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Determining Optimum Soil Type and Salinity for Rearing the Federally Endangered Salt Creek Tiger Beetle, *Cicindela* (*Ellipsoptera*) *nevadica lincolniana* Casey (Coleoptera: Carabidae: Cicindelinae)

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ABSTRACT: Effective rearing methods are needed to recover the federally endangered Salt Creek tiger beetle, *Cicindela* (*Ellipsoptera*) *nevadica lincolniana* Casey, a subspecies that occurs exclusively in saline wetlands and seeps along Little Salt Creek in Lancaster County, Nebraska. Experiments were initiated to determine soil type and salinity concentrations appropriate for stimulating female oviposition in laboratory settings to produce larvae and/or adults for reintroduction to native habitats. In 2013, there were highly significant differences between native soil and a sand/loess soil mixture, but no differences between two salinity levels, 0.354 M and 0.5 M. In 2014, using only a sand/loess soil mixture, there were again no differences between the test salinity levels. A sand/loess soil mixture of either 0.354 M or 0.5 M salinity was determined to be optimum for egg production.

KEY WORDS: Cicindelidae, breeding, husbandry, Endangered Species Act

The Salt Creek tiger beetle, *Cicindela* (*Ellipsoptera*) *nevadica lincolniana* Casey, is one of the rarest insects in North America. Limited to a single stream system and adjacent saline seeps and wetlands near Lincoln, Lancaster Co. Nebraska, estimated numbers are precipitously low. Estimates of adults have been taken yearly since 1991, and numbers have fluctuated from a low of 115 in 1993 to a high of 777 in 2002. The 2014 estimate was 143 adults. The Salt Creek tiger beetle (SCTB) was listed as state endangered in 2000 (Nebraska Game and Parks Commission, 2000), federally endangered in 2005 (U. S. Fish and Wildlife Service, 2005), and critical habitat was designated in 2014 (USFWS, 2014a).

A goal of the Endangered Species Act is to protect threatened and endangered species and pursue their recovery. An objective toward achieving this goal is to increase numbers and populations of the species to levels where it is no longer threatened or endangered. One method of doing this is by breeding individuals in the lab and introducing their progeny into new suitable habitats or by reintroductions into presently- or previously-occupied habitats. Several endangered and threatened insects, including the American burying beetle, *Nicrophorus americanus* (Olivier), and the Oregon silverspot butterfly, *Speyeria zerene hippolyta* (W. H. Edwards), are being reared by zoos and universities and reintroduced into the wild with some positive results (Amaral *et al*., 1997; USDA, 2011; USFWS, 2014b; WAZA, 2015). In addition, the Northeastern Beach tiger beetle, *Cicindela* (*Habroscelimorpha*)

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dorsalis dorsalis Say has been successfully reintroduced into the wild (Kapitulik, 2012). In 2011, a coalition composed of the City of Lincoln, Lower Platte South Natural Resource District, U. S. Fish and Wildlife Service, Nebraska Game and Parks Commission, Lincoln Children's Zoo, Omaha's Henry Doorly Zoo, and the University of Nebraska set out to rear the SCTB with the goal of reintroductions and increased number of wild populations on multiple streams and saline wetland systems where suitable habitat is available.

Attempts by other researchers to get tiger beetles to oviposit have been somewhat successful. Palmer (1979) used several types of containers as oviposition chambers: plastic shoeboxes, bread boxes, and glass terraria. She used tops of canning jars, with the metal plates replaced with window screen, and filled them with soil from the species' native habitat. Gwiazdowski *et al*. (2011) used sterilized native soil at a depth of 7 cm in polystyrene containers. They placed one pair of adults in each container and kept a 14:10 L:D schedule with a temperature of 17–18°C, provided humidity with a pan of water, and fed crickets. Brust *et al.* (2012) also used plastic containers with a native soil depth of 7–13 cm and fed the adults termites or *Tribolium*. Knisley and Schultz (1997), in their review of rearing methods, noted that some species may have very specific conditions for oviposition that may be difficult to duplicate in the laboratory.

One problem to overcome in rearing saltmarsh-dwelling tiger beetles is determining what type of soil and salinity is required for the female to oviposit. Allgeier (2005) and Brosius (2010) previously determined that sifted topsoil (commercially available), saturated with either a 0.5 M or 0.354 M NaCl solution was sufficient to induce oviposition. Prior to Allgeier's work, we had extracted soil from actual SCTB habitat and used that to induce oviposition and rear larvae. However, the clay-saturated habitat soil created problems with uneven moisture gradients and wet topsoil often promoted mold growth. Lab mortality was sometimes quite high. Our goal in these experiments was to determine the best soil type and salt concentration to maximize oviposition potential.

Materials and Methods

In 2011, we began preliminary experiments with soil types and determined that a mixture of topsoil and sand appeared superior to topsoil alone for egg production, although statistically, it was significantly different at only the 0.10 level of probability. In 2012, we used a higher sand ratio and females laid almost no eggs. Topsoil from commercial sources was found to be inconsistent in texture and content. In 2013, we reduced the sand ratio and added native loess soil (from a bluff at Henry Doorly Zoo in Omaha) rather than topsoil from commercial sources. In addition, we used soil from SCTB habitat along the banks of Little Salt Creek. We used the following treatments in 2013: (1) habitat soil + 0.354 M NaCl solution, (2) habitat soil + distilled water, (3) 50/50 loess:sand (by weight) + 0.354 M NaCl solution, and (4) 50/50 loess:sand (by weight) + 0.5 M NaCl solution. In 2014, we used only treatments 3 and 4 above. Soils were added to square plastic dishes (2.16 cm high $\times$ 10.16 cm^2) lined with plastic wrap and solutions were added until soils were saturated. Each of the four treatments were set into a layer of small, natural stone aquarium gravel so the tops of the square dishes were level with the gravel, and randomized within a clear plastic shoe box (31.1 cm long $\times$ 23.5 cm wide $\times$ 12.7 cm

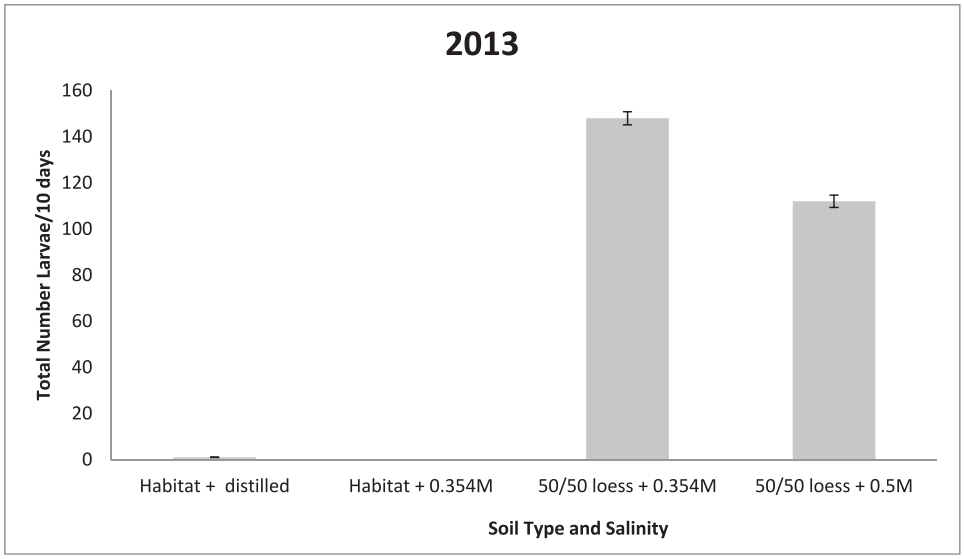

Fig. 1. Total ± SE number of SCTB larvae produced in ten days by 15 females with two soil types and two salinity concentrations, 2013.

tall; glassy shoe box, Nam Ngai Hong Co., Thailand) with a lid. Shoe boxes came pre-drilled with five, 0.3 cm diam. holes on each side. These holes were covered with 51 × 21 mesh plastic screen (Farm Tek, Dyersville, IA) to prevent fly or parasite infestation. Fifteen pairs of SCTB were collected as soon as possible after emergence in the field (13–18 June 2013; 11–18 June 2014) and transferred to the 15 shoe boxes (one pair per box) containing the four soil treatments described above. Males were kept with the females for two days to ensure mating, then removed and released into the habitat where they were collected. Females were allowed to remain in the box for ten days; they were then also removed and returned to the wild. Captive adults were fed a mixed diet of crickets, mealworms, and *Drosophila melanogaster* every other day and moisture was provided using Cricket Quencher (Fluker Farms, Port Allen, LA). Soils were kept moist (but not wet) by misting daily with distilled water. Temperature was room temperature (25–28°C) and light was ambient (sunlight and fluorescent).

Because eggs are very fragile and will often burst when handled, fecundity was determined by counting first instars. After eggs hatched, soil dishes were allowed to dry a few days then turned upside down. The plastic wrap was removed, and the soil was teased apart and the larvae were counted and transferred to overwintering plastic tubes for continual rearing. Data were analyzed using PROC GLIMMIX (SAS Institute, 2001) and mean treatment means were separated using LSD (least square differences) at $P \leq 0.01$ and 0.05.

Results and Discussion

In 2013, there were significant differences in numbers of larvae between treatments ($F = 6.54$, d.f. $= 3, 56$; $P < 0.001$) (Fig. 1). The two native habitat soil treatments

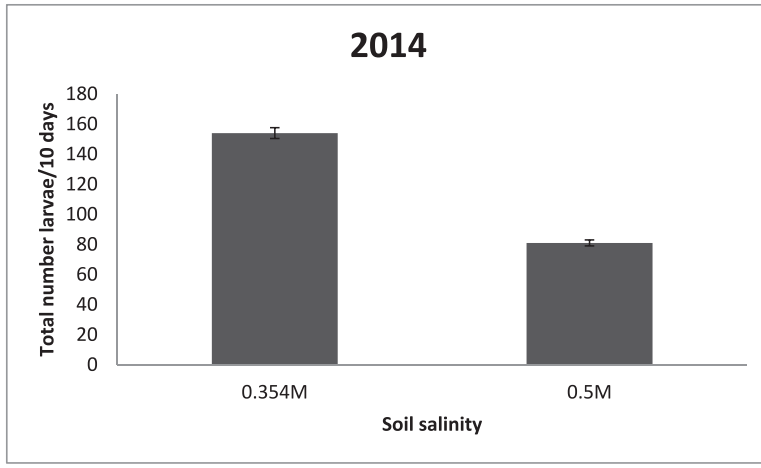

Fig. 2. Total ± SE number of SCTB larvae produced by 15 females in ten days using a 50/50 loess/sand soil mixture and two salinities, 2014.

were not significantly different from each other, but were both different from the 50/50 loess/sand mixtures.

The loess/sand mixture was superior for oviposition; however, there were no significant differences in larval numbers between the 0.5 M ($n = 112$) and 0.354 M ($n = 148$) saline solution additions to the loess/sand. Likewise, there were no differences between native soil ($n = 1$) and native soil plus 0.354 M ($n = 0$) saline solution. An additional 52 larvae were obtained from the loess/sand mixtures, but no record of molarity was recorded, so these were not included in the statistical analysis. These additional larvae were counted in overall numbers obtained however, producing a total of 313 larvae/15 females with a mean of 20.9 larvae/female. Individual females produced from 0–85 larvae.

In 2014, as in 2013, there were no significant differences in numbers of larvae between 0.5 M ($n = 81$) and 0.354 M ($n = 154$) saline solution additions to the loess/sand mixture ($F = 0.99$, d.f. = 1, 28; $P < 0.3274$) (Fig. 2). An additional 11 larvae were found underneath the soil dishes, so molarity could not be confirmed and these were not included in the statistical analyses. Fifteen females produced a total of 246 larvae with a mean of 16.4 larvae/female. Individual females produced from 0–66 larvae.

Based on these results, the potential for high fecundity is present, although it is unknown why some females laid many eggs while others refused to lay any eggs. Allgeier (2005) observed that captive SCTB females laid from 0 to 35 eggs in seven days in the laboratory. Pearson and Vogler (2001) stated that female tiger beetles can lay from 10 to 20 eggs a day in captivity. Shelford (1908) estimated that fecundity ranged from 10 to 50 eggs for a few North American tiger beetle species, and Knisley (unpublished) reported 20 to 50 eggs for several Arizona species. Mating was assured in our studies because males were not separated from the females until actual mating (not just mate guarding) was witnessed by an observer, and mating always occurred at least once by the end of the second day. Lighting was from ambient sources to assure that there was no light during nighttime hours when females oviposit.

According to Allgeier (2005), SCTB only oviposits at night, and the senior author's observations for 25 years agree with this. Nighttime temperatures in the laboratory were probably slightly higher than field temperatures, but field temperatures also vary greatly, and this still would not explain why some females produced many eggs while other produced zero.

Another interesting observation is the almost complete lack of oviposition in native soil. The soil used was taken directly from a site where SCTB occurs. In previous years, we had used native soil for oviposition, sometimes with good results. However, at that time, the females were not given a choice of soils and salinities. We may have had better results with the native soil if the females were not given a choice. But the question remains: why do females prefer a loess/sand mixture when the native soil contains much more clay? In nature, SCTB females are very specific where they lay their eggs, but salinity may be a more important cue than soil composition.

In conclusion, these experiments provided some essential information necessary for lab propagation of SCTB. Future experiments will focus on refining these techniques.

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