EFFECTS OF HUMIDITY AND TEMPERATURE ON

BURYING BEETLE (COLEOPTERA: SILPHIDAE)

SURVIVAL AND FLIGHT

By

DAN ST. AUBIN

Bachelor of Science in Entomology and Plant Pathology

Oklahoma State University

Stillwater, Oklahoma

2017

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2017

EFFECTS OF HUMIDITY AND TEMPERATURE ON

BURYING BEETLE (COLEOPTERA: SILPHIDAE)

SURVIVAL AND FLIGHT

Thesis Approved:

_____

Dr. W. Wyatt Hoback - Thesis Adviser

_____

Dr. Kris Giles

_____

Dr. George Opit

_____

Dr. Amy Smith

ACKNOWLEDGEMENTS

FORWARD

In the United States and the UK, the word 'sexton' has evolved from the medieval Latin translation, vaguely referencing custodial positions, to describing specific duties associated with watching graveyards. Graveyards are considered sacred in almost every culture. Within the insect word, the value of burials is not overlooked. There are scarab beetles that bury animal dung (dung beetles), and sexton beetles that bury small dead animals (burying beetles); these scavenged resources are irreplaceably valuable reproductive catalysts for the parent beetles to rear their larval offspring. The burial also demonstrates care and respect, as humans allow for peaceful 'rest' or protection during sleep. Within the insect world, a semblance of reincarnation is beheld as the next generation of sexton beetles arises from the resources provided in the death of a small animal. mors e vita, vita e morte

The United States, is home to among the largest species of Silphidae, the American Burying Beetle, *Nicrophorus americanus*. It is with pride that Americans should gain knowledge and pursue a future where this beetle continues to serve its role as a sexton beetle.

Name: DAN ST. AUBIN

Date of Degree: DECEMBER, 2017

Title of Study: EFFECTS OF HUMIDITY AND TEMPERATURE ON BURYING
        BEETLE (COLEOPTERA: SILPHIDAE)
        SURVIVAL AND FLIGHT

Major Field: ENTOMOLOGY AND PLANT PATHOLOGY

Abstract: Terrestrial arthropods must prevent lethal desiccation during activity in dry environments. The small size of arthropods and high surface area to volume ratios increase the tremendous challenges to limit desiccation especially at warmer temperatures and lower humiditys. Among the insects, beetles in the family Silphidae include most species with shortened elytra and exposed abdomens. The silphids occur in communities that contain between 5 and 20 closely-related species that use carrion for food and reproduction. In the subfamily Nicrophorinae, burying beetles must locate appropriately sized, recently deceased vertebrates and bury them in the ground before they are discovered and consumed by predators or competitors. This activity requires the capacity for long sustained flights. The largest and most studied Silphidae is the endangered American burying beetle (ABB), *Nicrophorus americanus*, which is nocturnal and uses the largest carcasses. This study compared field data of activity patterns of the ABB with closely related species in Nebraska and Oklahoma. Laboratory studies of flight were conducted on *Nicrophorus orbicollis*, a proxy for ABBs, at temperatures between 10ºC and 40ºC, at three different relative humidity settings (INSERT THESE). The lowest and highest temperatures affected short flight activity while humidity did not. Desiccation rates were tested for three Silphinae and five Necrophorinae with different ecological and morphological traits at 23ºC. Water loss rates varied among species and were not tightly linked to subfamily, size, or activity pattern, suggesting that other ecological factors are important for members of the Silphidae. These data are useful for modeling Silphidae niches and niche overlap based on environmental factors. Based on the results, increasing daily temperatures could affect the activity of day-active species, but are unlikely to limit the activity of ABB and other night—active species. Shifts in weather pattern and droughts are likely to affect carrion beetle communities, increasing some species while eliminating others.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Terrestrial Arthropods: Adaptations for Life on Land

The oldest fossils of terrestrial animals occur during the Ordovician Period in strata that is approximately 450 million years old (Jeram *et al*. 1990). The transition to land by invertebrates offered opportunities to escape competition and predators while exploiting unfilled niches and gaining access to more-abundant oxygen, which can be limiting in aquatic ecosystems. The movement of invertebrates from seawater to land required profound changes in anatomy and physiology (Little 1983). The first terrestrial animals were myriopods and chelicerates, including or related to centipedes, scorpions, modern spiders, and horseshoe crabs (MacNaughton *et al*. 2002), followed by hexapods.

The movement of the first terrestrial animals to land was facilitated by rigid exoskeletons that could overcome the effects of gravity in the new environment where they are much denser than their surroundings. These early terrestrial organisms faced moisture loss to transpiration across membranes and through pores or openings. The small size of arthropods and high surface area to volume ratios increased desiccation (Hadley 1994). Many insects have waxy exoskeletons comprised of chitin and a surface wax layer that limit water loss from transpiration (Hadley 1994); reducing moisture loss.

Water loss also occurred through excretion. Excretion includes fluids lost in molting, salivation or external pre-digestion, release of waste, water lost while mating, and release of pheromones (Hadley 1994). Insects that inhabit dry environments have modifications including Malpighian tubules and modified hindguts. (Cloudsley-Thompson 1975; Hadley 1994).

Compared to the aquatic environment, the terrestrial environment's air has about one thousand times more oxygen (21%) and is less dense; providing less structural support and thermal capacity (Hadley 1994). Because of the higher availability of oxygen, less oxygen-exchanging-surface-area is required, but water loss through respiration is a major constraint. In insects, expansive cutaneous gills were replaced by a closed tracheal system characterized by branched internal tubes lined with cuticle and regulated by spiracles that open and close strategically to limit water loss (Lighton 1996; Hadley 1994).

Terrestrial life requires access to water and environments with lower humidity result in higher water losses. Death from desiccation results when insects cannot maintain water balance. Insects with morphological, physiological, and behavioral adaptations suited to overcome these water-regulatory challenges were successful. Most often, insects obtain water by drinking or by consuming foods with high liquid content, including nectar, xylem, and blood. Other insects can absorb moisture from the air when humidity is high (Beament 1964).

Adaptation to land also required the ability to survive an environment with rapid temperature changes. Because air has one three-thousandth of the thermal capacity of water, it fluctuates in temperature much more quickly than water, especially in the

temperate zone (Hadley 1994). Changes in temperature affect all physiological processes of terrestrial arthropods and species possess morphological, physiological, and behavioral adaptations to survive both daily and seasonal temperature fluctuations (Clarke 1996). On a broad scale, the effect of temperature on invertebrates is demonstrated by the latitudinal clines in species diversity (Bradshaw, *et al*. 2004) where invertebrate diversity generally decreases with increasing latitudes from the tropics, with little temperature change, to the artic which fluctuates from -34 °C to 12 °C (NOAA 2017).

Carrion beetle overview

Silphidae, is a small family of beetles that are commonly called carrion beetles. Unlike most beetles that have hardened elytra that cover the entire abdomen and reduce water loss, silphids are recognizable by having shortened elytra that exposes several abdominal segments. The beetles also have clubbed antennae. Within the family, there are two subfamilies; Nicrophorinae (burying or sexton beetles) and Silphinae (carrion beetles). Approximately 220 species of Silphidae and 70 species of *Nicrophorus* spp. have been identified worldwide, with 15 occurring in North America (Peck and Kaulbars 1987).

The sub-family Silphinae contains several genera and more diverse morphology among species than Nicrophorinae (Ratcliffe, 1996). The adults of Silphinae feed on a large diversity of carrion and their larvae feed on maggots. The Silphinae can be abundant and exhibit activity patterns, suggesting niche partitioning. One common North American Silphinae is the red-lined carrion beetle, *Necrodes surinamensis.* Morphologically, *Ne. surinamensis* is similar to *Nicrophorus*; however, it has thin elytra

and does not bury carrion. It is a nocturnal species. In contrast, the American carrion beetle, *Necrophila americana*, is a smaller diurnal species that occurs near moist environments (Bishop *et al*. 2002). The smallest silphid is *Thanatopilus lapponicus* or northern carrion beetle. Occurring from coast to coast, *T. lapponicus* is most common in the western United States with populations as far north as Alaska, and a southern range including large populations in New Mexico, Arizona, and California (Anderson and Peck 1985).

The subfamily, Nicrophorinae, has a single genus, *Nicrophorus*, known from fossils dating back to the Cretaceous (Sikes and Venables 2013), approximately 110 million years ago. *Nicrophorus spp.* are primarily found in the northern hemisphere including Europe, Asia, and North America. However, a southern species was recently described from specimens collected near Papua New Guinea (Sikes *et al.* 2006). The group is likely of Eurasian origin (Sikes *et al*. 2008).

Nicrophorinae are commonly refered to as "burying beetles" because of their behavior of burying small dead vertebrates as a food source for their offspring (Pukowski 1933; Scott 1998). These beetles search for the patchy and ephemeral resource of a small dead vertebrate to use as a reproductive resource. Burying beetles detect butyrate; and other volatile compounds released during decomposition using very sensitive olfactory receptors located on their clubbed antennae (Hoermann *et al*. 2011). Leasure (2014) estimated traps baited with rotten carrion to have a 1 km (0.5 mile) radius range of attraction. Beetles cover long distances by flying and these flights may generate substantial thoracic heat (Merrick and Smith 2004). The ability to fly for extended

periods of time may be driven by heat loss via the exposed abdomen similar to what is used by sphinx moths in the family Sphingidae (Heinrich 1996).

After discovering and assessing the corpse to determine it is of appropriate size, a male and female pair of the largest species of beetles bury the carcass. This eliminates the access of competitors to the resource (Scott 1998). With the carcass secured, the pair create a brood-chamber. Theyprepare the carcass by stripping it of hair or feathers, and then covering it with anal and oral secretions which slow or prevent bacterial and fungal growth on the carrion (Scott 1998).

Multiple species of burying beetles often exist in a single environment, exhibiting niche partitioning through daily and seasonal activity, use of different carrion size, and habitat characteristics (Anderson and Peck 1984; Ratcliffe 1995; Bishop *et al*. 2002). Species of *Nicrophorus* are attracted to carrion including birds, mammals, and reptiles (Bedick *et al*. 2000). One species, *N. pustulatus*, was recently discovered to be a parasitoid of snake eggs (Blouin-Demers and Weatherhead 2000; Keller and Heske 2001). The size of carcass determines the number and size of offspring produced. Some species utilize larger carrion by communally breeding (Scott 1998).

Activity patterns are broadly classified as either diurnal or nocturnal. However. the examination of one species *N. marginatus* which is diurnal, shows that it avoids the hottest times of the day, and lives in habitats where water can be easily obtained; a necessity due to rapid water loss documented in the species (Bedick *et al*. 2006). The activity of *N. marginatus* in August can be described as bimodal, with activity in the morning and afternoon. In contrast *Nicrophorus orbicollis* and *Nicrophorus americanus* are nocturnal, flying when the air temperatures and the risk of desiccation is reduced

(Bedick *et al*. 2006). The differences in temporal activity between silphid species are potentially driven by interspecific competition (Scott 1998); however, research to characterize physiological and morphological differences is limited.

With increased heat comes increased rates of desiccation (Bedick *et al.* 2006). Body size, morphology, the rate at which heat can be transferred from thoracic muscles to abdominal muscles, and the environmental temperature all affect the ability to fly without sustaining heat damage in *Nicrophorus* (Merrick and Smith 2004). Among the montane species *N. guttala, N. investigator,* and *N. hybridus*, the largest species, *N. hybridus*, has the best thermoregulatory ability (Merrick and Smith 2004). Short flights were recorded for all the species and showed increases in the thoracic muscle temperature over the abdominal region. During long flights, *N. hybridus* had the least thoracic temperature change compared to the abdomen (Merrick and Smith 2004).

Although most *Nicrophorus* species are active in the summer in the northern range (Bishop *et al.* 2002), variation in seasonal activity has been observed. For example, *Nicrophorus tomentosus* is a bumblebee mimic with a thorax covered in yellow hairthat is active early and late in the year. The hair on the thorax both mimics a bumble bee and also allows better thermal regulation (Merrick and Smith 2004).

The most studied species of North American silphid is the federally endangered American burying beetle (ABB), *Nicrophorus americanus*, which is the largest North American species with adults between 25 and 45 mm in length (Holloway and Schnell 1997; USFWS 2014). The ABB was listed as endangered in 1989 as its range declined from 35 temperate eastern states to 7 states, occupying less than 10 percent of its original range (Jurzenski *et al.* 2011).

The ABB is thought to have diverged from *Nicrophorus orbicollis* which is also nocturnal (Sikes and Venables 2013), and share many similarities (Kozol 1988). The ABB is distinguished from other *Nicrophorus* by an orange marking on the pronotum that is shaped similar to a police badge. Males are distinguished from female by the dark-orange markings on their clypeus; males have a large rectangular marking while females have a smaller triangular marking (Bedick *et al.* 1999). Teneral beetles (recently emerged adults) tend to be vibrant, with shiny eleytra and bright orange markings. The senescent (older beetles) are a Texas-University dark-orange with dull color compared to teneral beetles. The change in appearance from teneral beetles to adult beetles is from a combination of aging, and wear on the exoskeleton from burrowing behavior. Age is not easy to distinguish (Ferrari 2014).

American burying beetles remain in the soil during the day and forage or look for mates at night. During inactive periods, ABBs burrow into moist soil or substrate (Bedick *et al.* 2004). *Nicrophorus,* including ABB, associate with wet loam and leaf litter with a strong preference for moist leaf litter (Willemsens 2015). Adults overwinter in the soil and become active when the nighttime temperature regularly exceeds 15°C (Bedick *et al.* 1999, Schnell *et al.* 2007).

The ABB can utilize larger carrion (weighing up to 300 grams) than other burying beetles (Kozol *et al.* 1988). It is a habitat generalist and can be found associated with prairie, woodlands and forests (Leassure and Hoback 2017). Although a habitat generalist, it needs large intact ecosystems to find suitable carrion. The habitats most likely to support ABB are those that are further away from edge disturbance, where scavengers reduce the number of carcasses available for ABB (Holloway and Schnell

1997; DeVault *et al.* 2011). The main competition for carrion is from large scavengers, including raccoons and the Virginia opossum (Jurzenski *et al.* 2014). Competition between beetles and flies is seasonal with the greatest numbers of flies occurring in the summer (Scott and Traniello 1990).

Soil is an important habitat requirement because burying beetles spend as much as 75 percent of their lives underground (Bishop *et al.* 2002). Soil that is too hard or too compact impedes rapid carrion burial and the construction of a brood chamber. However, if the soil is loose sand, it may not support the brood chamber walls (Willemssens 2015). Higher moisture may allow survival of adults, but may be unfavorable for reproduction if the chamber becomes flooded (Pukowski 1933) and thus, well-drained soil is preferred.

For ABB, Lomolino and Creighton (1996) measured higher reproductive success in forested habitats than grasslands. Wooded areas have a complex soil composition; often comprised of loose soil with a detritus layer, often topped with a leaf layer. The loose undergrowth is better suited for burying carrion than compact grasslands, though burials in grasslands also occurred and the number of larvae per brood was unaffected (Lomolino and Creighton 1996).

The survival of ABBs is influenced by air and soil temperature, and moisture (Kozol *et al.*1988; Scott 1998; Bedick *et al*. 2006; Willemssens 2015). Deaths observed in pitfall traps in hotter and drier environments (Bedick *et al*. 1999) suggest that temperature and humidity could be range limiting factors. The historical range of ABBs appears to stop near the transition to short grass prairie in the central US (USFWS 2014). Although the species once occurred from Texas to Canada, current populations occur

from Oklahoma to South Dakoda (Leisure and Hoback 2017). This reduction in historic range may have resulted from a combination of factors including rainfall, soil characteristics and vegetation.

The decline of ABB distribution is attributed to a combination of potential factors including 1) pesticide, i.e. the widespread use of DDT (Jurzenski *et al.* 2011); 2)carrion availability, notably the extinction of the passenger pigeon and the reduction in range for the greater prairie-chicken- both of which were very abundant in the 19th century (Peck and Kaulbars 1987; USFWF 1991); and 3) habitat alteration, including light pollution (Ferrari 2015) and fragmentation (Fujita *et al.* 2008). They also face competition with other carrion feeders including flies, large scavenging mammals, and other carrion beetles. Year to year fluctuations in population are affected greatly by carrion availability which is made scarcer by the competition.

Climate change and burying beetles

Carbon dioxide (CO_2) levels have fluctuated during earth's history but have not exceeded 300 ppm in the last 100,000 years prior to the 1950s (NOAA 2017). Beginning with the Industrial Revolution, human technology has increased the release of CO_2. Measurements of climatic conditions over the past 150 years show increased levels of CO_2 in the atmosphere. Today CO_2 levels are approximately 405 ppm compared to 330 ppm in 1970 (NOAA 2017). Increases of CO_2 affect many environmental and biological cycles including atmospheric temperature and water cycles, pH of aquatic systems, and plant photosynthesis (NOAA 2017). Nearly all climate scientists attribute these changes to anthropogenic origins.

Increased CO_2 has triggered a rising average temperature of earth's crust resulting in warmer oceans, shrinking ice sheets, decreased snow cover, rising sea levels, more frequent extreme weather events, an increase of acidity in the oceans, and a decline in the extent and thickness of the artic sea ice (NOAA 2017). Areas have experienced changes in rainfall patterns, with some areas experiencing drought and others facing flood events. In North America, the Great Plains have been experiencing a rise in temperature and a decline in average precipitation (Shafer 2014). Models suggest increases in sustained extreme droughts and heavy rainfall events (Seager *et al*. 2007; Shafer 2014). Frequent heatwaves and diminished precipitation will decrease soil moisture, leading to dry deep-soil layers (Schlaepfer *et al*. 2017) which alter plant and microbe communities.

Higher soil temperatures can affect other aspects of burying beetle life history. For example, high temperatures can limit the effectiveness of the secretions used to slow the decomposition of carrion resources (Jacques *et al*. 2009). In Korea, *Nicrophorus quadripunctatus, Nicrophorus maculifrons,* and *Nircophorus concolo* have been monitored in Mt. Sokrisan National Park since 2004 (Kwang-Sik *et al.* 2011). Numbers have decreased within the peak months of July and August, potentially as a result of increasing temperatures.

The rate of climate change, the location, the size of a population, amount of genetic variation, and the extent of environmental change should all be considered as significant variable in models to examine the potential of a species to adapt to change or be introduced to new areas (Dobrowski *et al*. 2012; Loarie *et al*. 2009). American burying beetle reintroduction efforts can be costly and success to date has been limited.

An ongoing reintroduction effort of ABB to Ohio and Missouri has yet to produce successful results (USFWS 2014). In a study on Nantucket Island, Massachusetts, ABB persisted only while carcasses were provided (Mckenna-Foster *et al.* 2016). Differences in environments among re-introduction locations should be considered when conducting reintroductions because the source populations are limited to small areas of Rhode Island, Oklahoma, Nebraska and Arkansas.

Autumn is a critical time for insects, including burying beetles, because as day length shortens insects prepare to overwinter. In the northern ranges, birds migrate south and many animals go into hibernation- limiting carcass availability. The first freeze kills the susceptible animals. After this initial kill, carrion is scarce. Then the ground freezes as autumn turns to winter. If climates shift results in a warmer autumn in southern ranges, large effects on the ecology of the region would be anticipated (Gallinat *et al*. 2015). Warmer weather could also prolong survival and potentially increase mutations in pathogens, reduce pathogen die offs, and alter pathogen dormancy, further affecting carrion availability (Gallinat *et al*. 2015). In addition to the effects of warming, limited moisture during the winter may also result in mortality. Most insects drink readily to replace lost moisture when they have access to water allowing them to regulate their water content (Hadley 1994; Bedick *et al*. 2006). During overwintering diapause, water lost to respiration and transpiration may not be replaced because the beetles are inactive and death from desiccation may be more likely than death to starvation.

Nebraska has two separated populations of ABB that produce one new generation per year (Beddick *et al.* 1999; Jurzenski *et al.* 2014). The southern population occurs in the Loess Canyons (McPherron *et al*. 2012; Jurzenski *et al.* 2014). Beetles overwinter in

the soil, below the frost line and then emerge in late May or early June in Nebraska

(Bedick *et al*. 1999). Less is known about the southern populations of ABB, which may

or may not overwinter and have been observed between early April and late October.

Schnell *et al.* (2008) revealed that overwintering ABB in Arkansas that were provided

with carrion had approximately two times higher survival than unprovisioned beetles.

The average depth of overwintering beetles was 6 cm; with beetles buried as deep as 20

cm. The survivability of provisioned and non-provisioned beetles was 77.1% and 44.6%,

respectively (Schnell *et al.* 2008). This result suggests that beetles maintained some level

of activity and feeding during the winter.

The temperature difference between Oklahoma and Nebraska should be

considered when studying ABB. During a lab study, Lomolino and Creighton (1996)

noted that five of their 8 pairs produce 2 generations of offspring. It is suspected that

ABB is capable of producing more than one generation per year when the ground does

not freeze in Oklahoma, assuming they can find carrion resources. If climatic norms

change for Nebraska under a high emission scenario, where increase temperatures occur,

then this may increase the likelihood of double broods.

Objectives

The objectives of this study are 1) to determine water loss rates and survival of different
species of silphids to add data on potential species changes in association with drought
and 2) determine the effects of temperature and humidity on flight capabilities of
Silphidae.

Chapter I Works Cited

Anderson, R.S. and S.B. Peck. 1985. The carrion beetles of Canada and Alaska. The
Insects and Arachnids of Canada,. Publication 1778, Research Branch Agriculture
Canada, Ottawa Part 13, pp. 1–121.

NESTA. 2017. "Arctic Weather." Arctic Weather - Windows to the Universe,
www.windows2universe.org/earth/polar/weather_arctic.html.

Beament, J.W.L. 1964. The active transport and passive movement of water in Insects.
Advances in Insect Physiology Volume 2. 67–129.

Bedick, J.C., B.C. Ratcliffe, and L.G. Higley. 2004. A new sampling protocol for the
endangered American burying beetle, *Nicrophorus americanus* Olivier
(Coleoptera: Silphidae). The Coleopterists Bulletin. 58: 57–70.

Bedick, J.C., B.C. Ratcliffe, W.W. Hoback, and L.G. Higley. 1999. Distribution,
ecology and population dynamics of the American burying beetle [Nicrophorus
Americanus Olivier (Coleoptera, Silphidae)] in South-central Nebraska. USA.
Journal of Insect Conservation. 3: 171–181.

Bedick, J.C., W.W. Hoback, and M.C. Albrecht. 2006. High water-loss rates and rapid
dehydration in the burying beetle, *Nicrophorus marginatus*. Physiological
Entomology. 31: 23–29.

Bishop, A.A., W.W. Hoback, M. Albrecht, and K. Skinner. 2002. A comparison of an
ecological model and GIS spatial analysis to describe niche partitioning amongst
carrion beetles in Nebraska. Transactions in GIS. 47.

Blouin-Demers, G., and P.J. Weatherhead. 2000. A novel association between a beetle
and a snake: parasitism of Elaphe obsoleta by *Nicrophorus pustulatus*
(Coleoptera) Silphidae. Ecoscience. 7: 395–456.

Bradshaw, W.E., P.A. Zani, C.M. Holzapfel. 2004. Adaptation to temperate climates.
The Society for the study of evolution 58(8): 1748-1762.

**Butler, S.R., R. Harms, K. Farnsworth-Hoback, K. Koupal, J. Jurzenski, W.W.
Hoback. 2012.** Standardized capture rates of the endangered American buring
beetle, Nicrophorus americanus Olivier (Coleoptera: Silphidae) using different trap
protocols. J Incest Conservation. 17: 607-613

Clarke A. 1996. The influence of climatic change on the distribution and evolution of
organisms. Pp. 377–407.

Cloudsley-Thompson, J.L., and F.T. Abushama. 1970. A guide to the physiology of
terrestrial Arthropods. Khartom University Press, The Democratic Republic of
Sudan.

Cloudsley-Thompson J.L. 1975. Adaptations of Arthropoda to arid
environments. Annual Review of Entomology 20: 261-283.

Devault, T.L., Z.H. Olson, J.C. Beasley, O.E. Rhodes Jr. 2011. Mesopredators
dominate competition for carrion in an agricultural landscape. Basic and Applied
Ecology. 12: 368-274.

**Dobrowski, S.Z., J. Abatzoglou, A.K. Swanson, J.A. Greenberg, A.R. Mynsberge,
Z.A. Holden, and M.K. Schwartz. 2012.** The climate velocity of the contiguous
United States during the 20th century. Global Change Biology. 19: 241–251.

Ferrari, T.N. 2014. Seasonal dynamics of the American burying beetle in Eastern Oklahoma. Master Thesis. Oklahoma State University.

Fleissner, G., G.N. Fleissner, and B. Frisch. 1993. A new type of putative non-visual photoreceptors in the optic lobe of beetles. Cell and Tissue Research. 273: 435–445.

Fugita, A., K. Maeto, Y. Kagawa, and N. Ito. 2008. Effect of forest fragmentation on species richness and composition of ground beetles (Coleoptera: Carabidae and Brachinidae) in urban landscapes. Entomological Science 11: 39-48

Gallinat, A.S., R.B. Primack, and D.L. Wagner. 2015. Autumn, the neglected season in climate change research. Trends in Ecology and Evolution. 30: 364.

Hadley, N.F. 1994. Water relations of terrestrial arthropods. Academic Press, San Diego.

Heinrich, B. 1996. The Thermal Warriors: Strategies of Insect Survival. Harvard University Press. Cambridge, Mass.

Hoermann, C.V., J. Ruther, S. Reibe, B. Madea, and M. Ayasse. 2011. The importance of carcass volatiles as attractants for the hide beetle *Dermestes maculatus* (De Geer). Forensic Science International. 212: 173–179.

Holloway, A.K., and G.D. Schnell. 1997. Relationship between numbers of the endangered American burying beetle *Nicrophorus americanus* Olivier (Coleoptera : Silphidae) and available food resources. Biological Conservation. 81: 145–152.

Jacques, B.J., M. Abe, S. Akahane, W. Middleton, and W.W. Hoback. 2009. Temperature and food availability differentially affect the production of

antimicrobial compounds in oral secretions produced by two species of burying beetle. Journal of Chemical Ecology. 35: 871–877.

Jeram, A.J., P.A. Selden, and D. Edwards. 1990. Land animals in the Silurian: Arachnids and myriapods from Shropshire, England. Science. 250: 658–661.

Johnston, I. A., and A. F. Bennett, eds. <u>Animals and Temperature: Phenotypic and Evolutionary Adaptation</u>. Cambridge University Press, Cambridge, U.K. 436 pp.

Jurzenski, J.D., C.F. Jorgensen, A.W, R. Grosse, J. Riens, and W.W. Hoback. 2014. Identifying priority conservation areas for the American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae), a habitat generalist. Systematics and Biodiversity. 12: 149–162.

Jurzenski, J., D.G Snethen, M.L. Brust, W.W. Hoback. 2011. New records of carrion beetles in Nebraska reveal increased presence of the American burying beetle, *Nicrophorus americanus* Olicer (Coleoptera: Silphidae). Great Plains Research. 21: 131-143.

Keller, W.L. and E.J. Heske. 2001. An observation of parasitism of black rat snake (*Elaphe obsoleta*) eggs by a beetle (*Nicrophorus pustulatus*) in Illinois. Transactions of the Illinois State Academy of Science 94: 167-169.

Kozol, A.J., M.P. Scott, and J.F.A. Traniello. 1988. The american burying beetle, *Nicrophorus americanus*: studies on the natural history of a declining species. Psyche: A Journal of Entomology. 95: 167–176.

Kwang-Sik, O., H.K. Min, S.J. Yoon, J.W. Ryu, Y.B. Cho. 2011. Study of beetles and butterflies in the area Dowon-Valley, Mt. Masan, Gangwon Province, Korea. Jornal of Korean Nature, 4: 7-10.

Leasure, D.R., and W.W. Hoback. 2017. Distribution and habitat of endangered American burying beetle in northern and southern regions. Journal of Insect Conservation. 21: 75–86.

Lighton, J.R.B. 1996. Discontinuous gas exchange in insects. Annual Review of Entomology. 41: 309–324.

Little C. 1983. The Colonization of Land: Origins and Adaptations of Terrestrial Animals, Cambridge University Press. Cambridge, UK. 300.

Loarie, S.R., P.B. Duffy, H. Hamilton, G.P. Asner, C.B. Field, and D.D. Ackerly. 2009. The velocity of climate change. Nature. 462: 1052–1055.

Lomolino, M.V., J.C. Creighton, G.D. Schnell, D.L. Certain. 1995. Ecology and conservation of the endangered American burying beetle. Conservation Biology. 9: 605-614.

Lomolino, M.V., J.C. Creighton. 1996. Habitat selection, breeding success and conservation of the endangered American burying beetle *Nicrophorus americanus.* Biological Conservation. 77: 235-241.

MacNaughton, R.B., J.M. Cole, R.W. Dalrymple, S.J. Braddy, D.E. Briggs, and T.D. Lukie. 2002. First steps on land: Arthropod trackways in Cambrian-Ordovician eolian sandstone, southeastern Ontario, Canada. Geology. 30: 391.

Mckenna-Foster, A., L. Perrotti, J.Blyth, E. LoPresti, S. Kennedy. 2016. Measuring Success of a reintroduced population of the American burying beetle (*Nicrophorus americanus* Oliver) to Nantucket Island, MA. Journal of Insect Conservation. 20: 895-904.

Merrick, M.J., R.J. Smith. 2004. Temperature regulation in burying beetles (*Nicrophorus spp*. Coleoptera: Silphidae): effects of body size, morphology and environmental temperature. Journal of Experimental Biology. 207:723-733.

McPherron, M.M., J. Jurzenski, M.C Albrecht, K.M. Farnsworth-Hoback, T.L. Walker, W.W. Hoback. 2012. Model of habitat suitability for American burying beetles in Nebraska's Loess Canyons ecosystem. Trends in Entomology. 8: 27-36.

(**NOAA Climate.gov | science & information for a climate-smart nation**) **NOAA Climate.gov | science & information for a climate-smart nation. 2017.** NOAA Climate.gov | science & information for a climate-smart nation. (https://www.climate.gov/).

Peck, S.B., and M.M. Kaulbars. 1987. A synopsis of the distribution and bionomics of the carrion beetles (Coleoptera: Silphidae) of the conterminous United States. Proceedings of the Entomological Society of Ontario, 118:47- 81.

Pukowski, E. 1933. Okologische Untersuchungen and Necrophorus. F. Z. Morph Okol Tiere. 27: 518-596.

Ratcliffe, B.C. 1996. The carrion beetles (Coleoptera: Silphidae) of Nebraska. University of Nebraska State Museum, Lincoln, Nebraska

Ratcliffe, B.C. 1995. American burying beetles. Nebraska's threatened and endangered species. NEBRASKALand Magazine. 73: 6.

Schnell, G.D., A.E. Hiott, J.C. Creighton, V.L. Smyth, and A. Komendat. 2008. Factors affecting overwinter survival of the American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae). Journal of Insect Conservation. 12: 483–492.

Schlaepfer, D.R., J.B. Bradford, ,W.K. Lauenroth, S.M. Munson, B. Tietjen, S.A. Hall, S.D. Wilson, M.C. Duniway, G. Jia, D.A. Pyke, A. Lkhagva, K. Jamiyansharav. 2017. Climate change reduces extent of temperate drylands and intensifies drought in deep soils. Nature Communications 8:

Scott M.P., J.F.A. Traniello. 1990. Behavioral and ecological correlates of males and female parental care and reproductive success in burying beetles (*Nicrophorus spp.).* Animal Behavior. 39: 274-283.

Scott, M.P. 1998. The ecology and behavior of burying beetles. Annual Review of Entomology. 43: 595–618.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H.-P. Huang, N. Harnik, A. Leetmaa, N.-C. Lau, C. Li, J. Velez, and N. Naik. (2007). Model projections of an imminent transition to a more arid climate in Southwestern North America. Science 316.5828 (2007): 1181-184.

Shafer, C.L. 2014. From non-static vignettes to unprecedented change: The U.S. National Park System, climate impacts and animal dispersal. Environmental Science and Policy. 40: 26–35.

Sikes, D.S., and C. Venables. 2013. Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). Molecular Phylogenetics and Evolution. 69: 552–565.

Sikes, D.S., S.M. Vamosi, S.T. Trumbo, M. Ricketts, C, Vanables. 2008. Molecular systematics and biogeography of Nicrophorus in part- the investigator species group (Coleoptera: Silphidae) Using mixture model MCMC. Molecular Phylogenetics and Evolution. 48: 646-666.

Sikes, D.S., R.B. Madge, and S.T. Trumbo. 2006. Revision of Nicrophorus in part: new

  species and inferred phylogeny of the nepalensis -group based on evidence from

  morphology and mitochondrial DNA (Coleoptera : Silphidae : Nicrophorinae).

  Invertebrate Systematics. 20: 305.

Sikes, D.S., and C.J. Rathel. 2002. A review of hypotheses of decline of the endangered

  American burying beetle (Silphidae: *Nicrophorus americanus* Olivier). 6: 103-

  113.

Sugiuri, S., R Tanaka, H. Taki, N. Kanzaki. 2013. Differential responses of scavenging

  arthropods and verebrates to forest loss maintain ecosystem function in a

  heterogeneous landscape. Biological conservation. 159: 206-213.

U.S. Fish and Wildlife Service. (2014). American burying beetle *Nicrophorus

  americanus* -Olivier. Retrieved from

  https://www.fws.gov/southwest/es/oklahoma/Documents/ABB/Conference%20M

  aterials/6_American%20Burying%20Beetle%20Biology.pdf

Willemssens K.A. (2015). Soil preferences of Nicrophorus beetles and the effects of

  compaction on burying behavior. Master Thesis. University of Nebraska –

  Lincoln.

CHAPTER II

TEMPERATURE AND HUMIDITY VS. NICROPHORUS FLIGHT

Effects of temperature and humidity on *Nicrophorus orbicollis* flight and comparison with field data on *Nicrophorus americanus*

Abstract

Flight allows insects to avoid poor environmental conditions and locate resources including food and mates. Despite being poikilothermic, insects are capable of flying at cool temperatures by generating thoracic heat. However higher temperatures may reduce capacity for flight by resulting in fatal heat stress. Carrion beetles in the family Silphidae are relatively large insects which search large areas to locate recently deceased vertebrates which are used for food and reproduction. Morphologically carrion beetles have shortened elytra which exposes the abdomen and may reduce heat gain but also contribute to observed high water loss rates. The ability to fly was tested for the nocturnal *Nicrophorus orbicollis* at 10^oC, 15^oC, 20^oC, 24^oC, 30^oC, 35^oC, and 40^oC. At each of these temperatures, low (5% to 30% RH), moderate (50% to 60% RH), and high humidity (75%-95% RH) conditions were simultaneously tested. Humidity did not impact flight. Both low and high temperatures reduced flight activity below 80% success. Laboratory results were compared to field sampling data for for *N. orbicollis* and the closely-related and federally endangered American burying beetle, *Nicrophorus americanus*. Field data were obtained from surveys at Camp Gruber near Braggs, Oklahoma between July 25, 2016 and October 15, 2017. Captures increased with increased temperatures for both species with many captures at the highest recorded midnight temperature of 28^oC. The lowest night temperature with capture of *N. americanus* was less than 10^oC. These results suggest that burying beetle flight activity is not reduced at current temperatures and that seasonal activity may span more months in Oklahoma than is currently documented. Increasing temperatures are unlikely to affect

nocturnally active burying beetles but other factors including completion with flies and other carrion beetles and microbial decay of carcasses should be tested.

Introduction

Insects were the first organisms to fly, evolving powered flight by about 350 million years ago. They remain the only invertebrates, and only poikilohermic organisms, capable of this activity. Insect flight has been extensively studied with an emphasis on determining minimum flight temperatures and characterizing physiology that allows insects to fly at cool environmental temperatures. A number of insects including Lepidoptera, Hymenoptera, and Coleoptera have been found to have morphological and physiological characteristics that allow warming and maintenance of thoracic heat through shivering, insulating setae, and counter-current heat exchange (Heinrich 1974). Often, minimum temperatures for flight take-off differ from temperatures that allow sustained flight. For example, the black bean aphid, *Aphis fabae* has been found to require 17°C for take-off (Johnson and Taylor 1957) but only 6.5°C for flapping of wings (Cockbain 1957).

For insects capable of retaining heat, the maximum environmental temperatures that allow flight are between 40°C and 45°C (Heinrich 1974). As a consequence, insects that generate high muscle temperatures during flight must regulate thoracic temperature within a relatively narrow range and are likely limited to activity periods that maximize heat loss or reduce potential for additional heating by the environment. Within hot environments, insects must regulate their body temperatures. The upper thermal limit of most tested insects is around 50°C (Church 1960). Direct sunlight can increase the heat of an insect (Merrick and Smith 2004). Compounded with heat generated by the flight

muscles of the flying insect, the sunbathed insect is at higher risk of death by heat stress, even at lower environmental temperatures (Merrick and Smith 2004). Less research has been conducted on the maximum air temperature at which an insect that does not retain thoracic heat can fly without overheating. Among the beetles, two groups that have been studied are the tiger beetles and carrion beetles.

Tiger beetles (Carbidae: Cicindelinae) are primarily diurnal species that inhabit open areas without vegetation where surface temperatures often exceed 60° C. Their thermal maxima is between 48 and 50° C (Hoback et al. 2001) and tiger beetles use a number of behaviors including stilting and shade-seeking to avoid overheating. In addition, tiger beetles can make short flights that promote convective cooling (Morgan 1985).

Carrion beetles (Silphidae) are larger and heavier than tiger beetles and are named for their behavior of feeding on carcasses. Within the Silphid family, the subfamily Nicrophorinae or burying beetles, have been studied for the effects of temperature on activity.

$Nicrophorus$ beetles heat up during flight and the heat is lost via convection or radiation from the surface of the insect into the environment. Heat must dissipate from the flight muscles to the outside of the insect for cooling to occur. Flights are limited by the amount of heat that the beetles build up in flight muscles (Church 1960; Merrick and Smith 2004), necessitating post-flight cooling to avoid lethal temperatures (Merrick and Smith 2004). $Nicrophorus$ beetles are more likely to reach lethal temperatures 46.15+/-0.6° C when flying during hot periods under full sun, even when air temperatures are 25°

C to 30º C (Merrick and Smith 2004). The diurnal species *Nicrophorus marginatus* has reduced activity during the hottest parts of the day (Bedick *et al.* 2006). Other species, including *N. americanus, N. orbicollis*, and *N. pustulatus*, are nocturnal, potentially as an adaptation to avoid overheating during flight.

In this study, I tested a close relative of the American burying beetle to determine the proportion of flying individuals at different environmental temperatures and different humidity levels. In addition, I compared the catch rates of both species in the field across 18 months and examined historic catch data of the American burying beetle in Oklahoma and Nebraska. I tested the hypotheses that both low and high temperatures limit activity of nocturnally active *Nicrophorus* and that environmental capture rates are similar to temperatures tested under laboratory conditions.

Materials and Methods

Field data

As part of an on-going study of the population of American burying beetles at Camp Gruber in Muskogee County, Oklahoma, data on *Nicrophorus orbicollis* activity were gathered. Field sampling occurred for a period of five days beginning July 25, 2016 at 20 sites using standard above ground pitfall traps (USFWS 2014) baited with rotten extra-large rats (Rodentpro.com), and placed a minimum of 1.5 kilometers apart. Traps were checked and captured beetles were identified to species and then released. Bait was changed after the third day and traps were removed after completion of sampling. Baited pitfall traps were placed in 10-15 areas and monitored for two day periods approximately

every other week until October 30, 2016. Sampling resumed April 1 and continued until

October 15, 2017 with twenty traps sampled for five consecutive days between July 11

and July 16. All traps were checked in the morning between 06:00 and 10:00 when both

target species of *Nicrophorus* are inactive.

Beetles per trap night were calculated by dividing the total number of beetles by

the number of traps that were set for each night. Weather information for each trap night

was obtained from weatherunderground.com for Braggs, Oklahoma. Midnight

temperatures were compared to beetle captures for each species through linear regression

using SigmaPlot 11.0 software.

On the last trap night, *N. orbicollis* were collected and transported in moist soil to

the laboratory for flight tests. In total, 456 *N. orbicollis* were individually lab-tested at

different temperature and humidity combinations to determine flight activity under

different conditions.

Amy Smith and Wyatt Hoback field trials – Contributed Data

To analyze the relationship between temperature and flight activity, an extensive

data set of *Nicrophorus americanus* captures spanning 2001-2012, encompassing samples

from Nebraska and Oklahoma were evaluated by Dr. Amy Smith and Dr. Wyatt Hoback.

Beetles were captured using standard pitfall trapping consistent with the region sampled

(USFWS 2005, 2015). Weather Underground data was used to determine trap night

temperature based temperature data from nearest weather meter for the period of 21:00 to

04:00 on the trap night. Only positive trap nights when *N. americanus* were captured

were used. Environmental conditions during surveys limited the tested temperatures,

thereby the amount of tests across specific temperatures is not uniform.

Data was visually inspected by developing a scatter plot and a line graph illustrating potential relationships between temperature and captures.. The line graph displays average captures in a trap night while the scatter plot displays a dot for every capture so that the dataset can be better visualized.

Laboratory trials

To determine the effects of humidity and temperature on flight activity, individual *N. orbicollis*, a proxy for American burying beetles, were exposed to experimental conditions in an environmental chamber. Within the environmental chamber, a 43 cm x 43 cm x 43 cm glass terrarium was used as a testing arena to control conditions and prevent escapes. The terrarium (figure 6) contained 9 cups (14 cm tall, 11 cm in diameter), each lined with Vaseline to prevent beetles from escaping by climbing. Beetles were inserted into individual cups using a pipe that was accessed and manipulated through the top of the terrarium. This allowed the terrarium to remain at the desired temperature and humidity by reducing air exchange that would result from opening and closing the terrarium.

Three small wooden dowels were anchored into 5 cm of clay at the bottom of each cup so that the top ends met in the center and extended approximately 1 cm above the height of the cup. Multiple dowels allowed a beetle walking in circles to regularly encounter a stick and be able to climb, a behavior often used by *Nicrophorus* prior to flight. Beetles were unable to reach the cup sides from the centered dowels and were only able to escape by flight from the dowels. A perforated cup containing soil soaked with rotten rat blood, a by-product of bait preparation, was suspended at the top of the terrarium to provide incentive for beetles to escape the cups. The chamber was unlit

during trials to represent nocturnal conditions when beetles are normally active. Beetles were left in the chamber for up to 2 days until they escaped, died, or were removed. Any beetle that escaped the cup was considered a pass and those that remained in the cup were considered a fail under the experimental conditions. Each beetle was only tested once and discarded if dead. Survivors were released.

Temperature was controlled within the environmental chamber with desired temperature set and allowed to stabilize for at least 24 hours. Flight was tested at 5° C, 15° C, 20° C, 24° C, 30° C, 35° C, and 40° C. At each of these temperatures, low (5% to 30% RH), moderate (50% to 60% RH), and high humidity (75% to 95% RH) conditions were tested. The readout on the environmental chamber provided temperature information. There were 3 real-time Imagitarium temperature gauges and 3 Imagitarium humidity gauges in the terrarium to confirm conditions among the test cups and aquarium were within the desired range.

High relative humidity (75-95 RH) was created by using a humidifier and adjusting the output to saturate the terrarium. This was maintained among all tested temperatures. The humidity of the testing room was approximately 55 RH on most days. Thus, for moderate humidity (50-60 RH) the ambient conditions of the chamber were used. On days when environmental humidity was observed to be below 50 or above 60, more easily manipulated conditions (low or high RH) were used in trials. For low humidity (5% to 30%), a half and half mix of regular and indicating calcium sulfate (drierite) was applied outside the testing cups within the testing terrarium. When the indicating $CaSO_4$ was saturated it turned blue, it was removed and restored to an anhydrous state in an oven set at 210° C for 2 hours as needed. Between 6 and 54 *N.*

orbicollis were tested under each condition and the percent of passes, in which beetles flew out of cups, was calculated for each condition.

Results

Field data

Across all sampling dates at Camp Gruber (2016-2017), a total of 2,730 *N. orbicollis* and 1,852 *N. americanus* were captured in baited pitfall traps. Beetles per trap night (TN) ranged from 21.08 *N. orbicollis* on 8/27/2016 to 0 on multiple dates in April and May 2017. For *N. americanus* the highest observed capture rate was 8.53 beetles per TN on 8/19/2017 and 0 on three dates (Figure 1). The peak in activity by *N. orbicollis* likely represents emergence of new individuals in 2016 but a similar pattern was not observed in 2017. No clear peaks in emergence for *N. americanus* were observed either year. The numbers of *N. orbicollis* and *N. americanus* show similar patterns of increase and decrease in 2017, suggesting that weather impacts both species similarly.

Comparisons of beetles per TN with temperature at midnight in 2016 and 2017 at Camp Gruber revealed that captures are positively correlated with temperature for both species (Figure 2). The minimum temperature at which capture occurred for *N. orbicollis* was 12.2º C (0.1 beetles per TN) with captures of 11.9 per TN at 14.2 º C and generally high numbers of beetles captured at the warmest temperatures. Similarly, *N. americanus* was captured at 11.7º C, and capture rates increased with increasing temperature. The greatest numbers of captures occurred at 25º C and higher. Both species were captured in high numbers at 27.78º C, the highest midnight temperature observed in the field studies (Figure 2).

Over the course of 18 months of sampling, the mean ($\pm$ 1 S.E.) number of *N. orbicollis* captured was 4.5 $\pm$ 0.67 per trap night compared to 2.4 $\pm$ 0.32 per trap night for *N. americanus*. Field data also reveal that activity of both species exhibit fluctuations that follow similar activity patterns (Figure 1). The fluctuations are likely a result of many factors including carrion availability and environmental factors such as temperature and water availability. The capture of both species increased with increasing temperatures (Figure 2). The relationship for *N. americanus* with temperature was stronger ($R^2 = 0.39$) than for *N. orbicollis* ($R^2 = 0.09$).

Amy Smith and Wyatt Hoback field trials – Contributed Data

American burying beetles were observed in traps when average temperatures exceeded 29.4º C and were below 15.5º C. The greatest density of captures was observed between 22.2 and 28º C (Figure 4 and 5). Across all samples, *N. americanus* were captured starting at approximately 5º C and larger numbers were captured at 9º C to 10º C (Figure 4 and 5). All *N. americanus* captured below 15º C were captured in Nebraska (Figure 4). The Oklahoma dataset doesn't include activity below 15.5º C because current USFWS protocols restrict sampling to periods when temperatures are >15.5º C between 21:00 to 04:00 for five consecutive nights.

Laboratory trials

In laboratory trials 8% and 11% of beetles flew in low humidity and high humidity respectively, while 21% flew under moderate humidity at the coolest temperature, 10º C At all other temperatures tested, between 57% and 93% of beetles flew. Humidity did not appear to influence flight behavior (Figure 3). Greater than 75% of tested *N. orbicollis* flew between 15º C and 30º C.

The highest and lowest temperatures affected beetle survival and the period of potential flight. At 40°C (104°F), many beetles died within the first hour, and within 3 hours most were dead or dying on their backs. At 10°C, beetles were sluggish and less likely to fly, although they survived more than 12 hours. Many were found with wings spread at the top of the dowels, but would often fall off or be unable to escape the cup by generating enough lift while flapping their wings.

Discussion

Field data revealed that both *N. orbicollis* and *N. americanus* were captured across the season with low levels of activity recorded in both the earliest (April 1) and latest (October 30) trapping efforts near Braggs, in Muskogee County Oklahoma. These data suggest that beetles are unlikely to overwinter in Oklahoma as they do beneath the frostline in Nebraska (Hoback unpublished data – Further testing needed to validate). Although activity occurred across all environmental temperatures, higher capture rates were observed with higher temperatures; possibly as a result of seasonality. However, caution should be used in interpreting this result because warmer temperatures may also affect the dispersion of scent from the carrion or other correlated factors such as bait decomposition stage (Hoermann *et al.* 2013).

Laboratory trials confirmed that *N. orbicollis* could fly at temperatures between 10 and 40° C with similar activity observed between 15 and 35° C at all levels of humidity. The beetles tested at 40° C died within short periods of exposure, suggesting that the temperatures are at their maximum even without the additional heat generated by powered flight. Similar results for lethal maxima were observed by Merrick and Smith (2004) for montane species.

Higher thoracic temperatures may require dissipation of heat and concurrent water loss. Although environmental humidity has often been found to correlate with beetle survival (Beament 1964; Cloudsley-Thompson 1975; Bedick *et al.* 2006), no effects of humidity on flight activity were noted lab trials (Figure 3).

In addition to niche partitioning occurring as *Nicrophorus* species fly during different times of the day, there are studies that suggest different tolerances to temperature. Competition can be dependent on temperature (Wilson *et al.* 1984; Merrick and Smith 2004) as is the case when *N. orbicollis* displaces *Nicrophorus defodiens* on carcasses in warmer weather. In cooler weather, *N. defodiens* wins competition for similar sized carcasses and may contribute to limits for *N. defodiens'* southern range where *N. orbicollis* becomes more prevalent (Wilson *et al.* 1984).

Fewer successful flights of *N. orbicollis* at 10° C in the laboratory compared to ABB captures in the field is potentially explained by field temperatures representing average conditions obtained from a nearby weather station and the effects of temperature at the time of flight. Because traps are open from dusk until dawn, temperatures could be warmer when beetles arrived at the trap and then cool to the point where beetles would not be able to fly. Alternatively, captures at the field traps could represent the proportion of the population that is motivated by hunger and capable of flight at temperatures that prevent less motivated or incapable individuals from attempting flight. In the field, beetles have been recaptured. The recapture rate for unprovisioned ABB is 19%-21%. When they are provided food, the capture rate drops to 10%. Prior to 2012, 8-cup transects were used to test for the presence of beetles and no food was provided (Amy

Smith – personal communication). Post 2012, rats in closed or open containers were commonly used.

Burying beetles, *Nicrophorus* spp., are persistent flyers that are capable of quickly covering large amounts of area (Eggert and Muller, 1997, Merrick and Smith 2004). They must locate ephemeral carcasses quickly regardless of weather conditions in order to secure the resources for successful reproduction.

In the future, tethered flight, such as in Merrick and Smiths' 2004 study would be helpful to determine the thoracic range necessary for flight in a number of species, and to continue to test extended flight across a range of non-montane species. Future tests should also include more low temperature trials to determine if there is a range at which shivering or preflight heat-building exercises can put species within thoracic temperature ranges necessary for flight. Furthermore, insulating the abdomen and testing the thermoregulatory abilities of silphids would address speculation on the function of the exposed abdomen in Silphidae.

Figure Legends

Figure 1. Beetles per trap night for *Nicrophorus americanus* (hollow circles) and *N. orbicollis* (filled circles) at Camp Gruber near Braggs, Oklahoma. Dates represent the first morning of checks for either 2 day or 5-day sample periods.

Figure 2. Graph of capture data for *Nicrophorus orbicollis* and *Nicrophorus americanus* with temperature at midnight for beetles captured at Camp Gruber near Braggs, Oklahoma.

Figure 3. Percentage (%) of *Nicrophorus orbicollis* that flew under different humidity and temperature combinations.

Figure 4. Scatter plot and line graph of midnight temperatures and capture of *N. americanus* for data collected between 2001 and 2012 in Nebraska.

Figure 5. Scatter plot and line graph of midnight temperatures and capture of *N. americanus* for data collected between 2005 and 2012 in Oklahoma

Figure 6. Photograph of flight-test terrarium. This beetles were inserted into the cups via a PVC pipe that was manipulated from outside the terrarium, in order to maintain conditions in the terrarium.

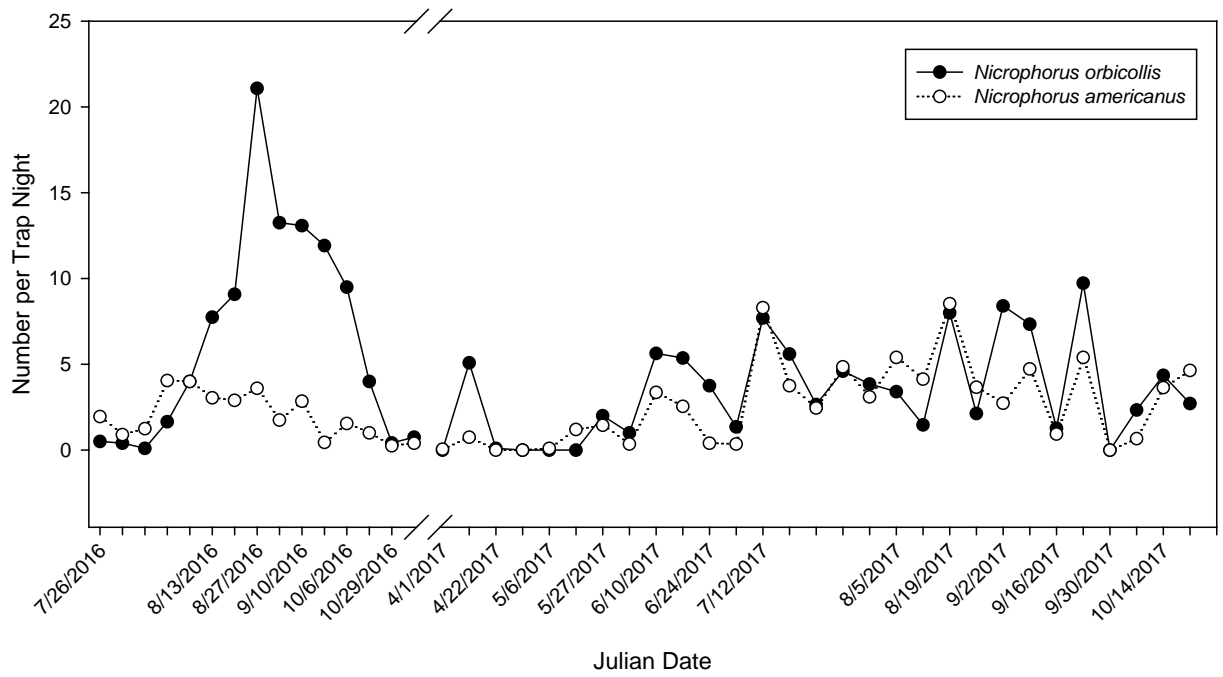Figure 7. Average July air temperature in Tahlequah, Oklahoma from 1994 to 2017.

Figure 1. Beetles per trap night for *Nicrophorus americanus* (hollow circles) and *N. orbicollis* (filled circles) at Camp Gruber near Braggs, Oklahoma. Dates represent the morning of checks for either 2-day or 5-day sample periods.
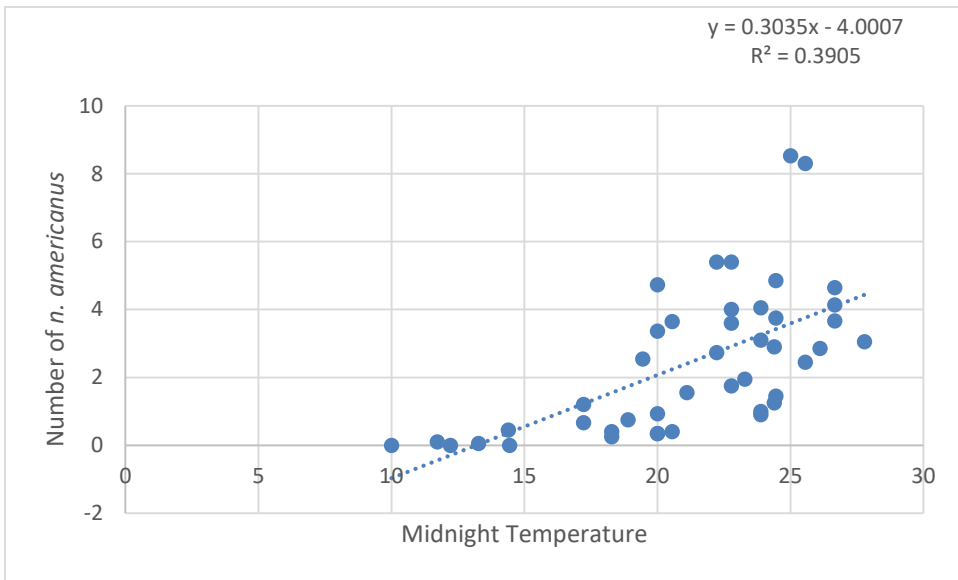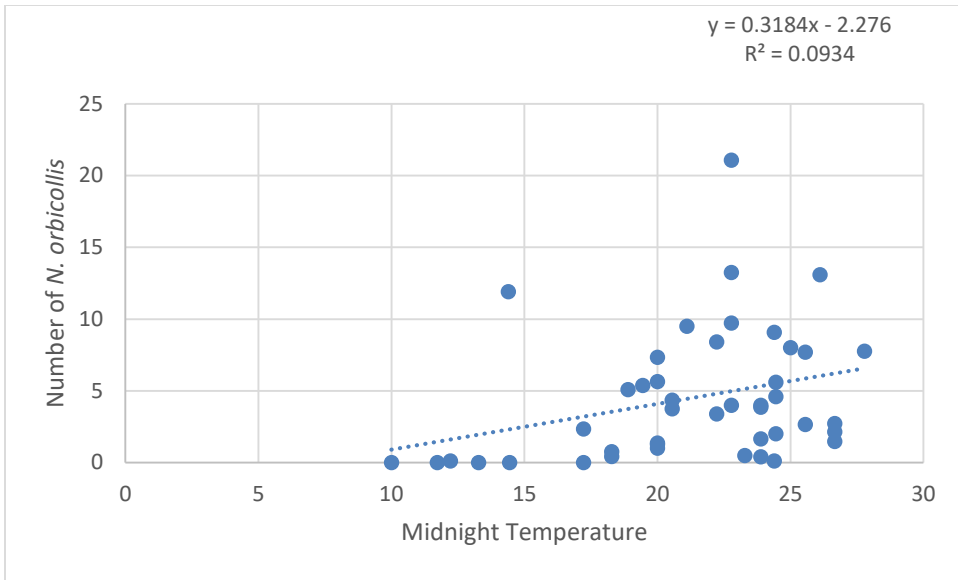
Figure 2. Graph of capture data for *Nicrophorus orbicollis* and *Nicrophorus americanus* with temperature at midnight for beetles captured at Camp Gruber near Braggs, Oklahoma.
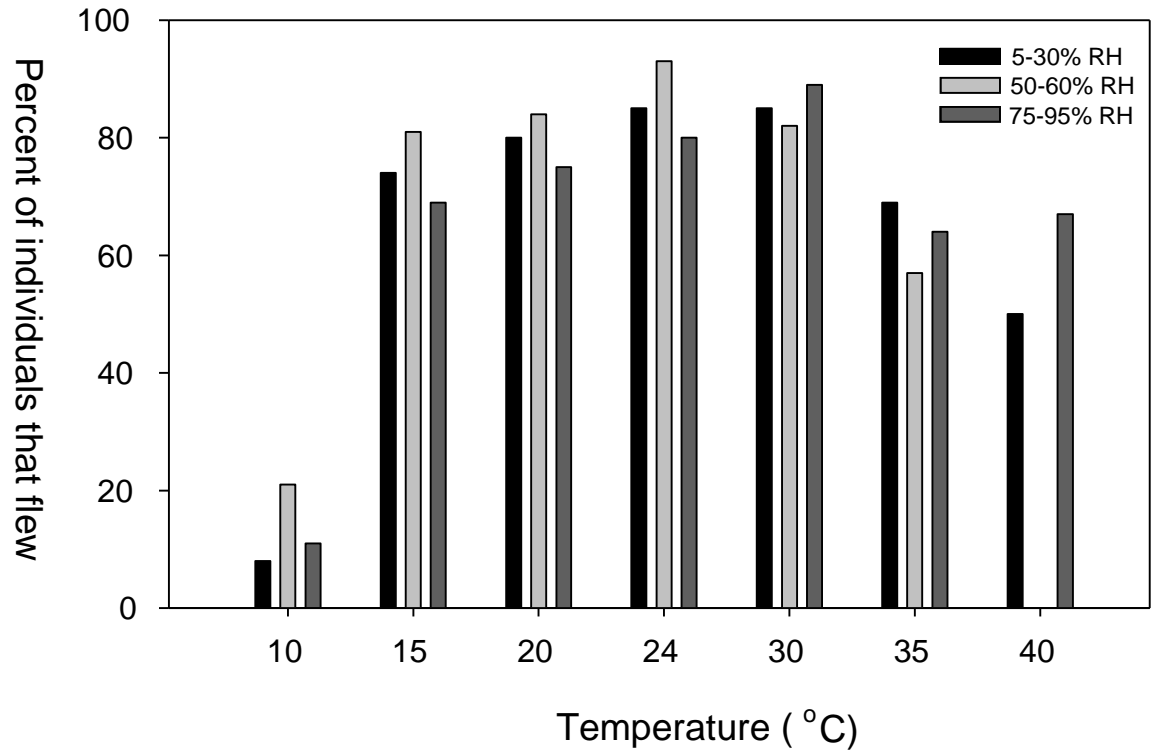
Figure 3. Percentage of *Nicrophorus orbicollis* that flew under different humidity and temperature combinations.

Figure 4. Scatter Plot and line graph of midnight temperatures vs captures of *N. americanus* for data collected between 2001 and 2012 in Nebraska. Data provided by Drs. Amy Smith and Wyatt Hoback.
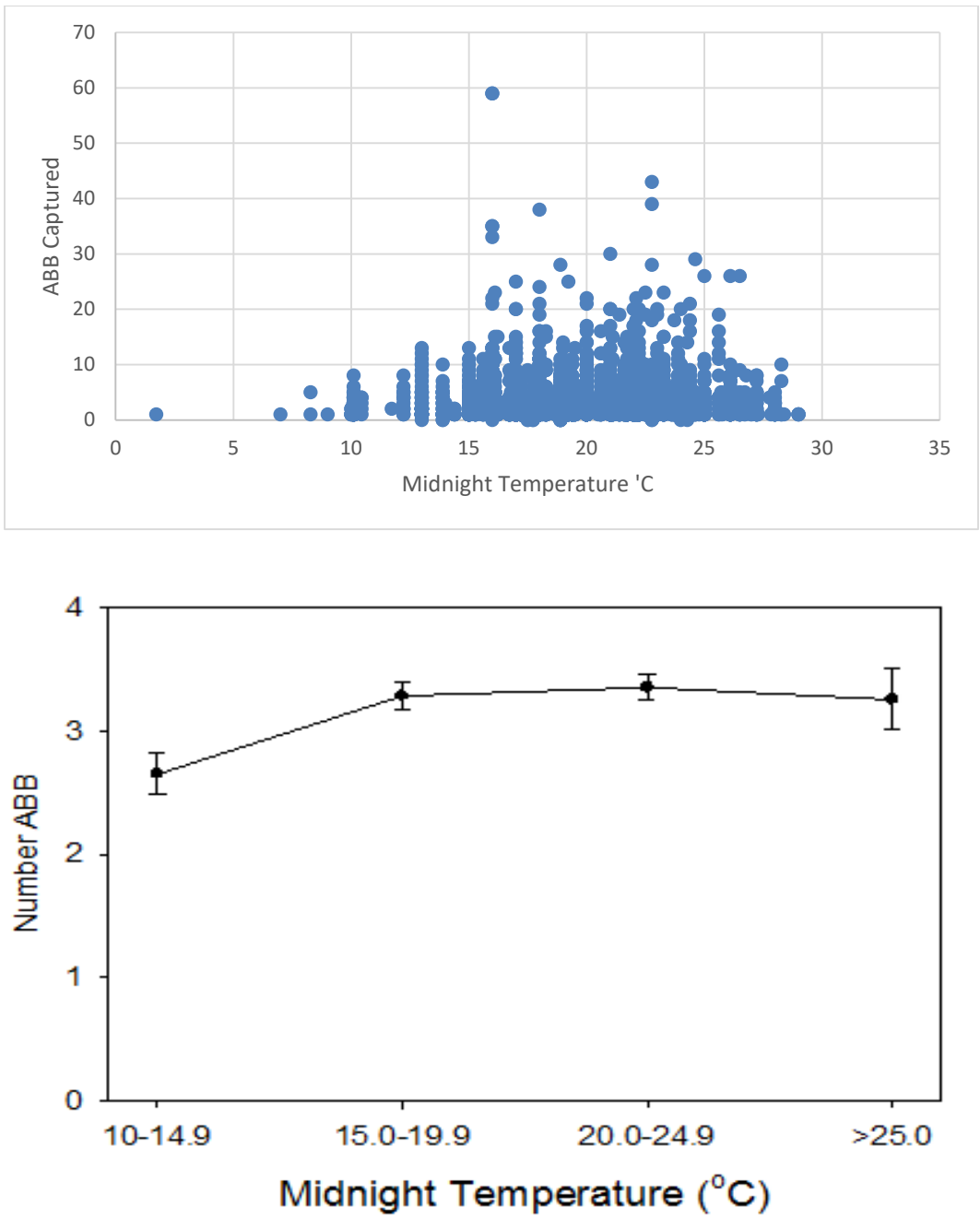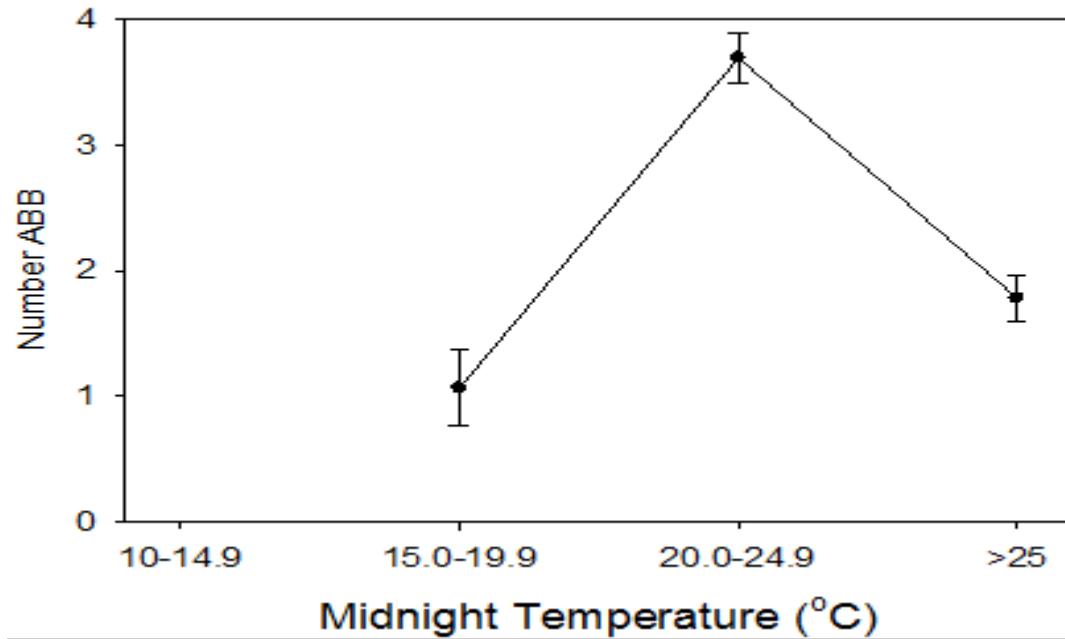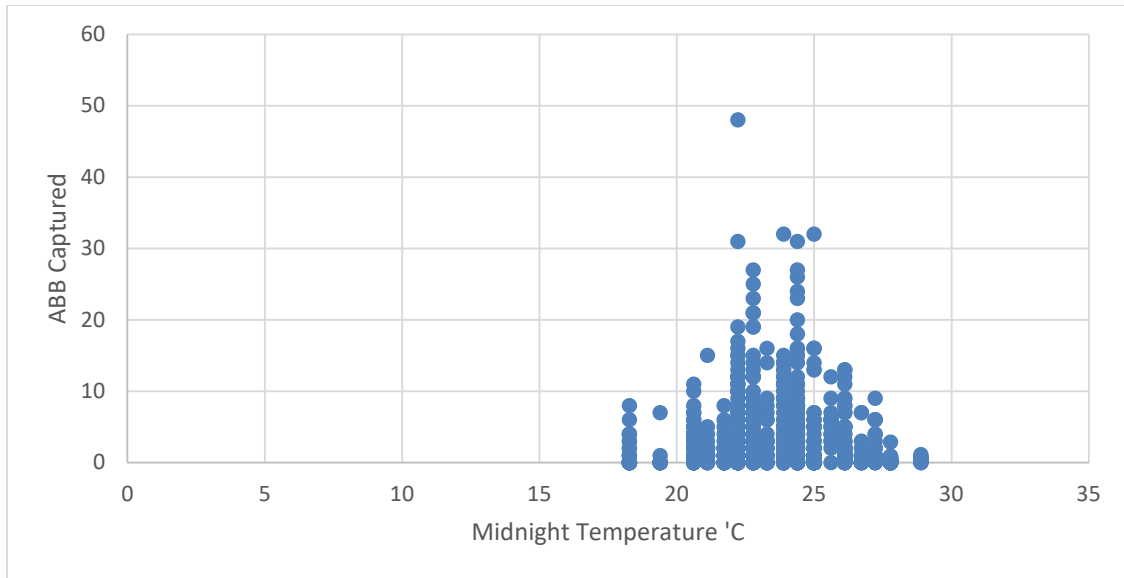
Figure 5. Scatter Plot and line graph of midnight temperatures vs captures of *N. americanus* for data collected between 2005 and 2012 at Camp Gruber in Braggs, Oklahoma. Data provided by Drs. Amy Smith and Wyatt Hoback.

Figure 6. Photograph of flight-test terrarium. This beetles were inserted into the cups via a pvc pipe that was manipulated from outside the terrarium, in order to maintain conditions in the terrarium.

Figure 7. Average July air temperature in Tahlequah, Oklahoma from 1994 to 2017

(From Mesonet)

Chapter II Works Cited

Anderson, R.S. and S.B. Peck. 1985. The carrion beetles of Canada and Alaska. The
  Insects and Arachnids of Canada,. Publication 1778, Research Branch Agriculture
  Canada, Ottawa Part 13, pp. 1–121.

Attisano, A., J.T. Murphy, A. Vickers. P.J. Moore. (2015). A Simple Flight Mill for
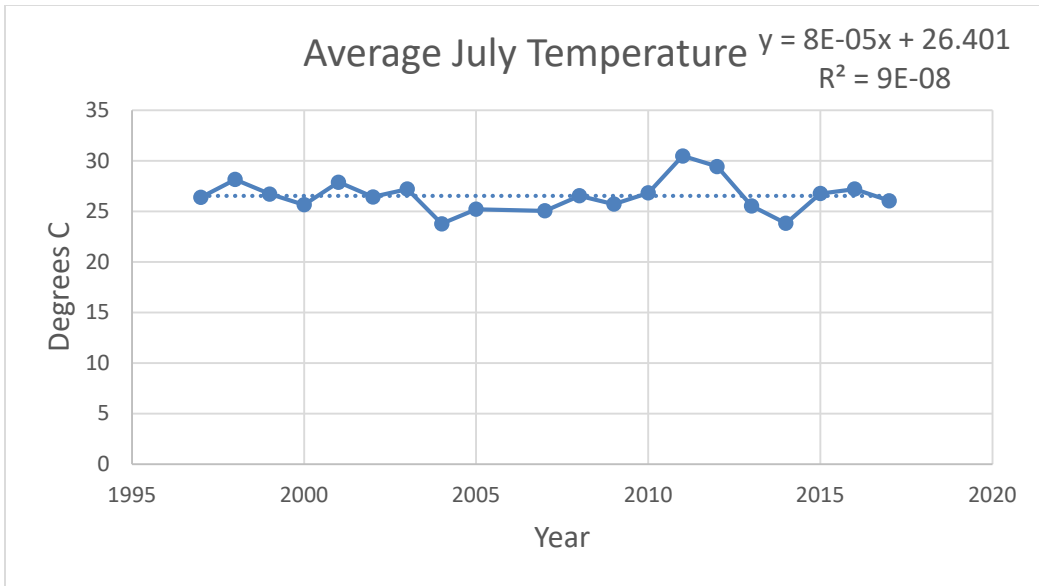 the Study of Tethered Flight in Insects. J. Vis. Exp. (106), e53377,
  doi:10.3791/53377.

Attisano, A., and R.M. Kilner. (2015). Parental effects and flight behaviour in the
  burying beetle, *Nicrophorus vespilloides*. *Animal Behaviour*, *108*; 91–100.

Beament, J.W.L. 1964. The active transport and passive movement of water in Insects.
  Advances in Insect Physiology Volume 2. 67–129.

Bedick, J.C., B.C. Ratcliffe, and L.G. Higley. 2004. A new sampling protocol for the
  endangered American burying beetle, *Nicrophorus americanus* Olivier
  (Coleoptera: Silphidae). The Coleopterists Bulletin. 58: 57–70.

Bedick, J.C., W.W. Hoback, and M.C. Albrecht. 2006. High water-loss rates and rapid
  dehydration in the burying beetle, *Nicrophorus marginatus*. Physiological
  Entomology. 31: 23–29.

Church, N.S. 1960. Heat loss and the body temperatures of flying insects. Department
  of Zoology, University of Cambridge, and Canada Agriculture Research Station,
  Lethbridge, Alberta.

Cloudsley-Thompson J.L. 1975. Adaptations of Arthropoda to arid
  environments. Annual Review of Entomology 20: 261-283.

Cockbain., A.J. 1961. Low temperature thresholds for flight in Aphis fabae Scop.

Entomologia Experimentalis et Applicata, 4: 211–219.

Creighton, J.C. and G. Schnell. 1998. Short-term movement patterns of the endangered

American burying beetle *Nicrophorus americanus*. Biological Conservation 86:

281-287.

DeVault, Travis L., Zachary H. Olson, James C. Beasley, and Olin E. Rhodes Jr.

2011. Mesopredators dominate competition for carrion in an agricultural

landscape. Basic and Applied Ecology. 12(3): 268-274.

Eggert, A.K., J.K. Muller. 1997. Biparental care and social evolution in burying beetles:

lessons from the larder. In *The Evolution of Social Behavior in Insects and*

Arachnids. New York: Cambridge University Press. 216-236.

Heinrich, B. 1974. Thermoregulation in endothermic insects. Science 185: 747-756.

Hoermann, C.V., S. Steiger, J.K. Müller, M. Ayasse. 2013. Too fresh is unattractive!

The attraction of newly emerged *Nicrophorus vespilloides* females to odour

bouquets of large cadavers at various stages of decomposition. PLoS One

8:e58524.

Hoback, W.W., L.G. Higley, and D.W. Stanely. 2001. Tigers eating tigers: evidence of

intraguild predation operating in an assemblage of tiger beetles. Ecological

Entomology. 26: 367-375.

Johnson, C.G. and L.R. Taylor. 1957. Periodism and energy summation with special

reference to light rhythms in aphids. Journal of Experimental Biology. 34; 209–

221.

Jurzenski, J.D., C.F. Jorgensen, A.W, R. Grosse, J. Riens, and W.W. Hoback. 2014.
Identifying priority conservation areas for the American burying beetle,
Nicrophorus americanus (Coleoptera: Silphidae), a habitat generalist. Systematics
and Biodiversity. 12: 149–162.

Kalinova, B., H. Podskalska, J. Ruzicka, M. Hoskovec. 2009. Irresistible bouquet of
death – how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by
carcasses. Naturwissenschaften 96: 889–899.

Merrick, M.J., R.J. Smith. 2004. Temperature regulation in burying beetles
(Nicrophorus spp. Coleoptera: Silphidae): effects od body size, morphology and
environmental temperature. Journal of Experimental Biology. 207:723-733

Morgan, K.R. 1985. Temperature regulation and terrestrial activity in the ectrothermic
beetle *Cicindela tranquebarica.* Physiological Zoology 58: 29-37.

Raithel, C.J., and H.S. Ginsberg. 2006. Population trends and flight behavior of the
American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae) on
Block Island, RI. Journal of Insect Conservation. 10: 317-322.

Scott, M.P. 1998. The ecology and behavior of burying beetles. Annual Review of
Entomology. 43: 595–618.

Scott, Michelle Pellissier, and James F.A. Traniello. 1990. Behavioral and Ecological
Correlates of Male and Female Parental Care and Reproductive Success in
Burying Beetles (Nicrophorus Spp.). Animal Behaviour 39.2: 274-83.

Smith, A. 2017. Personal communication. OSU master thesis committee.

U.S. Fish and Wildlife Service. (2014). American burying beetle *Nicrophorus
americanus* -Olivier. Retrieved from

https://www.fws.gov/southwest/es/oklahoma/Documents/ABB/Conference%20Materials/6_American%20Burying%20Beetle%20Biology.pdf

Wilson, D.S., W.G. Knollenberg, and J. Fudge. 1984. Species packing and temperature dependent competition among burying beetles (Cilphidae, *Nicrophorus*). Ecological Entomology 9: 205-216.

CHAPTER III

COMPARISON OF WATER LOSS RATES AND SURVIVAL OF DESICCATION

AMONG THE CARRION BEETLES (COLEOPTERA: SILPHIDAE)

Abstract

In arthropods physiological processes, including respiration and excretion, contribute to water loss and most terrestrial insects have adaptations to reduce these losses. Among the beetles, the family Silphidae has shortened elytra. The exposed abdomen has been suggested to contribute to high water loss rates reported for *Nicrophorus marginatus*, a day-active species. In this study, the rates of water loss were compared for 8 species in the family Silphidae under desiccating conditions at 23°C. The tested species belonged to *Nicrophorus* (*N. orbicollis, N. tomentosus, N. pustulatus, N. carolinus,* and *N. marginatus*) and three other genera, *Necrophila americana*, *Necroides surinamensis*, and *Thanatopholes lapponicus*. The rates of water loss varied among all tested species of *Nicrophorus* with *N. orbicollis,* a nocturnal species, suffering fatal water loss the fastest. The day active *N. carolinus* had the slowest water-loss rates. While water loss rates varied among other species tested, they were not strongly associated with activity pattern or size. The data generated through these experiments predicts changes in carrion beetle assemblages based on changing moisture conditions. Because the federally endangered American burying beetle, *N. americanus* is the most similar to *N. orbicollis*, it is likely that the species will be limited by drying conditions.

Introduction

   Water is essential for all living organisms including insects and access to fresh

water limits the occurrence of many species in terrestrial environments. Insects exposed

to low humidity environments experience water loss. This water must be replaced at a

rate that is greater or equal to water loss or the insect will desiccate and die (Hadley 1994).

A physiological contributor to water loss is excretion. Excretion in insects includes fluids lost through molting, salivation, external pre-digestion, release of waste, exchange of fluids and resources while mating, and release of pheromones (Hadley 1994). Terrestrial arthropods exhibit many adaptations to reduce desiccation and to replace lost water through drinking and absorption from the environment. For example, tenebrionid larvae can absorb water though the rectum or through the cuticle, stopping when "saturation" is achieved (Beament 1964). Thysanura can absorb or collect water from air with 70 percent or greater humidity across their body wall and transport it into their hemolymph (Beament 1964). However, this adaptation also prevents these insects from inhabiting very dry environments where they lose water rapidly.

Many species also exhibit behaviors to reduce water loss and survive in otherwise inhospitable environments. For example, three separate species of tenebrionids from the Namib desert, expose their bodies to fog which collects on their surface and channels to their mouthparts (Seely and Hamilton 1976). Long-legged silver ants (*Cataglyphis bombycina*) take advantage of the afternoon heat, leaving borrows in search of arthropods that have succumbed to moisture loss or heat exhaustion from the 60° C sand and arid atmosphere (Heinrich 1996). They move quickly on thin, stilt-like legs as their metallic bodies reflect the sun's heat. These ants have tightly sealed cuticles that reduces significant moisture loss, and they do not shed water as a primary means of cooling off. Their spiracles remain closed as the ants survey the terrain, only opening when internal carbon dioxide builds up (Heinrich 1996). The ants generate water as a byproduct of

metabolism and cool themselves by running quickly, increasing locomotory convection (Heinrich 1996).

Low humidity and higher temperatures causes greater water loss and increased chances of desiccation. Thermal maxima for most insects is between 40° and 50° C (Heinrich 1981). Insects prone to high rates of water loss may use adaptive behaviors, increasing their chances of survival in environments where moisture is scarce or hard to obtain. Day active individuals, including the carrion beetle *Nicrophorus marginatus*, may avoid the hottest parts of the day (Bedick *et al.* 2006), leading to a bimodal pattern of activity.

Carrion beetles (Coleoptera: Silphidae) are a unique family of beetles which utilize resources found in dead vertebrates for feeding and reproduction. They have been extensively studied because of their unique behaviors and co-occurrence of multiple species.

Unlike most beetles, the family Silphidae is characterized as having shortened elytra that expose multiple segments of the abdomen. The exposed abdomen potentially provides a means of cooling during the long flights in search of the ephemeral carrion resources they seek (Merrick and Smith 2004). Although characterized as persistent fliers, individuals have been observed resting after intense short flights. Burying beetles have been observed to drink frequently and access to moist soil substantially reduces trap mortality on hot days (Bedick *et al.* 2002).

Among the *Nicrophorus,* co-occurring species differ in adult size and often differ in seasonal and daily activity patterns, likely as a result of niche partitioning (Scott 1998). Species have also been found to differ in habitat associations with some species occurring

in more arid environments than others (Anderson and Peck 1985, Bishop *et al*. 2002). However, the impacts of abiotic factors on the differences in activity patterns and reasons for habitat association have not been tested for most *Nicrophorus* nor has there been tests conducted on the more diverse subfamily Silphinae.

This study was conducted to test the hypothesis that all Silphidae rapidly lose water when in desiccating environments. The data will help further explore the abiotic constraints on silphid beetle distributions.

Materials and Methods:

Study sites and capture techniques for insects

The Silphidae used in this study were collected during surveys conducted during May, June, and July, 2017 from three different sites in Oklahoma and Nebraska. The sites were: Camp Gruber near Braggs, in north-central Oklahoma, the Oklahoma State University Lindley Research and Demonstration Farm near Valliant, in Southcentral Oklahoma, and near the town of O'Neill, Nebraska, in north-central Nebraska. All sampling was conducted in accordance with the U.S. Fish and Wildlife (USFWS 2014) protocol. Traps consisted of 5-gallon buckets topped with pitfall-trap lids. Traps were provided with moist peat moss for substrate and baited with previously frozen rotten rats to attract carrion beetles. Traps were checked in the morning between 6am and 10am. The beetles brought from the field were maintained in the laboratory with substrate and water and were fed ground beef, for no more than one week prior to testing.

Table 1. Species and number of individuals tested for tolerance of desiccation.

Subfamily	Species	Individuals tested
Silphinae	*Necrodes surinamensis*	21
	Necrophila americana	12
	Thanatophilus lapponicus	20
Necrophorinae	*Nicrophorus orbicollis*	48
	Nicrophorus tomentosus	20
	Nicrophorus pustulatus	24
	Nicrophorus carolinus	20
	Nicrophorus marginatus	35

Figure 1: Species tested during desiccation trials

Water-loss rates

Anhydrous calcium sulfate 'Drierite' desiccant was used to decrease the relative humidity in the testing chamber. The test chamber allowed 40 individuals to be tested each time. The experimental group contained 40 randomly selected individuals among all beetle species.

Prior to experiments, the beetles were kept in moist peat moss substrate with food and water provided. At the beginning of an experiment, live weight was measured after substrate was removed using dry brushes and paper towels to gently swab the beetle. Individual beetles were placed into small plastic 60 ml (2-ounce) mini-cups with matching lids which were marked with reference numbers. The weight of the cups and lids was recorded. Individual beetles were inserted into a cup and weighed to the nearest milligram using a shielded Mettler Toledo PB303-S scientific scale.

The test beetles, were placed into a temperature-controlled test chamber at 23°C (77°F). A tray containing Drierite was placed at the bottom of the chamber to produce extremely low humidity. Beetles were checked for survival and re-weighed each hour at which time the Drierite was stirred to insure consistent moisture control. Living beetles were reweighed every hour until all had died (beetle on its back, no movement in

response to agitation of the chamber) or the experiment reached 48 hours. During the experiments, an empty cup and lid was reweighed every time the beetles were weighed to ensure that the weight of the plastic cup had not changed from exposure to experimental conditions. All tests were repeated five times using different beetles for a total of 200 individuals tested. Ten individuals for each species served as controls.

Beetles in the control group were placed into the same small plastic perforated cups with lids, and placed into the chamber containing a tray of Drierite. Each of the control beetles was provided a water-soaked cotton-ball from which they could drink. Control beetles were checked each hour and deaths were recorded. The cotton balls for the control beetles were rewetted at least once every 12 hours. Control beetles were not reweighed but were checked for mortality.

All tests stopped at 48 hours although a few individuals were still alive. At the conclusion of trials, remaining live beetles were euthanized by placing into a freezer. All beetles were placed in a drying oven set at 40° C and left for two weeks and then re-weighed to measure dry weight.

Statistical analysis

The dried weights of the beetles were recorded and used to compare with wet weight. 'Percent water loss at death' was calculated by subtracting the weight of a fully dried beetle from the starting live weight to get total water content. The amount of water lost at death was calculated and compared to total water content to provide the percent water-loss at death. The average survival time while exposed to extremely desiccating conditions was compared among species using Analysis of Variance (ANOVA) followed by a Tukey test to separate means. Percent water loss resulting in death was also

compared among species using ANOVA. Percent water loss rates were analyzed by calculating mean change in mass ($\pm$ 1 S.E.) per hour for each tested species and then using linear regression to determine the relationship between time and mass loss.

Results:

No mortality occurred in control beetles over the course of experiments. Beetles in the control, with wet cotton balls, group exhibited fewer signs of stress compared to experimental (desiccating) groups, which exhibited heightened activity and regular attempts to escape. Individuals in the test group were often observed clinging to the holes in the cups and lids or running in circles in the cup.

Within the test groups the average survival time varied by species (Figure 12). The most susceptible was *Nicrophorus orbicollis* which is night-active. Surprisingly, the next two most susceptible species were the day active *N. marginatus* and *N. tomentosus* followed by *N. pustulatus*. The most resistant species was *N. carolinus*, a day-active species that occurs in dry sandy soils (Bishop *et al.* 2002).

We compared day active species to each other. *Nicrophorus carolinus, N. marginatus.* and *N. tomentosus* varied slightly in water loss rates (Figure 9 a-c). We also compared the two nocturnal species; *N. orbicollis* and *N. pustulatus (Figure 10 a and b)* for desiccation rates. We found apparent differences though they are not consistent with size or activity patterns. *Nicrophorus. tomentosus* and *N. marginatus* differ in size, but had very similar slopes (around 0.011) in the regression (Figure 9 a-c).

Among the Silphinae, *Necrophila americana*, a species associated with moist habitats, died rapidly. The other two species tested, *T. lapponicus* and *Necrodes surinamesis* had similar survival times despite differing greatly in mass.

The mass of beetles used in these experiments ranged from 0.087 grams for *T. lapponicus* to 1.296 grams for *N. carolinus*. The average percent water lost at death ranged between 45% (*N. orbicollis*) and 57% (*N. marginatus*). Most individuals died around 53% water loss (Figure 8). Although *N. carolinus* is the largest species tested and had the greatest survival times (Figure 12), it was no more tolerant of water loss than most of the other species.

The rate of water loss varied among species tested. The *Nicrophorus* that lost water at the slowest rate was *N. carolinus* while *N. pustulatus* and *N. marginatus* had similar rates of loss. The greatest water loss rates were observed in *N. orbicolllis* (Figure 8). Among the Silphinae, *N. americana* had the greatest water loss rates (Figure 11 a-c).

Discussion

These experiments tested the survival of dehydration and water loss rates of five species of *Nicrophorus* and three species of carrion beetles that differ in size, activity pattern, and habitat association. All species were similar in the amount of desiccation that resulted in death (Figure 2) with species of carrion beetles tolerating approximately 50% loss of mass before expiring. Despite similarities in lethal water loss, the time of exposure to desiccation that produced lethal water loss significantly varied among species (Figure 12). The most resistant species was *N. carolinus*, followed by *N. marginatus*

which are both diurnal burying beetle species. The night-active species of *Nicrophorus* varied, with *N. orbicollis* being susceptible but *N. pustulatus* being resistant. Among the other carrion beetles, the night active *Necroides surinamensis* was more resistant than day active *Necrophila americana*.

Laboratorystudies that characterize the rate of water loss for a species assemblage can help predict survival likelihood in different environments and improve models of species distribution while improve the understanding of niche partitioning (Bishop *et al.* 2002). During periods of daily and seasonal inactivity, silphids will seek refuge in the soil (Willemsens 2015; Bedick *et al.* 2002).

Although water loss rates were similar among individuals tested) the percent water loss leading to mortality varied among individuals tested), likely because of variance in water and fat content and potentially as a result of differences between the sexes. A well fed beetle with large body mass and access to water can store excess nutrients and water while a smaller beetle with higher surface area to volume ratios may be at a disadvantage. All experimental beetles were provided ground beef and water during housing so the nutritional state and hydration prior to experiments should have been similar. During testing, control individuals all lived for over two weeks with access to water but without being fed.

The Necrophorinae have truncate elytra that exposes the abdomen which may be a factor in high water loss rates (Bedick *et al.* 2004). Within Silphinae, there are greater morphological differences, and diversity of genera; however, all possess an exposed abdomen similar to *Nicrophorus*. The smallest silphid tested was *Thanatophilus lapponicus*, which occurs in the same habitats as *N. marginatus* and *N. carolinus*

53

(Anderson and Peck 1985, Jurzenski *et al.* 2011), which were the two *Nicrophorus* species that had the longest survival times in desiccating conditions. Reasons for its resistance to desiccation and what other factors limit its range should be investigated.

Staphylinids and Meloids also have exposed abdomens. Future tests should be conducted on these families to determine similarities with Silphids. Staphylinids are also regularly recovered from traps baited with carrion. If the exposed abdomens of all these species serve similar functions, then data should be obtained and compared.

Nicrophorus orbicollis and *N. pustulatus* are similar in size and morphology, yet orbicollis loses water and dies faster. This difference could be driven by a variety of factors, including the lack of microbial activity in oral secretions of *N. pustulatus* (Jacques *et al.* 2009). *N. pustulatus* is a brood parasitoid and it often takes over the brood ball of another species. The production of expensive chemicals may take a water toll on the species that produce antimicrobial secretions.

In the diurnal species, *N. carolinus* loses water at a slower rate than *N. marginatus* and *N. tomentosus*. Jacques *et al*. (2009) noted that, similar to *N. pustulatus*, *N. carolinus* did not produce a secretion that reduced microbial activity in their tests. More research on *Nicrophorus* physiology and chemical ecology should be conducted to determine what drives differences in desiccation rates among silphids.

A major reason for studying water relations for silphid species is to determine optimal protocols for detection, including the use of landsat to drive sampling efforts. Leasure (2017) aimed to perfect detection using landsat and predictive models. Wind speed, in combination with dew point and temperature, can affect desiccation rates. Landsat images, in conjunction with GIS, can help determine ideal sampling areas.

CHAPTER III FIGURES

Figure 8: The percent of total water content at death is reported in this figure. *N. orbicollis* was most susceptible to death by desiccation, dying when it lost under 45 percent of its total water content. *N. marginatus* was the hardiest, capable of losing greater than 55 percent of its water content before death to desiccation. Note the scale of the Y axis.

Figure 9a-c. Mean ($\pm$ 1 S.E.) water loss rates by time for three diurnal species of *Nicrophorus*.

9a. *N carolinus*
9b. *N. maginautus*
9c. *N. tomentosus*

Figure 10 a-b. Mean ($\pm$ 1 S.E.) water loss rates by time for two nocturnal species of *Nicrophorus*.

10a. N. orbicollis
10b. N. pustulatus

Figure 11 a-c. Mean ($\pm$ 1 S.E.) water loss rate of three species of Silphidae: Silphinae.

11a. *Thanatophilus lapponicus*
11b. *Necrodes surinamensis*
11c. *Necrophilla americana*

Figure 12: Hours to death varied in Silphidae. *T. lapponicus* survived longer than expected, as it was the smallest tested beetle. *N. orbicollis* and *N. americana* died very quickly. *N. marginatus, N. surinamensis,* and *N. carolinus* were among the longest living at the tested conditions. Groups with the same letters are not statistically different from each other.
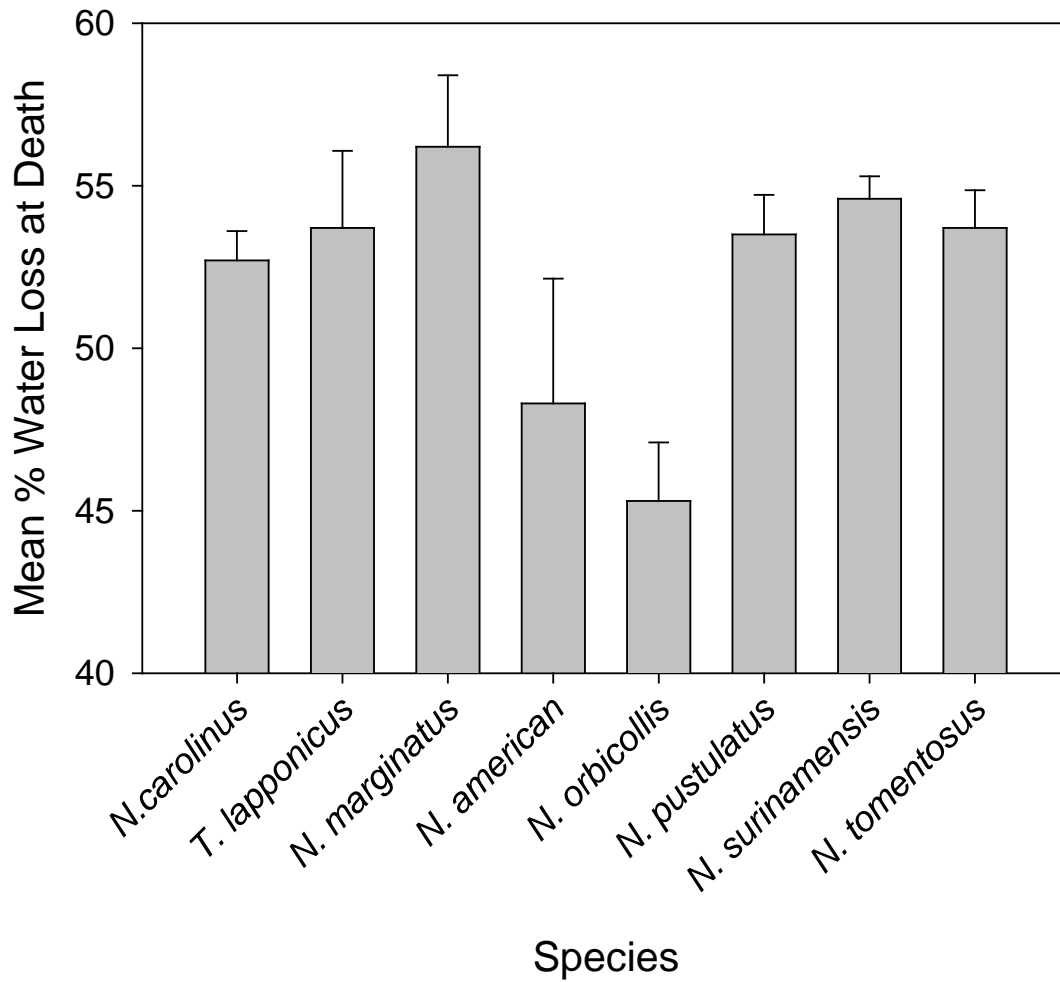
Figure 8: The percent of total water content at death is reported in this figure. *N. orbicollis* was most susceptible to death by desiccation, dying when it lost under 45 percent of its total water content. *N. marginatus* was the hardiest, capable of losing greater than 55 percent of its water content before death to desiccation. Note the scale of the Y axis.
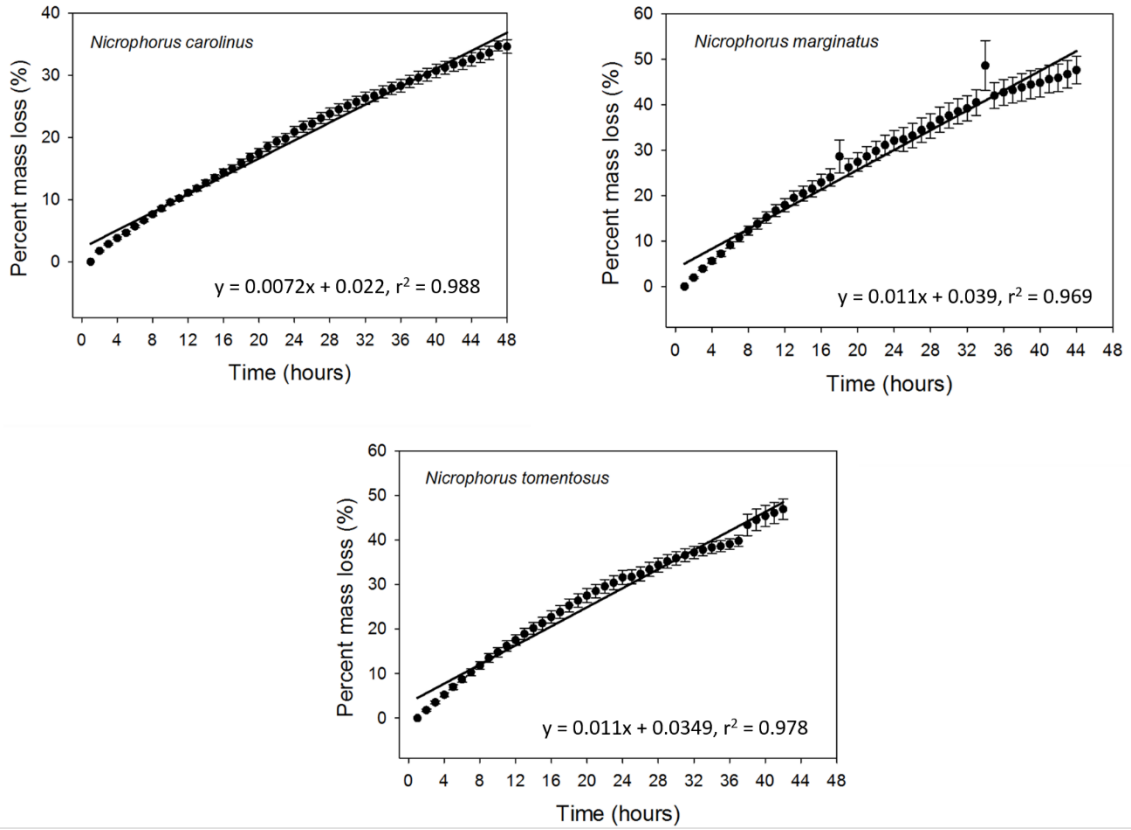
Figure 9a-c. Mean ($\pm$ 1 S.E.) water loss rates by time for three diurnal species of

Nicrophorus.

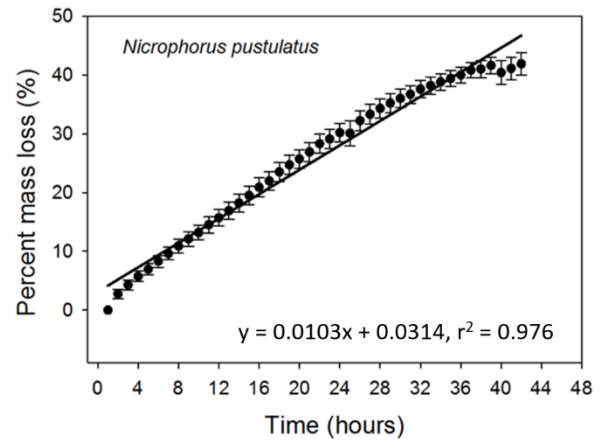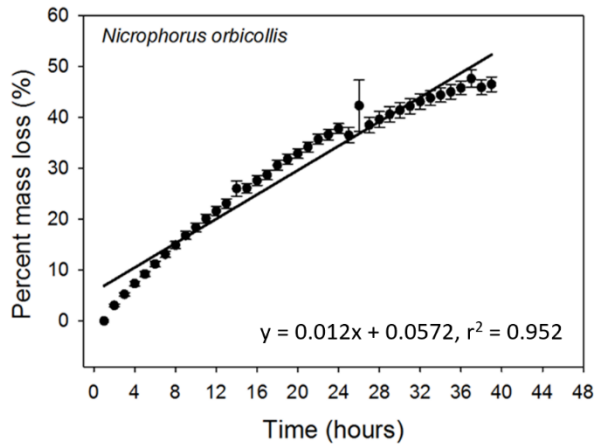9a. *N carolinus*

9b. *N. maginautus*

9c. *N. tomentosus*

Figure 10 a-b. Mean ($\pm$ 1 S.E.) water loss rates by time for two nocturnal species of

Nicrophorus.

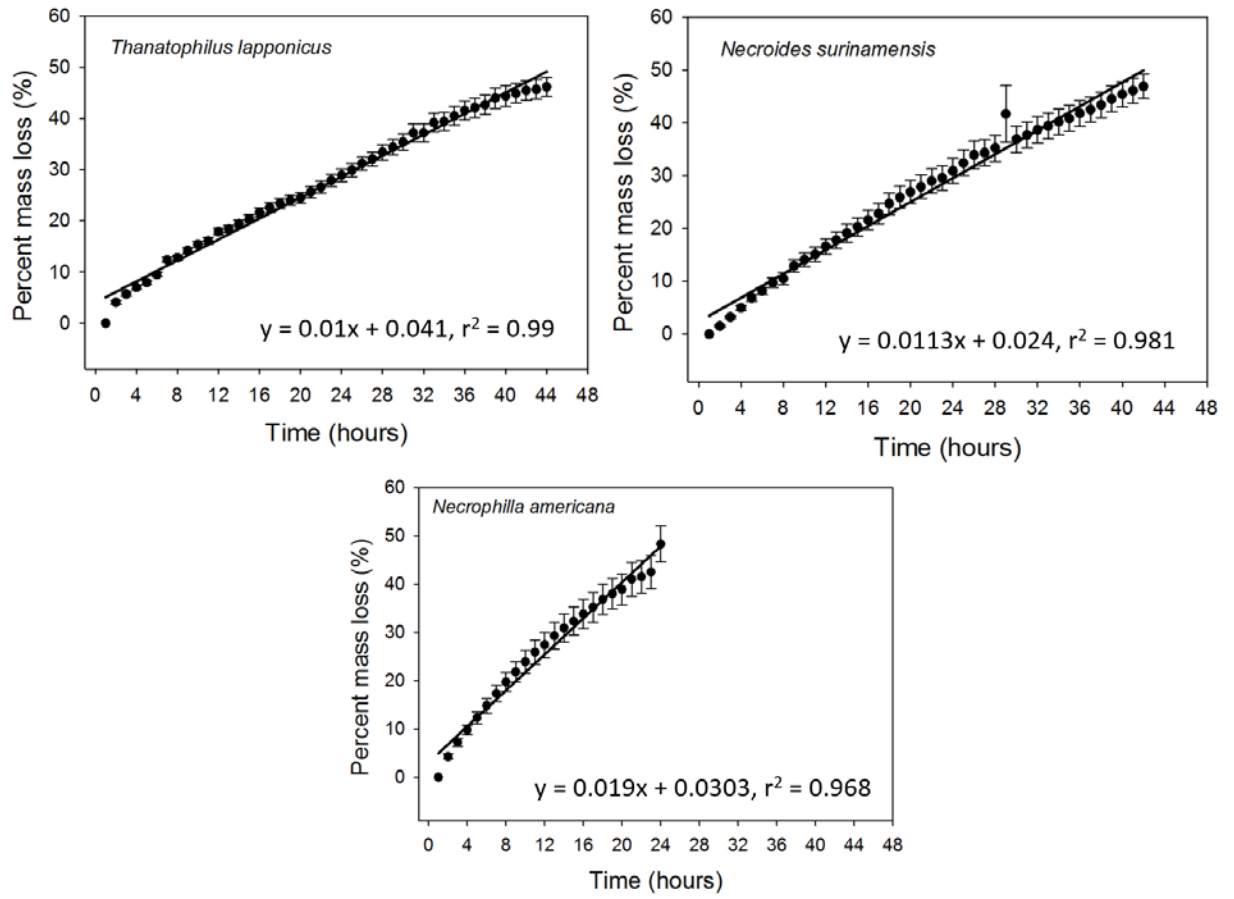*10a. N. orbicollis*
10b. N. pustulatus

Figure 11 a-c. Mean ($\pm$ 1 S.E.) water loss rate of three species of Silphidae.

11a. *Thanatophilus lapponicus*

11b. *Necrodes surinamensis*
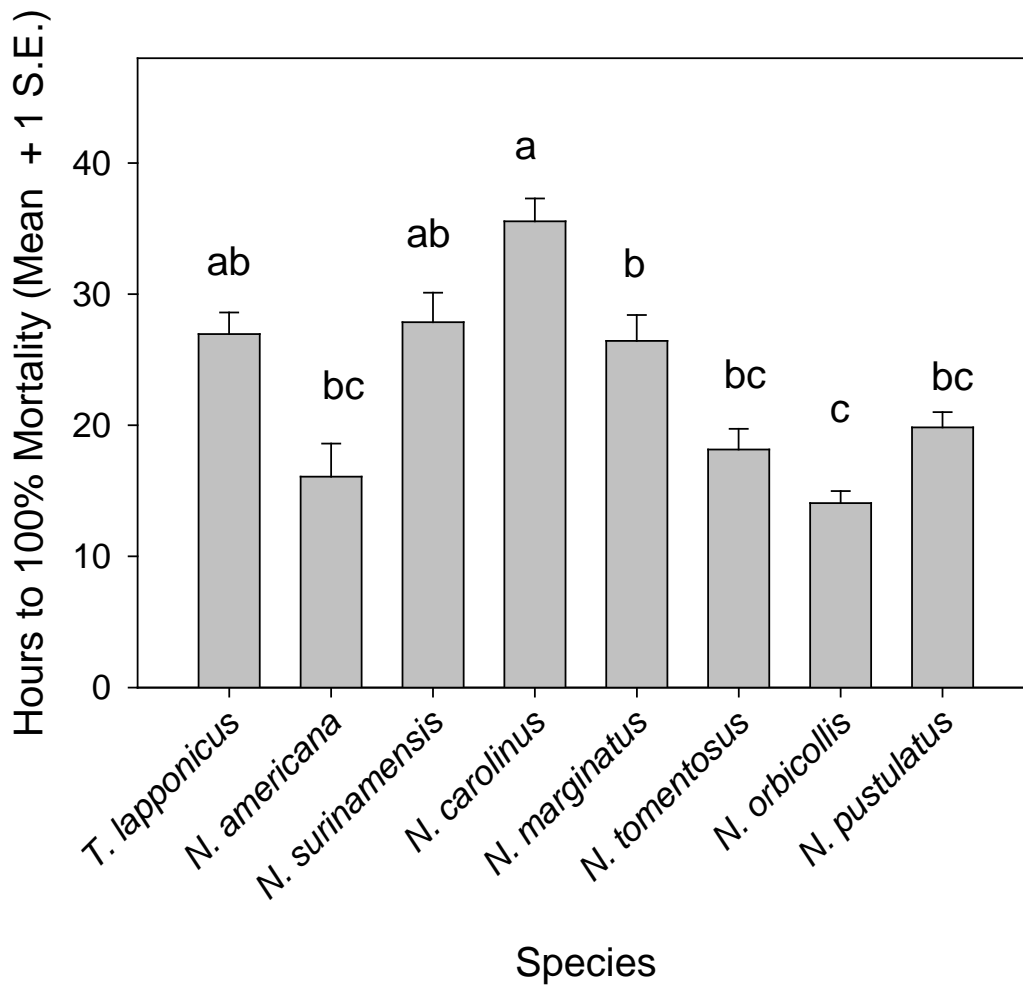
11c. *Necrophilla americana*

Figure 12: Hours to death varied in Silphidae. *T. lapponicus* survived longer than expected, as it was the smallest tested beetle. *N. orbicollis* and *N. americana* died very quickly. *N. marginatus, N. surinamensis,* and *N. carolinus* were among the longest living at the tested conditions. Groups with the same letters are not statistically different from each other.

CHAPTER III WORK CITED

Anderson, R.S. and S.B. Peck. 1985. The carrion beetles of Canada and Alaska. The Insects and Arachnids of Canada,. Publication 1778, Research Branch Agriculture Canada, Ottawa Part 13, pp. 1–121.

Beament, J.W.L. 1964. The active transport and passive movement of water in Insects. Advances in Insect Physiology Volume 2. 67–129.

Bishop, A.A., W.W. Hoback, M. Albrecht, and K. Skinner. 2002. A comparison of an ecological model and GIS spatial analysis to describe niche partitioning amongst carrion beetles in Nebraska. Transactions in GIS. 6: 457-470.

Bedick, J.C., W.W. Hoback, and M.C. Albrecht. 2006. High water-loss rates and rapid dehydration in the burying beetle, *Nicrophorus marginatus*. Physiological Entomology. 31: 23–29.

Hadley, N.F. 1994. Water Relations of Terrestrial Arthropods. Academic Press, San Diego.

Heinrich, B. 1996. The Thermal Warriors: Strategies of Insect Survival. Harvard University Press. Cambridge, Mass.

Heinrich, N. 1981. Ecological and evolutionary perspectives; Insect Thermoregulation. Wiley, New York. 236-302.

Jurzenski, J., D.G Snethen, M.L. Brust, W.W. Hoback. 2011. New records of carrion beetles in Nebraska reveal increased presence of the American burying beetle, *Nicrophorus americanus* Olicer (Coleoptera: Silphidae). Great Plains Research. 21: 131-143.

Leasure, D.R. 2017. Landsat to monitor an endangered beetle population and its habitat:
Addressing annual life history and imperfect detection. Insect Conservation and
Diversity .

Jacques, B.J., S. Akahane, M. Abe, W. Middleton, W.W. Hoback, J.J Shafer. 2009.
Temperature and food availability differentially affect the production of
antimicrobial compounds in oral secretions produced by two species of burying
beetle. Journal of Chemical Ecology. 35: 871-877.

Maruyama, M., K. Kambara, H. Naka, and M. Azuma. 2015. Insect water-specific
aquaporins in developing ovarian follicles of the silk moth *Bombyx mori*: role in
hydration during egg maturation. The Biological Bulletin 229.1: 58-69.

Merrick, M.J., R.J. Smith. 2004. Temperature regulation in burying beetles
(Nicrophorus spp. Coleoptera: Silphidae): effects of body size, morphology and
environmental temperature. Journal of Experimental Biology. 207:723-733.

Seely, M.K., and W.J. Hamilton. 1976. Fog catchment sand trenches constructed by
Tenebrionid beetles, Lepidochora, from the Namib Desert." Science. 193.4252:
484-86.

U.S. Fish and Wildlife Service. 2014. American burying beetle *Nicrophorus americanus*
-Olivier. Retrieved from
https://www.fws.gov/southwest/es/oklahoma/Documents/ABB/Conference%20M
aterials/6_American%20Burying%20Beetle%20Biology.pdf

Willemssens K.A. (2015. Soil preferences of Nicrophorus beetles and the effects of
compaction on burying behavior. Master Thesis. University of Nebraska –
Lincoln.

VITA

Daniel Benjamin St. Aubin

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF HUMIDITY AND TEMPERATURE ON BURYING BEETLE
(COLEOPTERA: SILPHIDAE)

Major Field: Entomology and Plant Pathology

Biographical:

Education:

Completed the requirements for the Master of Science in Entomology and Plant
Pathology at Oklahoma State University, Stillwater, Oklahoma in December,
2017.

Completed the requirements for the Bachelor of Science in Entomology at
Oklahoma State University, Stillwater, Oklahoma in 2017.

Experience:

Field Collection
Lab Study

Professional Memberships:

Entomological Society of America
Sanborn Entomology Club
EPPGSA