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Niche Specialization and Conservation Biology of *Cicindela nevadica lincolniana*

by Tierney R. Brosius

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
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For the Degree of Doctorate of Philosophy

Major: Entomology

Under the Supervision of Professor Leon G. Higley

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Niche specialization and conservation biology of *Cicindela nevadica lincolniana*

Tierney Rose Brosius, PhD

University of Nebraska, 2010

Advisor: Leon G. Higley

As with many organisms across the globe, *Cicindela nevadica lincolniana* is threatened with extinction. Understanding ecological factors that contribute to extinction vulnerability and what methods aid in the recovery of those species is essential in developing successful conservation programs. Here we examine behavioral mechanisms for niche partitioning along with improving techniques for captive rearing protocol and increasing public awareness about the conservation of this local insect. Ovipositional selectivity was examined for *Cicindela nevadica lincolniana*, *Cicindela circumpecta*, *Cicindela togata*, *Cicindela punctulata*, and *Cicindela fulgida*. Models reflect that these species of co-occurring tiger beetles select different ranges of salinity in which to oviposit thereby reducing the potential for interspecific competition. In a second study, thermoregulatory niche partitioning was examined for the same complex of tiger beetle species. Time spent in the sun, on different substrates, and engaging in various behaviors associated with thermoregulation were significantly different during different parts of the day and between species. I continued along a previous line of study to develop a viable captive rearing program. So far fourteen adult *Cicindela nevadica lincolniana* have been successfully reared in captivity. Overwintering mortality has been determined as a key factor in the mortality of this species in captivity. Finally, I examined the potential for using the visual arts to promote the conservation of *Cicindela nevadica lincolniana* and

associated saline wetlands. The results from surveys conducted at the exhibit suggest that art exhibits can have a strong positive impact on members of the community.

Dedication

Mike and Deb Berger

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Introduction

Across the world multiple species are in danger of becoming extinct from habitat destruction. Habitat conservation often occurs after the habitat has already been drastically reduced and fragmented. How individual organisms cope with this loss has long been an area of interest for conservation biologists. The most common cause of extinction is the reduction and modification of habitat (Hilton-Taylor 2000).

Understanding what happens to ecological communities as their habitats become reduced and fragmented is an important key to understanding how to preserve what is remaining, reconstruct new habitat, and prevent reoccurrences of the same destruction in other areas.

Ecologically, the question of susceptibility to extinction is related to species requisites. All individuals reside within a species-specific niche. What determines the width of the organism's niche is an important component in determining how environmental factors interrelate to produce the population's realized niche. Evidence suggests that variability within an ecologically functional trait is relevant to determining the cause of niche breadth (Roughgarden 1972, Kammer et al 1997, Kotiaho et al. 2005). High variability in a trait suggests a wide niche width, whereas low variability may suggest a narrow niche width (Roughgarden 1972). Some suggest that those individuals residing in a narrow niche are the first individuals most likely to succumb to extinction (Kammer et al. 1997, Kotiaho et al. 2005).

The eastern saline wetland of Nebraska supports a community of saline-adapted tiger beetles that provide a unique opportunity to investigate niche partitioning as it contributes to extinction vulnerability, as well as the population structure of highly restricted invertebrates. Unfortunately, over the past 100 years over 90% of these saline

wetlands have been lost. Aquatic ecosystems are divided into many different classifications, each composed of specific wetland communities. Among the most endangered of these aquatic ecosystems are the eastern saline wetlands of Nebraska (Johnsgard 2001). Frequently, within these wetlands, salt concentrations in the soils and wetlands are so high that the only saline-adapted plants and animals can survive. As a result of the disappearance of these wetlands, many plants and animals endemic to this region are disappearing. Willis (1967) suggested that *Cicindela nevadica lincolniana* Casey (Salt Creek tiger beetle) of Lancaster County, Nebraska might be in decline and this insight was confirmed by population and range estimates (Spomer and Higley 1997). After additional research, *C. n. lincolniana* received state (2000) and federal listing (2005) as an endangered species (Spomer and Higley 1993). Previously, little was known about *C. n. lincolniana*'s biology, but what was known is that its populations were intimately tied to the quickly disappearing saline wetlands of Southeast Nebraska. The growth of the city of Lincoln and the modifications of the rivers and tributaries have destroyed much of the beetle's habitat. Additionally, populations disappeared from some saline wetlands with urban encroachment.

Over the last 15 years, census data has only indicated a maximum of 800 individuals and in some years this number has dropped to as low as 155 individuals (Table 1 and Fig. 1) (Spomer and Higley 1993, Allgeier 2005). There are only 14 distinct populations of which seven have been extirpated (Table 1). More habitat destruction or any sort of catastrophic event could easily lead to complete extinction within just a few years. Many other endangered insect species still have population numbers that range in the thousands (Morgan et al. 2000, Knisley and Hill 2005) not hundreds as we see in this

population. Consequently, *C. n. lincolniiana* is currently considered to be one of the most endangered insect in North America. The population ecology of organisms under this magnitude of pressure from habitat destruction has rarely been examined. The three largest impacts on this habitat are; 1) invasive species, 2) human development of the landscape, and 3) changing ecological communities.

Invasive species are considered one of the biggest potential threats to endemic wildlife populations (Sakai et al. 2001). The natural history of North America is greatly shaped on what one may refer to as invasive species. Throughout its history this continent has been in a constant state of flux as species migrated from Eurasia and South America. The difference between natural immigration and the modern introduction of what we refer to as invasive species is the frequency and volume. Humans have transported organisms around the globe at a greater rate than has ever been seen in the history of this planet. Invasive species destroy native habitats primarily by outcompeting native species. The loss of native species creates a domino effect that can adversely affect multiple organisms within the ecosystem. The tiger beetles within this study area are adapted to saline wetlands that exist among tall grass prairie. This native tall grass is considered to be composed of several types of bunchgrass species. Bunchgrass is the common name for perennial grass species that tend to grow in discrete clumps rather than in sod-like carpets. The area between these clumps provided open spaces for tiger beetles to forage. Cool-season sod grass species were introduced to many areas of North America to increase hay production and help prevent erosion. Unfortunately, many species of sod grass became invasive and began to outcompete native warm-season bunch grass (DiTomaso 2000). In this case the introduced species is causing much of the landscape to

be covered in a monoculture of grass, free from natural disturbances. Additionally, the manipulation of waterways has made many of these wetlands less saline which has made the salt basins more favorable to many invasive and non-saline native species.

Tiger beetles are found in many diverse habitats throughout around the world (Knisley and Schultz 1997, Pearson and Vogler 2001) and as a result they are considered to be useful as biological indicators. Biological indicators are organisms whose presence indicates certain well-defined environmental conditions. The greater the precision these conditions can be defined (life history requirements, habitat features, natural enemies, etc.) the more useful the bioindicator (Wilson 1994).

Conspicuous insects are often used as biological indicators by serving as surrogates for lesser known or visible groups (Samways 2005). For example, dipteran species richness has been found to be slightly correlated with hymenopteran species richness and significantly correlated with the total number of insect orders (Hughes et al. 2000). Pearson and Cassola (1992) illustrated the potential value of tiger beetles as bioindicator species. Geostatistical methods were used to test the usefulness of tiger beetles as a predictor of butterfly species across North America. Their results were significant, suggesting that tiger beetles may be useful indicators of species richness. These examples suggest that insect taxa may be reasonable surrogates for one another when sampling is done across habitat types (Samways 2005).

The tiger beetle communities associated with the Eastern saline wetlands of Nebraska, including the endangered *C. n. lincolniana*, present a unique opportunity to study invertebrate populations in the context of a severely damaged ecosystem. The documented success of using tiger beetles as indicators of habitat health helps to

emphasize the importance of such studies. Understanding the life history and population structure of *C. n. lincolniana* will allow an in-depth investigation into how the destruction of the saline wetlands has impacted this population. The broad implications of this research will help to explain what happens to populations as they approach the brink of extinction due to loss of habitat and why some populations seem more susceptible to extinction.

The most apparent question that arises when one focuses on the tiger beetle assemblage occurring within the eastern saline wetlands is the question of why *C. n. lincolniana* is disappearing. Populations of *C. n. lincolniana* have dropped to perilously low numbers; however, populations of other tiger beetle species, with apparently identical life history traits, associated with the same habitat continue to exist in relatively high numbers. Is the disappearance of *C. n. lincolniana* a result of habitat (niche) destruction and could the success of sympatric species an indication of *C. n. lincolniana* residing in a narrower niche?

Past work with *C. n. lincolniana* and other tiger beetle species has shown evidence of ovipositional niche partitioning among sympatric tiger beetle species (Hoback et al. 2000, Allgeier 2005). It is likely that oviposition is not the only niche partitioning taking place within these tiger beetle populations. Food relations among species, in the study of evolutionary ecology, are among the most important aspects of biology (Hutchinson 1959). Tiger beetles are highly sensitive to food availability in both larval development and fecundity (Pearson and Knisley 1985, Knisley and Juliano 1988), therefore, it is likely that sympatric tiger beetles in this system are competing for food resources.

The thermal ecology of tiger beetles is another area where niche partitioning has been suggested. It has been demonstrated that sympatric tiger beetles are active at different temperatures (Pearson and Lenderhouse 1987). This temporal separation is another mechanism for niche partitioning which is probably not just a result of the lethal temperatures affecting tiger beetles but perhaps also predator activity levels. The saline wetlands are a harsh environment with very high temperatures from June into August. Personal observations indicate that *C. n. lincolniana* are active at higher temperatures when compared to their activity at lower levels. It appears that abdominal dipping, a behavior in which adult tiger beetles dip their abdomen into a water source and then carry a droplet of water under their bodies, may have evolved an adaptive trait for thermoregulation.

To gain a clear understanding of the factors driving competition between species and how the population structure of *C. n. lincolniana* has been affected as a result of its personal niche we must first answer a few questions. What are the ecological characters of *C. n. lincolniana* and how do they differ from other tiger beetle species found in these salt marshes? Ecological characters such as dispersal ability, fecundity, thermoregulation, prey sources, predators, and behavior all may contribute to the realized niche of an individual species.

Captive Rearing

The development of a captive rearing program is essential in a successful conservation plan for *C. n. lincolniana*. There is a long history of rearing tiger beetles in the lab. Shelford (1908) is responsible for providing the foundation for what we know about the life history of many North American tiger beetles. As part of his research on the

life histories of North American tiger beetles Shelford (1908) reared at least 11 species of tiger beetles in the lab. Due to his success many still use his methods today. Shelford (1908) used well ventilated glass-roofed vivariums. Adult tiger beetles were introduced to the cage that contained the soil where he collected the specimens. Of the 11 species reported in this paper all could be induced to lay without difficulty. Shelford (1908) also created cages made from glass plates separated by glass tubing that corresponded to the width of the prothorax of the species he was studying. These cages were filled with dirt and sealed at the bottom with cloth saturated with paraffin. He used this method to describe larval habits. Several years later Soans and Soans (1972) reported successfully rearing large numbers of tiger beetles in glass jars. Palmer (1978, 1979) developed a method for raising individual larvae in glass tubes. Tubes were packed with soil, leaving an inch at either end empty. The bottom of the tubes were plugged with foam fruit fly bottle stoppers. Hori (1982) examined the population dynamics of *Cicindela japonica* which included captive rearing. In his study he created a rearing tube to be used in releasing and monitoring lab reared individuals to the field. With these he was able to study survival and adult emergence. More recently Knisley (1997) provided a large summary of rearing many species of tiger beetles. An important link between food availability and female fecundity was discovered (Pearson and Knisley 1985). Successful translocations of wild *Cicindela dorsalis* larvae establish the likelihood that the same methods could be used to introduce captive reared tiger beetles (Knisley et al. 2005). Currently the Elkinton lab at the University of Massachusetts at Amherst is working to develop a system for mass rearing tiger beetles as part of their research on physiology

and development (Elkinton lab Web 2010). The techniques and data that result from their research will be used in conservation programs for tiger beetle species.

Attempts to rear *C. n. lincolniana* in captivity before we began a formal captive rearing program were marginally successful but were very important in developing these techniques. These preliminary methods for rearing are described in Allgeier (2005). In July of 2002 (towards the end of the adult activity period), thirty adult *C. n. lincolniana* were collected from the field. Beetles were placed into five 5-gallon terrariums filled to within 2 inches of the top with substrate from one of the sites where *C. n. lincolniana* was historically found. Two females and one male were placed in each of these five terrariums. After three weeks the beetles were taken out and transferred to 18.9 L terrariums filled with sifted greenhouse soil sifted through a # 10 screen. This soil was moistened with a saline solution of 30 ppt NaCl based on the work from Hoback et al. (2000). Terrariums were kept in environmental chambers at 29° C, with a photoperiod of 16 hours light: 8 hours dark. Soil was kept moist with distilled water. Larvae that resulted were fed small insects that were collected from sweep nets every two days. In 2002, the only viable eggs were those in the treated greenhouse soil. Three larvae successfully constructed burrows. Out of these three 2 successfully reached the third instar but because the larval burrows were constructed in close proximity the burrows collapsed. It is possible that cannibalism may have been part of the reason for mortality.

In 2003, two 37.9 L terrariums were filled with 20 cm of soil. One terrarium was filled with soil from *C. n. lincolniana* habitat and the other with sieved greenhouse soil moistened with 1 L of 0.354 M of NaCl. Two males and 5 females were placed in each

terrarium for two weeks to allow for oviposition. No eggs resulted from these two terrariums in 2003.

In 2004 methods were improved by using Petri dishes (5.5 cm X 1.1 cm) filled with 140 grams of sieved greenhouse soil. The soil in the Petri dishes was moistened with 50 ml of 0.354 M NaCl solution. Petri dishes were placed in Gladware® containers and aquarium gravel was placed around the outside of the dishes so the beetle could gain access to the dishes. A female beetle was placed in each container. Females were fed one mealworm daily and watered. The rearing containers were kept in environmental chambers at 29° C with 16:8 light:dark photoperiods. After two weeks soil in the Petri dishes was broken apart and 12 larvae were recovered. The larvae were moved to a 10 gallon terrarium that had been filled with soil from *C. n. lincolniiana* habitat. These terrariums were established 90 days earlier to allow for the soil to settle and allow for the salinity in the soil to concentrate at the surface of the soil to replicate conditions found on the salt flats. After the larvae successfully established burrows, the terrarium was placed in an environmental chamber at 29° C with a 16 light:8 dark photoperiod. One individual failed to successfully establish a burrow, the remaining 11 successfully became second instars by the end of August. In October all 11 burrows became plugged. In mid-November 5 third stage burrows appeared. Third instars were fed fruit flies (*Drosophila melanogaster*), and European corn borer (*Ostrinia nubilalis*) larvae. The 6 individuals that never re-opened their burrows were assumed dead. Out of the remaining 5 individuals 1 adult emerged.

Dissertation Objectives

The saline wetlands of Lancaster county and associated biota are severely threatened by habitat loss and degradation. The collection of detailed ecological data is necessary for the successful conservation of this habitat including *C. n. lincolniiana*. This dissertation is focused on the assemblage of sympatric congeneric tiger beetles inhabiting these wetlands. One of the main objectives of this dissertation is to determine if niche specialization has contributed to *C. n. lincolnina*'s ecological vulnerability. By examining egg laying preferences and behavioral thermoregulation we will be able to understand more about two important ecological characteristics that make up this organism's ecological niche. Requirements for successful reproduction and oviposition, behavioral thermoregulation, niche specialization, and means for effective public outreach were all examined.

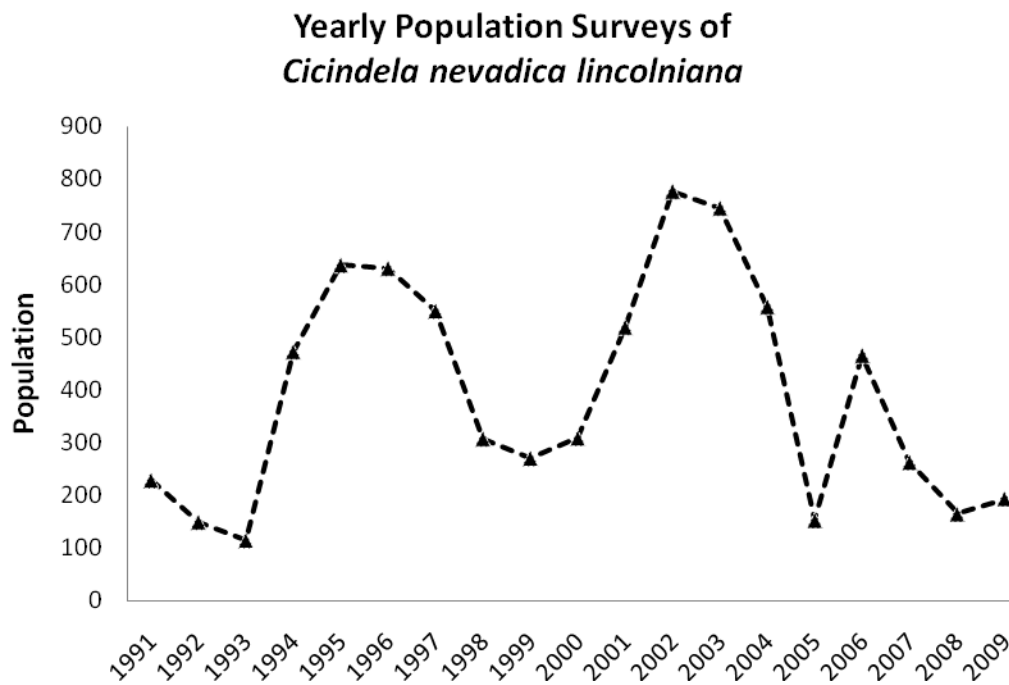


Figure 1. Yearly census data for the total population of *C. n. lincolniana* beginning in 1991. Visual counts taken by S. Spomer at the University of Nebraska Lincoln.

Table 1. Yearly visual counts for *C. n. lincolniiana* over the last 19 years at all known locations.

Locality	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Capitol Beach & I-80	12	8			0			4	0	
Jack Sinn W.M.A	15	11	1	0	0	1		1	0	
Arbor Lake South	15	8		25	8	1	7	55	41	20
Arbor Lake		24		51	36	7	33	11	25	4
Little Salt Creek N of I-80	156	62	62	300	415	429	366	188	142	201
Mill Rd.	7	5	4	8	3	0	0	0	0	0
NW 12th St	24	15	4	0	1	3	0	0	0	0
1st St		17	44	34	13	39		4	8	4
Little Salt Fork Marsh				1				0		
Raymond Rd.										
Little Salt Creek S of I-80				54	161	151	144	44	55	80
Whitehead Mitigation								1	0	0
Roper										
King Acquisition										
Totals	229	150	115	473	637	631	550	308	271	309

Locality	2001	2002	2003	2004	2005	2006	2007	2008	2009
Capitol Beach & I-80	0	0	0	0	0	0	0	0	0
Jack Sinn W.M.A	0	0	0	0	0	0	0	0	0
Arbor Lake South	58	43	65	5	6	0	13	1	2
Arbor Lake	1	30	10	1	4	46	17	3	0
Little Salt Creek N of I-80	375	438	508	386	105	293	167	105	146
Mill Rd.	0	0	0	0	0	0	0	0	0
NW 12th St	0	0	0	0	0	0	0	0	0
1st St	0	7		12	16	24	33	39	25
Little Salt Fork Marsh		0	0	0	0				
Raymond Rd.		1	0	0	0	0			
Little Salt Creek S of I-80	57	154	64	74	4	46	9	3	1
Whitehead Mitigation	0	0	0	0					0
Roper	28	104	98	77	17	51	23	14	20
King Acquisition				3	1	6	1	0	0
Totals	519	777	745	558	153	466	263	165	194

Chapter 1

Extinction Vulnerability in Tiger Beetles as a Result of Ovipositional Niche Breadth

Abstract

Competition and species requirements are central issues to conservation biology. Understanding how these issues affect the endangered *Cicindela nevadica lincolniana* and other sympatric tiger beetle species is essential to understanding their natural histories and in the development of conservation programs. Between 2007 and 2009 five species of mated adult tiger beetles (*C. n. lincolniana*, *Cicindela circumpecta*, *Cicindela fulgida*, *Cicindela togata* and *Cicindela punctulata*) were given a choice to lay in Petri dishes filled with sieved top soil treated with a gradient of molar solutions of NaCl based on salinities taken from the field. Once eggs were laid the soil was carefully sifted and larvae were counted. Larvae of *C. n. lincolniana* were then reared in topsoil treated with no saline solution. Species differed in not only their preferred salinities but also in the range of salinities they were willing to oviposit in. Ovipositional preferences suggest that larval competition has been a key selection pressure for tiger beetles. Additionally, the ability of *C. n. lincolniana* to successfully reach maturity in non-saline soils suggests that salinity is not a requirement for development but that it serves only as an ecological marker.

Introduction

Darwin (1859) introduced a fundamental ecological concept that the more closely related the individuals, the greater the potential for competition between those individuals. A logical expectation from this concept is that competition will drive specialization ultimately leading to speciation. A related expression of this idea is the competitive exclusion principle: the argument that two species cannot co-exist with exactly the same set of requisites (Gause 1934). Because these are core concepts of ecology and evolutionary biology, examining sympatric congeneric species (those species with overlapping ranges belonging to the same taxonomic genus) provides an important test of these fundamental concepts. Moreover, characterizing sympatric, congeneric species is key to understanding how communities are structured when species share similar behaviors, physiology, morphology, and ecologies (Pianka 1983).

Competition and species requirements also are issues central to conservation biology. What causes one species to fail when other, similar species thrive? Is extinction risk an issue of overspecialization, or is it more often a consequence of altered competitive relationships? The complex of co-occurring tiger beetles of the eastern saline wetlands of Lancaster County Nebraska provides excellent opportunities to address these questions. This saline ecosystem supports a community of ten species of tiger beetle (Spomer et al. 1997) and within this community four species are found only in saline habitats (Spomer and Higley 1993). These four species include *Cicindela circumpecta*, *C. fulgida*, *C. nevadica lincolniana* and *C. togata*. Initial examination of this group of tiger beetles reveals that these species appear to inhabit the same habitats as larvae and forage in the same habitats as adults. Out of these four species, populations of *C. n. lincolniana*

and possibly *Cicindela togata* are in decline. This decline is thought to be directly tied to habitat loss and degradation which is associated with both the growth of the city of Lincoln and agricultural practices in the surrounding area. One of the main changes in to this ecosystem has come with the channelization of the streambeds associated with these wetlands. Annual population estimates of *C. n. lincolniana* adults have been falling over the past 15 years, with current totals of only a few hundred individuals. These low numbers and dramatic reductions in geographic distribution, led to a federal endangered species listing in 2005.

Given their biology, tiger beetle communities have been valuable in examining many basic questions in ecology and evolution (Pearson and Vogler 2001). Tiger beetles are intimately tied to topography, hydrology, soil chemistry, plant communities, and prey diversity of their habitats (Pearson and Vogler 2001). For example, soil moisture, larval competition, parasitoids, and prey abundance all have large impacts on larval survival (Palmer 1976, Palmer 1979, Knisley and Pearson 1981, Hori 1982, Pearson and Knisley 1985, Knisley and Juliano 1988, Hoback et al. 2000, Allgeier 2005). Additionally, tiger beetles are sessile predators as larvae; therefore, the location of oviposition is essential for larval success. As is the case with most invertebrate organisms, the larval stages of tiger beetles are the most vulnerable to resource competition. Indeed, because sessile organisms compete for space more intensively than most species (Connell 1980), tiger beetle larvae are likely to be especially susceptible to resource limitations, which may lead to greater niche differentiation than non-sessile species.

An organism's risk of extinction is linked to the width of its ecological niche (Kammer et al. 1997, Kotiaho et al. 2005). Organisms that are highly specialized tend to

be more susceptible to extinction when their habitats are disrupted (Kammer et al. 1997, Kotiaho et al. 2005). For example, Diogo et al. (1999) examined a complex of co-occurring *Cicindela* species and found that one species (*Cicindela deserticoloides*) was less common than other co-occurring tiger beetles. Diogo et al. found that *C. deserticoloides* is limited to dry areas that are either less common or more directly affected by development than areas used other members of this *Cicindela* complex.

Previous work in our lab strongly indicates ovipositional-niche partitioning (egg laying selectivity) occurs among the saline-adapted tiger beetles in the Lancaster County tiger beetle complex (Hoback et al. 2000, Allgeier 2005). Past studies focusing on *C. circumpicta*, *C. nevadica lincolniana*, and *C. togata* in this ecosystem establish that these species have significantly different ecological requirements regarding both ovipositional soil salinity and moisture, as well as adult prey selectivity. Hoback et al. (2000) showed that two of these co-occurring species were selecting significantly different soil salinities for egg laying. *Cicindela circumpicta* laid eggs in soils containing significantly lower levels of salinity than *C. togata* in laboratory trials. Allgeier (2005) compared egg laying by *C. circumpicta* and *C. nevadica lincolniana* in the laboratory, using salinities based on field readings. Again, significantly different soil salinities were used by these two species. Although these studies show that ovipositional niche partitioning may occur, more precise measurements of oviposition of niche breadths have yet to be examined.

Comparing ecological characters of threatened and nonthreatened organisms could lead to a deeper understanding of what causes a species to become extinct (Kotiaho et al. 2005). Given the strong association of certain tiger beetles with saline habitats and the demonstration that ovipositional differences occur among species in relationship to

soil salinity, ovipositional preferences may represent a key ecological character driving speciation and extinction risk among the saline tiger beetles of eastern Nebraska.

Consequently, the primary question addressed in this study is if the endangered *C. n. lincolniana* occurs in a narrower ovipositional niche than other co-occurring tiger beetles with similar life history traits.

Methods and Materials

Between 2007 and 2009 ovipositional salinity preferences were compared between five species of co-occurring tiger beetles found in the eastern saline wetlands of Lancaster County, Nebraska: *Cicindela nevadica lincolniana*, *Cicindela togata*, *Cicindela fulgida*, *Cicindela circumpecta* and *Cicindela punctulata*. *Cicindela punctulata* is a common tiger beetle not associated with strictly saline environments, so it was included to provide a comparison with a non saline-associated species.

Salinity gradients for ovipositional soils for *C. circumpecta* and *C. togata* were based on ovipositional preferences determined by Allgeier (2005). Salinity gradients for *C. n. lincolniana* and *C. circumpecta* were determined by field measurements near larval burrows, and calibration tests to obtain soil salinities with comparable electroconductivities as in the field. The average preferred pore water conductivities for *C. n. lincolniana* and *C. circumpecta* oviposition sites were determined in the field using a Delta-T WET Sensor (Delta-T Devices Ltd., Burwell, UK) at the exact site of observed oviposition. In the laboratory, repeated aliquots of 150 ml of different molar solutions of NaCl solutions were introduced to 450 grams of sieved topsoil (#10 mesh screen), until the molarities were identified that produced electroconductivities close (< 0.05%) to those observed in the field. Using this technique, we found that 150 ml of 0.35 and 0.85 molar

NaCl solutions added to 420 grams of topsoil resulted in the preferred electroconductivities determined by Allgeier (2005) (2,992.1 mS/m and 7,873.9 mS/m, respectively). These amounts translated to 27 ml of saline solution per 75 grams of topsoil in 90 mm x 13 mm sterile plastic Petri dishes. Gradients divided among were developed based on these two previously determined preferred salinities.

With this information we conducted oviposition choice experiments, to determine preferred soil salinities for egg laying. Eight Petri dishes were filled with sieved top soil and moistened with 75ml of a NaCl solution corresponding to the salinity gradient (0.0, 0.1, 0.25, 0.35, 0.5, 0.85, 1.0, 1.25 M). The molar solutions of 0.35 and 0.85 were previously determined preferences for *C. circumpecta* and *C. togata*. The eight Petri dishes were randomly placed in a single drawer of a Sterilite™ 7-Drawer Craft Cart (dimensions: 38 cm x 38 cm x 61.1 cm, individual drawers 3.6 cm in height) and set level with aquarium gravel so the research subjects to climb up the side of the dish to reach the soil. A small dish for water was also included. Consequently, the drawer with 8 Petri dishes (for choice tests) was a blocked replicate, and the experimental unit was a petri dish. Eight to ten drawers (depending on the availability of adult pairs) were placed in environmental chambers set at 28° C with 16:8 light:dark photoperiods.

From 2007 to 2009 at the peak of the breeding season for each species of tiger beetle compared in this study, adult males and females of all species except *C. punctulata* were collected from the Arbor Lake area (Table 1-1). *Cicindela punctulata* was collected from the University of Nebraska East Campus. For 24 hours, males and females were held together and fed wingless fruit fly adults (*Drosophila melanogaster*) from a lab colony and field-collected worker termites (*Reticulitermes flavipes*) *ad libitum*. After the

24 hour holding period where individuals were fed and observed to mate, a single male/female pair was introduced to each container. Subjects were fed fruit flies, termites, and/or mealworm larvae (*Tenebrio molitor*) every other day and Petri dishes were misted with distilled water to keep the soil damp. After 7 days males were removed from the containers, and after 14 days females were removed. Because eggs are delicate and larvae from these studies were used for rearing, no attempt was made to remove eggs from soil. Instead, the undersides of the Petri dishes were examined daily for active larvae, which were observed between 10 and 14 days after females were removed. During this time the Petri dishes were lightly misted with distilled water daily to prevent eggs from desiccating. Once larval activity was observed, larvae were found by carefully breaking apart the soil in each Petri dish with a larval forceps and hand lens. After hatching, resulting larvae were counted and recorded for each Petri dish

In 2007 ovipositional salinity range preferences were examined for *C. n. lincolniana* and *C. circumpecta*. In 2008 ovipositional range preferences were examined for *C. togata*, *C. fulgida*, and again for *C. circumpecta*. In 2008, the range of salinities used for *C. circumpecta* included higher salinities in an effort to determine the maximum salinity acceptable for oviposition (specifically, a range of 75 ml of 0, 0.5, 0.85, 1.25, 1.5, 1.75, 2.0, 2.25 M was used). In 2009 ovipositional range preference studies were conducted using the same techniques as described above with *C. togata*, *C. fulgida*, and *C. punctulata*.

The relationship between the mean number of larvae recovered and the molar solution in the Petri dish was highly significant for *C. circumpecta*, *C. n. lincolniana*, and *C. togata*. Because the data range was different between species (2008 when *C.*

circumpicta range was expanded) we fit the models separately for each species. Larval distribution across molarities was modeled using a generalized additive model (generalized additive model using a smoothing function and a Poisson distribution) (Figs 1-1 to 1-3). Each of these models was significantly different from a null model (Table 1-2). Salinity was the fixed effect. The parameters were fit using model selection method with (library mgcv in R 2.10.0, R Development Core Team).

Results

The observed salinity preferences determined for *C. n. lincolniana* and *C. circumpicta* support Allgeier's (2005) observations in which *C. circumpicta* laid in soils that had a higher electroconductivity than *C. n. lincolniana* (Figs. 1-4a - 1-4c). Results from 2007 indicated that the upper salinity limit for *C. circumpicta* exceeded the salinity range tested. Because *C. n. lincolniana* did not lay any eggs in Petri dishes treated with the highest two molar solutions (Fig 1-4 b), we were confident that their upper limit of acceptable conditions had been met. This result (along with the concern that *C. n. lincolniana*'s endangered status makes conducting research that does not maximize potential fecundity of wild caught females an unwise choice for the success of a captive rearing program) is why we were unwilling to repeat this experiment with higher salinity choices. Figure 1-4c includes the expanded range for *C. circumpicta*. From these results it appears that we have not reached the upper threshold of what *C. circumpicta* finds as acceptable for oviposition, however, it is clear that *C. circumpicta*'s acceptable range for oviposition is much broader than that of *C. n. lincolniana*.

Data collected from *C. togata* in 2008 were inconclusive, because we had insufficient eggs to produce a reliable assay. The low oviposition rate may be a

consequence of when females were collected. Females collected too late after initial emergence seem to lose their ability to lay eggs (TB, personal observation). In 2009 *C. togata* successfully laid eggs but at much lower numbers than observed for *C. n. lincolniana* and *C. circumpecta*. Nevertheless, numbers were sufficient to show that oviposition increased with greater salinity (Fig. 1-1), within the limits of the tested salinities. This result is consistent with data from Hoback et al. (2000), but neither Hoback nor our results establish an upper salinity limit for oviposition by *C. togata*. In comparing patterns of oviposition, it appears that *C. togata* follows a pattern similar to that of *C. circumpecta* in that they are willing to lay in a wide range of salinities (at least at the lower limit of their range). Unfortunately, the low egg numbers with *C. togata* do not provide sufficient resolution to describe egg distribution in response to salinity.

We predicted that *C. punctulata* would either show no preferred salinity or choose to lay in low salinities. *Cicindela punctulata* did not lay large numbers of eggs but from the data collected it is clear that they chose to lay eggs in the Petri dishes containing no or very little saline solution (Fig. 1-4d).

Discussion

In 2008 and 2009 experiments conducted with *C. fulgida* were unsuccessful because females did not oviposit. *Cicindela fulgida* adults occur in the spring through early summer, and we collected adults for experiments as soon as possible in the spring. With the other species tested, the greatest oviposition occurred with newly emerged females, but the lack of oviposition from *C. fulgida* seems to indicate that oviposition is either delayed until substantially after first emergence or that our protocols (adult feeding, mating opportunities, and ovipositional substrates) somehow missed an essential

requirement. The one conclusion we can draw is that ovipositional behavior of *C. fulgida* is different from that of the other salt marsh tiger beetles we examined.

The results of our study strongly suggest that *C. n. lincolniana*, *C. circumpecta*, and *C. togata* are selecting suitable oviposition locations based on levels of soil salinity. Each species of tiger beetle shows preferences for salinities within a distinct range and avoids laying in soils that fall outside of that range. *Cicindela punctulata*, unlike the other tiger beetle species in this study, appears to have a negative association with salinity (Figure 1-4d). *Cicindela togata*, *C. nevadica lincolniana*, and *C. circumpecta* all exhibit a positive preference for saline soils but each of these species exhibits a distinctly different range of preferences. Curves fit to each species' ovipositional choice result in significant differences in each species ovipositional preference appears to be distributed normally which allows us to make reasonable predictions about upper and lower limits of their range even if we didn't provide a range of salinity that was completely outside of the species' range of preference as in the case with *C. circumpecta* (Fig. 1-4a and 1-4c). The width of *C. circumpecta*'s range of acceptable salinities for oviposition (ranging from 0.0 M to more than 2.25 M with a preferred salinity of 1.25 M) as compared with *C. nevadica lincolniana* (ranging from 0.0 M to 1.25 with a preferred salinity of 0.5 M) is much broader. The larger ecological breadth indicates that *C. circumpecta* is acting as a generalist species when it comes to ovipositional behavior.

Why the preference for salt exists poses an interesting ecological question. Is the salt physiologically necessary for these species? For many species of marine and freshwater organisms salinity is a physiologically constraining factor to the organism's survivorship and growth. It seems unlikely that saline soil provides a physiological

benefit to these salt flat tiger beetles because we have successfully reared *C. n. lincolniana*, *C. circumpicta*, and *C. fulgida* in non-saline soils, without significant larval mortality. In the absence of any indication of a physiological need for saline soils, selection of oviposition sites based on soil salinity seems to be a behavioral adaptation not directly related to physiological requirements. Futuyma and Moreno (1988) point out that behavior is often a mechanism in which specialization is genetically exercised. Because soils of differing salinities can be found within a relatively small distance (less than a meter) and because we haven't observed any obvious associations of other insects (e.g., potential tiger beetle prey) with soil salinity, ovipositional specificity doesn't seem to be associated with differences in prey preferences by larvae. Indeed, like other tiger beetle larvae (Pearson and Vogler 2001) from our field and laboratory observations larvae of *C. n. lincolniana*, *C. circumpicta*, and *C. fulgida* seem to be generalist predators, accepting any potential prey that is active and of manageable size.

For the simple choice of saline versus non-saline soils, soil salinity likely serves as an environmental marker -- a mechanism an organism uses to associate with a habitat possessing required features. We know that tiger beetles use other forms of environmental markers. Slope and particle size both serve as environmental markers in some tiger beetle species (Pearson and Vogler 2001). In a small experiment with *C. n. lincolniana* we observed no changes in ovipositional preference based on angle of slope. Similarly, in small ovipositional choice tests we saw no indication that soil particle size is a factor in ovipositional choice.

Allgeier (2005) determined that time of day, temperature, soil humidity, and soil salinity all influenced oviposition behavior in both *C. n. lincolniana* and *C. circumpicta*. If

temperatures were too low ($< 24^{\circ}\text{C}$) or soil humidities were too low ($< 30\%$ water), females would not lay eggs. Thus, air temperature and soil humidity requirements seem to represent thresholds that must be met for oviposition to proceed. In contrast, for the saline-adapted tiger beetles, oviposition is not based on just meeting a threshold of suitability, but follows a distribution around a specific salinity. Moreover, the distribution and preferred salinities differ among species.

Soil salinity could reflect many attributes of habitat suitable for oviposition. Where there are salt concentrations one finds very little vegetation. We know that lack of vegetation affects the surface temperature of the salt flats due to lack of shade. Surface temperatures can reach as high as 50°C during the day (TB, per. obs). These high temperatures certainly affect egg viability and larval activity. It is also possible that vegetation could impact biotic factors such as prey availability and/or predator and parasitoid numbers.

Riparian ecosystems are intimately tied to seasonal flooding. Salinity could be a predictor of this flooding. When spring flooding occurs in these salt marshes, non-saline water inundates the salt flats. Brust and Hoback (2009) determined that riparian species of tiger beetles are able to be underwater for extended periods of time; however, it is unlikely that salinity is being used as an environmental indicator for seasonal flooding. When ability to withstand extended periods of flooding was examined across species no correlation was found between survival times and habitat risk of flooding (Brust and Hoback 2009). Even non-riparian specific tiger beetle species, including *C. punctulata*, have a remarkable tolerance for flooding. Salt concentrations may have a negative correlation with seasonal flooding. Spring flooding is associated with the dilution of salt

concentrations at the surface (E.Harvey, UNL. per. com.). The salt content found in the soil could be affecting the evaporative quality of the surface. Evaporative soil mechanics could impact the temperature at the soil air interface thereby affecting the tiger beetle larva's foraging microclimate. In extreme environments such as these salt flats a few degrees can have dramatic consequences on an organism's reproductive success.

Along with these saline adapted species we chose to work with *C. punctulata* because it is a widely distributed species found within this saline wetland ecosystem and other local non-saline habitats. We predicted *C. punctulata* to exhibit one of two behaviors. Because the range for *C. punctulata* includes habitat where there are and are not saline soils present we predicted that *C. punctulata* would not show a preference based on soil salinity or this species would select soil with little or no saline content. Salinity does have a strong negative influence on where *C. punctulata* chooses to lay their eggs. This avoidance behavior indicates that non-saline associated tiger beetle species test for soil salinity as part of selecting suitable habitat for oviposition and that this behavior is not unique to saline adapted species. The oviposition behavior of *C. punctulata* suggests that all *Cicindela* may be able to detect salt.

As an environmental marker, discriminating between saline and non-saline soils would be sufficient to identify suitable larval habitat (open areas, areas with potential flooding, areas with high summer soil temperatures, etc.) However, the saline-adapted tiger beetle species go beyond a simple salinity threshold response: species have different preferred salinities and different ranges (distributions) of acceptable salinities. These different saline specificities imply that saline-adapted tiger beetles are using salinity to partition the habitat. The obvious reason for such partitioning would be to avoid larval

competition, either for prey or even from direct interactions (intraguild predation). Given that tiger beetle larvae are sessile, oviposition would be the most direct mechanism for selection to minimize larval competition.

From an evolutionary standpoint partitioning of larval habitats suggests that those species living in the harsh saline wetland habitat have adapted a way to use the ability to use salinity as a token stimulus. Based on our findings with *C. punctulata*, salinity is being used as an environmental marker habitat suitability. If closely related species are under strong selection pressure from competition then it is reasonable to predict that the competition between these congeneric species of tiger beetle could be the driving force behind this ecological community.

Gause's Law of competitive exclusion (1934) establishes that it would be impossible for two species of tiger beetles to exploit the same ecological niche without forcing one of the species to go extinct. It appears that these species of tiger beetles, especially *C. circumpecta* and *C. n. lincolniana*, are co-existing as larvae. Potentially competing species are able to coexist if they specialize in different resources. The data from this study indicates that the more common species, *C. circumpecta*, accepts a wider range of salinities for oviposition than the endangered *C. n. lincolniana*. Thus, *C. circumpecta* is acting as more of a generalist species when it comes to what salinities are acceptable for oviposition. It is likely that because *C. circumpecta* is serving as an ovipositional generalist it is better able to deal with the environmental changes caused by human-caused habitat degradation.

Due to the larval tiger beetle's sessile nature sufficient prey availability could be a very limiting factor. It is likely that adults are under strong selection pressure to

behaviorally reduce competition between larvae by carefully selecting sites for oviposition. In most cases avoidance of interspecific competition is driven by natural enemies or limitations in prey availability. Tiger beetles are commonly parasitized by *Bombyliidae* and hymenopteran parasitoids. Strong selection pressure from predation and parasitism and resource acquisition can be responsible in creating specificity (Futuyma 2001). This sort of pressure could be responsible for drastic selection in a population but that doesn't appear to be the case with these tiger beetles. The parasitoids found in this system are thought to be serving as generalists and where tiger beetle burrows are located there are often many burrows from multiple species found within close proximity out in the open on the salt flats.

It is likely that intraspecific and interspecific competition between larvae is having a larger impact on oviposition site selection by females. Not only would it be detrimental to have a burrow located in an area where there is a high density of neighboring burrows because of an increased competition for prey items, it is possible that neighboring burrows may intersect beneath the soil. In the lab we have had incidences of cannibalism by larval tiger beetles at the same developmental stage and we have had difficulties with captive reared larvae burrows intersecting which results in the death of one or both of the larvae. Eggs laid in close proximity could be at a high risk of being cannibalized by their neighbor. It is common to find groupings of first and second instar burrows but third instar burrows are often found with fewer neighboring burrows (per obs.) This observation may suggest that burrows found in close proximity of one another may succumb to both intraspecific and interspecific competition. Overlapping generations could prove to be problematic if eggs are being deposited near larvae that

were deposited in the previous season due to the larvae being older and therefore much larger.

This study demonstrates the points made by Pearson and Vogler (2001) about the value of tiger beetles for examining key issues in evolution and ecology. Ovipositional preferences suggest that larval competition is or has been a key selection pressure for this group. Meeting ovipositional preferences is essential not only for successful egg laying but also for improved larval survivorship. Many important directions for future research have emerged from this work. In the area of physiological and behavioral ecology the relationship between ovipositional preferences and physiological adaptation needs to be further explored. This study also brings up several important topics in the area of population ecology. Determining how organisms fit within their environment is important in understanding how to prevent their extinction. The narrow ecological niche of *C. n. lincolniana*'s egg laying preference is only one dimension of this species's realized niche that could cause drastic reduction in range and population. Here we see evidence of the importance of competition within this ecological community. Understanding what role prey availability and interspecific and intraspecific predation plays in the population dynamics of *C. n. lincolniana* and other co-occurring species is an important component of their natural history and plays an essential part in the successful conservation of these species.

Table 1-1. Species included in ovipositional choice experiments, salinity range (molar solutions) tested for each species, and date individuals were collected from the field.

Species	Salinity Range Tested	Date collected from the field
<i>C. n. lincolniana</i>	0.0-1.25	6/11/2007
<i>C. togata</i>	0.0-1.25	6/27/2008, 6/26/2009
<i>C. circumpicta</i>	0.0-2.25	6/14/2007, 6/23/2008
<i>C. fulgida</i>	0.0-1.25	5/18/2008, 5/19/2009
<i>C. punctulata</i>	0.0-1.25	6/25/2009

Table 1-2. Test of significance for generalized additive model of oviposition versus salinity. The test indicates significance versus a null model; edf = estimated degrees of freedom.

Species	Chi sq	P	n	edf	Intercept
<i>C. n. nevadica</i>	201.3	<.0001	8	4.79	1.63
<i>C. circumpecta</i>	379.4	<.0001	18	2.30	2.26
<i>C. togata</i>	60.64	<.0001	20	3.98	1.92

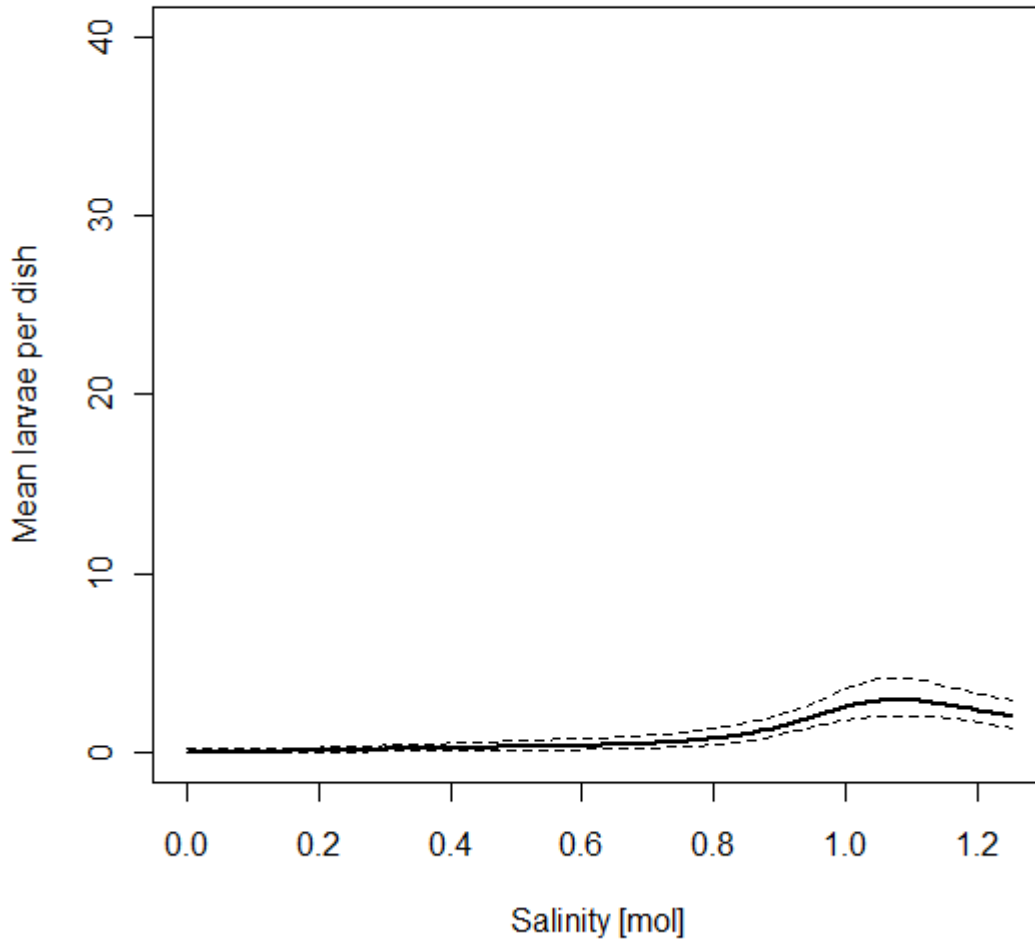


Figure 1-1: Oviposition preference models for *C. togata*. Data was collected in 2008 and 2009. First instar distribution across molarities was determined by using a generalized additive model (generalized additive model using a smoothing function and a Poisson distribution). The relationship between the mean number of larvae collected and the molar solution in the Petri dish was examined. Models were run individually to account for the difference in data range between species (2008 when *C. circumpecta* salinity range was expanded). Salinity was the fixed effect with species and year as the random effects. The parameters were fit using model selection method with (library mgcv in R 2.10.0, R Development Core Team).

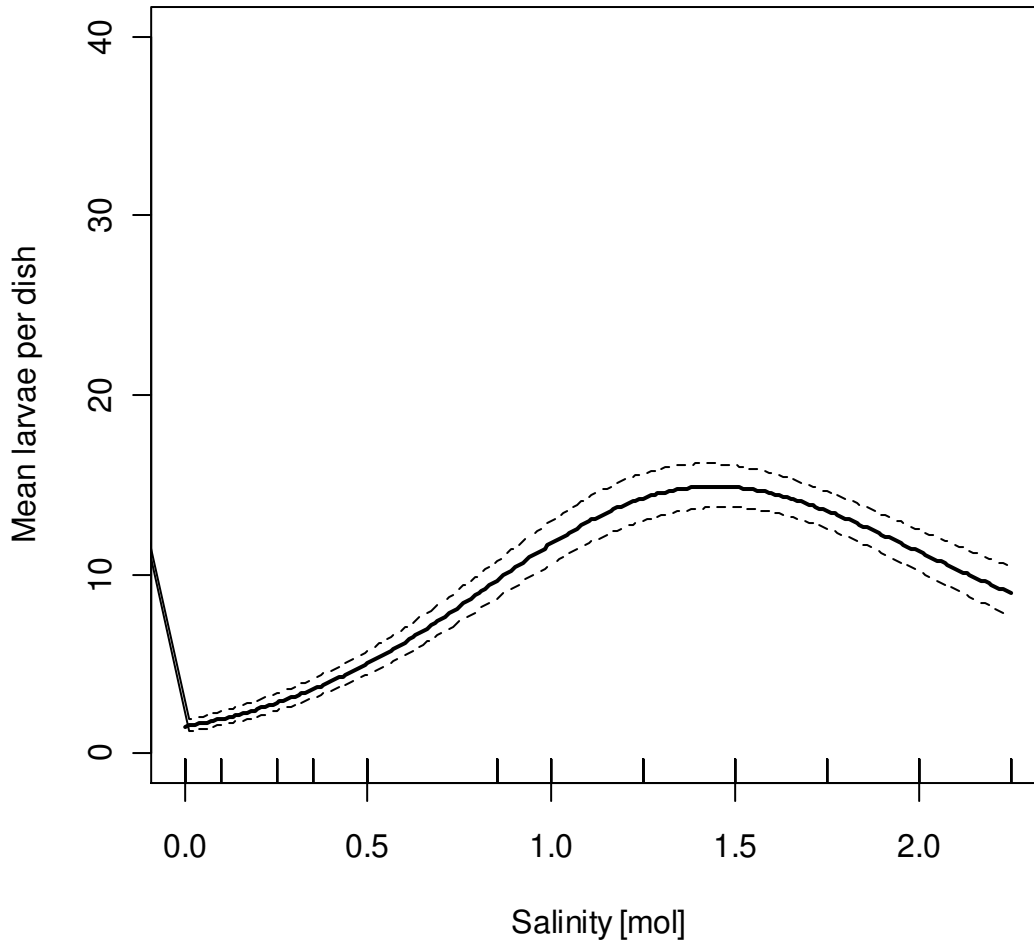


Figure 1-2. Oviposition preference models for *C. n. lincolniiana*. First instar distribution Oviposition preference models for *C. circumpecta*. Data was collected in 2007 and 2008. First instar distribution across molarities was determined by using a generalized additive model (generalized additive model using a smoothing function and a Poisson distribution). The relationship between the mean number of larvae collected and the molar solution in the Petri dish was examined. Models were run individually to account for the difference in data range between species (2008 when *C. circumpecta* salinity range was expanded). Salinity was the fixed effect with species and year as the random effects. The parameters were fit using model selection method with (library mgcv in R 2.10.0, R Development Core Team).

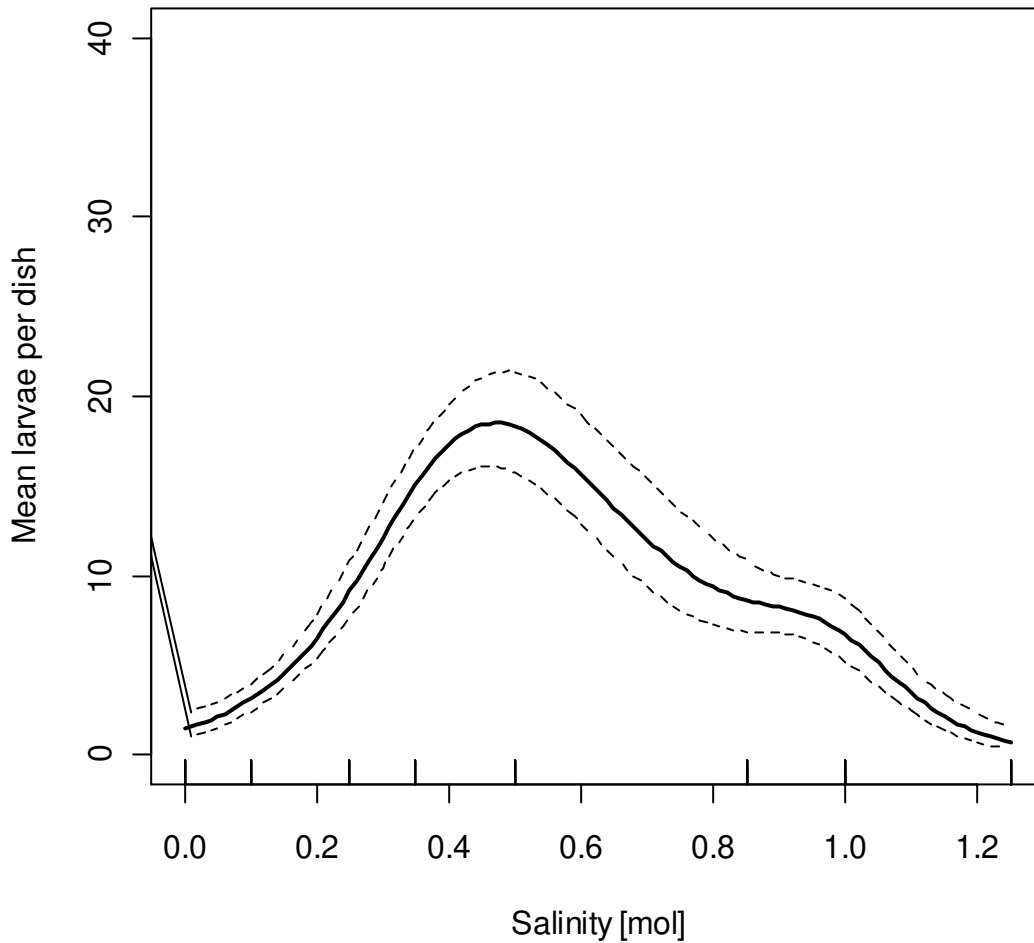


Figure 1-3. Oviposition preference models for *C. n. lincolniiana*. Data was collected in 2007. First instar distribution across molarities was determined by using a generalized additive model (generalized additive model using a smoothing function and a Poisson distribution). The relationship between the mean number of larvae collected and the molar solution in the Petri dish was examined. Models were run individually to account for the difference in data range between species (2008 when *C. circumpecta* salinity range was expanded). Salinity was the fixed effect with species and year as the random effects. The parameters were fit using model selection method with (library mgcv in R 2.10.0, R Development Core Team).

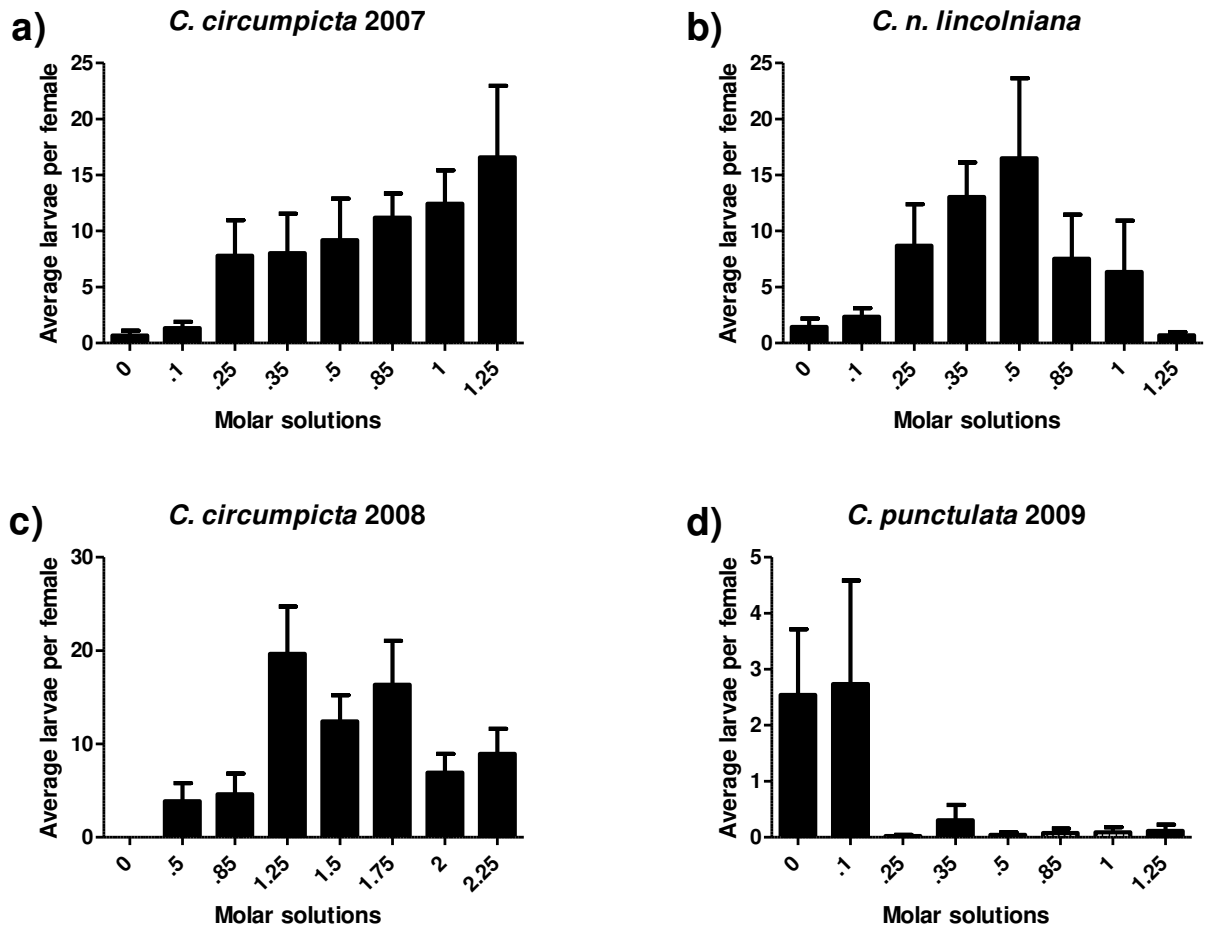


Figure 1-4. Data for *C. circumpicta* (2007 and 2008), *C. n. lincolniana*, and *C. punctulata* with the average number of larvae found per adult female in each soil filled Petri dish treated with 75 ml molar solution of NaCl.

Chapter 2

Captive Rearing and Mortality Analysis of *Cicindela nevadica lincolniana*

Abstract

Captive rearing is being used as a tool for the conservation of many organisms threatened with extinction. The development of a successful captive rearing program for *C. n. lincolnia* is part of a larger conservation effort. The population of this species has dropped to precariously low numbers due to habitat destruction associated with human development. Starting in 2004 procedures were developed to rear *C. n. lincolniana* from eggs in the lab. Many species of tiger beetles have been successfully reared from egg to adult by amateurs and by other labs. Although considerable progress has been made in determining how to obtain viable eggs in the lab and how to successfully raise these eggs to adult beetles, close examination of mortality factors clearly indicate that attention needs to be paid to mortality associated with overwintering **w**hich is responsible for over 51% of overall mortality. The key remaining issues are how to reduce mortality factors and how to release captive reared individuals to the wild in such a way that will most effectively increase the total population.

Introduction

Captive rearing is an increasingly common strategy used by conservation biologists to help preserve and restore populations of organisms that are threatened with extinction. Because captive rearing programs are a relatively new strategy, they are undergoing much scrutiny to determine if the limited funds available for species conservation are being used in a way that will optimize the survivorship of a species (Beck et al. 1994, Snyder et al. a 1996, Tenhumberg et al. 2004, Crone et al. 2007). While it is true that not all species will benefit from a formal, long-term captive rearing program, it is likely that rearing these organisms in captivity will give researchers insights into the organism's biology and therefore will be of major benefit in the long term. Once vital knowledge is gained about the organism's life history informed decisions can be made on how or if a captive rearing program will fit into a conservation plan for the endangered species in question.

Both captive rearing and conservation itself has been problematic for less charismatic organisms like invertebrates, despite their often profound importance to ecosystem function. Invertebrates make up over 99% of the world's biodiversity and are key indicator and regulator species in most terrestrial ecosystems (Samways 2005). Currently, the United States Fish and Wildlife Service lists only 199 species of invertebrates in the United States as threatened or endangered; of those, 58 are insects.

Many different traditional mechanisms for species conservation have been used to help preserve invertebrates, including habitat conservation and habitat reconstruction. Captive rearing also has been used to try to preserve insect species that are severely threatened with extinction. Rearing insects in captivity has a long history in human

culture. Captive-reared insects have provided everything from textiles to dyes, food, and biological control. Biologists commonly rear insects in captivity to gain insight into their natural history, to produce large numbers for biological control, and to produce specimens for laboratory experiments. Not until recently have insects and other invertebrates been reared in captivity as part of conservation efforts. The most common examples of captive rearing endangered insects are Lepidoptera rearing programs. Butterflies have gained much attention due to their aesthetic appeal as well as their value as biological indicators. Of the 58 federally listed insect species, 19 are lepidopteran species. *Lycaeides melissa samuelis* (the Karner Blue butterfly) has been captive reared by the Toledo Zoo since 1998 (Toledo Zoo Web 2010) and more recently the Butterfly Conservation Initiative, the Nature Conservancy, the United States Fish and Wildlife Service, as well as Game and Parks has become involved in developing captive rearing programs. Currently, most large scale rearing programs for insects in North America are for lepidopteran species.

We are working to determine if a long term rearing program for the endangered Salt Creek tiger beetle, *Cicindela nevadica lincolniana*, is a viable option as part of a larger conservation effort. *Cicindela nevadica lincolniana* adults are diurnal visual predators found only in the saline wetlands of Lancaster County, Nebraska. Adult beetles lay eggs in the saline soil associated with these wetlands in late June and early July. The predacious, sessile larvae hatch about 14 days after they are laid and develop into second instars before the winter of that year. We believe that this species' life cycle is typically two years with adult beetles emerging from a cohort two years after the initial eggs are laid. *Cicindela nevadica lincolniana* was listed by the U.S. Fish and Wildlife Service as

endangered in 2005 because surveys conducted since the 1990s determined populations to be dangerously low, dropping to as low as 155 in 2005 (Spomer and Higley 1993, Spomer UNL, unpublished data). These low numbers are attributed to the destruction of the saline wetlands of Lancaster County in Southeast Nebraska. Years of habitat destruction due to agriculture and urban development has left only 1% of the original saline wetlands intact (Farrar and Gersib 1991). The development of a captive rearing program will enable groups involved to reach many goals in the successful conservation of *C. n. lincolniana* populations along with their disappearing habitat.

Attempts to rear *C. n. lincolniana* in captivity before we began a formal captive rearing program were marginally successful but were very important in developing the techniques found in this paper. These preliminary methods for rearing are described in Allgeier (2005). In July of 2002 (towards the end of the adult activity period), thirty adult *C. n. lincolniana* were collected from the field. Beetles were placed into 18.92 L terrariums filled to within two inches of the top with soil from one of the sites where *C. n. lincolniana* was historically found. Two females and one male were placed in each of these five terrariums. After three weeks the beetles were taken out and transferred to 18.9 L terrariums filled with sifted greenhouse soil sifted through a # 10 screen. This soil was moistened with a saline solution of 30 ppt NaCl based on the work from Hoback et al. (2000). Terrariums were kept in environmental chambers at 29° C, with a photoperiod of 16 light: 8 dark. Soil was kept moist with distilled water. Larvae that resulted were fed small insects that were collected from sweep nets every two days. In 2002 the only viable eggs were those in the saline treated greenhouse soil. Three larvae successfully constructed burrows. Out of these three, two successfully reached the third instar but

because the larval burrows were constructed in close proximity the burrows collapsed. It is possible that cannibalism may have been part of the reason for mortality.

In 2003, two 37.9 L terrariums were filled with 20 cm of soil. One terrarium was filled with soil from *C. n. lincolniiana* habitat and the other with sieved greenhouse soil moistened with 1 L of 0.354 M of NaCl. Two males and 5 females were placed in each terrarium for two weeks to allow for oviposition. No eggs resulted from these two terrariums in 2003.

In 2004 methods were improved by using Petri dishes (5.5 cm X 1.1 cm) filled with 140 grams of sieved greenhouse soil. The soil in the Petri dishes was moistened with 50 ml of 0.354 M NaCl solution. Petri dishes were placed in Gladware® containers and aquarium gravel was placed around the outside of the dishes so the beetle could gain access to the dishes. A female beetle was placed in each container. Females were fed one mealworm daily and watered. The rearing containers were kept in environmental chambers at 29° C with 16:8 light:dark photoperiods. After two weeks soil in the Petri dishes was broken apart and 12 larvae were recovered. The larvae were moved to a 10 gallon terrarium that had been filled with soil from *C. n. lincolniiana* habitat. These terrariums were established 90 earlier to allow for the soil to settle and allow for the salinity in the soil to concentrate at the surface of the soil to replicate conditions found on the salt flats. After the larvae successfully established burrows the terrarium was placed in an environmental chamber at 29 C with a 16 light:8 dark photoperiod. One individual failed to successfully establish a burrow, the remaining 11 successfully became second instars by the end of August. In October all 11 burrows became plugged. In mid-November 5 third stage burrows appeared. Third instars were fed fruit flies (*Drosophila*

melanogaster), and European corn borer (*Ostrinia nubilalis*) larvae. The 6 individuals that never re-opened their burrows were assumed dead. Out of the remaining 5 individuals 1 adult emerged. While these methods (Allgeier 2005) were successful it was clear that improvements to his techniques were necessary in developing a successful captive rearing program for *C. n. lincolniana*. Along with increasing the number of successfully reared individuals it is also important to understand what issues are causing mortality in the lab.

Mass rearing *C. n. lincolniana* in captivity will provide valuable information on the natural history of this insect that will contribute to the development of a viable conservation plan in accordance with the requirements of the Endangered Species Act. Along with the gain of important data will come a potential safety net for this species. One of the biggest dangers for populations that drop to such precariously low numbers is the high probability of a catastrophic event leading to the extinction of the species. These salt marshes are subject to many risks including mass flooding through artificially channelized waterways that causes the banks where tiger beetles spend at the very least 90% of their life cycle as a sessile predator to become unstable, high numbers of parasitoids that could potentially drive the population even lower, and due to the proximity of these populations to urban areas and agriculture the likelihood of exposure to high levels of chemicals is potentially a serious threat. The availability of captive reared individuals provides a mechanism for reintroduction to areas where the population has been extirpated.

Consequently, our objectives here were (1) to develop and improve techniques for captive rearing of *C. n. lincolniana*, and (2) develop procedures for evaluating the

success of captive rearing, emphasizing the identification of limiting steps (particularly those with high mortality).

Methods and Materials

On 13 June 2007 16 adult *C. n. lincolniana* were collected from the field with aerial nets. Male and female adult *C. n. lincolniana* were kept together for 24 hours in a circular container (20cm x 10cm). During this time frame *C. n. lincolniana* were fed an unlimited amount of termites and wingless *Drosophila* (fruit flies). Males were marked using a paint pen (DecoColor, Uchida of America). After the 24 hour period 8 male, female pairs were introduced to the egg laying arena. This arena was comprised of eight Petri dishes that were randomly placed within a single drawer of a Sterilite™ 7-Drawer Craft Cart (38 cm x 38 cm x 61.1 cm, individual drawers 3.6 cm in height), and set level with aquarium gravel so the research subjects had easy access to soil filled dishes. Arenas were placed in environmental chambers. Environmental chambers were set at 28° C with 16:8 light:dark photoperiods. Soil was moistened with a range of salinities (0.0, 0.1, 0.25, 0.35, 0.5, 0.85, 1.0, 1.25 Molar solutions of NaCl). This range was based on salinities found in the saline wetlands sites. These substrate tests indicate that salinities determined by Allgeier (2005) are appropriate, with electroconductivities of 2504.1 mS/m (a 0.354 molar solution of NaCl/75 gm of soil).

Male and females were kept together in the arena for five days, and then males were removed. Females were left in the arena for an additional five days without males due to concerns that males may be a distraction to egg laying. *Cicindela nevadica lincolniana* were fed every other day to lessen our disturbance impact. The food source consisted of wingless *Drosophila melanogaster* (fruit flies), *Tenebrio molitor* larvae

(mealworms), and *Acheta domestica* nymphs (crickets). . Crickets' hind legs were excised to make for easier capture by the larva. Fluker's Cricket Quencher ® (Fluker Farms, Port Allen, LA) was used for their water source. A small amount of the quencher was placed in a small dish and included within the arena. Petri dishes containing soil were misted at feeding time with distilled water to prevent desiccation of eggs. Adult female *C. n. lincolniana* were removed from the arena and returned to the field per USFWS permit requirements.

The eggs hatched approximately two weeks after removal of adult females. At 12 days post removal, Petri dishes were examined for larval tunneling and burrow openings, which appeared perfectly round. By allowing the eggs to hatch we reduced the possibility of destroying fragile eggs. However, larvae had to be removed within a few days to avoid larval cannibalism. Once it appeared that most eggs had hatched we carefully went through the soil in each Petri dish with a hand lens and chemical spatula to gently break apart soil, removing all viable larva with larval forceps.

Larval *C. n. lincolniana* were introduced to soil filled tenite butyrite tubing (U.S. Plastic Corp, Lima OH). The tubes were filled to 2cm from the top of the tube to allow for a space for feeding live prey species. Drosophila vial plugs (Carolina Biol. Supply Co., Bwellington NC). were used to plug the opposite end of the tube. The foam plug allowed for water uptake and dispersal from the bottom of the tube. The top of the tubes were covered with a square piece of mesh netting (5.5cm x 5.5cm) and secured with a rubber band. This netting prevented potential escape of larva, and or prey. Tubes were placed upright in a polystyrene tray (Stuewe & Sons, Inc, Coreallis, OR) in plastic bins with handles. Placing the tray inside the bin allowed us to add water from the bottom of

the tubes. Having handles on the bins allowed for moving tubes in and out of environmental chambers while larvae were being maintained and observed.

Larvae were fed at three to four day intervals, when burrows were open. At each feeding first instars were fed fruit flies, second instars were fed one cricket nymph, and third instars were fed two cricket nymphs. Larval stage was recorded twice a week. Two thousand mL of water was added to the plastic bins at varying intervals (approx. 10-15 days) depending on when the soil in the tubes needed rehydrated.

In the first year of rearing, tubes were kept at room temperature until September 1st 2007. Tubes were then introduced to chambers set at a 16:8 light:dark photoperiod at 28° C. In 2008-2009, over wintering period light:dark photoperiods, and temperatures were changed weekly to mimic the natural photoperiod and temperature declines.

Temperature was slowly decreased weekly starting in October from 28°C high day, and 23°C high night temperatures to 14°C Low day and 10°C night temperatures. Although all burrows remained closed during the winter, environmental chambers and tubes were monitored daily. This monitoring consisted of checking temperature gauges, lights and humidity, and observing tubes for any abnormalities (e.g., mold).

In the spring of 2009 starting March 18th, temperatures and light: dark photoperiods were incrementally increased to coincide with natural temperatures and photoperiods. *Cicindela nevadica lincolniana* burrows began to open in the following weeks. These third instars were fed two cricket nymphs every two to three days until they entered the pupal stage. When adults emerged they were carefully removed from the tubes with larval forceps and place in a circular container (20cm x 10cm). These adults were fed cricket nymphs or termites daily and provided Fluker's Cricket Quencher® as

their water source. Males from the wild population were mated with females from the captive reared population. These pairs were placed in egg laying arenas as described previously. Larvae from these matings were also processed as described previously.

Life table and death probability analysis

Conventional and multiple decrement life tables were calculated for reared *C. n. lincolniana*, following procedures described by Carey (1993). With this information probabilities of death in the absence of other factors could be determined, as well as probabilities of death for combinations of mortality factors. Egg mortality was not estimated because the method used for laboratory oviposition did not allow a direct estimate of eggs laid. Consequently, stage-specific mortality began with the first larval stage, and each stage thereafter (i.e., L1-L3, and pupa).

Using the same data from eggs laid in 2007, life table and probabilities were calculated separately for two situations: cohorts with a 2-year life cycle and all cohorts (2-year and >2 year life cycle). These separate analyses provide a method for identifying key mortality factors based on potential differences in generation time. Additionally, once the multiple decrement life tables and death probability tables were calculated, it was possible to weigh hypothetical mortality to examine potential impacts on rearing success.

Results

Out of the 141 tubes, 110 had sieved topsoil and 31 were used to test rearing in habitat soil (Little Salt Creek bank) and 24 tubes were removed for reintroduction experiments. So, although 141 live larvae were obtained, only 86 could be observed through the entire life cycle. Of the 31 tubes containing soil from *C. n. lincolniana* habitat

6 larvae did not survive initial introduction to the tube and of the remaining 25 only one larva survived the first 2007-2008 overwintering period, and died during over wintering 2008-2009. Of the 110 tubes containing sieved topsoil 9 larvae did not survive the initial introduction to the tubes. Of the remaining 101 larvae, the first observed second instar occurred on 16 Aug. 2007. By 6 Sept. 2007 more than half of all remaining larvae were 2nd instar. In September 2007, 12 second instars were reintroduced to the banks of Little Salt Creek, and none of these produced adults. Therefore, 89 second instars entered diapause in the fall of 2007. From these, 70 survived over winter, and 12 of these were reintroduced to the banks of Little Salt Creek in the spring. Six progressed to the third instar, two survived over winter in 2008-2009, but by summer 2009 all of these reintroduced larvae were presumed dead when burrows did not open. Tubes were removed and examined for larvae during August of 2009, none were recovered. Of the 58 larvae remaining in the laboratory, all survived to enter overwintering in fall 2008. From these 58 entering the winter, 38 successfully overwintered as 3rd stage larvae, and 16 pupated in the spring of 2009. Of these 16, 2 died during pupation and 14 adults (11 males and 3 females) emerged. These females and males mated and have produced eggs. The remaining 22 individuals remained as third stage larvae, and overwintered in 2009-2010. We are currently waiting to determine the ultimate fate of these larvae.

A life table illustrating mortality and survivorship of reared *C. n. lincolniana* by stage and by time is shown in Table 2-1. As seen here, although some first-stage larvae failed to establish burrows and died, the highest mortalities occurred in the second and third larval stages during the winters of 2007-2008 and 2008-2009. A more detailed analysis through multiple decrement life table and death probabilities (Tables 2-2 - 2-3,

Fig. 2-1) provides an insight into key limitations to current rearing efforts. Excluding individuals with >2-yr generation times, the overall mortality was 58.1%, and overwintering mortality was principal cause of death (Table 2-2, Fig 2-1.a). Including all individuals and assuming remaining individuals fail to reach adulthood, overall mortality climbs to 83.7% (Table 2-3, Fig. 2-1b). Here, factors associated with failing to complete development are most important.

Discussion

Based on results to date, we have adequate oviposition procedures established, we have larval soil and food requirements (in a general sense) established, and we have determined that overwintering mortality is the key limitation to survivorship in our rearing method. We are exploring other important rearing variables include photoperiod, thermal cycling, and alternative prey in an effort to reduce mortality. We have not yet determined an appropriate method for larval reintroduction to the field, but we can state that spring reintroduction seems preferable to fall reintroduction since those that we introduced in the spring of 2008 were the only reintroduced larvae that successfully reached 3rd instar.

It is widely thought that captive rearing programs should be done only as an interim solution for species preservation (Beck et al. 1994, Snyder et al. 1996 b, Tenhumberg et al. 2004, Crone et al. 2007) but what constitutes as a viable program is still up for debate. Because captive breeding programs are relatively expensive it is imperative for proper evaluation of success and need. Snyder et al. (1996 a) argues that the use of captive breeding programs has grown without a concurrent growth in the appreciation of their limitations. Potential problems with captive rearing programs

include, 1) establishing self-sufficient captive populations, 2) poor success in reintroductions, 3) high costs, 4) domestication, 5) preemption of other recovery techniques, 6) disease outbreaks, and 7) maintaining administrative continuity.

It is important to evaluate the effectiveness of individual programs. Snyder et al. (1996 a) described seven limitations to captive breeding programs. Here we examine how a captive rearing program for *C. n. lincolniana* is affected by these limitations.

1) Achieving Self-Sustaining Captive Populations. It still holds true that a very small percentage of endangered or threatened taxa have been bred in captivity. Breeding *C. n. lincolniana* in captivity is subject to many of the same limitations due to the lack of knowledge about their specific physiological, physical, and environmental requirements to successfully reach adulthood. *Cicindela* as a group of insects is well studied and many rearing techniques have become well established for a variety of species (Shelford 1908, Soans & Soans 1972, Palmer 1978, Palmer 1979, Hori 1982, Knisley 1997, Knisley and Hill 2005, Allgeier 2005). Because current plans are to use wild caught females to provide eggs each year we will be able to avoid the problems associated with inbreeding depression (Ralls and Ballou 1983, Danielle and Murray 1986). Most insects have a high rate of fecundity, which should help us produce many viable offspring if mortality can be reduced. In 2007 we successfully hatched 144 *C. n. lincolniana* larvae, a large percentage of the total population, wild and captive reared. Once we are able to reduce mortality we should be able to produce a relatively high number of larvae in relation to the wild population. For a completely self-sustaining rearing program, the entire life cycle should be accomplished in the laboratory. Although we have had successful mating and reproduction with one individual, our program currently depends on field-collected

individuals for eggs. Consequently, developing a truly self-sustaining rearing program remains an objective.

2) Reintroduction. Reintroduction and translocation is a part of most captive rearing programs. Beck et al. (1994) reviewed 145 reintroduction programs and found only 16 programs that were able to establish wild populations (11%). One of the major causes of failure in reintroductions of captive reared animals appears to be behavioral deficiencies for many animals. Because *Cicindela* do not have learned behavior this may be a small concern for this program. One potential concern would be behaviors associated with predator avoidance. *Cicindela* have many predators including parasitic wasps and flies as well as birds. One of their most basic defenses against these threats is to rapidly move back down into their burrows. Personal communications (M. Brust, Chadron State College) indicate that some species become habituated to being fed by hand and may lose the instinctive behavior to move back down inside their burrows when an object approaches from above. It appears that some species readily associate movement above their burrows with food availability. We have not witnessed this behavior with *C. n. lincolniana*, probably because larvae have evolved to quickly descend into their burrows after prey capture (likely to avoid becoming prey themselves). Snyder et al. (1996 a) suggest that captive breeding programs are best when behavior is instinctive and the organism is at the top of the food chain or is introduced to a predator free environment. While it is true that all *Cicindela* appear to have instinctive behavior it is not possible to introduce them into an environment where the threat of predation is low.

Another major factor in the failure to establish wild populations is the failure to fix previously occurring problems. If there is no acceptable habitat to reintroduce the

species than the program is ultimately doomed to failure. Fortunately there is work currently underway to restore habitat along *C. n. lincolniiana*'s current range (US Fish and Wildlife 2009).

3) Domestication. In many organisms the genetic or phenotypic change in an organism can be very rapid (Snyder et al. 1996 a, Lewis and Thomas 2001, Heath et al. 2003, Dzurisin 2005,). These changes can make organisms unsuited for reintroduction into the wild after only a couple of generations and are a serious consideration for many captive breeding programs. Snyder et al. (1996) points out that genetic changes can be very rapid in certain fish and invertebrate populations (Bryant and Reed 1999, van Oosterhout et al. 2000, Lewis and Thomas 2001, Ford 2002, Dzurisin 2005), given their underlying genetic diversity and reproductive rates. However, with *C. n. lincolniiana* reductions in range and numbers over the past 20 years have undoubtedly reduced genetic diversity. Consequently, there may be less potential for artificial selection with populations on the brink of extinction, like *C. n. lincolniiana*, than with other threatened species. In our initial rearing efforts we did note that reared individuals seemed smaller than wild individuals; however, such differences are more likely the result of suboptimal diet or overwintering conditions than they are as reflections of artificial selection. Nevertheless, artificial selection remains a concern, and we plan to use only wild-caught females for obtaining eggs for rearing, as long as this is possible.

4) Disease. It is well established that with decreased genetic diversity most populations have an increased susceptibility to disease (Cunningham and Frank 1993, Cunningham and Daszak 1998, Pearce-Kelly et al. 1998, Mattoni et al. 2003). Additionally, populations in captivity are at an even greater risk of being exposed to exotic pathogens

(Cunningham et al. 1997). The risk of being exposed to exotic pathogens and then releasing infected organisms accidentally as part of a reintroduction program is a real threat to already low populations. To address these problems, Snyder et al. (1996) points out that basic veterinary principles should be maintained, specifically:

- a) Captive populations should be maintained in isolated single-species facilities that do not regularly exchange stocks with other facilities. Currently *C. n. lincolniana* are reared in a facility with other invertebrates. Crickets and wingless fruit flies are being reared in close proximity to be used as a food source for larval *C. n. lincolniana*; however, each species is reared in a separate growth chamber. With invertebrates, separate rearing chambers and consistent use of sterilization techniques to minimize the potential for disease transmission seem to meet the intent of this requirement. A second rearing facility is being established at the Lincoln Children's Zoo, to provide spatial separation and duplication to minimize the risk of a single catastrophic event destroying all reared beetles.
- b) Captive breeding should be conducted within the natural range of the species to reduce exposure to exotic pathogens and in at least two geographically separate facilities. Rearing of *C. n. lincolniana* is currently conducted within the city of Lincoln, Nebraska which is located just south of its native range. As previously mentioned, the establishment of a second rearing location at the Lincoln Children's Zoo, meets the need for geographic duplication of rearing facilities.
- c) Founder stock should not be drawn from open multi-species facilities, but should be taken directly from the wild or from single-species facilities within the natural range that have good histories of disease prevention. *Cicindela nevadica*

lincolniana females are taken directly from the wild each year, and eggs are obtained from these females. Unfortunately, it is possible that wild numbers will diminish to the point that laboratory breeding is essential for the continued existence of *C. n. lincolniana*.

- d) Facilities should be closed to the public, and staff should practice rigorous disease-prevention methodology, including strict avoidance of contact with other captive stocks. Currently rearing facilities are closed to the general public, although opportunities for public viewing of rearing are being explored. With a non-charismatic species (like most invertebrates), establishing and maintaining public interest in species conservation is essential for long term sustainability. Consequently, we think isolation and disease-prevention procedures must be developed that allow opportunities for public viewing of endangered species, and we think this effort is especially important for endangered insects.
- 5) Financial and Physical Resources – logistics (include labor, extended rearing, e.g., prey species). The limitations brought on a captive rearing program by finances and resources can directly limit the success of any program. Over ten years ago some species were estimated to cost up to a half-million dollars per year per species (Derrickson and Snyder 1992). Luckily the cost of rearing invertebrates often is less expensive than costs for rearing most vertebrates. Because most invertebrates are K selected they produce many more offspring than vertebrates. If captive rearing programs can keep mortality to a minimum, potentially they could produce thousands of individuals for reintroduction in a very short time frame. The financial and physical resources for rearing *C. n. lincolniana* are very minimal. Currently, the main costs of rearing this species in captivity are

associated with environmental chambers and hourly help to feed the larvae. Some rearing programs are able to cover some of the costs by the revenue from public viewing of the animal. Snyder et al. (1996) recognized, however, that most endangered species are “visually unspectacular” and will not pay for their own keep. Unfortunately this is true in our case. It is only in recent years (since *C. n. lincolniana* was federally listed as endangered) that financial support became available for a captive rearing program. We are working with the Lincoln Children’s Zoo with captive rearing of *C. n. lincolniana*. Their involvement will not only help expand the rearing program but will also increase public education. Currently, financial support is on a competitive, annual basis, so there is no framework for long term rearing efforts. Given that uncertain resources are available even for a federally and state listed endangered species, like *C. n. lincolniana*, the prospect for long term conservation of other listed and unlisted endangered insects clearly remains tenuous.

6) Preemption of Other, Better Techniques. Keeping a conservation plan for an organism balanced between resources being spent on research, habitat, and captive rearing is very important. All of these components are vital to the successful reestablishment of any endangered organism. Luckily, the balance between these components in the case of *C. n. lincolniana* has so far been maintained. Currently we maintain an active research program along with our captive rearing efforts. Along with this there has been work done towards habitat reconstruction along *C. n. lincolniana*’s historic range that has seen some promising results (population increases in this location over the last two years since the start of the reconstruction).

7) Ensuring Administrative Continuity. Ensuring continuity in any program is a challenge. Although our group has been working with *C. n. lincolniiana* for over 15 years, in 2009 our most experienced team member was lost to budget cuts. Developing captive rearing procedures for *C. n. lincolniiana* has involved work by multiple individuals including a research technologist and three graduate students over the last 8 years.

The multiple decrement life table and determination of death probabilities in the absence of other causes (Tables 2 and 3, Fig. 1) provide insights into mortality and indicate the most important areas of research. Without this information, it might be tempting to focus on increasing oviposition or improving first stage larval establishment. However, our analysis indicates that the overwhelming importance of overwintering mortality would swamp any gains in mortality at earlier stages. For example, as shown in Fig. 2-1, completely eliminating establishment mortality only changes expected total mortality 2.3% (to 58.1% from 55.8%). We use spreadsheets for calculating life tables and death probabilities, so it is simple to examine different assumptions in stage-specific mortality and project changes on overall mortality.

What survival rate would constitute success in captive rearing? The simple answer is any survival rate higher than that in the field. While we don't have direct measures of field survival rates, declining population numbers indicate that the overall mortality is greater replacement rate. In our situation, we had 141 first-stage larvae from 8 females, to give 17.6 or about 18 viable larvae per female. Assuming that a male-female pair must be replaced by their offspring, a survival rate of $2/18$ or 11% is needed. Given that most insect species have survival rates in single digits (Carey 1993), this is a surprisingly high rate, and is - an artifact of measured oviposition in the laboratory. We

saw great differences in eggs laid per female, likely a consequence of variability in time of field collect versus time of adult emergence. We have observed with *C. n. lincolniana* and other cicindelid species that oviposition rates decline dramatically after the first week post-emergence. So the actual replace rate for *C. n. lincolniana* in the field is likely much less than 11%.

Relative to captive rearing requirements, we must exceed 11% survivorship to have a minimally acceptable rearing program for *C. n. lincolniana*. So we have met this standard (assuming all larvae > 2 yrs old fail to complete development, our survivorship rate is 16.3%). More realistically, to allow for potential mortality associated with reintroduction and to build field populations, higher survivorship rates are needed. However, captive rearing of endangered species is a court of last resort, essentially undertaken only when the likelihood of extinction in the field is so great that intervention is necessary. As long as that intervention, through captive rearing, can be sustained and prevents the continued loss of individuals, we believe it should be regarded as successful.

Another important issue illustrated through this analysis is the question of individuals with generation times > 2 years. Because we have reared *C. n. lincolniana* to the adult stage in a single year, our current thinking is that generation times are largely based on food availability, with a two-year life cycle most common in nature. Extended generation times, therefore, would imply food deficiencies. Alternatively, generation times may be based on meeting nutritional requirements as well as environmental conditions associated with overwintering. Results from our analyses of individuals with 2-year generation times versus those with >2 year generations times shows that extended generation time is potentially itself a more important mortality factor than overwintering.

That said, it is possible, even likely, that extended generation times are directly related to problems in overwintering conditions during captive rearing. However, our data and analysis show that we need to consider this question as a separate issue from overwintering mortality alone. Otherwise we run the risk of seeing gains in reduced overwintering mortality being swamped by mortality associated with extended generation times.

Life table analysis is not currently a standard procedure in captive rearing but we are convinced that it should be. Our understandings of key mortality factors in rearing *C. n. lincolniana* were not revealed by simple mortality data. As others have noted (e.g., Carey 1993, Peterson et al. 2009), although use of multiple decrement life tables and death probabilities is commonplace in human demography, its application continues to lag in biology. Captive rearing of endangered insects clearly represents a new area of application that would benefit both our understanding of rearing limitations and increasing efficiency and potential success in focusing new research efforts.

The use of life tables is sometimes criticized over meeting assumptions necessary for its use. The key assumptions associated with use of life tables are that individuals are exposed to mortality factors throughout each time interval (in our example, each life stage), and that mortality factors don't interact (the occurrence of one mortality factor doesn't enhance or diminish the likelihood of death from a difference mortality factor) (Carey 1993, Southwood 1968). The first assumption is largely methodological – does the measurement of mortality alter mortality rates being measured. This is usually an issue where mortality is measured by removing some proportion of individuals from a population – not an issue in our determinations of mortality. The second issue is more

problematic, because it can be difficult to determine interactions among mortality factors., and this issue is not unique to lifetable analysis. Interactions among mortality factors are typically associated with nutrition, parasitism, genetic impairments, or disease, where the presence of a non-lethal condition (e.g., genetic fitness or disease) increases the mortality from a different factor, (e.g., predation). In captive rearing, genetic factors (given a limited gene pool) and disease (always a potential issue with insect rearing) seem to be the greatest concerns, however, in a rearing environment (given the absence of predation and parasitism as potential mortality factors), interactions in mortality seem less likely than in field situations. Consequently, assumptions required for life table use should be met in most captive rearing programs for insects.

Because of this species' long life cycle we need to work improvements on a continual basis. Increasing survivorship in the lab should be a key focus but our we should strongly focus on improving overwintering survivorship. Currently we lose over 50% due to overwintering. This is probably less than what occurs in the wild but it is a key point to improving our numbers in the lab. Along with improving overwintering survivorship increasing wild-caught female fecundity will increase our numbers. From past years of research it is clear that the timing in which female beetles are caught in the field is very important to the amount of eggs laid. It is also clear that the amount of food (and potentially quality of food) consumed by the female beetle is also of vital importance for egg production (TB per obs, Pearson and Knisley 1985). These improvements must be coupled with successful reintroductions for the ultimate success of this program. So far introductions have not been successful but the knowledge gained will be used to continue to try to develop successful methods. Determining when the best

time for reintroductions of larvae and what techniques work should be a priority in future research.

Developing a viable captive rearing program for *C. n. lincolniana* does have potential challenges yet it is a promising component of the successful conservation program for this species. Three of the main goals of this program are to stop extinction, reestablish the range of the species, and to have self-sustaining populations. One of the biggest challenges for invertebrates is their life histories are often complex and poorly understood. Unfortunately this means that many questions cannot be answered before a captive rearing program is begun. Some of the biggest challenges in rearing larger vertebrates are found in replicating mating behavior, providing adequate food sources, and genetic depletion. With invertebrates one has these issues along with understanding distinctively different life stages and diapause requirements. Fortunately success has been achieved even with difficult species whose life histories are not fully understood.

Captive rearing has high scientific value beyond simply preserving endangered species. A great deal of scientific value can be attained during the development of a captive rearing program. In the case of *C. n. lincolniana* the exploration of oviposition requirements and nocturnal oviposition behavior has added to the body of knowledge of tiger beetle ecology and the techniques developed will add to the increasing number of invertebrate captive rearing programs. Captive rearing will become an increasingly important technique for preserving endangered insects.

Table 2-1. Life table for *Cicindela nevadica lincolniiana* captive rearing from 2007-2010. Cohort began with eggs laid in the summer of 2007 from field collected (and subsequently released) females. Abbreviations: x = stage, l_x = # surviving at start of stage, d_x = # dying in stage, $d_x F$ = factor causing death in stage x , $100q_x$ = fraction of deaths from all causes in stage, al_x = fraction of starting cohort alive at start of stage, S_x = survival rate in stage, cS_x = cumulative survival rate across stages.

Period		Within Stage						Cumulative
Year	Season	x	l_x	d_x	$d_x F$	$100q_x$	$100S_x$	$100cS_x$
2007	fall	1st-2nd	86					
			77	9	failed to establish			
		Totals	77	9		10.5%	89.5%	89.5%
2007	winter	2nd	77					
			58	19	overwintering			
		Totals	58	19		24.7%	75.3%	67.4%
2008	spring	2nd-3rd	58					
			58					
		Totals	58	0		0.0%	100.0%	67.4%
2008	summer	3rd	58					
			58					
		Totals	58	0		0.0%	100.0%	67.4%
2008	fall	3rd	58					
			58					
		Totals	58	0		0.0%	100.0%	67.4%
2008	winter	3rd	58					
			38	20	overwintering			
		Totals	38	20		34.5%	65.5%	44.2%
2009	spring	3rd-pupa	38					
		14 adults	38					
		Totals	38	0		0.0%	100.0%	44.2%
2009	summer	3rd-pupa	38					
			36	2	Pupation	-	-	-
		Totals	36	2		5.6%	94.4%	41.9%
2009	fall	3rd-pupa	36					
			36					
		Totals	36	0		0.0%	100.0%	41.9%
2009	winter	3rd-pupa	36					
			36					
		Totals	36	0		0.0%	100.0%	41.9%
2010	spring	3rd-pupa	36					
			36					
		Totals	36	0		0.0%	100.0%	41.9%

Table 2-2. Conventional and multiple decrement life tables and death probability tables for reared *Cicindela nevadica lincolniana* from eggs laid in 2007, excluding 22 individuals with > 2 year generation times. Procedures prevented determination of egg mortality, so the egg stage is excluded. Abbreviations: x = stage, l_x = # surviving at start of stage, d_x = # dying in stage, ad_x = fraction of starting cohort deaths in stage, aq_x = fraction of deaths from all causes in stage, al_x = fraction of starting cohort alive at start of stage.

Conventional Life Table						
x	l_x	d_x	Cause of Death			
			establishment	molting	overwintering	other
first instar	86	9	9	0	0	0
second instar	77	19	0	0	19	0
third instar	58	20	0	0	20	0
pupae	38	2	0	2	0	0
adults	36					

Multiple Decrement Life Table						
x	al_x	ad_x	Fraction of Deaths			
			establishment	molting	overwintering	other
first instar	1.0000	0.1047	0.1047	0.0000	0.0000	0.0000
second instar	0.8953	0.2209	0.0000	0.0000	0.2209	0.0000
third instar	0.6744	0.2326	0.0000	0.0000	0.2326	0.0000
pupae	0.4419	0.0233	0.0000	0.0233	0.0000	0.0000
adults	0.4186					
Total		0.5814	0.1047	0.0233	0.4535	0.0000

Probability for Cause of Death						
x	l_x	aq_x	Probability for Cause of Death			
			establishment	molting	overwintering	other
first instar	86	0.1047	0.1047	0.0000	0.0000	0.0000
second instar	77	0.2468	0.0000	0.0000	0.2468	0.0000
third instar	58	0.3448	0.0000	0.0000	0.3448	0.0000
pupae	38	0.0526	0.0000	0.0526	0.0000	0.0000
adults	36					
Total			0.1047	0.0233	0.4535	0.0000

Probability for Cause of Death in the Absence of Other Causes						
x	l_x	Total q_x	Probability for Cause of Death in the Absence of Other Causes			
			establishment	molting	overwintering	other
first instar	86	0.1047	0.1047	0.0000	0.0000	0.0000
second instar	77	0.2468	0.0000	0.0000	0.2468	0.0000
third instar	58	0.3448	0.0000	0.0000	0.3448	0.0000
pupae	38	0.0526	0.0000	0.0526	0.0000	0.0000
L1 to adult		0.5814	0.1047	0.0526	0.5065	0.0000

Table 2-3. Conventional and multiple decrement life tables and death probability tables for reared *Cicindela nevadica lincolniana* from eggs laid in 2007, including all individuals (2-year and > 2 year generation times) and assuming that those with >2 yr generations times ultimately die. Procedures prevented determination of egg mortality, so the egg stage is excluded. Abbreviations: x = stage, l_x = # surviving at start of stage, d_x = # dying in stage, ad_x = fraction of starting cohort deaths in stage, aq_x = fraction of deaths from all causes in stage, al_x = fraction of starting cohort alive at start of stage.

Conventional Life Table						
x	l_x	d_x	Cause of Death			
			establishment	molting	overwintering	other
first instar	86	9	9	0	0	0
second instar	77	19	0	0	19	0
third instar	58	20	0	0	20	0
pupae	38	24	0	2	0	22
adults	14					

Multiple Decrement Life Table						
x	al_x	ad_x	Fraction of Deaths			
			establishment	molting	overwintering	other
first instar	1.0000	0.1047	0.1047	0.0000	0.0000	0.0000
second instar	0.8953	0.2209	0.0000	0.0000	0.2209	0.0000
third instar	0.6744	0.2326	0.0000	0.0000	0.2326	0.0000
pupae	0.4419	0.2791	0.0000	0.0233	0.0000	0.2558
adults	0.1628					
Total		0.8372	0.1047	0.0233	0.4535	0.2558

Probability for Cause of Death						
x	l_x	aq_x	Probability for Cause of Death			
			establishment	molting	overwintering	other
first instar	86	0.1047	0.1047	0.0000	0.0000	0.0000
second instar	77	0.2468	0.0000	0.0000	0.2468	0.0000
third instar	58	0.3448	0.0000	0.0000	0.3448	0.0000
pupae	38	0.6316	0.0000	0.0526	0.0000	0.5789
adults	14					
Total			0.1047	0.0233	0.4535	0.2558

Probability for Cause of Death in the Absence of Other Causes						
x	l_x	Total q_x	Probability for Cause of Death			
			establishment	molting	overwintering	other
first instar	86	0.1047	0.1047	0.0000	0.0000	0.0000
second instar	77	0.2468	0.0000	0.0000	0.2468	0.0000
third instar	58	0.3448	0.0000	0.0000	0.3448	0.0000
pupae	38	0.6316	0.0000	0.0555	0.0000	0.6100
L1 to adult		0.8372	0.1047	0.0555	0.5065	0.6100

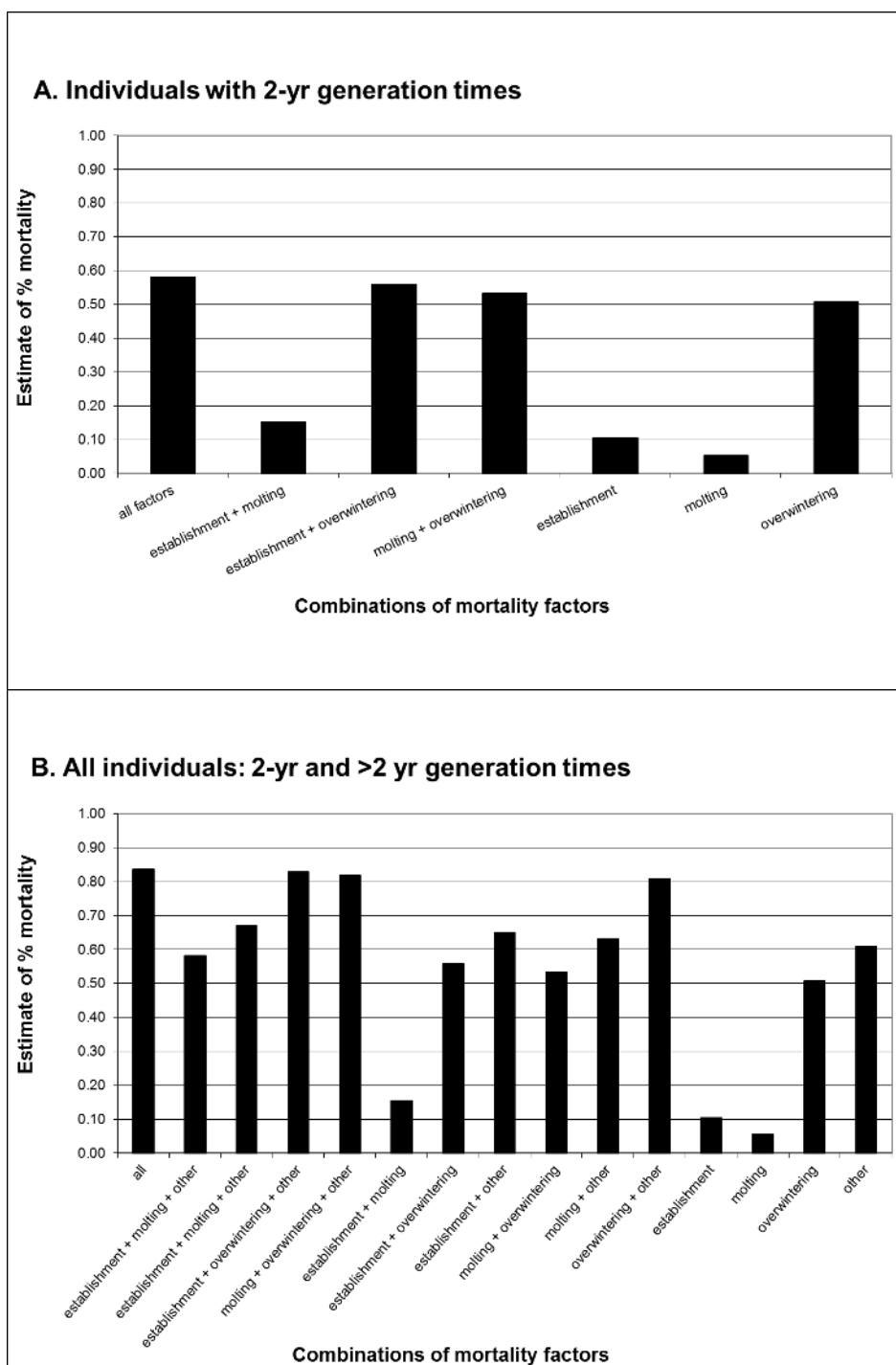


Figure 2-1. Death probabilities for multiple mortality factors in the absence of other causes for reared *Cicindela nevadica lincolniana* from eggs laid in 2007. Chart A illustrates mortality excluding individuals with >2 year generation time; chart B illustrates death probabilities for all individuals (and 2-year and >2-year generation times). Because the fates of the 22 individuals with > 2-year generation times is not yet known, assigning all to “other” cause of mortality represents a worst case projection

Chapter 3

Behavioral niche partitioning in a sympatric tiger beetle assemblage

Abstract

How behavioral patterns are related to niche partitioning is an important question in understanding how closely related species within ecological communities function. Behavioral niche partitioning associated with thermoregulation is well documented in tiger beetles as a group. Co-occurring species of salt flat tiger beetles have adapted many thermoregulatory behaviors to cope with this harsh ecosystem. On first examination these beetles appear to occur in overlapping microhabitats and therefore compete for resources. To determine if behavioral niche partitioning is allowing multiple species to occur within the same harsh salt flat ecosystem we observed *C. n. lincolniana*, *C. circumpecta*, *C. fulgida*, and *C. togata* between 8:00 hours and 21:00 hours and recorded all behaviors related to thermoregulation using a digital voice recorder. Results of this study strongly indicate that competition among these species for resources has been reduced by the adaptation of different thermoregulatory behaviors such as spending time in shallow water, avoiding the sun during the hottest parts of the day, and by positioning their body against or away from the soil. It is clear that there are behavioral differences between species that relate to their ability to acquire food resources.

Introduction

Theoretically, competition is one of the main forces driving niche partitioning and, consequently, evolution. Variation in behavior of sympatric species is considered to be a mechanism for niche partitioning. Interspecific competition may lead to segregation among habitats followed by physiological adaptations to these different microhabitats (Schultz and Hadley 1987). Due to their high visibility and long history of ecological research, the study of tiger beetles has provided an excellent opportunity to examine how behavior reduces interspecific competition. Many assemblages of tiger beetles have been studied for this reason. Behaviors that serve to partition resources and reduce physiological stressors have been extensively examined for many species of tiger beetles yet a great deal remains to be discovered about the diversity and functions of tiger beetle behavior (Pearson and Mury 1979, Pearson 1980, Pearson and Stemberger 1980, Pearson and Knisley 1985, Ganeshiah and Belavadi 1986, Schultz and Hadley 1987, Hoback et al. 2000, Hoback et al. 2001, Romey and Knisley 2002).

The saline wetlands of eastern Nebraska are home to a unique biota of salt adapted organisms. Along with these organisms' unique ability to tolerate high levels of salt they are also well adapted to tolerate the harsh, desert-like environment typically associated with this habitat during the summer months. Within this environment exists a large assemblage of congeneric, sympatric tiger beetle species. Because of the uniqueness of this environment and the endangered status of one of these species of tiger beetle much attention has been given to this group of tiger beetles.

Past and current research supports the theory that these tiger beetles are using oviposition as a mechanism for niche partitioning (Hoback et al. 2000, 2001, Allgeier

2005). Tiger beetle larvae do not move more than a few cm from where their eggs are originally deposited by the female. This unique life history trait along with the larva's dependence on limited prey resources for proper development (Mury Meyer 1987) emphasizes the importance of the location chosen for oviposition. Prey resources are vital for adult female fecundity. The amount of prey items consumed by adult females is directly tied to their ability to lay larger numbers of eggs (Pearson and Knisley 1985). The ability to lay higher quantities of eggs could be very important to the success of individual populations due to high mortality in the larval stages of most insects. Shelford (1913) documented the mortality of some populations of *Cicindela scutellaris* to be as high as 80% due to the parasitoid *Anthrax sp.* Knisley and Shultz (1997) documented a mortality rate upwards around 63% due to tiphiid wasps. Because it is so important for females to gain enough caloric resources for egg development it is likely that adult tiger beetle assemblages have evolved behaviors to reduce interspecific competition as adults. Although it is suggested that *C. n. lincolniana* is reducing competition through oviposition selection it is unclear how adult *C. n. lincolniana* behavior that is associated with reducing adult interspecific competition.

There is a long history of thermoregulation studies that focus on tiger beetles and that link thermoregulation behaviors to resource partitioning (Dreisig 1980, Dreisig 1981, Dreisig 1984, Dreisig 1985, Morgan 1985, Pearson and Lederhouse 1987, Schultz and Hadley 1987, Schultz et al. 1991, Schultz 1998, Hoback et al. 2001, Romey and Knisley 2002). Many cicindelids are found in environments where temperatures are capable of exceeding 60°C, a lethal temperature for most insect species (Hadley 1994). From these studies it is clear that *Cicindela* are capable of regulating their body

temperatures by changing their body orientation and shuttling between microclimates. Cicindelids spend a high percentage of their day balancing foraging behavior with behaviors associated with thermoregulation. Pearson and Stemberger (1980) determined that the gain of one hour of additional foraging could increase the biomass of ingested prey items by as much as 20%. This increase in prey items would translate into an increase in egg production for adult females. In this situation where resources such as time, space and food may have limited the evolution of behaviors that could lead to an increase in foraging time yet a decrease in competition between species is very likely.

Co-occurring species use their habitat and behaviors in distinctly different ways. One reason for these differences could be directly associated with the physiology of the insects. Physiological character divergence in species' ability to cope with temperatures could be a mechanism to reduce intraguild predation. Differences in heat tolerances between species is a likely mechanism to reduce competition, however, a close examination of lethal maximum temperature of 13 tiger beetle species near Willcox, Arizona, USA revealed very few differences between upper heat tolerances among species (Pearson and Lederhouse 1987). Out of the 13 species examined in this study only 2 were found to be significantly different and the difference was less than one degree from the overall mean of 48.1° C. Hoback et al. (2001) determined the lethal maximum temperature for two species of tiger beetles (*Cicindela circumpecta* and *Cicindela togata*) within the complex of co-occurring species in the Eastern saline wetlands of Nebraska and found no differences between species and found almost identical temperature values as Pearson and Lederhouse (1987). While tiger beetle species may have some variation in their ability to cope with varying degrees of humidity

and temperature (Pearson and Lederhouse 1987, Schultz and Hadley 1987, Schultz et al. 1991) it does not appear that physiological differences do account for all of the behavioral differences that were seen in the field. In an effort to determine the evolutionary cause for such differences, Hoback et al. (2001) investigated lethal high temperatures, prey base, prey size, and mobility, and the effect of direct predation of *C. togata* by *C. circumpicta*. Hoback et al. (2001) found strong evidence that *Cicindela circumpicta* and *Cicindela togata* were subject to the effects of interguild predation. Laboratory studies that investigated feeding behavior of *C. togata* in both the absence and presence (separated by a clear pane of glass) of *C. circumpicta* indicated that *C. togata* feeding behavior was negatively affected by the presence of *C. circumpicta*.

In the case of *C. n. lincolniana* and its co-occurring *Cicindela* species it is likely that similar evolutionary forces as seen in earlier studies are at work. Past research strongly suggests that behaviors associated with the reduction of predation, thermoregulation, foraging, and predator avoidance may reduce competition among sympatric, adult tiger beetles (Pearson and Mury 1979, Schultz and Hadley 1987, Pearson and Juliano 1991, Romey and Knisley 2002).

This study will not only answer important questions about the behavior of a federally endangered species, it will help answer questions regarding the significance of behavior as a mechanism for niche partitioning. My objectives were to quantify behavioral differences among co-occurring tiger beetle species, determine how these differences in behavior reduced competition between co-occurring species, and to gain a greater understanding of microhabitat use by the endangered *C. n. lincolniana* for use in future habitat reconstruction.

Methods and Materials

During the summer of 2007 initial observations were made of three species of saline adapted tiger beetles: *C. circumpecta*, *C. togata*, and, *C. n. lincolniana* at the Arbor Lake Complex, Lincoln, NE. From these initial observations it was clear that these tiger beetles exhibit a large variety of behaviors and use a wide range of habitats. Behaviors associated with thermoregulation were of particular interest. Based on the 2007 data, an ethogram or catalogue of discrete behaviors was developed. Observations were classified specifically as behavior, events, and states. Behaviors included running, stilting, basking, and standing. Stilting occurs when the tiger beetle holds itself up off of the substrate with its front two legs extended straight downward. Often the beetles appear to be standing at a 45° angle from the ground. Stilting is done during the hottest time of day in an effort to reduce surface area and keep their bodies away from the hot surface (Pearson and Vogler 2001). Basking occurs when the tiger beetle presses its body up to the substrate in an effort to warm its body in the early morning hours (Pearson and Vogler 2001). Events are recorded when individuals exhibit a behavior that has measurable no time duration. Events include mandible dipping (dipping mandibles into the substrate they are standing on), wing pumping (a quick opening and closing of their elytra), flight (this event has a measurable time duration but we almost always lost track of the individual), and abdomen dipping (the individual would dip its abdomen into the water by doing what appeared to be a quick pushup). Categories that include states were both light and substrate. Light includes if the subject was standing in the sun or shade and substrate includes if the subject was occurring on dry soil, mud, or water.

Behaviors of four co-occurring species of tiger beetles were examined on 20 June 2008, 30 June 2008, 7 July 2008, 23 June 2009, and 2 July 2009.. Species included *C. circumpicta* and *C. togata*, *C. n. lincolniiana*, and the spring-fall species *C. fulgida* (which occasionally occurred in early summer). Behavior, states, and events were recorded using digital voice recorders and later transcribed using the program JWatcher™ (Version 1.0). One recording was made for each hour in the field. For each hour three or four observers were randomly assigned a species to observe. Recordings were made from the first individual of that species the observer could locate using close-focus binoculars. At the start of each recording the observer noted the date, time, species, and, observer. The observer watched one individual as long as they could in a 30 minute period. If recordings were less than 10 minutes in length the observer recorded a second individual of the same species for that hour. On the rare occasion that no species of that individual could be found (at least 15 minutes of looking) the observer selected the first tiger beetle that they could find for recording observations. Along with these behaviors hourly temperatures were recorded. The surface temperature was taken from the area near where the recordings were being made. Temperature measurements were taken at 1 meter and 1 cm elevation from the same location. Because tiger beetle's bodies are approximately 1 cm above the soil surface I used the 1 cm measurement to reflect the temperatures being experienced by the beetles.

Observations were recorded between 08:00 hours and 21:00 hours on 20 June 2008, 30 June 2008, 11 July 2008, 23 June 2009, and 2 July 2008. The data was offloaded into the computer and entered into the program JWatcher using keypunches that represented each behavior, state, and event as the voice recordings were played back.

For each block of time, we averaged time spent exhibiting individual behaviors (basking, running standing, and stiling), time spent in sun or shade and time spent on type of substrate (mud, dry soil, and water). Because times that each individual was watched varied we converted the time spent to percentages that could be compared between time and species. Events were averaged by hour by species.

Results

Temperatures increased until 15:00 hours in all measurements (Fig. 3-1). As a result of the absorption of solar radiation, soil temperature became higher than ambient and 1 cm air temperatures after 11:00. At 08:00 hours the surface temperature was more than 1°C cooler than both 1 cm above the surface and ambient temperature. By 15:00 the surface temperature was 7.9°C higher than at 1 cm above the surface. At 08:00 hours there was almost no difference between ambient temperature and 1 cm above the soil surface (0.12°C). At 16:00 hours the difference rose to 1.9°C. Temperatures dropped dramatically between 20:00 and 21:00. At 1 cm above the salt flat surface, temperatures dropped 5.4°C.

Time spent in the sun was directly linked to the time of day and therefore temperature. For all species time spent in the sun was significantly higher in the morning hours (8:00-11:00) when the salt flat temperatures were the coolest (Fig. 3-2, Table 3-1). As temperatures rose the amount of time spent in the shade increased for all species (Fig. 3-2). Significant differences in percentages of time spent in the sun were found between species. *Cicindela nevadica lincolniana* spent the most time in the sun throughout the day (77.1% of their total time).

Cicindela togata spent the most amount of time on dry soils (Fig. 3-3, Table 3-2). *Cicindela nevadica lincolniana* spent significantly more time standing on the damp surfaces and in the shallow water of the seeps than the other species observed (Fig. 3-3, Table 3-2). Both *C. circumpecta* and *C. fulgida* spent the majority of their time on damp substrate as opposed to dry soil (Figs 3-3a & 3-3c, Table 3-2). *Cicindela circumpecta* spent more time on dry substrate when temperatures rose in the late afternoon (Fig. 3-3a, Table 3-2). *Cicindela fulgida* spent the most amount of their time on damp substrate (60.1% total) and time spent on damp substrate increased with temperature (Fig. 3-3c, Table 3-3).

Behaviors were also examined by percentages (Fig. 3-4). All four species of tiger beetle spent a large portion of their time alternating between standing and running. This reflects typical tiger beetle foraging behavior where they alternate between running in short bursts and standing watching for prey. *Cicindela togata* had the largest shift between running and standing in the middle of the day (Fig. 3-4d). Basking was almost always associated with morning hours (before 11:00) and evening hours (after 16:00). This lined up with the coolest hours of the day. Stilting was almost completely associated with the middle parts of the day for all four species. *Cicindela circumpecta* spent more time basking in the early hours (Fig. 3-4a).

Events related to thermoregulation were examined. The total events per hour were averaged over the entire day for all four species (Table 3-4). *Cicindela nevadica lincolniana* averaged 78.05 mandible dips per hour, which was far greater than *C. circumpecta* which was the next highest at 5.44 mandible dips per hour. *Cicindela togata* averaged 0.88 flights per hour, which was greater than *C. fulgida* which was the next

highest at 0.51 flights per hour. *Cicindela togata* appeared to make many short flights during the 8:00 time block but short flights seemed to have no other significant correlation to time of day for any other tiger beetle species (Fig. 3-5). Wing pumping occurred in greater frequency in *C. n. lincolniana* earlier in the day but appeared to occur with greater frequency later in the day for *C. circumpecta* (Fig. 3-6).

Discussion

These results present evidence that co-occurring species of adult tiger beetles within this system could be segregating by behavior in relation to temperature. The high surface temperatures reached by the salt flats in the middle of the day are a challenge for organisms living in this ecosystem, including tiger beetles. The results of this study suggest that these species have evolved multiple behavioral and physiological ways of dealing with the high temperatures found on the salt flats. When one looks at the temperature being experienced by these tiger beetle species throughout the day in conjunction with their behavior it becomes clear that temperature is a driving force behind tiger beetle diurnal activity. Shade seeking behavior during the heat of the day is a common behavior seen in many organisms. Interestingly, *C. n. lincolniana* is very active during the hottest part of the day while other species of salt flat tiger beetles spend much of their time seeking refuge in the shade. This difference is probably not due to differences in physiology but due to differences in their behavior. *Cicindela nevadica lincolniana* has a very different foraging behavior; a great deal of their time is spent foraging in the shallow seeps. What we observed as mandible dipping is probably two different types of behavior. Tiger beetles drink water by sinking their mandibles into a damp substrate. We observed tiger beetles taking a few seconds to hydrate using this

behavior. *Cicindela nevadica lincolniana* would mandible dip rapidly in shallow water and would often come up with a small insect larva that it would quickly consume. Even in the hottest part of the day *C. n. lincolniana* was able to forage while in the shade of the salt grass growing in the seeps. This difference in foraging behavior allows *C. n. lincolniana* to be active during the hottest part of the day due to the cooling effects of the shallow water. This unique foraging behavior probably explains the large difference in mandible dipping frequency between species (Table 3-4, Fig. 3-7). Another interesting observation made for the behavior of *C. n. lincolniana* was how they would move out of the water in the later part of the day and further out onto the salt flats as evening approached. This could be due to the absence of other tiger beetle species. It was difficult to find other tiger beetle species to observe after 19:00 hours.

Cicindela fulgida was the only species of tiger beetles we observed that is classified as a spring-fall species. Spring-fall species of tiger beetle emerge as adults in the fall and overwinter as adults in contrast to summer species which over winter as larvae and emerge as adults in the spring. The adult beetles of this species that we were observing had overwintered as adult beetles and had been active as early as March (possibly even warm days in February). We predicted that this species would have a more difficult time dealing with extreme temperatures. As predicted *C. fulgida* spent the most time on damp substrate and in the shade (Fig. 3-2c and 3-3c). This species also spent a large proportion of the morning hours basking (Fig. 3-4c), presumably in an effort to warm their body temperature.

Cicindela circumpicta spent more time on dry substrates as temperatures rose during the day. Most of the shade on the salt flats is provided by vegetation which grows

on the perimeter of the flat. This area has dry substrate because of the distance from the seep. As the temperatures dropped later in the day *C. circumpecta* appeared to move back onto damp surfaces to forage (Figure 3a). *Cicindela circumpecta* also spent a large proportion of their time basking in the early morning hours (Fig. 3-4a). It is possible that the larger body mass of *C. circumpecta* requires more basking to raise its body temperature.

Cicindela togata spent the most time on dry surfaces. Unlike *C. circumpecta* who moved to dry surfaces during the hottest part of the day and moved back to damp surfaces *C. togata* appears to spend all of its time on dry soils. The time spent on damp soils in the morning is likely a result of the lack of dry soil due to morning condensation. *Cicindela circumpecta* and *C. togata* have the same tolerance to heat yet they forage in different microhabitats on the flats (Hoback et al. 2001). Hoback et al. (2001) theorized that this was due to *C. togata* avoiding being preyed upon by *C. circumpecta*. In the lab *C. togata*'s behavior was modified by the presence of *C. circumpecta*. Because *C. circumpecta* is a larger, more aggressive species of tiger beetle it is possible that other species may have adapted different foraging behavior as a way of avoiding contact with this species. Wing pumping is thought to be a thermoregulatory behavior to release heat (source) but *C. togata* (which had the highest per hour average) pumped its wings at a greater frequency in the morning. *Cicindela fulgida* and *C. circumpecta* both appeared to pump their wings with greater frequency in the middle of the day but did not show a direct association with temperature (Fig. 3-6).

How tiger beetles allocate their time in relation to temperature has been studied extensively (Dreisig 1980, Dreisig 1981, Dreisig 1984, Dreisig 1985, Morgan 1985,

Pearson and Lederhouse 1987, Schultz and Hadley 1987, Schultz et al. 1991, Schultz 1998, Hoback et al. 2001, Romey and Knisley 2002). There were many similarities and differences between species in this study. Temperature appears to be tied into what substrate beetles chose to occur on, whether or not they chose to spend time in the sunlight, and what behaviors they exhibited. *Cicindela nevadica lincolniana* appears to rely most heavily on the shallow water of the seeps for their diurnal foraging behavior.

The endangered status of *C. n. lincolniana* requires that those involved with the conservation of this insect examine its habitat requirements closely. Because this beetle appears to have reduced competition over food resources by adapting to forage in a unique environment they may be more susceptible to habitat destruction. Organisms that are highly specialized, such as in the case of *C. n. lincolniana*, are thought to be more susceptible to extinction due to habitat destruction (Kammer et al 1997, Kotiaho et al. 2005).

The shallow seeps found in saline wetlands have been destroyed by the channelization of these water ways over the last 100 years. Consequently, the close association of *C. n. lincolniana* with seeps and associated shallow pools seems to let *C. n. lincolniana* adults forage at times when temperatures may limit foraging for other saline-adapted tiger beetles. Ironically, this association also may help explain *C. n. lincolniana*'s susceptibility to extinction: beyond the loss of saline wetlands generally, limited seeps and pools even within remaining saline habitat may represent a further habitat limitation within an already limited habitat.

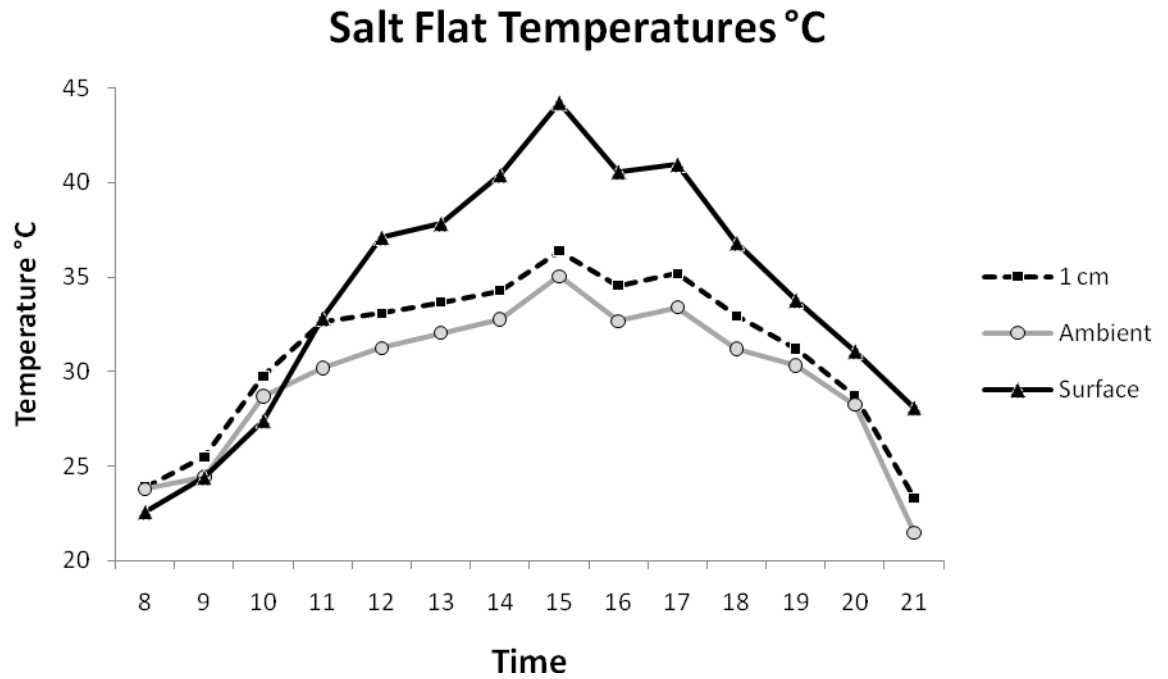
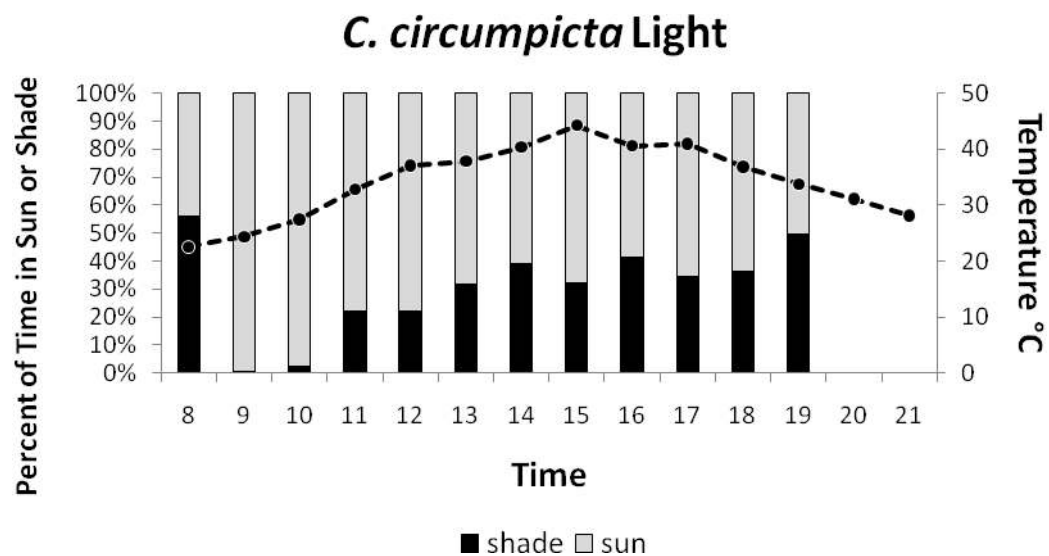
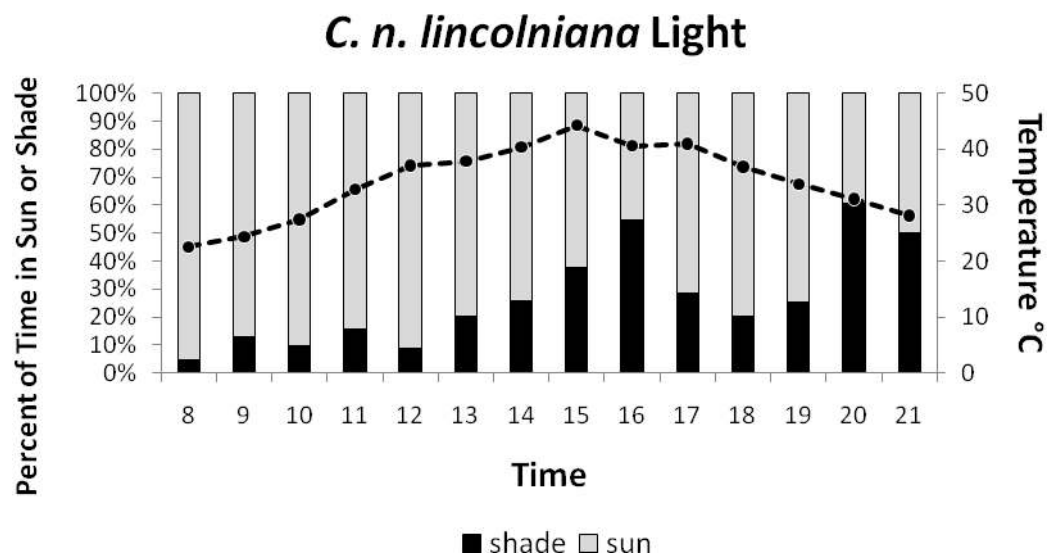


Figure 3-1. Temperatures were recorded on the salt flats where observations were made for adult tiger beetles. Temperatures were recorded one meter above the soil surface to determine ambient temperature, one cm above the soil surface to determine the air temperature the subjects were experiencing, and at the soil surface.

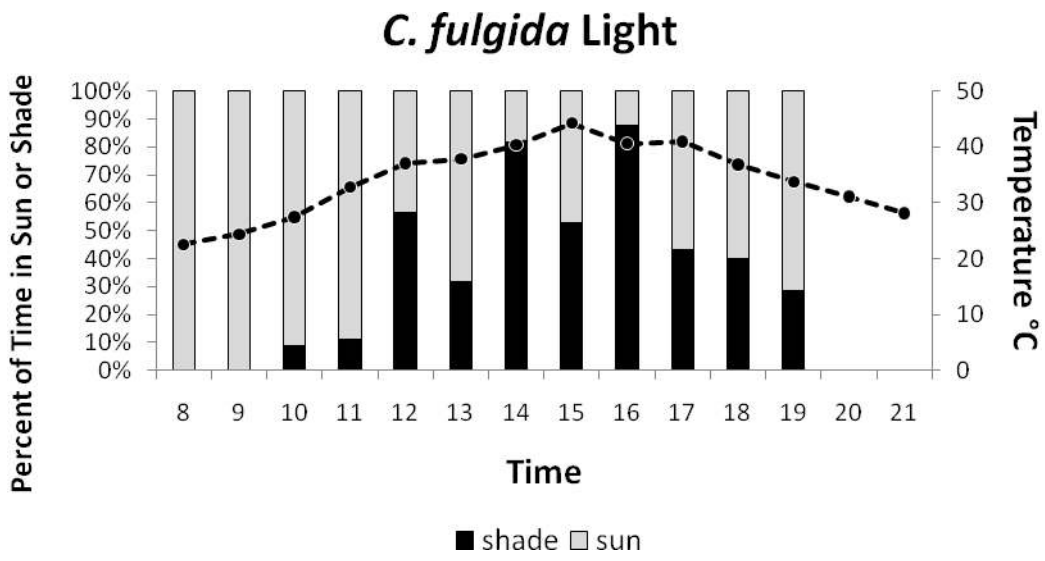
a)



b)



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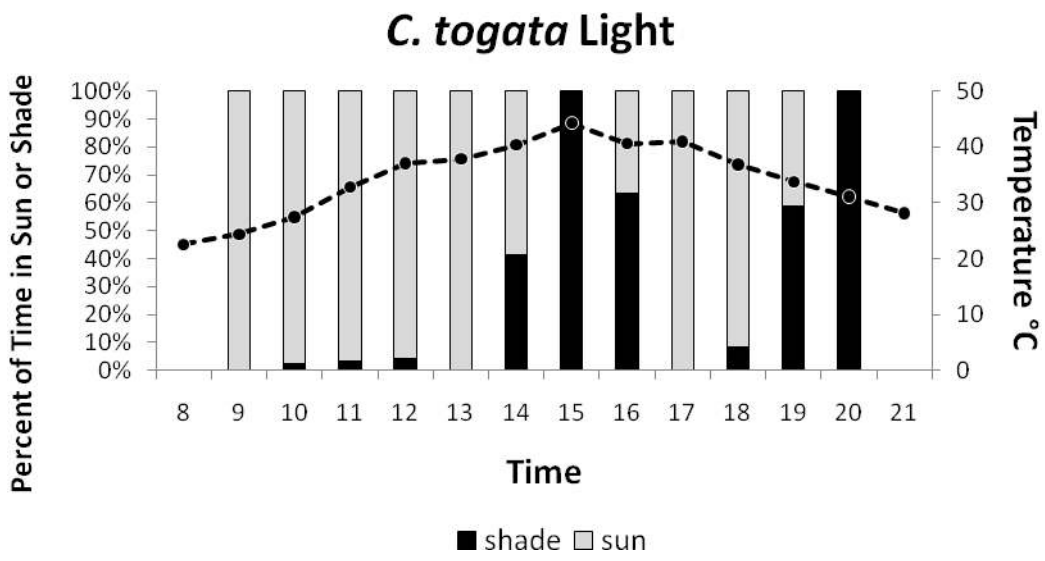
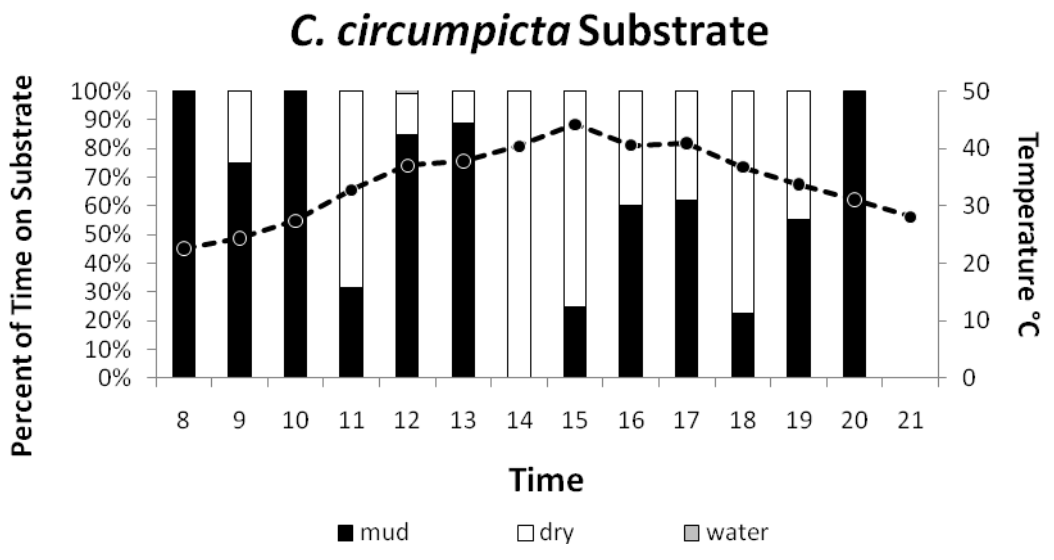
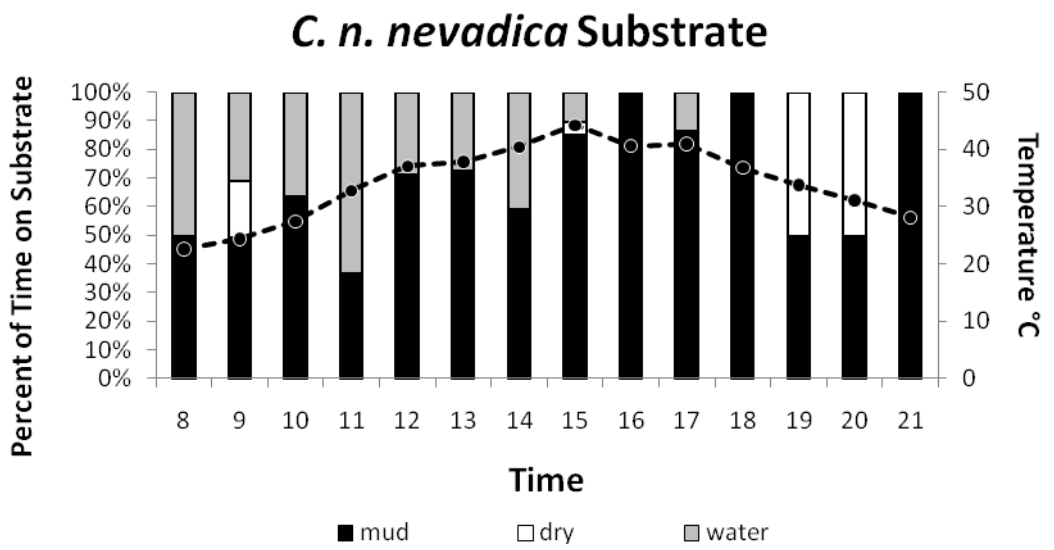


Figure 3-2. Proportion of time spent in the sun or the shade between 8:00 and 21:00 hours and surface temperature of the salt flats over those hours for four species of salt flat tiger beetle: a) *C. circumpicta*, b) *C. n. lincolniana*, c) *C. fulgida*, and d) *C. togata*.

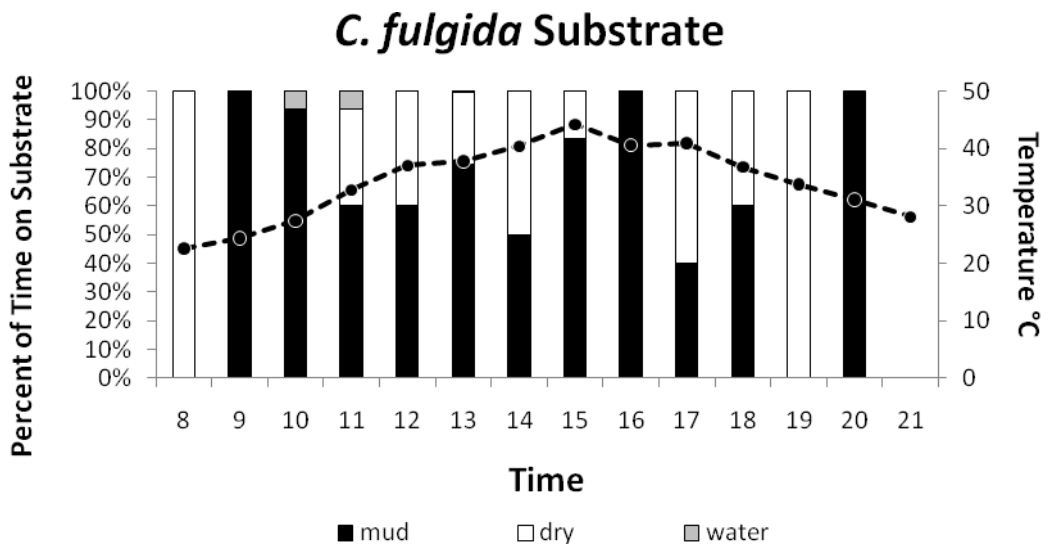
a)



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d)

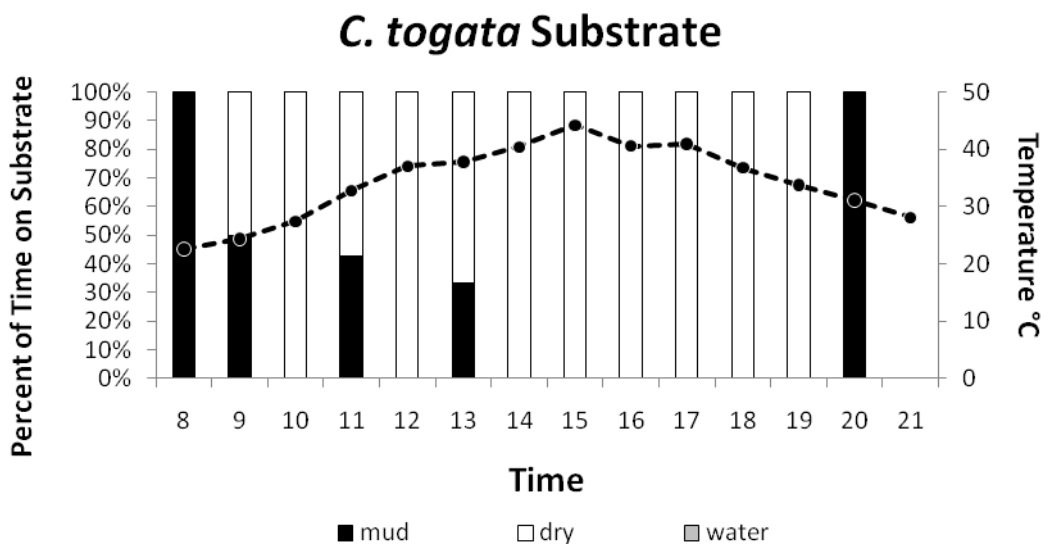
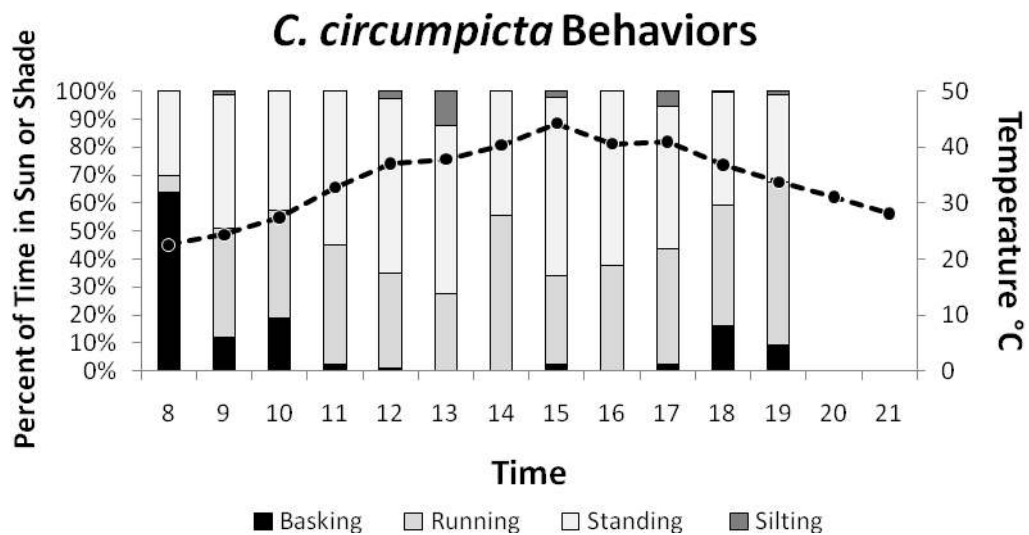
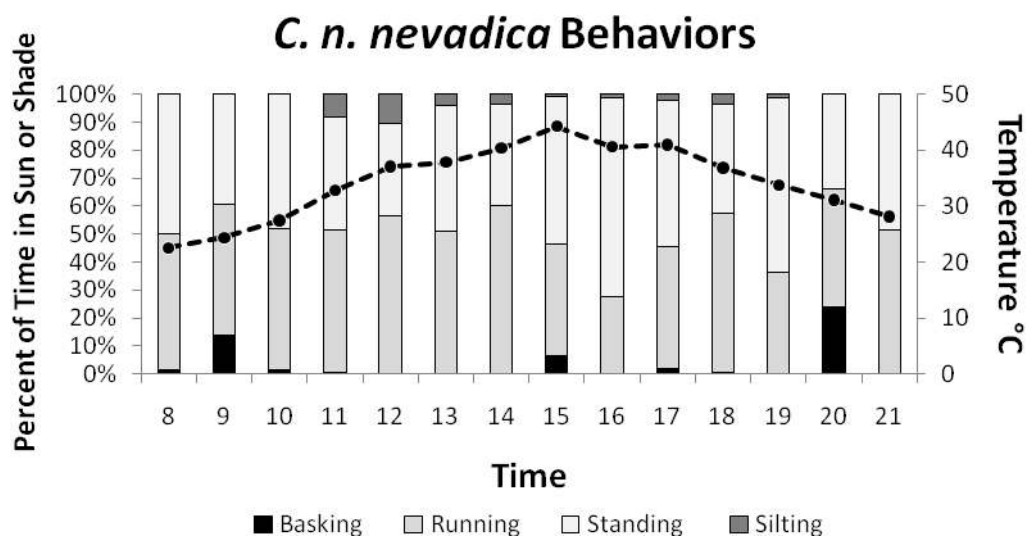


Figure 3-3. Proportion of time spent on individual substrates between 8:00 and 21:00 hours and surface temperature of the salt flats over those hours for four species of salt flat tiger beetle: a) *C. circumpecta*, b) *C. n. lincolniana*, c) *C. fulgida*, and d) *C. togata*.

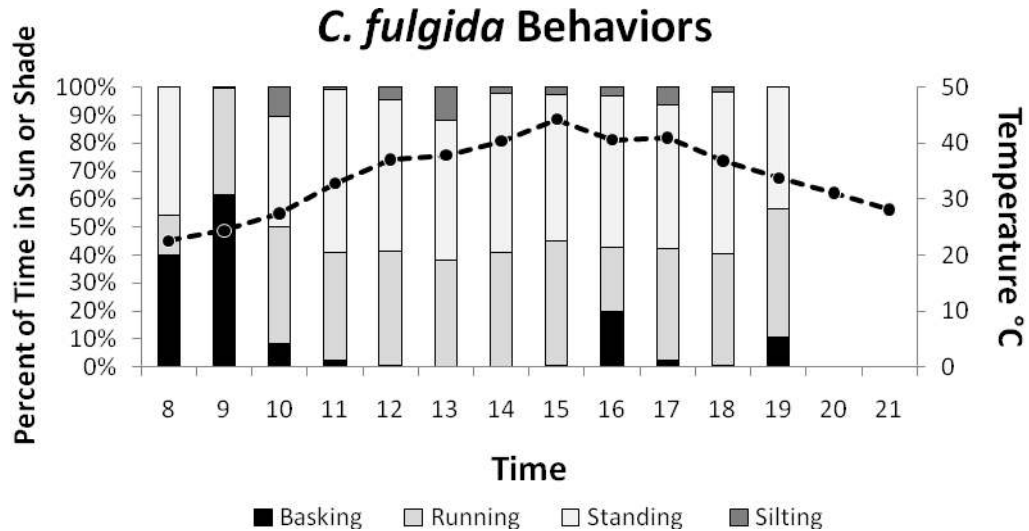
a)



b)



c)



d)

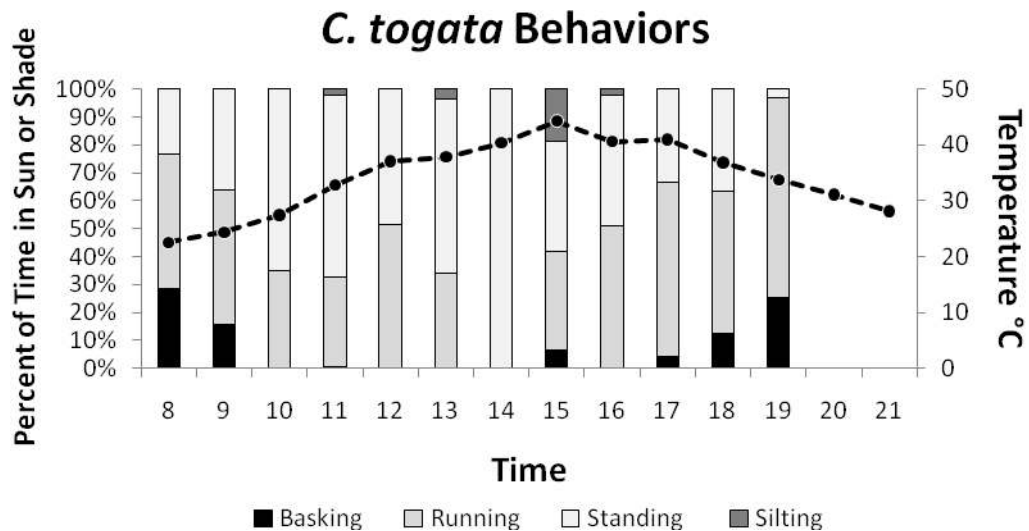


Figure 3-4: Proportion of time spent in distinct behaviors between 8:00 and 21:00 hours and surface temperature of the salt flats over those hours for four species of salt flat tiger beetle: a) *C. circumpicta*, b) *C. n. lincolniana*, c) *C. fulgida*, and d) *C. togata*.

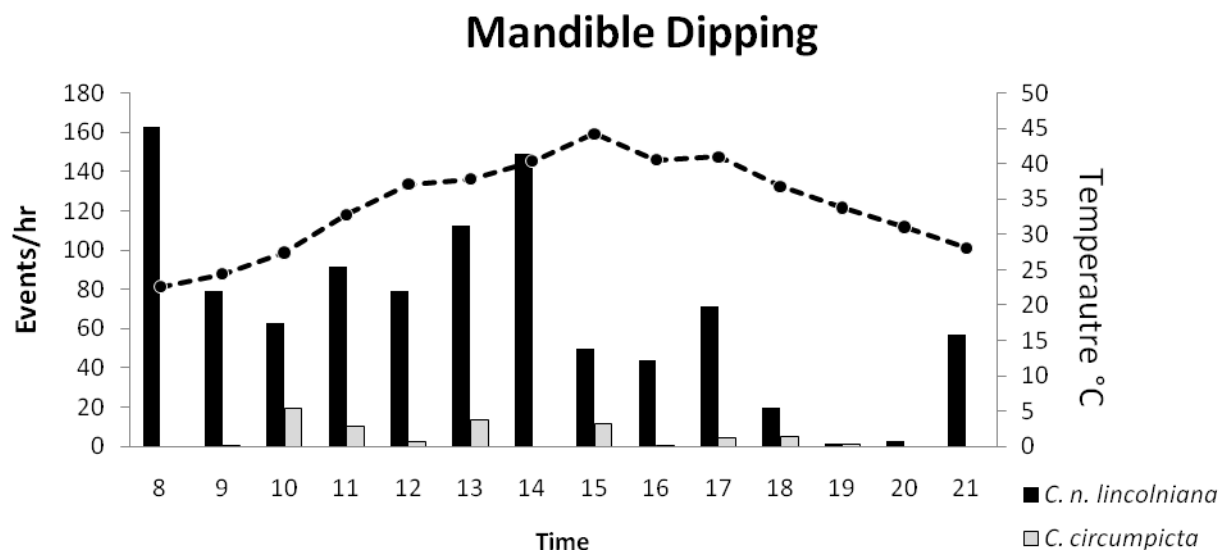


Figure 3-5. Average recorded mandible dipping events per hour of observation for *C. n. lincolniana* and *C. circumpicta* and surface temperature of the salt flats over those hours.

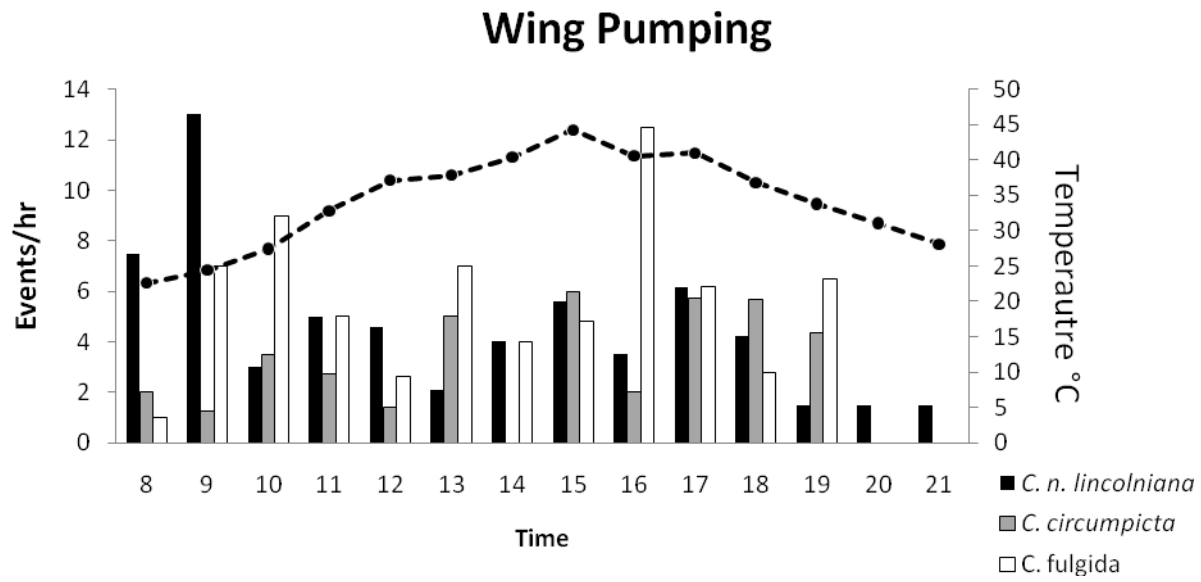


Figure 3-6. Average recorded wing pumping events per hour of observation for *C. n. lincolniana*, *C. fulgida*, and *C. circumpicta* and surface temperature of the salt flats over those hours.

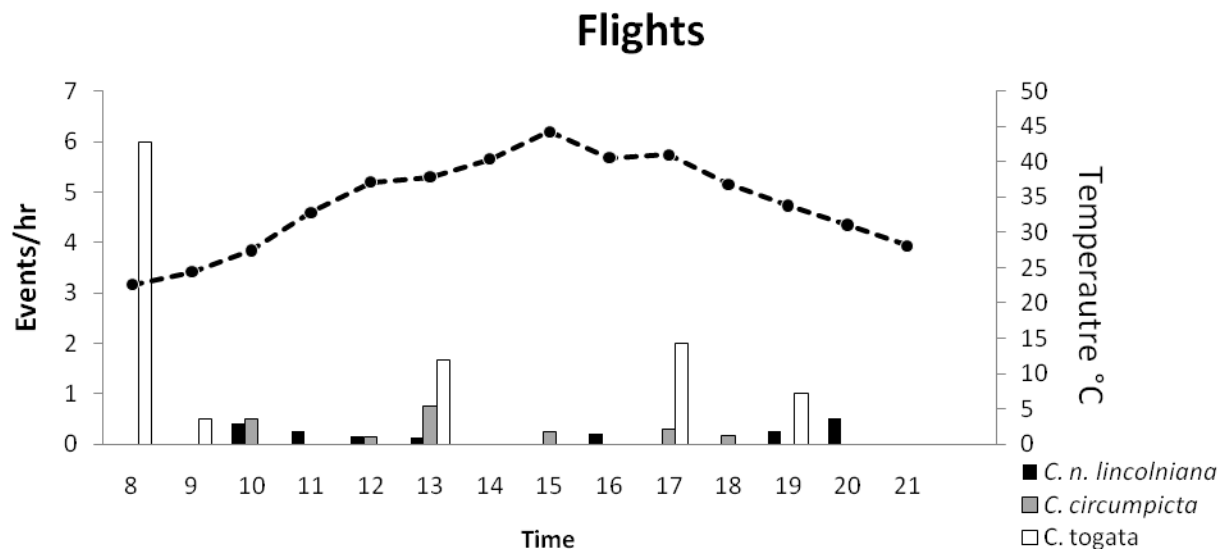


Figure 3-7: Average recorded flight events per hour of observation for *C. n. lincolniana*, *C. togata*, and *C. circumpicta* and surface temperature of the salt flats over those hours.

Table 3-1. Comparison of proportion of time spent in the sun by *C. n. lincolniiana*, *C. circumpicta*, *C. togata*, and *C. fulgida*. Significance examined between species and between morning (between 8:00 and 11:00 hours) and afternoon/evening (between 11:00 and 21:00 hours).

Species	Proportion of Time in Sun					
	Comparison	Mean	sd	F	df	P>f
Total	8:00-11:00	0.92	0.14	27.67	1,165	<.0001
	11:00-21:00	0.65	0.29			
<i>C. n. lincolniiana</i>	8:00-11:00	0.89	0.13	5.06	1,59	0.028
	11:00-21:00	0.74	0.26			
<i>C. circumpicta</i>	8:00-11:00	0.91	0.24	9.33	1,46	0.0038
	11:00-21:00	0.67	0.39			
<i>C. togata</i>	8:00-11:00	0.98	0.03	2.65	1,15	0.12
	11:00-21:00	0.62	0.41			
<i>C. fulgida</i>	8:00-11:00	0.94	0.10	13.98	1,39	0.0006
	11:00-21:00	0.54	0.31			

Table 3-2. Comparison of proportion of time spent on dry surfaces by *C. n. lincolniana*, *C. circumpicta*, *C. togata*, and *C. fulgida*. Significance examined between species and between morning (between 8:00 and 11:00 hours) and afternoon/evening (between 11:00 and 21:00 hours).

Species	Proportion of Time on Dry Surfaces					
	Comparison	Mean	sd	t value	df	P>f
Total	8:00-11:00	0.14	0.36	-2.05	165	0.04
	11:00-21:00	0.33	0.46			
<i>C. n. lincolniana</i>	8:00-11:00	0.09	0.30	0.63	59	0.53
	11:00-21:00	0.04	0.20			
<i>C. circumpicta</i>	8:00-11:00	0.14	0.38	-1.67	46	0.1
	11:00-21:00	0.46	0.48			
<i>C. togata</i>	8:00-11:00	0.33	0.58	-2.22	15	0.04
	11:00-21:00	0.86	0.34			
<i>C. fulgida</i>	8:00-11:00	0.14	0.38	-1.21	39	0.23
	11:00-21:00	0.38	0.49			

Table 3-3: Comparison of proportion of time spent on muddy substrates and in the water by *C. n. lincolniana*, *C. circumpicta*, *C. togata*, and *C. fulgida*. Significance examined between species, within substrates and between morning (between 8:00 and 11:00 hours) and afternoon/evening (between 11:00 and 21:00 hours).

Proportion of Time on Mud and Water							
Species	Time	Substrate	Mean	sd	t-value	Df	P>f
Total	8:00-11:00	Water	0.15	0.30	1.46	165	0.15
	11:00-21:00	Water	0.08	0.22			
	8:00-11:00	Mud	0.70	0.42	1.35	165	0.18
	11:00-21:00	Mud	0.58	0.45			
<i>C. n.lincolniana</i>	8:00-11:00	Water	0.36	0.40	6.57	60	<.0001
	11:00-21:00	Water	0.23	0.32			
	8:00-11:00	Mud	0.55	0.42	15.48	60	<.0001
	11:00-21:00	Mud	0.73	0.35			
<i>C. circumpicta</i>	8:00-11:00	Water	0.00	0.00	1.00	47	0.32
	11:00-21:00	Water	0.00	0.01			
	8:00-11:00	Mud	0.86	0.38	8.66	47	<.0001
	11:00-21:00	Mud	0.54	0.47			
<i>C. togata</i>	8:00-11:00	Water	0.00	0.00	NS	16	NS
	11:00-21:00	Water	0.00	0.00			
	8:00-11:00	Mud	0.67	0.58	2.20	16	0.04
	11:00-21:00	Mud	0.13	0.33			
<i>C. fulgida</i>	8:00-11:00	Water	0.05	0.12	1.59	40	0.12
	11:00-21:00	Water	0.01	0.03			
	8:00-11:00	Mud	0.81	0.38	8.68	40	<.0001
	11:00-21:00	Mud	0.61	0.49			

Table 3-4. Total events per hour averaged throughout the day for *C. circumpecta*, *C. n. lincolniana*, *C. fulgida*, and *C. togata*.

Species	Event Type		
	mandible dipping	flying	wing pumping
<i>C. circumpecta</i>	5.44	0.19	3.67
<i>C. n. lincolniana</i>	78.05	0.13	4.74
<i>C. fulgida</i>	2.95	0.51	5.59
<i>C. togata</i>	7.41	0.88	4.71

Chapter 4

Using the visual arts to promote the conservation of the Salt Creek tiger beetle

Abstract

The arts have been used for the promotion of the environmental movement and to increase awareness of conservation efforts. Increasing public opinion of less aesthetically pleasing organisms, such as the endangered Salt Creek tiger beetle, has been a difficult challenge for those working on the conservation of such organisms. By using the visual arts to promote an organism with a negative cultural stigma, such as in the case of insects, we will be able to examine the true ability of art to sway public opinion. I developed an art exhibit and educational publication to promote the conservation of the Salt Creek tiger beetle and associated saline wetlands. Exhibit attendees were given voluntary surveys were taken. Results from the survey suggest that exhibits such as these have a positive effect on those that attend them, especially on those who have not had previous experiences with conservation. Being able to make an emotional connection with a local conservation effort could help individuals relate to conservation efforts on a global scale.

Introduction

Current art-based outreach programs often focus on an organism or ecosystem that is already well supported by the public. Because much of the focus of our lab's research has been on the federally protected Salt Creek tiger beetle and because invertebrates have a clear negative cultural stigma (Kellert and Berry 1980) the Salt Creek tiger beetle is the perfect subject to examine how effectively the arts can be used to disseminate knowledge of a local non-charismatic species living in a local non-charismatic environment.

The Earth is in the middle of an environmental crisis caused directly and indirectly by human activity. There are many challenges that lie ahead of mankind's efforts to reverse the damage that has been done. Two of these challenges are tied to the apathy of our culture towards the environment on which we depend. The first challenge is the complexity of instilling values of nature in a civilization that is becoming increasingly detached from the natural world while the second challenge is the reality that the vast majority of biodiversity is seen as unimportant therefore not worth our resources to preserve.

Humans are now more detached from nature than ever. On a recent outing to native prairie near Lincoln Nebraska with a 3rd grade classroom I was shocked at a comment made by one of the students. "Why isn't someone mowing this grass?" It is concerning that a child growing up in a city of only 226,000 is unfamiliar with the native habitat that historically covered 99% of their home state. We have not always been so disconnected from the environment. One simply has to open a book on early humans to get a sense of the importance nature played in the everyday lives of early man. Religions

were based on the natural world and it is commonly thought that early belief systems were set in place in an attempt to bring order to the chaos of the environmental conditions man could no control. Roszack (1992) coined the term ecopsychology, the connection between ecology and human psychology and maintains that this alienation has had negative psychological consequences on humanity. One of the greatest writers about the value of nature Thoreau (1906) wrote, “We can never have enough of nature. We must be refreshed by the sight of inexhaustible vigor, vast and titanic features...the wilderness with its living and decaying trees, the thunder cloud, and the rain in which lasts three weeks and produces freshets. We need to witness our own limits transgressed, and some life passing freely where we never wander.”

The majority of biodiversity on our planet is phylogenetically distant from humans and considered to be non-charismatic. Psychologically mankind may downplay the significance of these groups of these groups of organisms but from a physiological standpoint our existence, along with the rest of the “charismatic” mega fauna, would not be possible without the presentence of what most would consider insignificant creepy crawlies. One study places the value of the services provided by insects at more than \$57 billion to Americans. This breaks down at 50 billion in value as food for wildlife that supports a \$50 billion recreation industry, 4.5 billion in pest control, 3 billion in crop pollination, and 380 million in the cleanup of grazing lands. (Losey and Vaughan 2006)

Humans are predisposed to assigning value to organisms in relation to that organism’s phylogenetic relation to themselves.(Kellert and Berry 1980). The more similar an organism is to humans physically the more they tend to evoke a positive affect or emotion. This phenomenon has been coined as the biophilia hypothesis (Wilson 1984).

Clearly, public perception is vital to the success or failure of any conservation effort. Yeffee et al. (1996) states that "...public opposition is the major constraint to implementing ecosystem management plans in the United States." More and more attention is being directed towards education and outreach when developing plans for species conservation. An informed and involved public is instrumental in conservation efforts (Jacobson et al. 2007). There is no difficulty in eliciting public support for charismatic species such as the Bengal tiger. A survey conducted by Kellert and Berry (1980) found that while 89% of people agreed that the Bald Eagle should be protected only 25% if those surveyed agreed that the Kawai wolf spider should be equally protected.

If it is true that insects and other invertebrates are not commonly perceived as worthy of protection, how can we address the challenge of a society that is seemingly uninterested in the preservation of over 99% of animal biodiversity? This challenge will force humanity to drastically shift the way they view the world around them. Paradigm shifts such as these are extremely difficult to achieve. It may be possible to foster significant changes in people's attitudes and behaviors through the arts. Curtis (2003) notes that the arts are commonly at the forefront of challenging dominant paradigms and that artists are often active participants in attempts to change society. The arts have a long tradition of being used to foster certain ideologies throughout history. As long as civilization has existed mankind has represented nature through the arts. The first symbols used by man were animals (Berger 1980).

More recently the arts have been used to promote conservation efforts. Art movements during the 1960s coincided with the awakening environmental consciousness

of the world. It is commonplace to see art being used as an outreach tool to promote conservation. Emotions play a central role in human decision making processes (Jacobson et al. 2007). Many people are aware of the larger global issues associated with conservation and have strong feelings associated with that knowledge but few are aware of issues in their own community.

Given my interest in scientific illustration and the focus of my research on invertebrate conservation, I was particularly concerned about how the community that shared the Eastern saline wetlands of Nebraska viewed the plight of the Salt Creek tiger beetle. The Salt Creek tiger beetle is a diurnal predator that actively seeks out its prey on the salt flats of saline wetlands of Lancaster County located in Southeastern Nebraska. Today there are less than 1% of these wetlands in existence making them one of the rarest ecosystems in the world. Populations of Salt Creek tiger beetles have been monitored since 1991 (Spomer and Higley 1993, Allgeier 2005). Visual counts of adult beetles have fluctuated over the last 17 years but it is clear that numbers are dangerously low. The low population of the Salt Creek tiger beetle is directly tied to habitat loss and the city of Lincoln encroaching on the last pockets of habitat where this beetle occurs. The Salt Creek tiger beetle received state of Nebraska and federal listing as endangered over the past 10 years, and I have been involved in the research, rearing, and recovering planning for the beetle.

So one of the rarest insects in North America in one of the rarest habitats occurs only in Lancaster County Nebraska, in or near the city of Lincoln. Moreover, the salt flats themselves were the reason the city of Lincoln (originally called Lancaster) was originally founded, based on the mistaken notion that the salt was associated with an

underlying salt dome that could be mined. However, the uniqueness of Salt Creek tiger beetle, its habitat, and the historical importance are not reflected in public opinion.

As with other invertebrates public perception of the Salt Creek tiger beetle has been largely negative. The small amount of land that has been protected as part of the habitat conservation plan being developed for the beetle can no longer be developed which has angered land owners and developers. This is largely because this land is considered to be in a prime spot for development. In her book "Hope for Animals and Their World: How Endangered Species Are Being Rescued from the Brink" Jane Goodall (2009) observed in reference to the Salt Creek tiger beetle "And of course, there can be no question of the importance of protecting an ecosystem and preventing the loss of biodiversity. Yet there are millions of people who simply 'don't get it.' Especially if the species concerned is an insect - 'Just a bug!'" She notes that "...while many (Lincoln Journal Star) readers welcomed the decision, many others were shocked and horrified; some, too, were genuinely mystified."

We have been involved in many public education efforts about Salt Creek tiger beetle and environmental education. Many of these focus on elementary students who have been responsive and even excited about having an endangered species almost literally in their backyards. A teacher from Fremont Elementary School spoke to a parent of one of her 3rd grade students during a parent teachers conference. This parent recounted a surprising dinnertime discussion with his daughter after he proclaimed that the idea of saving the Salt Creek tiger beetle was a "stupid idea." Having just discussed the importance of this beetle to the Salt Creek wetlands in class his daughter quickly corrected her father by pointing out that the tiger beetle was an important component in

that ecosystem's web of life. This proud parent promptly took his child out for ice-cream as a celebration of her intelligent rebuttal. This is a clear example of how speaking to youth can have a domino effect in the community. To seek broader audience and explore the use of art in environmental education, I developed and curated an art exhibit featuring the Salt Creek tiger beetle and its native habitat. The Nebraska Environmental Trust, the Harden Art Center, Pioneers Park Nature Center, Insect Ecology Project of UNL, Nebraska Game and Parks, the U.S. Fish and Wildlife Service, Roots and Shoots, the Great Plains Chapter of the Guild of National Scientific Illustrators (GNSI), the Saline Wetland Partnership and the Xerces Society, a society for invertebrate conservation, were all involved in the creation and support of of this exhibit. Because the impact of art on social acceptance is relatively unknown we saw this as an opportunity to explore how effective this sort of event is on changing public perception.

Methods and Materials

During July 2009 an art exhibit by 15 local artists and another 9 from outside the state of Nebraska featuring the Salt Creek tiger beetle was held at the Haydon Art Center in downtown Lincoln. Artists were asked to participate based on their interest in conservation, talent, and for the diversity of their mediums. Artwork included origami, fabric arts, sculpture, oil paints, watercolor, and photography. Artists were encouraged to include a personal artist's statement that reflected their thoughts on the Salt Creek tiger beetle and the conservation of this local wetland. In the center of the exhibit a collage of mounted insects were displayed. These insects were collected as part of a survey being conducted by the University of Nebraska to assess the diversity of insects found within these wetlands along with a description of the survey was displayed. Informational

posters were hung in the exhibit that featured some of the research being conducted by the University of Nebraska Lincoln.

Along with the art gallery's normal advertising for a new exhibit, the exhibit also was promoted throughout the city of Lincoln during the month prior to the exhibit. An article about the art exhibit was featured in a local publication and a full color art catalogue was created that also included educational information about the Salt Creek tiger beetle and its native wetland ecosystem, and was distributed without charge to attendees. Pioneers Park Nature Center, a local nature education center, teamed up with the educational coordinator for the Haydon Art Center to create a nature focused art unit for a local summer camp series offered to 3rd grade students within the city of Lincoln. These students were taken to the art exhibit as well as the actual saline wetlands where they learned about the native plants and animals first hand.

Another aspect of exhibition promotion were an opening reception and a reception with the artists. The opening reception was held on 10 July 2009 in conjunction with the city of Lincoln's First Friday art walk where most of the city's 15 local art galleries are open. The second reception held on July 31st 2009 was held as an artists' reception where all of the artists included in the show and all those involved with the creation of the exhibit were invited, as well as members of the general public.

Volunteers at the Haydon Art Center recorded the number of individuals that attended the exhibit on a daily basis and encouraged individuals to sign the guest book. Along with these methods of keeping record of attendance a voluntary survey was conducted (Fig. 4-1). Ideally we would have had attendees fill out a before and after survey but because of the nature volunteer surveys it would have been difficult to

encourage participation with no incentives available. The survey collected demographic information such as age, gender, occupation, and hometown along with asking them to recall their knowledge of and feelings toward the Salt Creek tiger beetle and its habitat before and after the exhibit. Surveys were conducted by giving subjects statements that they could agree or disagree with by using a nine point scale beginning with “disagree extremely” and ending with “agree extremely.” Subjects responses were categorized into three different categories: students, academics, and non-academic adults.

Data from surveys were categorized by subject group, and analyzed within each group. Statistical analysis was by one-tail, paired t-test (response for before and after exhibit attitudes). We used a one-tail test based on the expectation that the exhibit couldn't make respondents less informed about conservation issues.

Results and Discussion

The artists' wide range in background provided for a very interesting and dynamic exhibit. Several works by artists from outside the Lincoln area drawn to the plight of the Salt Creek tiger beetle were included. These included “Teri” the Salt Creek tiger beetle by Jessa Huebing-Reitinger (Fig. 4-2), an original print of Salt Creek tiger beetle by British artist Christopher Cockburn (Fig. 4-3), a new tiger beetle watercolor by Dr. John Cody (Fig. 4-4), and an origami Salt Creek tiger beetle by Dr. Robert Lang (Fig. 4-5) were among the pieces that were shown. Jessa Huebing-Reitinger is co-founder of the educational traveling art show “Project Insect” in which insects and insect conservation are explored. As part of her artist's statement, Huebing-Reitinger wrote, “Accepting the challenge to paint the dire need of this very small, yet most important Salt Creek tiger beetle came with many personal sacrifices. Our passion to assist the smallest of creatures

in peril required our extreme devotion. We vowed to return to Nebraska and paint portraits of the beetle as many times as necessary in support of educating local communities and seek awareness through media and press in assistance of the tiger beetle achieving Federal Listing status.” (Brosius and Helm 2009)

Christopher Cockburn is a British artist who emphasizes the aesthetic appeal of insects through his work. His contribution to the exhibit included a linoleum block print of the beetle where he tried to “portray the feeling of the animal.” Dr. John Cody is the premier living watercolorist of insects, who has exhibited throughout the United States, including the Smithsonian. In his artist’s statement he humorously noted the insignificance of the Salt Creek tiger beetle “I have to say that never before have I ever painted anything so pusillanimous. When you said ‘tiger beetle’ I pictured something orange, striped and tremendously menacing about a third of a foot long, practically a ‘frumious bandersnatch’. Not a bit of it. Instead I found myself on the very verge of dislocating my eyeballs as I tried to get them to focus on a timid looking creature that was absolutely teensie bordering on weensie!” (Brosius and Helm 2009). Dr. Lang is a world-renowned origami artist who was featured in the February 19, 2007 issue of The New Yorker. In the article by Susan Orlean, Lang expresses that his “...favorite commission was to fold an endangered Salt Creek tiger beetle for an entomologist who collects Salt Creek tiger beetle art. ‘For me, that commission was like manna from Heaven,’ he said. ‘I’ll never be done with bugs.’

The exhibit also included works of local artists such as Joel Sartore, noted photographer for National Geographic. Mr. Sartore has taken personal interest in the tiger beetle. During public lectures, he has referred to the beetle as “the last of the least,”

making the point that caring enough to save a single insect may better ensure our own future well-being.

Many members of the Great Plains Chapter of the Guild of Natural Science Illustrators GNSI, Inc. had original works in the exhibit. At the national level, Guild members carry on a long partnership of art visualizing science to help viewers interpret complex scientific information. Their works appear in scientific journals and texts, educational materials, museum exhibits, and in such varied places as on television, in newspapers, and on stamps. The Great Plains Chapter is a regional group of Nebraskans, Iowans, and Kansans who are scientists as well as gifted artists with specialized degrees in science and/or art who run illustration businesses, paint, and teach.

This unique exhibit also included amateur artists with impassioned interests in tiger beetle preservation to be showcased alongside professional artists. Dr. Leon Higley a professor of applied ecology at the University of Nebraska, Lincoln included a photograph taken during a hot day of field research. He has been researching the Salt Creek tiger beetle since 1993 and was a member of the group that succeeded in getting the endangered listing for the tiger beetle in 2005. Dr. Phyllis Higley, a professor of biology at the College of St. Mary's in Omaha, included a piece of fabric art depicting the Salt Creek tiger beetle. Because this exhibit focused on public perceptions we wanted to be sure to include the young voices of Lancaster County. The Bright Lights summer enrichment classes, "Art: Animal & Nature Drawing" included a mural of the salt creek habitat that was created in the 2008 classes. This mural drew in the 30 or so 3rd-6th grade students and their parents to the exhibit. The booklet that was created as part of the

exhibit is currently being used in Bright Lights Animal and Nature Drawing classes as a reference to the salt creek marsh environment and tiger beetle.

A total of 72 surveys were completed (approximately 13% of those that attended the exhibit). A t-test was performed to look at the statistical significance of changes in perceptions (Table 4-1) Overall subjects were affected positively by the survey with an average point change of their attitudes of 1.3. When split into categories those in academia changed the least (0.7) followed by students (1.1) and then adult non-academics (1.4). The academic category saw no significant change in any of the four categories that the subjects were surveyed on (Table 4-1). It is not surprising that those subjects in academia, who were primarily from the biology department, already had prior knowledge of the Salt Creek tiger beetle and had a positive view of insects. Those who were listed as academia had a more positive overall score than the two other categories with an overall average before score of 7.8 (adult-non academic 6.5, students 6.6). Most students were from Lincoln which means that they have probably been exposed to the tiger beetle in a classroom setting. The largest changes that we saw were in the adult non-academics group which is a positive outcome. Those individuals in the adult non-academics group are not exposed to ideas about the environment or the value of all organisms on a regular basis. The categories with the largest changes within this group were if they had adequate knowledge of the saline wetlands and the Salt Creek tiger beetle (2.4) followed by their awareness for endangered habitats located in Nebraska (1.6). This is an encouraging result from the exhibit. We think that this increased knowledge and awareness of the Salt Creek tiger beetle and associated wetlands could be the result of participants being inspired to read information provided in the exhibit.

Some comments written on the survey:

“It is neat to see art from biologists”

“The Salt Creek tiger beetle was at the heart of our class: marine scientist in NE, Loved the display!”

“An outstanding exhibition – so many pieces with such strength and compelling beauty. Thank you”

“Wow! Who knew ?!”

“Really weird in a good way”

“This is a truly beautiful and inspiring display! I have never so convincingly seen artists as advocates for causes and ideas - more as expressions of impressions and experiences. If as a very flattering demonstration of their skill and effectiveness in this regard. So glad I made the trip down to see it for myself.”

“Beautifully and thoughtfully illustrated. Take these to the schools!”

“This was a great exhibit, especially in the summer where tourism brings more people to possibly see it. We need to protect our endangered species. You never know, we might become one someday. God created all life for a purpose and we shouldn't nonchalantly let any of it becoming extinct.”

It was clear that those individuals with a biology background were more educated about endangered species within the state of Nebraska but many of them still felt that their knowledge of the Salt Creek tiger beetle was increased by the exhibit. The most interesting results came from the sector of the public that did not have a background in biology. This exhibit and catalogue clearly helped people look at this insect in a new light and made them really consider what makes an organism worth saving.

Overall this event was very successful and reached many of its goals. A wide sector of the public was educated about the status of the Salt Creek tiger beetle in a new and innovative way. Many organizations worked together for the first time and the art exhibit fostered collaboration between groups that normally do not come into contact.

The benefits from this exhibit will continue in the future as collaborations continue to expand among all of the groups involved. However, the benefits from this exhibit could have been more significant. Unfortunately having an exhibit like this up for only a single month at a single location severely limits the magnitude of potential impact. Additionally, having more educational material on display might have been useful in building upon enthusiasm generated by the art and would have increased the overall intended educational goal of this exhibit. However, too much educational emphasis could have destroyed the sense of aesthetic appreciation for nature that was embodied in the exhibit.

In *Alice through the Looking Glass* the gnat asked “ What sort of insects do you rejoice in where you come from?” Scientists and artists can have a unique connection to their subjects: understanding, appreciation, and often an emotional bond. It is clear from this exhibit that the arts can play a vital role in the education of not only the charismatic species but also the invisible or despised species. Perhaps the value of combining art and science is to give people access to insects and ecosystems in which they can rejoice.

Salt Creek Environment: Local and Endangered Exhibit Impact Survey:

The University of Nebraska-Lincoln Department of Entomology is currently sponsoring a survey of the impacts of this art exhibit. Asking questions about what was learned from this exhibit is important for identifying what best motivates and educates people about endangered species and how art may or may not influence attitudes towards conservation.

Your answers are completely confidential and will be used to evaluate the impacts of this art exhibit. The survey should take about 5 minutes to complete and all information will be used in summaries in which no individual's answers can be identified. There are no benefits or risks to you for participating. This survey is voluntary and there is no requirement for your participation.

This project has been approved by the Institutional Review Board (IRB) at University of Nebraska-Lincoln (IRB#**). If you have questions about this research, you can contact Tierney Brosius at (402) 472-8698 or the IRB at (402) 472-6965.

Age _____ Gender (circle one) M F
 Occupation _____
 Hometown _____ State _____



	Disagree extreme	Disagree very	Disagree moderat	Disagree slightly	Neither agree or disagree	Agree slightly	Agree moderat	Agree very	Agree extreme
BEFORE THIS EXHIBIT (X in box)									
... you had adequate knowledge about Lancaster County's saline wetlands and the Salt Creek tiger beetle.									
... you considered insects to be beautiful									
... you considered Insects to be economically and environmentally important									
... you were aware of critically endangered habitats located in Nebraska.									
AFTER THIS EXHIBIT (X in box)									
... you had adequate knowledge about Lancaster County's saline wetlands and the Salt Creek tiger beetle.									
... you considered insects to be beautiful									
... you considered Insects to be economically and environmentally important									
... you were aware of critically endangered habitats located in Nebraska.									

Thank you for your participation! Please feel free to write any comments on the back of this survey.

Figure 4-1. Voluntary survey conducted at the Haydon Art Gallery in 2009. Participants were asked to rank questions on a 9 point scale varying between extremely disagree to extremely agree.



Figure 4-2. Oil painting by Jessa Huebing-Reitinger for Salt Creek tiger beetle art exhibit. 60 inches by 44 inches



Figure 4-3. Linoleum block print by Christopher Cockburn included in the Salt Creek tiger beetle art exhibit. 12 inches by 12 inches.



Figure 4-4. Water color by John Cody painted for Salt Creek tiger beetle art exhibit. 10.75 by 7.5 inches.



Figure 4-5. Origami by Robert Lang commissioned by Leon Higley. Included in Salt Creek tiger beetle art exhibit. 6 inches

Table 4-1. Analysis of survey responses for all respondents and by individual respondent categories (student, non-academics, academics). Statistical analyses were by one-tail, paired t-test (response for before and after exhibit attitudes). We used a one-tail test based on the expectation that the exhibit couldn't make respondents less informed about conservation issues.

Paired Two Sample for Means								
	"Wetland Knowledge"		"Insects are beautiful"		"Insects are important"		"Awareness of ecosystem"	
	Before	After	Before	After	Before	After	Before	After
All Individuals Surveyed								
Mean	5.11	7.22	7.16	7.76	7.58	8.35	6.67	8.08
Var.	6.84	2.80	3.48	3.56	3.63	1.61	4.73	1.57
df	73		73		71		71	
t Stat	-8.96		-4.72		-4.74		-6.52	
P(T≤t)	>0.001		>0.001		>0.001		>0.001	
Academics								
Mean	6.50	7.50	8.83	9.00	8.83	8.83	6.83	8.33
Var.	10.30	3.90	0.17	0.00	0.17	0.17	7.37	1.47
df	5		5		5		5	
t Stat	-1.22		-1.00		NS		-1.57	
P(T≤t)	0.138		0.182		NS		0.089	
All Non-Academic Adults								
Mean	4.85	7.13	7.28	7.91	7.49	8.34	6.51	7.98
Var.	6.86	2.73	3.21	2.86	3.60	1.54	5.10	1.52
df	52		52		52		52	
t Stat	-7.96		-5.02		-4.20		-5.50	
P(T≤t)	>0.001		>0.001		>0.001		>0.001	
Students								
Mean	5.77	7.46	6.08	7.00	7.38	8.15	7.23	8.38
Var.	5.36	2.77	4.24	5.83	4.92	2.64	2.36	1.92
df	12		12		12		12	
t Stat	-4.25		-2.22		-2.38		-3.64	
P(T≤t)	>0.001		0.023		0.017		0.002	

Summary and Conclusions

Understanding what factors contribute to extinction and what techniques (whether they be scientific or sociological) aid in preservation are fundamental in conservation. Niche partitioning has been closely examined as a driving force in natural selection. How ecologically specialized an organism becomes directly impacts the size of their niche and therefore their vulnerability to habitat disturbances. Our findings support other research that suggest that organisms that have become highly specialized, assumedly to reduce competition, are more likely to suffer declines in population than more generalized species.

Ovipositional range selection is significantly different between individual species occurring in the saline wetlands of Lancaster County, Nebraska. *Cicindela nevadica lincolniana* had an ovipositional range selectivity much narrower than the more common *C. circumpecta*. This selectivity could be one reason populations of *C. n. lincolniana* have been in decline. As saline wetlands are disturbed by alterations to waterways, invasive plant species, and human development soil surfaces with acceptable margins of salinity become scarcer. Because *C. circumpecta* finds a wider range of salinities acceptable for oviposition their overall population seems to be much stronger than the more saline restricted *C. n. lincolniana*. Interestingly salinity does not seem to be a requirement for larval development. In the lab *C. n. lincolniana* were successfully reared in topsoil with no added salinity. This suggests that salinity is being used for an ovipositional queue rather than a developmental requirement.

Rearing *C. n. lincolniana* in captivity has provided much insight into the natural history for this species as well as provided another tool to aid in its recovery.

Creating a multiple decrement life table and the determination of death probabilities in the absence of other causes provide insights into mortality and indicate the most important areas of research. Without this information, it might be tempting to focus on increasing oviposition or improving first stage larval establishment. However, this research indicates that addressing overwintering mortality is a critical component to the development of a viable captive rearing program. This is a clear example of why it is so important to examine different assumptions in stage-specific mortality and project changes on overall mortality with any captive rearing program that aims to restore a population of organisms.

Thermoregulatory behavior has been well studied in tiger beetles but not within a complex of tiger beetles that includes a tiger beetle species on the brink of extinction. Understanding how species reduce completion while coping with the extreme temperatures found on these salt marshes will play a key part in understanding what microhabitats are important to adult *C. n. lincolniana*. From this research it is clear that maintaining shallow seeps for *C. n. lincolniana* to use for foraging is important, especially since we know that food acquisition by adults is directly tied to female fecundity.

No conservation program can be completely successful without the support of the community. Most individuals are aware of the growing need for conservation but do not associate this need with their community. Rather, they associate conservation with far off places like the Great Barrier Reef and the Amazonian rain forests. Using art as a means of reaching the public about local conservation issues appears to be a successful way of increasing public perceptions. Using an accessible medium such as art breaks down

barriers that develop between science, which can often come across as intimidating, and the general public.

Future research should focus on ways of increasing viable habitat as well as linking existing and historical habitat. Reintroductions of captive reared individuals would help in this process. Optimizing captive rearing by decreasing mortality in the lab as well as determining the best way to reintroduce lab reared individuals will an essential component in *C. n. lincolniana*'s recovery program. Continued focus on public education will help to ensure continued support from the local community.

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