2015

AN UPDATE OF THE CAPTIVE MANAGEMENT AND REINTRODUCTION OF THE SALT CREEK TIGER BEETLE, *CICINDELA NEVADICA LINCOLNIANA* (COLEOPTERA:CARABIDAE) AT OMAHA'S HENRY DOORLY ZOO & AQUARIUM

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INTRODUCTION

Tiger beetles are a high speed predator, both as larvae and adults. There are over 2,600 species found worldwide. They are so fast that their eyes can't gather enough light to process visual information while running and the beetles have to pause during pursuit to regain sight of their target. The fastest known tiger beetle can run up to 8 kilometers per hour (5 mph) which is comparable to a human running 772.5 kph (480 mph) when adjusted for body length (Yong, 2014). The adults are sexually dimorphic, with males possessing short white hairs on the inside of the tarsi of the prothoracic legs whereas females do not have these hairs. They display a unique breeding behavior called mate guarding wherein males continue to hold onto the female after copulation to prevent other males from mating with her.

Tiger beetles are considered an indicator species or sentinel organisms for environmental health, due to their diet of small invertebrates and specialized breeding requirements. An estimated 15 percent of the 255 described species and subspecies of North American tiger beetles are now threatened with extinction (Pearson, 2011). The saline wetlands of Lancaster county in eastern Nebraska are home to one such beetle, an endemic subspecies of the Nevada Tiger Beetle, *Cicindela nevadica* (Palmer & Klatt, 2014). This beetle is aptly named the Salt Creek tiger beetle, *Cicindela nevadica lincolniana*, due to its presence only along Little Salt Creek and associated tributaries (Spomer, *et al*. 2007).

Fig1: Adult Female *Cicindela nevadica lincolniana*, photo by Jeremy Dixon.
Third instar *C. n. lincolniana* larva, photo by Joel Sartore.

The following paper gives a brief overview of the natural history of the Salt Creek tiger beetle, *Cicindela nevadica lincolniana*, and the current progress in the captive management and reintroduction of Nebraska's endangered tiger beetle.

NATURAL HISTORY

The Salt Creek tiger beetle, *C. n. lincolniana,* is found on small patches of land 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 underground, in the larval stage. The adults emerge in early June in most years and are active until about mid-July (Brosius, 2010). Within a few days of eclosion, the adults are ready for breeding. After copulation, the female will deposit a single egg into soil of suitable salinity. This is repeated for 50 – 200 eggs. Eggs hatch after 10-14 days, then the larvae dig a cylindrical burrow which can be up to one meter in length. There are three larval instars, each with a correspondingly larger burrow opening. In captivity, the larvae are typically overwintered as third instars. Once ground temperatures rise, the larvae begin pupation. Adults begin to eclose in June and start the process over again.

The wild population ranges from 100 – 750 adults per season (Spomer). The species was federally listed as endangered in 2005. In 2014, a final critical habitat revision was made, setting aside 449 hectares (1,110 acres) for Salt Creek tiger beetle conservation.

RECOVERY PROJECT & CAPTIVE MANAGEMENT

The Salt Creek tiger beetle recovery project has many partners: United States Fish & Wildlife Service, Nebraska Game & Parks, University of Nebraska at Lincoln, Lincoln Children's Zoo, Nebraska Master Naturalists, City of Lincoln, Saline Wetlands Conservation Partnership. Omaha's Henry Doorly Zoo & Aquarium (OHDZA) has been involved in the captive management of the Salt Creek tiger beetle since 2010. By breeding 15 wild pairs, the team at the Berniece Grewcock Butterfly and Insect Pavilion produced 27 larvae in 2011 and only 2 larvae in 2012, both years had high mortality during overwintering.

In 2012, a series of experiments were carried out at OHDZA using the White-cloaked Tiger beetle, *Cicindela togata globicollis,* as a surrogate species to troubleshoot low larval numbers. *C. t. globicollis* is a common, saline-dependent species found to inhabit similar niches to the *C. n. lincolniana* (Spomer, *et al*. 2007). The results of the experiments with *C. t. globicollis* helped to find ideal substrate mix, moisture levels, salinity, ways to reduce larval cannibalism, pairing times, and alternative methods for individual larval housing. The number of larvae produced has skyrocketed with the implementation of these findings (Figure 2).

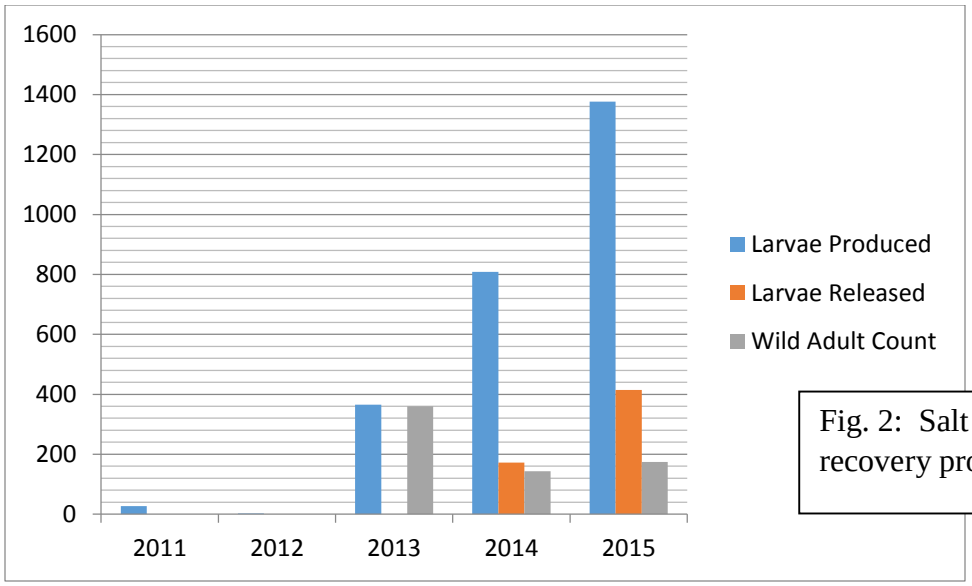

Fig. 2: Salt Creek tiger beetle recovery project history

In 2014, larval production was augmented by breeding zoo-eclosed adults in addition to the 15 wild pairs. The first ever fall release of larvae was done in 2014. Heavy rainfall in the spring of 2015 resulted in a completely flooded habitat and there were no wild pairs available when we normally begin the breeding process. For 2015, we used 15 captive-eclosed pairs for breeding and kept them for three rounds of breeding.

CURRENT STATUS

April of this year, we had our largest larval release to date of 415 larvae. This was quickly followed by torrential rains in May that resulted in flooding of the city of Lincoln and the habitat. In order to prevent damage to human habitation, a decision was made to allow the emergency dumping of raw sewage into the Salt Creek for about 12 hours at the rate of 300 gallons per second (Hicks, 2015). Not surprisingly, the adult Salt Creek tiger beetles did not emerge in their normal time frame. About 100 L3 larvae had been held back from the release to eclose in captivity at University of Nebraska Lincoln. Most of these larvae did eclose in the expected time frame. Fifteen pairs were from these captive-eclosed beetles were used for this year's breeding. These pairs were placed in Oviposition Box for 5 days and then the males were removed and held individually. The females were given another 5 days to oviposit undisturbed. On the tenth day, the females were paired with a different male in a new Oviposition Box and the process was repeated. We started a third round of breeding, but it was cut short due to an overabundance of larvae. All surviving adults were marked and released at Little Salt Creek. By late June, wild adult Salt Creek tiger beetles were present in the habitat. Our final larvae count for 2015 is 1376!

PROCEDURE FOR CAPTIVE REARING

The following is a simplified procedure for rearing the *C. n. lincolniana*. Percival environmental chamber temperatures are adjusted periodically to coincide with temperatures from habitat

monitoring to synchronize captive and wild populations. The complete list of materials, with manufacturer, can be found in Appendix A.

1. Collect 15 male and 15 female *C. n. lincolniana*. (or captive-eclosed adults)
2. Set up 1.1 beetles in an Oviposition Box (Figure 3)
   Oviposition Box:
   3 plastic-wrapped petri dishes of 50:50 soil mix and 35 ml of 0.354 M saline solution
   3 plastic-wrapped petri dishes of 50:50 soil mix and 35 ml of 0.5 M saline solution
   Gravel to fill non-petri dish areas
   Portion cup lid with water moat and cricket quencher
   Aquarium plant (for shelter)
3. Remove male after five days and return to the wild.
4. Allow female to lay eggs for 5 more days (10 days total).
5. Remove females from the Oviposition Boxes at 10 days 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, by inserting a thread into the burrow, wiggling, and pulling out a larva
8. Set larvae up individually in 59 ml (2 oz.) portion cups and place inside Percival chambers
9. Feed larvae three times weekly until diapause. Alternate between pinhead crickets and fruit flies (L1=3 items, L2=4 items, L3=6 items, closed=1 item)
10. Once larvae go into diapause, check larvae once per week and offer 1 prey item each only if an open burrow is present.
11. In spring, resume normal feeding schedule once burrows begin to open.
12. Release: Carefully remove soil from cup and search for larva.
13. Place larva in a portion cup with a small amount of soil 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.

Fig. 3: Oviposition Box set-up;
Top left: wrapping petri dish with plastic wrap,
Top right: adding saline solution to soil mix,
Bottom left: completed Oviposition Box interior,
Bottom right: completed Oviposition Box exterior

SUMMARY

Captive management of the Salt Creek tiger beetle had some rough starts, but has been perfected over the last few years to the point that we have too many larvae to manage by ourselves this year. The wild adult population is still extremely low, even with our release efforts. Moving forward, we need to address the timing of our releases and determine if a more successful strategy can be found. Overwintering continues to be an area where more research is required. Despite autoclaving all substrates, the mold *Aspergillus* has been found in larval substrate and growing on deceased prey items. Additionally, we need to come up with a plan for larvae produced that are beyond our ability to manage. We currently have three locations that house and feed larvae: OHDZA, Lincoln Children's Zoo, and University of Nebraska at Lincoln. New

partners are needed to accommodate more larvae. We look forward to the day when *Cicindela nevadica lincolniana* is removed from the endangered species list.

Appendix A: A list of materials, with manufacturer or vendor.

Incubator and Cup Storage

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

Oviposition Boxes

(15) Ziploc Weathertight Boxes XS, 44.2 x 30.0 x 17.0 cm (B00MWTJXHI).
http://www.amazon.com/ with 10 holes of 5 mm diameter drilled into the sides for ventilation and covered with mesh
(90) 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 fine gravel or aquarium gravel, 11.3- 22.6 kg. Be sure to rinse thoroughly before use. Available at local hardware stores or pet stores.
(6) 18.9 L 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) scale to measure weights of sand and loess.
Fill 2 18.9 L buckets with a 50:50 (by weight) mix of sifted play sand and sifted Loess. Sift through at least a U.S. standard No.30 size sieve. Autoclave soil mix.

Larval Rearing Cups

(1 per larva) 59 ml (2oz) polystyrene portion cup and lid (S-20149, S-20151)
http://www.uline.com/ or any food service supply store. Poke 4-6 holes in the lids and around top edges of cups for ventiliation
Approximately 52 g of 50:50 substrate mix and 7 ml reconstituted reverse osmosis water per portion cup. Allow water and substrate to sit, covered, for 24 hours prior to use and then gently push moistened soil mix against bottom and sides of cup.

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