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lincolniana (COLEOPTERA: CARABIDAE).

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**THE NATURAL HISTORY AND CAPTIVE HUSBANDRY OF THE
SALT CREEK TIGER BEETLE, *Cicindela* (= *Ellipsoptera*) *nevadica lincolniana*
(COLEOPTERA: CARABIDAE).**

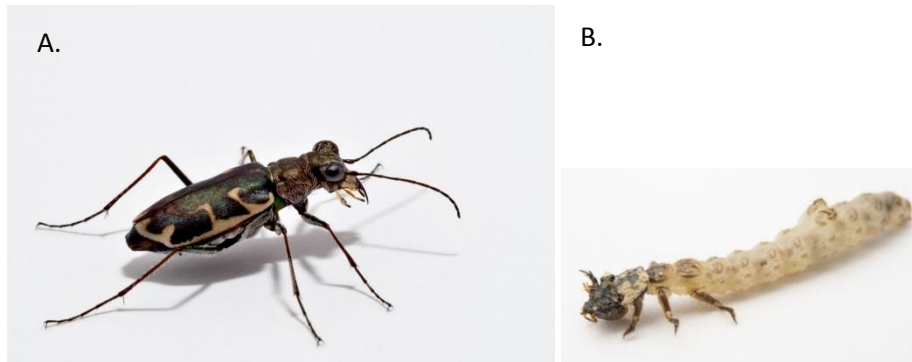


Fig1: A. Adult Female *Cicindela* (= *Ellipsoptera*) *nevadica lincolniana*, photo by Jeremy Dixon. B. Third instar *C. n. lincolniana* larva, photo by Joel Sartore.

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INTRODUCTION

Tiger beetles have long been admired for their mix of beauty, speed, and ferocious hunting abilities (Pearson 2011). The tiger beetles are some of the fastest insects on the planet with the Australian species, *Cicindela hudsoni* being clocked at 2.5 meters per second (Merritt 1999). The tiger beetles run so fast that they are temporarily blinded when they are engaged in the high speed pursuit of their prey (Friedlander 2014). However, despite their speed, and the joy they bring to many enthusiasts, tiger beetles are unable to outrun the destruction and degradation of their habitats by human activities, and an estimated 15 percent of the 255 described species and subspecies of North American tiger beetles are now threatened with extinction (Pearson 2011). Several species of tiger beetle are very niche specialized and will only inhabit certain areas where the conditions are just right for their survival (Pearson 2006).

The saline wetlands of Lancaster and Saunders counties located in eastern Nebraska are home to one such beetle. An endemic subspecies of the Nevada Tiger Beetle, *Cicindela* (= *Ellipsoptera*) *nevadica*, calls these saline wetlands home. This beetle is aptly named the Salt Creek Tiger beetle, *Cicindela* (= *Ellipsoptera*) *nevadica lincolniana*, due to its presence only along Little Salt Creek and associated tributaries (Spomer, *et al.* 2007). The olive-colored, 5mm long beetles are dependent on the saline wetlands, and due to the rapid destruction of their habitat by farmers and developers, are now threatened with extinction (Spomer, *et al.* 2007). The population numbers for the *C. n. lincolniana* range from 150-1000 adults a year, making this species one of the most endangered insects in North America (Higley & Spomer 2001).

The following paper gives a brief overview of the natural history of the *C. n. lincolniana* and outlines the efforts of the Omaha's Henry Doorly Zoo and Aquarium in its attempt to captive rear this species, and save Nebraska's native tigers and the wetlands they call home.

NATURAL HISTORY

The Salt Creek Tiger beetle, *C. n. lincolniana*, is a small, predatory beetle native to the eastern saline wetlands of Nebraska. These beetles are found on small patches of land, most 30 yards or less in length scattered along the Little Salt Creek, inside of the city limits of Lincoln, Nebraska (Brosius 2010). This species takes one to two years to complete its lifecycle and spends a majority of this time in the larval stage.

The adults of these summer active beetles emerge in early June in most years and are active until about mid-July (Brosius 2010). Female beetles usually emerge a few days after the first males and are ready to be bred upon emergence from their pupal cells (Spomer personal communication 2014). The males are quick to mount any female that they see and grab them firmly around the thorax with their large mandibles. The females usually respond to the male's advances by violently rolling and bucking in attempts to shake the male. If the male is able to hold on for a few seconds then he is allowed to copulate. After engaging in this "vigorous copulation" the male will continue to hold onto the female to insure that his spermatophore does not get displaced by a competing male (S.M. Spomer personal communication 2014). This behavior is called mate-guarding and can last several minutes to more than an hour and a half. Shortly after breeding the females begin to probe the soil seemingly searching for a substrate that meets the correct specifications for oviposition. When the females locate suitable substrate they will lay one 2mm oval shaped, white egg into the soil (Higley & Spomer 2001). Females are believed to lay a majority of their eggs in the first few days of their life, in a process called egg dumping (S.M. Spomer personal communication 2014). The female *C. n. lincolniana* oviposits at night to avoid the extreme temperatures that occur at ground level in the saline wetlands (Higley & Spomer 2001).

These beetles are very niche specific and have only been found to lay their eggs in soils that have concentrations of sodium chloride (NaCl) that are between 0.354M and 0.5M NaCl (Brosius 2010). This niche specialization has been evolved to reduce the larval competition among the species of tiger beetle that inhabit these small fragmented habitats (Hoback *et al.* 2000, Allgeier 2005). A study done by Hoback, *et al.* (2000), found that while different species of adult tiger beetles are often found together along the salt flats associated with the saline wetlands, the larvae of each species are often found separated, and are confined to specific salinity ranges.

The reduction of larval competition is important for the tiger beetle species present in these areas, due to the sessile habits of the larvae. The larvae of the tiger beetles have a unique hunting strategy, in which they dig a burrow and sit at the entrance waiting for an insect to pass by (Pearson 2006). When an unfortunate insect gets close to the burrow the larva of the tiger beetle lunge out and grabs the unsuspecting victim and then quickly retreats down the burrow to consume its quarry (Pearson 2006).

C. n. lincolniana larvae use this feeding strategy to prey upon small invertebrates (Higley & Spomer 2001). This strategy is also beneficial because the larvae of *C. n. lincolniana* are vulnerable to predation themselves (Brosius 2010). Natural enemies of *C. n. lincolniana* in the

larval stage include birds, spiders, predatory bugs, hisster beetles (Coleoptera: Histeridae), and other tiger beetle adults and larvae (Ratcliffe & Spomer 2002). Mites, several species of wasps and bee flies (Diptera: Bombyliidae) have also been shown to be important natural enemies of the *C. n. lincolniana* larvae, due to their specialization as parasitoids of *Cicindela* spp. larvae (Higley & Spomer 2001). Adult *C. n. lincolniana* have only ever been recorded to be predated by robber flies (Diptera: Asilidae), and other tiger beetle species that inhabit the saline wetlands (Ratcliffe & Spomer 2002). However, it is assumed that large dragonflies, birds, predatory bugs, and spiders will also prey upon the adult *C. n. lincolniana* (Ratcliffe & Spomer 2002).

C. n. lincolniana adults and larvae tend to be found in moist conditions closer to the waters of Little Salt Creek (Higley & Spomer 2001). This habit may be one of the biggest threats to the survival of this species. During Nebraska's frequent, heavy spring rains there is a large amount of flooding along the eastern saline wetlands, due to the channelization of the Little Salt Creek and the increased runoff volumes from the city of Lincoln (Farrar & Gersib 1991). This flooding can be devastating for the populations of *C. n. lincolniana* (Brosius 2010). During these flooding events, large amounts of soil, where *C. n. lincolniana* larvae are present, are eroded from the banks of the Little Salt Creek and washed away. In 2014 an entire reintroduction site which included 20 reintroduced third instar larvae and an entire HOB0® data logger system was washed away during one such flooding event.

CAPTIVE BREEDING EFFORTS

THE BEGINNINGS

Entomologists at the University of Nebraska-Lincoln (UNL) spearheaded the captive breeding initiative for *C. n. lincolniana* in 2002 (Brosius 2010). Efforts were made to bring wild collected males and females into the lab in an attempt to get the beetles to oviposit so that a jumpstart initiative could be established (Allgeier 2003). The first attempt in 2002 consisted of introducing groups of *C. n. lincolniana* into 10-gallon aquaria filled halfway with greenhouse soil wet with a NaCl solution (Allgeier, *et al.* 2003). This method produced three larvae and unfortunately these larvae died after reaching their third instar (Allgeier 2003). Revisions were made to the captive rearing effort for the 2003 season and when it came time the entomologists at UNL used two aquaria: one that was filled halfway with soil from the *C. n. lincolniana*'s native habitat and the second filled with sifted greenhouse soil moistened with a NaCl solution. This time, no larvae were produced (Allgeier, *et al.* 2003).

In 2004 the UNL entomologists working with the *C. n. lincolniana* recovery project decided to try filling petri dishes with sifted greenhouse soil with a NaCl solution added to them (Brosius 2010). The petri dishes were then placed in 16-quart shoeboxes and gravel was placed around the petri dishes until it was level with the top (Brosius 2010). After the breeding boxes were assembled, a pair of adults, one male and one female, were added to each box. The adult beetles were watered and offered one mealworm larvae, *Tenebrio molitor* daily (Brosius 2010). This method showed signs of improvement, yielding 12 larvae (Brosius 2010). These larvae were then transferred to a 10-gallon terrarium, which was filled with habitat soil with a NaCl solution and allowed to settle for 90 days prior to larval introduction (Brosius 2010). The

terrarium was then placed in an incubator so that light cycle and temperatures could be controlled (Brosius 2010). Of these 12 larvae, 11 established burrows, 5 successfully molted into third instar, and 1 adult eclosed (Brosius 2010).

The 2005 attempt replicated the procedures carried out in 2004, only this time they added different NaCl solutions to the petri dishes to determine the range of salinity *C. n. lincolniana* required for oviposition (Brosius 2010). The NaCl solutions were based on soil salinity data collected from *C. n. lincolniana* habitat. 144 larvae were produced and this time they were set up in Tenite Butyrate tubes filled with soil (Brosius 2010). Most of the tubes were filled with sifted greenhouse soil but 31 were filled with habitat soil from Little Salt Creek (Brosius 2010). These tubes were placed in a tub that was fitted with a polystyrene tree seedling tray to hold the tubes upright (Brosius 2010). The larval tubes were then placed in the incubator with a set light-cycle and fed every three to four days (Brosius 2010). When spring came 24 larval tubes were reintroduced to the wild, and 58 larvae had survived the 2007-2008 diapause and were now in second instar (Brosius 2010). The 58 larvae were looked after in the lab and reached third instar in summer 2008 (Brosius 2010). These remaining 58 larvae returned to diapause during the winter 2008-2009 and when they were looked at in the spring 38 third instars survived overwintering, and 16 of those third instars successfully pupated (Brosius 2010). Of the 16 *C. n. lincolniana* that pupated 14 eclosed to adulthood (Brosius 2010). Of the 14 adults that eclosed, 11 were male and only 3 were female (Brosius 2010). The females and males that were produced were paired and even though the females laid eggs no larvae were produced (Brosius 2010). The following year, 2009 was not a good year for the wild populations of *C. n. lincolniana* (Laukaitis 2010). Heavy rains and severe erosion of the habitat dropped the wild population of *C. n. lincolniana* to 194 individuals and no larvae were produced from the females brought into captivity (Laukaitis 2010).

Lincoln Children's Zoo, which joined the Salt Creek Tiger beetle Recovery effort in 2010 received half of the larvae produced that year to help care for them over the winter diapause, and to ensure that one catastrophe would not wipe out all of the larvae (Brosius 2010).

THE OMAHA ERA

In 2010 the Omaha's Henry Doorly Zoo and Aquarium was approached by representatives from the United States Fish and Wildlife Services (USFWS) about taking on the challenge of trying to produce sustainable numbers of *C. n. lincolniana* larvae in captivity. The team at the Berniece Grewcock Butterfly and Insect Pavilion was eager to accept, and in 2011 the first 30 adults were brought in from the wild. The first year yielded a less-than-impressive 27 larvae, of which only 11 survived the winter diapause. The second year at the Omaha Zoo, 2012, was also not a very productive year. Only two larvae were produced, and both larvae died during overwintering. This prompted the Omaha Zoo keepers to meet with UNL, USFWS, and Nebraska Game and Parks officials to talk about the shortcomings of the *C. n. lincolniana* captive husbandry program. This meeting prompted a series of experiments to be carried out using the White-cloaked Tiger beetle, *Cicindela* (= *Eunota*) *togata globicollis*, as a surrogate species. *C. t. globicollis* is a common,

saline-dependent species found to inhabit similar niches to the *C. n. lincolniana* (Spomer, *et al.* 2007).

The experiments with *C. t. globicollis* included testing preferred soil composition for oviposition and larval rearing, which salinity range yielded greatest numbers of larvae, what was the proper amount of water to add to the petri dishes used for oviposition, and ways to reduce larval cannibalism in the petri dishes. *C. t. globicollis* were collected and set up in the same shoeboxes (aka Honeymoon Suites) as *C. n. lincolniana* were housed. The shoeboxes had four petri dishes set in the center of the box that contained different soils. The empty space in the shoebox around the petri dishes was filled in using small-sized pea gravel.

The soils used for the tests included habitat soil, topsoil, play sand, loess, and a 50:50 mixture of sand and loess by weight. The salinities used in the soils were based on the findings of Brosius's 2010 study on the salinity preference of the species of tiger beetle found in the eastern Nebraska Saline Wetlands, and were 0.354M NaCl, 0.5M NaCl, 0.75M NaCl, and 1.0M NaCl. Different amounts of water were added to each petri dish and it was quickly realized that 50ml of reverse osmosis water was the perfect amount.

In the substrate tests we found that the 50:50 mix of the sifted play sand and sifted loess were the preferred substrates for *C. t. globicollis*, which showed a significant preference for this type of substrate in statistical tests. The salinity tests showed that the preferred salinities were 0.354M NaCl and 0.5M NaCl, with statistical tests showing no significant preference for either salinity. It was also decided that 0.75M NaCl should be attempted on *C. n. lincolniana* due to *C. t. globicollis*'s activity in this salinity, but this group was later thrown out based on the findings of Brosius (2010) and an unexpected early emergence of the adult *C. n. lincolniana*. Once the larvae of *C. t. globicollis* started to emerge the Omaha Zoo team began to think of ways to separate the larvae before they got a chance to cannibalize one another. The idea struck the team when they googled "tiger beetle larvae" on the Internet. A video of a person "fishing" tiger beetles from their burrows popped up in the search and it was decided that this method should be attempted. The Omaha Zoo crew went fishing and very quickly realized that this was the answer to the cannibalization question. The results from the experiments done on the *C. t. globicollis* yielded just under 100 larvae, which gave the program a much-needed second wind.

In 2013 the Omaha Zoo Entomologists prepared the honeymoon suites with four cellophane wrapped petri dishes of 50:50 substrate. As the Omaha Zoo was waiting to receive the saline solutions from the UNL Chemistry department *C. n. lincolniana* made an early appearance. The adult beetles emerged the twenty-second of May due to an unseasonably warm winter. This put a rush on the project and it was decided to just use 0.354M NaCl and 0.5M NaCl solutions in the 50:50 substrates. Two dishes of each salinity were added to the honeymoon suites and adult *C. n. lincolniana* were introduced.

All eyes were on the beetles, and it was suggested by the entomologists at the Omaha Zoo that the male beetles be removed after five days instead of after the traditional seven days so that the females could lay eggs without constant advances from the males. After the males were removed the number of oviposition holes went up dramatically. Soon, tiny one-tenth-of-an-inch

larval burrows started to appear and the fishing began. The 2013 season yielded an outstanding 365 larvae!

The larvae were set up in the tubes and were fed every other day. The diet consisted of fruit flies (*Drosophila melanogaster*), week-old crickets (*Acheta domesticus*), and springtails (Collembola: Entomobryidae). A set number of prey items was determined in order to ensure a level of consistency between the Omaha and Lincoln zoos' feeding schedules. First instar larvae receive three prey items per feeding, second instars receive four prey items, and third instars receive six prey items. The feeding schedule allowed the beetles to quickly go through their first two larval instars and a majority of the larvae were third instar by the time they entered diapause. Entering diapause in the third instar instead of the first or second increased the survival rates during this period. The larvae are checked twice a week during diapause and are offered one prey item during this time, so in case the larval burrows were opened the larvae would have access to prey.

175 third instar larvae were reintroduced into the wild in May of 2014, and 20 third instar larvae were held back to see if they would eclose to adulthood in captivity. As of this writing, thirteen adults have eclosed (7.6) and five pairs are set up in honeymoon suites. These captive-reared adults are part of an experiment to see if there could potentially be a future captive assurance colony for the *C. n. lincolniana*. The F₁ generation has proven to be fecund, as there were several incidences of mate-guarding and copulations that have been observed, a large number of oviposition holes have been made in the substrate, and 44 F₂ larvae have been recovered to date.

This season, 2014 promises to be another big year for *C. n. lincolniana* program with 250 larvae and counting produced at the time this was published.

PROCEDURE FOR CAPTIVE REARING

The following is a simplified procedure for rearing the *C. n. lincolniana*. The complete list of materials, with manufacturer, can be found in Appendix A.

1. Collect 15 male and 15 female *C. n. lincolniana*.
2. Set up 1.1 beetles in Honeymoon Suites
3. Remove male after five days and return to the wild.
4. Allow female to lay eggs for 5-7 more days.
5. Remove females from the honeymoon suites and return to the wild.
6. Check petri dishes daily for larval burrows.
7. When larval burrows are seen attempt to 'Fish' the larvae from the burrows.
8. Set larvae up individually in Tenite butryte tubes.
9. Feed tubes every other day until diapause. (L1=3, L2=4, L3=6)
10. Once larvae go into diapause check larvae every 3 days and offer 1 prey item per tube.
11. In spring increase temperature in chamber and begin every-other-day feeding schedule.
12. Carefully cut tubes along seam to expose larvae
13. Place larvae in a portion cup with a small amount of soil in to transport to the reintroduction site.

14. At reintroduction site poke a small hole in the soil with a half-an-inch diameter rod and coax larvae into the hole. Place holes at least six inches apart to ensure larval tunnels don't cross and to minimize competition for food and other resources.
15. Monitor release sites and hope for the best.

THE FUTURE OF THE PROJECT

While the Salt Creek Tiger beetle recovery effort is showing signs of success there is still much work to be done with the program. For everything we know about *C. n. lincolniana* there are several more questions yet to be answered.

The plan for coming years is to attempt to synchronize the eclosion of wild and captive adults so that it can be confirmed that wild individuals are capable of producing offspring with the captive reared beetles. The synchronized eclosion of captive and wild beetles also promises to increase success rates of reintroductions into the wild, because instead of releasing larvae that are very hard to monitor, the Salt Creek Tiger beetle team can release adults that can immediately breed and lay eggs to hopefully increase the wild populations.

The overwintering/diapause stage is also going to be reviewed as a large percentage of mortality occurs during this stage. Plans include attempting to overwinter the larvae in the tubes that are placed outside in protected areas, and to use refrigerators for overwintering larvae in addition to the Percival Chambers ®.

The use of portion cups filled with soil instead of the Tenite butryte tubes has been shown to increase the rate of development and reduce the amount of space needed to house larvae in experiments done on *C. t. globicollis*. The use of portion cups for *C. n. lincolniana* larvae hopes to also prove successful.

Lastly, alternative substrates are being explored that would allow the larval stage of the tiger beetles to be visible throughout development. These substrates would allow the team to keep better track of the larval progression and health. Substrates such as ant gel® (<http://antgel.com/>) have been tested on *C. t. globicollis* larvae and have yielded little success. The larvae developed, but problems with the substrates occurred such as shrinking, molding, and use as food by *D. melanogaster* larvae that always result in the death of the tiger beetle larvae. Further research is needed in order to make this a feasible option.

CONCLUSION

Since it was listed as an endangered species in 2005, *C. n. lincolniana* has caused much controversy within the city of Lincoln, NE. The critical habitat designation has put restrictions on the development potential for the northeastern corner of the city. Many people oppose the existence of this "little bug" and question why any effort should be made to conserve it. Those that oppose the Salt Creek Tiger beetle project fail to realize that this species not only offers a valuable service to these people as one of the top predators in its microcosm, eating things such as biting insects and crop pests, but is also the only thing standing between the onslaught of the city of Lincoln and the complete annihilation of one of Nebraska's most endangered habitats.

The survival of Nebraska's eastern saline wetland ecosystem rests solely on the shoulders of this tiny insect. As many as 250 migratory birds use these wetlands as either a feeding area

along their way or as an important breeding area (Farrar & Gersib 1991). Several other rare species of insect also call the eastern saline wetlands their home (Harvey, *et al.* 2007). The saline wetlands are represented today as only broken fragments of a once-thriving ecosystem (Farrar & Gersib 1991). With more than 90 percent of this habitat destroyed it is going to be an uphill battle to restore the eastern saline wetlands (Farrar & Gersib 1991). But thankfully there is a little tiger that stands, and protects the beauty of this unique habitat. The entomology staff at Omaha's Henry Doorly Zoo, and all of our partnering organizations, are proud to say that we are helping to conserve not only *C. n. lincolniiana* but its home and all of the organisms with which it shares its habitat. We are "Beetle Huggers" and aren't ashamed to admit it.

Appendix A: A list of materials, with manufacturer.

Incubator and Tube Storage

(2) Percival® Incubator (Model:I-30BLL). <http://www.percival-scientific.com/>

(2) Tree seedling Trays with cells (3098A). <http://www.bioquip.com/>

(2) Solid Euro-Fix Stackable Container (EF6220) <http://www.schaefershelving.com/>

Salt Solution

(1kg) 99.5% pure Sodium Chloride Crystals (BP358-1). <http://www.fishersci.com/>

(18.9L) Distilled Water

Honeymoon Suites

(15) Shoe Boxes, 21.5x30.5x11.6cm (887). <http://www.hktdc.com/>

(60) Sarstedt Petri Dish Square, 100 x 100 x 20 mm (82.9923.422).

<http://www.practicalcalibrationresources.com/Petri-Dish-p/82.9923.422.htm>

(1) Roll Food Service Film, 45.7cm x 609.6 m (BWK7204). <http://www.supplycloset.net/>

(3) Bags small stone pea gravel, 11.3- 22.6 kg. Be sure to rinse thoroughly before use. Available at local hardware stores.

(6) 18.9L buckets, Available at local hardware stores.

(4) 11.3- 22.6 kg Bags play sand, Available at local hardware stores.

(1) U.S. Standard No. 30 size sieve

(4) 18.9L buckets of unsifted loess soil.

(1) balance to measure weights of sand and loess.

Fill 2 18.9L buckets with a 50:50 (by weight) mix of sifted play sand and sifted loess. Sift through at least a U.S. standards No.30 size sieve.

Larval Rearing Tubes

1.82 m lengths of Tenite Butyrate Tubing, 25 x 25.4 x 1.58 mm (42127).

<http://www.usplastic.com/>

* Cut the 1.82m sections into 30.48cm sections and split them down the center.*

(3) Packs Drosophila Vial Plugs (173122). <http://www.carolina.com/>

25.4 mm PVC caps, 1 per tube.

(1) Roll Plastic Insect Netting 0.25mm x 0.8mm (GNE-029).

<http://www.harrodhorticultural.com/>

(1) Pack 1000 Large Rubber bands

Enough 50:50 substrate to fill the 30.48cm tubes to within 25.4mm of the top.

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