavioral flexibility that enables them to successfully respond to rapid environmental change. Before exploiting novel resources, an individual must first explore them. Neophilia, therefore, is a behavioral trait that may facilitate learning and exploitation of novelty in individuals. We examine neophilia in a species known to be highly invasive, the cane toad (*Rhinella marina*), in order to test the hypothesis that a species known to be capable of coping with many different habitats exhibits neophilic tendencies. To do this, individuals were placed in an arena and given a choice between novel and familiar prey items. Our results show that cane toads do not preferentially eat novel prey; however, toads exhibited increased latency to eat familiar prey items when compared to novel prey items. Although cane toads do not appear to be neophilic, this is the first study to examine neophilia in anurans, so it is unclear whether this strategy is widespread within this taxa. Understanding behavioral flexibility and its relation to response to
novelty, however, can provide insight into the invasive ability of a species and to the ability of individuals to cope with rapidly changing environments.

**Introduction**

Habitats worldwide are experiencing change due to anthropogenic influence. Through altered land-use patterns, humans are increasing levels of urbanization and decreasing natural habitat. Furthermore, urbanization is facilitating human-mediated species introductions, which threaten biodiversity and cause economic concern (reviewed in Pimentel, Zuniga, and Morrison 2005). Because of such influences, species across the globe, native and introduced alike, are often faced with unfamiliar situations and changing habitats. Anthropogenic-driven environmental change typically occurs rapidly, so evolutionary responses often are not an option. Within a species, however, individuals may possess behavioral flexibility that enables them to cope with such rapid change (Fragaszy and Mason 1978). A species, therefore, may harbor the ability needed to respond to rapid environmental change. Individuals able to alter their behavior to take advantage of novelty, for instance, are expected to survive in changing environments (Wright et al. 2010). Exploration is a precursor to innovation and, along with learning, enables individuals to develop new behaviors for exploiting the environment (Reader and Laland 2003; Russell 1983). For instance, worldwide invasive bird species with greater behavioral flexibility are more successful than species unable to innovate behaviors and exploit novel food items, indicating the importance of such flexibility in different habitats (Sol et al. 2005).
Because exploration and learning are critical to success in changing environments, multiple behavioral traits relating to boldness have been examined. Two behavioral traits that have received increasing attention are neophilia and neophobia (Greenberg and Mettke-Hofmann 2001). Neophilia, the “love” of novelty, describes the exploratory interest of individuals towards a new object or place in the environment (Greenberg 2003). Neophilic individuals are thus more likely to approach novel objects, which allows them to gather information and innovate behaviors for exploiting novel situations and opportunities (Lefebvre et al. 1997). At the other end of the spectrum is neophobia, or fear of novelty. Neophobic individuals are less likely to explore novel environments and situations (Greenberg 1992). Neophobia and neophilia thus represent the ends of a continuum of possible outcomes to an approach-avoidance conflict (Montgomery 1955). In new environments, this trade-off favors approach because individuals unable to overcome fear of novelty are unlikely to survive (Sol et al. 2011).

Neophilia has been studied in species that live in proximity to humans and are therefore likely to experience rapid environmental change. Juvenile common ravens (Corvus corax) living in Maine, for instance, are preferentially attracted to novel objects both when given a choice between novel and familiar items and when presented each separately (Heinrich 1995). Similarly, neophilic tendencies have been examined in introduced species. As these species expand their range across new habitats, they are under selection to exploit novelty and are thus expected to be less neophobic (Sol et al. 2011). For example, resident house sparrows (Passer
domesticus) are more hesitant to approach novel objects than actively invading individuals (Martin and Fitzgerald 2005). It is unclear, however, whether house sparrows from invading populations are preferentially attracted to novelty. In comparison with neophobia, the role of neophilia has received relatively little attention. In this study, we investigated neophilia in a highly invasive species that often lives in proximity to humans, the cane toad (Rhinella marina, formerly Bufo marinus). Cane toads are successful in disturbed habitats modified by human activity as well as forest (Lever 2001), suggesting they have an ability to cope with novel situations and changing environments. We hypothesize that cane toads, which are capable of coping with different types of habitat, exhibit neophilic tendencies. We predict that cane toads will be likely to explore novel prey items. To our knowledge, this is the first study examining neophilia in anurans and contributes to increase much-needed knowledge about the behavior of this highly successful species (Burghardt 2003).

**Methods**

Sixteen adult male cane toads were collected in Gamboa, Panama (9°7'0.46"N, 79°42'2.79"W), around the facilities of the Smithsonian Tropical Research Institute. Gamboa is a small town surrounded by mature forest as part of the Soberania National Park. All toads were found near buildings in town. Collection occurred during the evening (1900-2300 hrs) and only males that were not actively mating or calling were collected. Upon capture, toads were housed in individual plastic containers lined with wet paper towel for moisture. Each container was supplied with a water
bowl. Two mealworms (*Tenebrio molitor*) were given to each toad the night of capture. After eating both mealworms and completing a prior experiment in which they were fed, individuals were housed for four days without food. On the fourth night, each toad was tested individually for neophilic tendencies. This schedule follows natural feeding behavior as cane toads eat every third to fourth night in the wild (Zug and Zug 1979).

Individuals were marked by toe-clipping to avoid retesting and released at the site of collection following the experiment. The Guidelines for the Use of Live Amphibians and Reptiles in Field Research compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists’ League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR) were followed when toe-clipping toads. No more than two toes were clipped on any individual and no thumbs on the forefoot were clipped due to their importance in amplexus.

**Experimental Set-up**

Each toad was placed at the beginning of a runway-style arena (122 X 15 X 46 cm; Fig. 1). Two small, plastic dishes were placed side by side at the opposite end of the arena. One dish contained a mealworm and the other contained a yellow powder-colored waxworm. Because individuals had been fed mealworms previously, these were considered a familiar prey item. Colored waxworms were used as the novel prey item. Waxworms are larvae of the greater wax moth (*Galleria mellonella*) and live exclusively in beehives. Thus, despite their wide distribution (Paddock 1918), waxworms would not have been encountered by toads prior to
experiments. Waxworms also differ from mealworms in their general shape and locomotion pattern. Yellow nontoxic, waterproof powder used for coloring fishing bait was chosen to add another layer of novelty to the waxworms (Candler and Bernal, unpublished data). Preliminary experiments showed that cane toads respond more strongly (i.e. leaning in and examining prey items) to yellow-colored mealworms compared to other colors such as pink. Although cane toad color vision has not been investigated in detail, toads are known to respond to blue and yellow visual components (Reuter and Virtanen 1976).

All trials were video-recorded using a Sony HDR-UX20 camera set on night shot function. We measured the time to hop down the runway to the feeding bowls and time spent looking at individual worms before consumption. The worm that was examined first was noted as well as which one was eaten first.

Data Analysis

Time to begin exploring was analyzed using a zero correlation test. Variables tested included time to begin exploring, whether or not the individual ate, type of prey eaten first, and latency to eat. Two-sided binomial exact tests were performed in order to determine if the proportion of individuals eating or examining a particular prey item first was significantly greater than by chance (probability of success=0.5). The latency to eat after the prey item was detected for those individuals that consumed prey were analyzed using a Mann-Whitney U test.
Results and Discussion

Of the 16 toads tested, half ate at least one prey item (3 ate one, 5 ate both). In addition, 12 examined at least one of the worms during experimental trials. Individuals were no more likely to examine either worm first than would be due to chance (binomial probability, n=12, p=0.774, power=0.109). Similarly, individuals that ate were no more likely to eat a particular worm first (binomial probability, n=8, p=0.726, power=0.298, Fig. 2a). The type of worm examined first had no relationship to the type of worm eaten first (binomial probability, n=8, p=0.726).

Time to begin exploring also did not correlate to the type of prey eat first (correlation coefficient=-0.123, p=0.651) or latency to eat (mealworm: correlation coefficient=-0.141, p=0.602; waxworm: correlation coefficient=0.064, p=0.813).

Although choice of prey eaten first was not different from random, there was a significant difference in the latency of individuals to eat mealworms versus waxworms (Mann-Whitney test, U=-2.197; n=8, p=0.028; Fig. 2c). Toads ate waxworms faster than mealworms, suggesting they were more attracted to the novel prey. This readiness to consume unfamiliar prey suggests that individuals eating novel prey were not hesitating as would be expected in neophobic individuals (Greenberg and Mettke-Hofmann 2001). Although this result suggests that cane toads exhibit neophilic tendencies, toads had no statistical preference for eating novel over familiar prey. Lack of preference for a particular prey item may be due to the inability of toads to detect differences between mealworms and waxworms. Latencies to eat each prey item indicate, however, that individuals do detect
differences. While it is possible that the colored waxworms are more conspicuous than mealworms and thus eaten faster, individuals were equally likely to first examine either prey item, suggesting potential differences in conspicuousness are not determining their choice. In agreement with this conclusion, preliminary data from laboratory studies using cane toads showed that prey coloration does not affect time to eat (Candler and Bernal, unpublished data). When toads were presented with prey of different colors (e.g., pink, blue, white), trials resulted in similar latencies across colors (pink: n=11, mean=16.74 +/- 4.92s; blue: n=12, mean=14.22 +/- 6.04s; white: n=13, mean=15.94 +/- 3.55s). This suggests that novelty, rather than conspicuousness, is the cause for different latencies to eat novel and familiar prey items in this study.

Our results indicate that native cane toads are neither neophilic nor neophobic. Instead, their behavior lies between the two ends of the continuum of potential strategies that result from an approach-avoidance conflict (Montgomery 1955). When faced with the choice of avoiding or exploiting novelty, cane toads at their native range are equally likely to eat familiar or novel prey. This suggests individuals have no preference for novelty. Although it is possible that there are consistent differences in neophilic tendencies among individuals, in this experiment individuals were tested a single time, so we cannot determine if such variation exists. Future studies examining consistent differences among individuals would provide valuable insights about whether individuals eating waxworms first are choosing at random or preferentially eating novel prey first. If individuals
consistently opt to eat novel prey first, neophilic individuals are present in native cane toad populations and could contribute to the success of cane toads in their invasive range (reviewed in Sih et al. 2010). Examining whether a larger proportion of individuals from the invasive range exhibit neophilic tendencies would further support this prediction. Neophilic individuals approaching novelty will have more opportunities to learn how to exploit different resources and, therefore, be more successful than less-neophilic individuals in a new range (Sol and Lefebvre 2000).

Overall our results indicate that native cane toads are not preferentially attracted to novel prey items; however, individuals eat novel prey faster than familiar prey. So, are cane toads neophilic? According to a power analysis, the current sample size is too small to detect an effect (power=0.298). This analysis indicates that a sample size of 62 toads would be necessary to achieve a power of 0.8. In their native range and in particular in Gamboa, where this study was performed, achieving such a sample size is unlikely since cane toads are not abundant. Although they expand quickly and reach high densities in most of their invasive ranges (Lever 2001), densities are much lower in their native range (Zug and Zug 1979). The need for such high sample size, however, suggests the effect would unlikely be biologically relevant.

Success in a novel environment can be in part due to the ability to gather information about the environment and adopt new behaviors (Greenberg and Mettke-Hofmann 2001). Thus, understanding behavioral flexibility could provide insight into the invasive capabilities of species. For example, bird species able to
innovate behaviors through increased ability to learn are more successful as invasive species than species less able to innovate (Sol et al. 2005). Although several studies have demonstrated neophilia in multiple bird and mammal species (e.g., domestic dogs, Kaulfuß and Mills 2008; baboons, Bergman and Kitchen 2009; ravens, Heinrich 1995; warblers, Mettke-Hofmann et al. 2009), this is the first study to examine neophilia in an anuran species. In light of worldwide decline of amphibians (Stuart et al. 2004), understanding the success of invasive species could improve conservation of native biodiversity. In addition, increasing our knowledge on the response to novelty in general could allow for better understanding of how native species cope with rapid human-induced environmental change.

**Figure Legends**

**Figure 1.** Schematic representation of the experimental set-up used to investigate neophilia in cane toads. Individuals were released in the experimental arena at one end (indicated by the toad) and presented with novel prey and familiar prey at the other end of the runway. The break indicates that the arena is not to scale in length.

**Figure 2.** Behavior of cane toads in response to novel (light gray bars) and familiar (black bars) prey. a) Number of individuals examining novel or familiar prey first; b) number of individuals eating novel or familiar prey first; and c) latency to consume prey items (Mean±SE); *=P<0.05.
Figure 3.1. Neophilia Experimental Set-up
Figure 3.2. Response to Prey
CHAPTER IV

BEHAVIORAL RESPONSES OF PREDATORS TO FIREFLIES: DO COURTSHIP SIGNALS ALSO FUNCTION AS APOSEMATIC SIGNALS?

Abstract

Aposematic signals are often multimodal and chemicals are typically paired with bright coloration or odors. Recently, Neotropical fireflies have been shown to contain many chemical compounds in their headspace, including pyrazines, which are toxic and considered to be noxious in odor and flavor. Because of this, flashing used in courtship signals in fireflies are hypothesized to also serve as an aposematic signal. Despite the longevity and popularity of this hypothesis, fireflies remain understudied, especially in the Neotropics, where they reach their highest abundance and diversity. In this study, we examine the responses of three common nocturnal predators, two species of insectivorous bats (*Myotis nigricans* and *Molossus molossus*) and the cane toad (*Rhinella marina*), to two species of Neotropical fireflies (*Photuris trivittatta* and *Bicellonycha amoena*). Predators were presented mealworms coated with pyrazine as well as live fireflies to determine how different predators respond to multiple signals. In addition, testing pyrazine separately provided insight into its role in aversive predator responses. We found that cane toads consumed all prey items with no differences in latency and no aversive behaviors. Although both bat species also consumed prey items with similar latency, fireflies were rejected in most cases. Our results suggest that
although pyrazine is not a deterrent for bats, fireflies themselves are unprofitable to bats but not cane toads. This study provides insight into the aposematic function of flashing in Neotropical fireflies and the evolutionary history between these prey and predator species.

**Introduction**

Prey species are under strong selection to reduce their attractiveness to predators (Siddall and Marples 2011). While palatable prey have adaptations to avoid detection or implement morphological defenses to deter a predator, unpalatable species often use different strategies. Aposematism, for instance, is present in a variety of toxic and unpalatable taxa. Aposematic signals are often multimodal, with coloration accompanied by odors, sounds, or aversive secretions (Rowe and Halpin 2013). Combining signals from different sensory modalities is expected to increase the likelihood of learned avoidance behavior by predators (Gittleman and Harvey 1980) and increase the longevity of such learned behaviors (Roper and Redston 1987). For example, domestic chicks (*Gallus gallus domesticus*) that learn to associate the color yellow with pyrazine exhibit an aversion that persists even after repeated exposure to palatable yellow food (Rowe and Guilford 1999a, 1999b). Multimodal signals, therefore, are often able to elicit stronger responses than solitary signals.

Fireflies (Coleoptera: Lampyridae) are well known for their use of photic emissions during courtship displays. It has been hypothesized that this flashing also serves as an aposematic signal (Lloyd 1973), and several studies have examined the
function of these signals (Eisner et al. 1977; Meinwald et al. 1979; Moosman et al. 2009; Long et al. 2012). European toads (Bufo bufo), for instance, are reluctant to eat glowing firefly larvae (DeCock and Matthysen 2003), suggesting that photic emissions serve as a warning to these predators. Circumstantial evidence is also consistent with this aposematic hypothesis. When disturbed, Neotropical fireflies flash and produce glandular secretions (Fred Vencl, personal communication). Such behaviors are similar to temperate species, which flash even when disturbed during daylight (McDermott 1964; Long et al. 2012). Furthermore, Photuris trivittata and many other firefly species have chemical compounds that make them unpalatable to predators (Eisner et al. 1978; Underwood et al. 1997; DeCock and Matthysen 2001). For example, mealworms treated with homogenized Photinus pyralis were rejected by four species of insectivorous bats (Moosman et al. 2009). Although several studies provide suggestive evidence supporting the aposematic role of flashing in fireflies, little is known about adult firefly defenses, especially in the tropics, among a suite of predators.

Recently, pyrazines have been found in Neotropical fireflies (Vencl, unpublished data). Pyrazines are volatile compounds that have a bitter taste and pungent odor, which can have an olfactory threshold as low as 0.002 ppb (Kaye et al. 1989). These compounds are present in a wide array of animal and plant taxa (Rothschild et al. 1984) and are thought to function as a ubiquitous warning (Moore et al. 1990). Many species learn avoidance behaviors when their food contains pyrazines (i.e. chickens, Guilford et al. 1987; laboratory rats, Kaye et al. 1989; wild
robins, Siddall and Marples 2011), which could influence the evolution of firefly flashing behavior (Lewis and Cratsley 2008). Anecdotal evidence suggests that while many taxa avoid flashing fireflies, some are attracted to it (reviewed in Lloyd 1973). Few studies, however, have examined the chemical defenses of Neotropical fireflies and how these defenses alter palatability of the fireflies to predators (Lewis and Cratsley 2008). In this study, we examine the feeding behavior of three common predators of Neotropical firefly species, cane toads and two species of insectivorous bats.

The cane toad (Rhinella marina, formerly Bufo marinus) is a voracious generalist predator; however, its diet consists mostly of ants and beetles (Zug and Zug 1979). Anecdotal evidence suggests there is variation in the responses of several species of frogs and toads to adult fireflies (reviewed in Lloyd 1973); however, there is no apparent pattern to whether or not anuran species will consume adult fireflies. Although some species like the European toad (Bufo bufo) consume fireflies, other species, such as the gray treefrog (Hyla versicolor), refuse to eat them (Lloyd 1973). As with cane toads, insectivorous bats typically encounter fireflies. We investigated the response to Neotropical fireflies by two common insectivorous bat species, Myotis nigricans and Molossus molossus. Although My. nigricans feeds mostly on moths and no beetles have been found in stomach contents of this species (Wilson 1971; Wilson and LaVal 1974), Mo. molossus feeds mostly on beetles. Although the reported prey for these bat species is within the size range of the firefly species examined here, no Lampyridae have been found in
stomach content analyses or fecal analyses (Howell and Burch 1973; Freeman 1979).

This study investigates the behavioral responses of three nocturnal predators to two species of Neotropical fireflies. In particular, we sought to examine the differences in responses of these predators to prey items coated in defensive chemicals produced by fireflies as well as live fireflies to examine how different predators respond to multiple signals. The volatile pyrazine was investigated separately to determine its role in aversive behavior. We tested the hypothesis that both pyrazine found in the headspace of adult Neotropical fireflies and the fireflies themselves deter some species of common nocturnal predators. Individuals from both species of bats were expected to be deterred by both fireflies and pyrazine. In contrast, we did not expect cane toads to be deterred by either. Because predators and prey alter their behaviors in response to one another, understanding the reactions toward the different components of firefly signaling by predators could provide insight into the function and evolutionary history of such signals.

**Methods**

**Cane Toads**

Cane toads were collected around buildings near the facilities of the Smithsonian Tropical Research Institute in Gamboa, Panama (9°7'0.46"N, 79°42'2.79"W). Gamboa is a small town surrounded by mature forest of the Soberania National Park. All individuals were collected during the evening (1900-2300 hrs). Sixteen adult male cane toads were collected. Adult male cane toads were
identified as such by being greater than 9 cm in length, with solid coloration, and prominent nuptial pads on the thumbs of the forelimbs (Zug and Zug 1979). No adults were actively mating or calling when captured. In addition, 22 juvenile cane toads were collected. Juveniles have a multi-coloration pattern and are less than 9 cm in length (Zug and Zug 1979). Upon capture, toads were housed in individual plastic containers lined with a wet paper towel for moisture.

Individual male cane toads were placed in a plastic arena (33 cm x 40.5 cm x 16.5 cm) covered with mosquito netting to prevent escape and allow video recording. Experiments consisted of four trials in which either mealworms or fireflies were dropped into the arena containing the cane toad. Individuals were given: 1) a mealworm, 2) a mealworm coated in mineral oil, 3) a mealworm coated in 0.9 ng/μl pyrazine in mineral oil, and 4) one of two species of fireflies (Photuris trivattatta or Bicellonycha amoena). Order of trials was kept constant except trials two and three, which were alternated with each experiment. At the end of the experiment, a mealworm was presented to ensure the individual was motivated to eat. Individuals that did not eat were excluded from analysis. Trials were recorded using a Sony® HDR-UX20 camcorder. Videos were analyzed for latency to eat, total trial time, and aversive behaviors during trials.

All juvenile cane toads were given a single Bicellonycha firefly due to their smaller size. Given that juveniles refused to eat in the testing arena, fireflies were presented in the individual’s home tank. The following day, individuals were checked for consumption of the firefly. Each individual was given a mealworm the
following evening to check for motivation to eat. Data on presence or absence of
prey items the following morning was recorded.

Following experimental trials, all toads were marked by toe-clipping to avoid
retesting and released at the collection site. The Guidelines for the Use of Live
Amphibians and Reptiles in Field Research compiled by the American Society of
Ichthyologists and Herpetologists (ASIH), the Herpetologists’ League (HL), and the
Society for the Study of Amphibians and Reptiles (SSAR) were followed when toe-
clipping toads. A maximum of two toes were clipped from each individual. No
thumbs on the forefeet were clipped given their importance in amplexus.

**Bats**

Male and female bats of both species were captured in mist nets as they
exited their roosts in Gamboa, Panama. Collection occurred during the evening
(18:15-20:30 hrs). Bats were kept separately in cloth bags following capture.
Individuals were acclimated to captivity for a minimum of 15 minutes prior to
experimental trials. Acclimation included being handfed three to seven mealworms.
Any individuals that did not eat during this period were excluded from experiments.
Trials began no sooner than one hour after feeding to ensure motivation. All trials
were recorded using a Sony® Handycam™ DCR-SR45 digital camcorder in Nightshot
setting illuminated with a 25 W red lightbulb and two Wisecomm® IR045 Infrared
LED lights.

Experiments consisted of four successive trials, in which bats were offered
mealworms or fireflies using forceps, and the bat’s behavioral response was
measured. The same four trials were performed with bats as were performed with cane toads. Bats were allowed 30 seconds to bite the mealworm or firefly offered before the trial was ended and scored as a rejection. If a bat bit the offered object within 30 seconds it was allowed to continue eating and the amount eaten was recorded. Trials were analyzed for latency to bite, time spent chewing, total time of trial, percent of time spent eating, and number of shakes using the editing program Video Edit Master. Percent of time spent eating was calculated as the time spent chewing divided by the total trial time. Less time spent chewing represents rejection by individuals. Shakes also represent rejection from individuals shaking their head when presented with a prey item. All bats were released on the same night that they were captured.

**Data Analysis**

A Fisher’s Exact Test was used to compare the proportions of juvenile cane toads that ate fireflies and mealworms. We performed repeated measures general linear models (GLM) to compare differences of variables across trials for the remaining experiments (adult cane toads and insectivorous bats). Differences between individuals were compared for toads and bats. Because two species of bats were used, species was included as an additional independent variable for bat analyses. Latency to eat was the only variable analyzed for adult toads because no aversive behaviors were noted during experimental trials. Variables measured for bats were: latency to eat, total trial time, time spent chewing, percentage of time spent eating, number of shakes, and amount of prey eaten.
Results

Toad Trials

All pairwise comparisons of trials within adult toads showed no difference in the latency to consume prey items (all p>0.071). There was, however, a trend toward significance in all trials when compared to the final mealworm (all p<0.093) due to longer latencies when eating the mealworm, which is suggestive of satiation of individuals at the end of experimental trials. The remaining trials, in contrast, were highly insignificant compared to one another (all p>0.610). There were also no differences in latency to consume prey items between individuals for all trials (F_{1,8}=0.235, p=0.641; Fig. 1a). In addition, there was no interaction between individual and latency (F_{4,32}=0.269, p=0.896).

As mentioned in the methods, juvenile toads did not respond to any prey when placed in the test arena. Individuals continually jumped at the mesh covering of the arena and attempted to climb the walls. These behaviors suggest anxiety rather than lack of hunger or motivation. All juveniles, however, readily consumed fireflies and mealworms when placed in their home container (Fisher’s Exact Test: N=22, p=1.0).

Bat Trials

We found no significant differences in the latency of bats to eat prey items (trial: F_{4,52}=0.326, p=0.859; species: F_{4,52}=1.022, p=0.404; individual: F_{4,52}=0.540, p=0.707; Fig. 1b). Time spent chewing, however, differed among trials (F_{4,52}=5.314, p=0.001) and among species (F_{4,52}=5.065, p=0.002; Fig. 2a). Pairwise comparisons
across trials reveal that the first uncoated mealworm was chewed for longer (70.72 +/- 11.47 s) than the second uncoated mealworm (25.73 +/- 5.19 s; p=0.003), suggesting satiation as individuals ate less during the final trial. Oil-coated mealworms were chewed longer (85.71 +/- 15.38 s) than pyrazine-coated mealworms (59.66 +/- 11.53 s; p=0.043), fireflies (2.60 +/- 2.33 s; p=0.035), and the second uncoated mealworm (p= 0.001). This again suggests satiation of individuals, as earlier trials resulted in more time spent chewing prey items. Fireflies were also chewed for less time than the second uncoated mealworm (p=0.006). Less time spent chewing the fireflies represents rejection, as most individuals did not eat when presented with a firefly. Species differences occurred in time spent chewing the first uncoated mealworm (F_{4,52}=5.823, p=0.031) as well as the mealworm coated with oil (F_{4,52}=6.461, p=0.025). *My. nigricans* chewed the first uncoated mealworm longer (107.5 +/- 11.38 s) than *Mo. molossus* (42.13 +/- 11.39 s). *My. nigricans* also chewed the oil-coated mealworm longer (143.5 +/- 13.92 s) than *Mo. molossus* (40.78 +/- 9.94 s).

No differences were found in the total trial time in individual bats across trials (F_{4,52}=0.879, p=0.482). There were also no differences between species (F_{4,52}=1.440, p=0.234) or among trial (F_{4,52}=2.129, p=0.090); however, pairwise comparisons among trials showed that individuals had longer trials when presented oil-coated mealworms (100.21 +/- 20.43 s) than when presented fireflies (6.75 +/- 4.01 s, p=0.018) and the final mealworm (47.78 +/- 8.63 s, p=0.002). This can be explained by the increased time spent chewing in these trials by individuals. In
addition, the total trial times during the oil-coated mealworm trial differed among species (F\textsubscript{4,52}=7.149, p=0.019). This difference between species is because *My. nigricans* had longer trials (176.5 ± 22.08 s) than *Mo. molossus* (40.86 ± 10.01 s), due to the increased time *My. nigricans* spent chewing in these trials.

Because of the differences in time spent chewing and total trial time, there were differences in the percent of time spent eating across trials (Fig. 2b). Pairwise comparisons among trials show that the percent of time spent eating was significantly shorter when eating fireflies (6.76 ± 6.24 s) than all other prey (first uncoated mealworm: 86.83 ± 4.88 s, p=0.015; pyrazine-coated mealworm: 84.02 ± 6.51 s, p=0.002; oil-coated mealworm: 92.79 ± 2.87 s, p=0.002; final mealworm: 70.50 ± 10.22 s, p=0.008). This is because most individuals rejected fireflies and spent little time chewing them during trials. We also found a difference between species in the percent of time the bats spent eating during the trials (F\textsubscript{4,52}=6.256, p<0.001). Bats from the two species differed in the percent of time they spent eating during the pyrazine trial (F\textsubscript{4,52}=9.471, p=0.009), with *Mo. molossus* spending more time (96.71 ± 5.599%) than *My. nigricans* (67.70 ± 12.55%).

The number of shakes differed among trials (F\textsubscript{4,52}=9.406, p<0.001), individuals (F\textsubscript{4,52}=3.435, p=0.014), and species (F\textsubscript{4,52}=6.461, p<0.001; Fig. 3). Shakes represent rejection of the prey, as individual bats shake their head either to avoid eating or in aversion as the researchers presented prey with forceps in front of them. There was a difference in the number of shakes between individuals during the firefly trial (F\textsubscript{4,52}=5.630, p=0.034), with some individuals shaking their head as
many as 24 times whereas others did not shake their heads. Pairwise comparisons of trials reveal that the number of shakes was significantly higher when fireflies where presented (6.25 +/- 2.10) compared to the other prey (first uncoated mealworm: 1.06 +/- 0.64, p=0.003; pyrazine-coated mealworm: 2.31 +/- 1.03, p=0.001; oil-coated mealworm: 0.81 +/- 0.41, p=0.001; final mealworm: 1.31 +/- 0.57, p=0.004). Increased rejection of fireflies explains the decreased time spent chewing, shorter trial times, and smaller percentage of time spent eating in individuals from both bat species. Species also differed in the number of shakes during firefly trials (F_{4,52}=9.219, p=0.010). *Mo. molossus* had more rejections (9.22 +/- 3.38 shakes) than *My. nigricans* (2.43 +/- 1.11 shakes).

The amount of prey consumed by the bats differed among trials (F_{4,52}=9.438, p<0.000) and between species across trials (F_{4,52}=3.891, p=0.008; Fig. 4). Pairwise comparisons among trials show that less of the firefly was eaten (6.3 +/- 6.3%) than all other prey (first uncoated mealworm: 97.8 +/- 1.64%, p=0.009; pyrazine: 87.5 +/- 7.57%, p=0.009; oil-coated mealworm: 99.4 +/- 0.63%, p=0.003; second uncoated: 58.1 +/- 11.64%, p<0.001). The results are similar to the time spent chewing because these two variables are directly related: less time spent chewing the prey resulted in less of the prey being eaten. In addition, the percent of second uncoated mealworm eaten was less compared to the first uncoated mealworm (p=0.012) and the oil-coated mealworm (p=0.019). Less of the second uncoated mealworm being eaten is again suggestive that individuals were becoming satiated as they neared the end of experimental trials. Species differed in the amount of prey
eaten during the second uncoated mealworm trial ($F_{4,52}=8.796$, $p=0.011$). This is because *Mo. molossus* ate more of the mealworm (90 +/- 10%) than did *My. nigricans* (17.14 +/- 10.23%), which may be due to the larger size of *Mo. molossus*.

**Discussion**

Our results show that adult cane toads eat both mealworms coated in pyrazine and live fireflies with the same latency. As expected, there is no difference in how adult toads respond to any prey type during all trials of the experiment. Adults readily consumed prey items and showed no signs of aversion to pyrazine or fireflies. In addition, all juvenile cane toads consumed fireflies in their home container. However, juveniles would not respond in the test arena and further details about their latency to eat and behavioral response to prey items cannot be determined without further study. Cane toads are unlikely to encounter large numbers of fireflies throughout their lives. Individuals forage on the ground and are often found in proximity to humans (Zug and Zug 1979). The fireflies examined in this study, however, are more abundant near the edges of forests and in open fields. The lack of rejection of fireflies by cane toads likely reflects rare encounters in nature. This suggests that either fireflies are not unprofitable to cane toads or that cane toads find fireflies distasteful but require repeated exposure to learn avoidance.

Toads have been shown to learn more slowly than other taxa (e.g., Williams 1967), requiring multiple exposures in order to learn (e.g., Van Bergeijk 1967; Schmajuk et al. 1980). For instance, only 48% of European toads (*Bufo bufo*) learned
avoidance of firefly larvae after 12 repeated exposures, with those that did not learn increasing their likelihood of consuming larvae (DeCock and Matthysen 2003).

Because our study did not examine learning and instead focused on the responses of predators based on their experience in nature, individual toads were only tested once. No individual exhibited any sign of distaste or aversion to adult fireflies during trials. If, however, cane toads are deterred by the taste of fireflies, it either takes many encounters to learn or the signal is not remembered for long periods of time.

Cane toads are voracious sit-and-wait predators; therefore, the cost of consuming prey items is typically low (Schoener 1971). Given this low input of energy required to acquire food as well as their broad diet, fewer prey are less likely to be unprofitable to cane toads than to more specialized, actively hunting predators. A preliminary taste aversion study found that adult female cane toads eat mealworms coated in up to 100% pure clove extract and 100% pure almond extract (Candler and Bernal, unpublished data). Concentrations of 25% of either extract caused a burning sensation on the mouth of the experimenter; however, cane toads showed no sign of distaste or increased latency to eat coated mealworms. These findings, along with the results of this study, suggest that cane toads have a wide tolerance of prey items. A broad tolerance for food items and a low propensity to learn combined with rare encounters with fireflies, suggest it is unlikely that cane toads have a strong effect on the evolution of firefly signaling.

In contrast to cane toads, the species of bats examined in this study avoided eating fireflies. Although there were no differences in latencies of both bat species to
eat the different prey items in this experiment, they differed in other behaviors that suggested aversion. *My. nigricans* individuals spent more time chewing in some trials than *Mo. molossus* individuals, which contributed to longer trials in the first compared to the latter species. These differences are likely due to the smaller size of *My. nigricans* (Wilson and LaVal 1974; Tamsitt and Valdivieso 1966), which probably needs more time to eat prey the size of an adult firefly. These bat species also differed in the number of shakes when presented with fireflies. *Mo. molossus* had more rejections of fireflies than *My. nigricans*, suggesting that the first species is more sensitive to fireflies than the latter species. Bats from both species ate less when presented with fireflies than with any other prey. Individuals also rejected fireflies more often than any other prey. These increased rejections combined with small amounts of fireflies eaten is consistent with the pattern seen in percent of time spent eating during trials, in which the bats spent the least amount of their time eating.

The species of bats examined in this study forage near forest edges and in open fields (Siemers et al. 2001; Mora et al. 2004), where the fireflies studied here are abundant. Unlike cane toads, bats are thus more likely to have encountered fireflies throughout their lives. This increased association may have promoted learned avoidance of fireflies or selected for innate aversion to fireflies. In addition, the cost of handling and consuming prey items is likely higher in bats than cane toads. Bats must actively hunt their prey before eating whereas cane toads capture prey as it passes. Investing energy in an unprofitable prey item is therefore often
more costly for bats than cane toads (Schoener 1971). Because of this, it is likely that selection has favored the ability of bats to avoid distasteful or harmful prey. In accordance, bats learn foraging tasks faster than amphibians. For example, Clarin et al. (2013) found that insectivorous bats (Myotis spp.) learned simple foraging tasks and successfully completed 8 of 10 trials within two days. Our results suggest that the species of firefly used in this study are unpalatable to My. nigricans and Mo. molossus. Such an aversion could probably be learned quickly; however, because these species of bats have a long evolutionary history together, innate aversion could have evolved. Studies that further investigate the mechanism underlying aversion of fireflies are needed to determine whether these behaviors are innate or learned.

In this experiment, decoupling pyrazine from the fireflies resulted in different responses from two common bat predators. Individuals of both species ate mealworms dipped in pyrazine but they did not eat live fireflies. This suggests that there are other chemical compounds present in fireflies that are influencing the response of bats but not the response of cane toads. It is known that fireflies contain a variety of chemical compounds in their headspace (Meinwald et al. 1979) in addition to pyrazine (Vencl, unpublished data). Although pyrazine is considered a ubiquitous warning compound (Moore et al. 1990), our results suggest there are additional compounds or an interaction between compounds that cause the rejection of fireflies by these two bat species. Studies including additional
compounds present in these fireflies are required to examine the role of additional compounds and their interactions on bat aversion.

To conclude, we found different responses in three common predator species to two species of Neotropical fireflies. Although fireflies themselves do not appear to warn predators directly of the presence of pyrazine alone, they seem to indicate unpalatability to the bat species examined here. This study suggests that signaling may function as an aposematic signal in Neotropical fireflies. However, it is still unknown whether *My. nigricans* and *Mo. molossus* associate flashing with unpalatability in the firefly species studied. Few studies to date have examined Neotropical fireflies despite their high abundance and diversity (Grimaldi and Engel 2005). Understanding the behavioral response of important predator species can provide insight into the evolution of flashing behavior in this group of fireflies as well as the evolutionary history between predator and prey species.

**Figure Legends**

**Figure 4.1.** Latency to eat different prey items by predators examined in this study.

a) Latency of cane toads to eat prey items. b) Latency of two bat species (black: *Myotis nigricans*, light gray: *Molossus molossus*) to eat prey items. Mean ± SE.

**Figure 4.2.** a) Time spent chewing individual prey items in *Myotis nigricans* (black) and *Molossus molossus* (light gray). b) Percent of time spent eating individual prey items in *Myotis nigricans* (black) and *Molossus molossus* (light gray). Mean ± SE; *=P<0.05.*
**Figure 4.3.** The number of shakes during trials in *Myotis nigricans* (black) and *Molossus molossus* (light gray). Mean ± SE; *=P<0.05.

**Figure 4.4.** Amount of individual prey items eaten in *Myotis nigricans* (black) and *Molossus molossus* (light gray). Mean ± SE; *=P<0.05.
Figure 4.1. Latency to Eat
Figure 4.2. Time Spent Chewing and Percentage of Time Spent Eating in *Myotis nigricans* and *Molossus molossus*
Figure 4.3. Number of Shakes in *Myotis nigricans* and *Molossus molossus*
Figure 4.4. Percent of Prey Eaten Across Trials in *Myotis nigricans* and *Molossus molossus*
CHAPTER V

CONCLUSION

Behavior has recently been identified as an important component in the success of invasive species (Sol et al. 2002; Martin and Fitzgerald 2005). In addition, the importance of behavior can be extended to individuals living in proximity to humans (Sol et al. 2013). Because of increasing human-induced environmental change, organisms must possess mechanisms to cope with novelty in order to survive. Acquiring food in changing environments is a crucial aspect of survival. The way in which individuals find and respond to prey, therefore, can provide insight into their ability to survive in new habitats. The experiments outlined in the second and third chapters of this thesis show that response to novel prey differs among populations of cane toads. Although cane toads do not exhibit neophilia, introduced populations are less neophobic than native toads. These findings are consistent with other studies that have used both novel food and a novel object to examine response to novelty (Thorsen et al. 2000; Sol et al. 2002; Martin and Fitzgerald 2005).

The cane toad is a generalist species known to be highly invasive. By understanding their ability to cope with novelty, insight can be gained into the reasons behind other successful invasive species. These are the first studies to focus on neophobia and neophilia in anurans. Future studies should be conducted on different ages of cane toads to determine the role of neophobia and neophilia (and possible experience) at multiple life stages. Other species of anurans should also be
examined for such behavioral tendencies to better understand strategies for coping with novelty in this group.

Although behavior influences the survival of predator species, it also impacts prey species. The behavior of a prey species can influence the response of predators, which then feeds back into the behavior of the prey. In this way, predators can shape the evolutionary trajectory of prey species and vice versa. The fourth chapter of this thesis focuses on a popular, yet understudied, hypothesis that courtship signals in Neotropical fireflies also serve as an aposematic warning signal to predators. Our results show that the insectivorous bats *Myotis nigricans* and *Molossus molossus* are deterred by live fireflies but not by pyrazine, whereas cane toads are not deterred by either, which is more anecdotal evidence of reduced neophobia in this species. Cane toads, however, are unlikely to come into contact with fireflies as often as bat species. These results suggest that flashing in fireflies does serve as an aposematic signal, but more research is needed to determine if predators associate flashing with unprofitability. Future studies focusing on different chemicals or combinations of chemicals present in fireflies are needed to determine the reason for rejection of fireflies by predators.

The studies presented in this thesis were designed to examine potential neophobic and neophilic behaviors. Because fireflies are understudied in the Neotropics, we provide the basis for future studies of Neotropical fireflies and their potentially aposematic signals. Examining behavioral response to fireflies further will reveal the deterrent to bat species and potentially the evolutionary history of
such signals. In addition, we provide a framework for future studies of behavior in the context of invasive ability within anurans, which has not been examined until now. By comparing multiple populations of cane toads in both their native and invasive range, we were able to establish a baseline for the response to novelty in this species. Because of increased urbanization worldwide, understanding the ways in which individuals respond to novelty has implications for not the only the success of introduced species, but also native species. Furthermore, increasing our knowledge of the mechanisms behind the success of introduced species can lead to better conservation of native biota and biodiversity.
BIBLIOGRAPHY


Kelly DJ, Marples NM. 2004. The effects of novel odour and colour cues on food acceptance by the zebra finch, *Taeniopygia guttata*. Anim Behav. 68, 1049-1054.


Moosman Jr. PR, Cratsley CK, Lehto SD, Thomas HH. 2009. Do courtship flashes of fireflies (Coleoptera: Lampyroridae) serve as aposematic signals to insectivorous bats? Anim Behav. 78, 1019-1025.


Paddock FB. 1918. The Beemoth or Waxworm. College Station, TX, US: Texas Agriculture Experiment Station.


