

Laboratory Rearing of Common and Endangered Species of North American Tiger Beetles (Coleoptera: Carabidae: Cicindelinae)

RODGER A. GWIAZDOWSKI,^{1,2,3} SANDRA GILLESPIE,^{1,2} RICHARD WEDDLE,⁴
AND JOSEPH S. ELKINTON^{1,2}

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ABSTRACT North American tiger beetles (*Cicindela* spp. L.) have been reared in the laboratory for more than a century, and here we summarize the relevant literature to develop a general rearing protocol. We used this protocol to experimentally overwinter adults in the laboratory and observe variation in oviposition and fecundity among several species. Overwintering experiments, involving five North East North American *Cicindela* species with spring-fall life histories—*Cicindela repanda* (Dejean), *Cicindela hirticollis* (Say), *Cicindela purpurea* (Olivier), *Cicindela scutellaris* (Say), and *Cicindela tranquebarica* (Herbst)—demonstrated that both a long cooldown (20 to 4°C by a degree a day) and a short photoperiod (8:16 [L:D] h) maximized survival and minimized overwintering weight loss, which varied between species and sex. Observations of oviposition, larval abundance and larval development involving five *Cicindela* species with summer life histories revealed that *Cicindela punctulata* (Olivier) produced more first-instar larvae than *Cicindela abdominalis* (F.), *Cicindela dorsalis dorsalis* (Say), *Cicindela puritana* (Horn), or *Cicindela unipunctata* (F.) and that high mortality due to accidental desiccation may be overcome by rearing larvae individually in tubes rather than in bins. We also present a first account of larval rearing of the federally threatened species *C. puritana* and the northern Martha's Vineyard population of the federally threatened species *C. d. dorsalis*. Notably, *C. d. dorsalis* produced fewer larvae than more common species reared in this study. We conclude that rearing large numbers of larvae is feasible with endangered as well as common species and we propose future improvements for rearing as part of conservation efforts.

KEY WORDS tiger beetle, captive breeding, laboratory rearing, *Cicindela puritana*, *Cicindela dorsalis dorsalis*

Populations of laboratory reared endangered insects represent an important tool for experimental research and can serve as a source for large-scale reintroductions (Pearce-Kelly et al. 1998). A high per-generation yield can be achieved from relatively little wild-take of adults (Remington 1968, Chambers 1977), but only if species specific rearing conditions can be adequately met (Singh and Ashby 1985). Determining these conditions is a resource-intensive process involving primary observation and preliminary experimentation that may be difficult to accomplish with rare or understudied species.

Tiger beetles (Carabidae: Cicindelinae) are a conspicuous nonpest insect group whose conservation status may benefit from research by using laboratory populations. This predatory, often brightly colored subfamily of ground beetles is considered a “global

flagship group for beetle conservation” (New 2007) for which many aspects of their biology (Pearson 1988), ecology (Pearson 1987), and phylogeny (Barraclough and Vogler 2002) have been well studied. The genus *Cicindela* (L.) is of such popular interest that North American species can be identified using one national (Pearson et al. 2006) or several regional field guides (Graves and Brzoska 1991, Knisley and Schultz 1997, Leonard and Bell 1999, Choate 2003). Despite being well known, new species and subspecies of North American *Cicindela* are regularly being described (Morgan et al. 2000, Knisley et al. 2008), and some taxa may have gone extinct soon after discovery (Knisley and Fenster 2005). Overall, Pearson et al. (2006) estimate at least 33 (15%) of the 223 named North American species and subspecies are in need of conservation management. Current management techniques include translocations of field-collected larvae, which have been used to supplement populations of threatened species such as *Cicindela puritana* (Horn) (Omland 2004) and create new, experimental populations of *Cicindela dorsalis dorsalis* (Say) (Knisley and Hill 1999; Knisley et al. 2005; Davis 2007; P. Nothnagle and T. Simmons [1990], Ecology of the northeastern beach tiger beetle, *Cicindela dorsalis*

¹ Department of Plant Soil and Insect Science, Fernald Hall, University of Massachusetts, 270 Stockbridge Rd., Amherst, MA 01003.

² Graduate Program in Organismic and Evolutionary Biology, 319 Morrill S, University of Massachusetts, 611 N. Pleasant St., Amherst, MA 01003.

³ Corresponding author, e-mail: rodger@psis.umass.edu.

⁴ Department of Animal Husbandry, Liberty Science Center, 222 Jersey City Blvd., Jersey City, NJ 07305.

dorsalis, in southeastern Massachusetts; unpublished report to the Massachusetts Natural Heritage Program, Massachusetts Natural Heritage Program, Boston, MA), suggesting that reintroduction of captive reared larvae may be a viable conservation strategy (Knisley et al. 2005).

Tiger beetles have been reared in captivity since the late 19th century (Schaupp 1885) and the work of ecologist Victor Shelford (Shelford 1908) established many basic laboratory rearing techniques. Notably, Shelford used these techniques to describe development for many North American species and elucidate two major phenological patterns of adult emergence, known as “spring-fall,” where larvae pupate and emerge in fall, overwinter as adults to mate in spring and “summer” where adults emerge only in the summer and overwinter as larvae. Subsequent rearing-based studies of North American species have added to the methods and materials used to rear both adults and larvae of *Cicindela* (Moore 1906; Macnamara 1922; Hamilton 1925; Willis 1967; Palmer 1978, 1979; Knisley and Schultz 1997). Outside of North America, only a few rearing methods have been proposed for *Cicindela* (Enock 1903, Soans and Soans 1972), most notably those made by Hori during a 7-yr study of *C. japonica* (Hori 1982). Collectively, these studies provided a robust foundation on which to develop our rearing protocols; however, several key aspects of rearing remain unexplored. For example, fall emerging adults of spring-fall species require a cold temperature-induced diapause before mating (Shelford 1908, Knisley and Schultz 1997, hereafter referred to as overwintering), and this has never been achieved in the laboratory. Furthermore, previous rearing attempts have generally been small in scope, conditions have varied widely and no studies have explicitly investigated species specific developmental differences under comparable laboratory conditions.

Here, we synthesize previous rearing methods to develop a general protocol for rearing North American *Cicindela*. We use this protocol to explore the effects of photoperiod and temperature on the variation of survival and weight loss in experimentally overwintered adults of the spring-fall species *Cicindela repanda* (Dejean). We observe species-specific survival and weight loss during overwintering in *Cicindela hirticollis* (Say), *Cicindela purpurea* (Olivier), *Cicindela scutellaris* (Say), and *Cicindela tranquebarica* (Herbst). We also used this protocol to observe differences in oviposition behavior and larval development in the common summer species *Cicindela abdominalis* (F.), *Cicindela punctulata* (Olivier), and *Cicindela unipunctata* (F.) as well as the threatened species *C. d. dorsalis* and *C. puritana*.

Materials and Methods

Field Collection. Adults were captured with aerial nets and transferred to 1.5- by 5.0-cm polyethylene tubes perforated with a \approx 4.0-mm hole and containing a \approx 0.5-cc volume of anionic polyacrylamide polymer gel, which adults chew to obtain moisture as they

would moist substrate (Willis 1967). During transport, the tubes were maintained at \approx 15–17°C (C. B. Knisley [1995] A study of laboratory propagation and fecundity of the tiger beetle, *Cicindela dorsalis*; unpublished report). In the field, native soil was collected within 2–4 cm of the surface adjacent to larval burrows at the site of adult collection. In the lab, this soil was first sterilized in a 1.6-kW microwave oven on the maximum setting for 10 min before use for oviposition and larval rearing (Schultz 1989, Knisley and Schultz 1997). *C. puritana* were collected under U.S. Fish and Wildlife regional endangered species blanket permit 697823 with additional permission granted from the Maryland Department of Natural Resources. *C. d. dorsalis* adults were collected under a Federal and State Mass Wildlife permit to Tim Simmons (Massachusetts State Natural Heritage and Endangered Species Program).

General Laboratory Conditions. Adults were maintained in clear polystyrene bins, measuring \approx 25 by 18 by 10 cm (part 195-C, Pioneer Plastics Co., North Dixon, KY). The rectangular center of the bin top was cut away and fitted with gray, 0.11-cm-diameter vinyl-coated fiberglass window screen with a mesh size of 18 by 16 threads per 2.5 cm. We filled bins with a flat layer of \approx 7 cm of sterilized native soil. A pair of adults was kept in each chamber as greater densities may contribute to adult mortality (Knisley [1995], unpublished report). Bins were held in environmental control chambers (Percival Scientific, Perry, IA) lit with 12-W fluorescent white/daylight tubes. The brightest light source was positioned directly above the bins as adults of most species will clamber toward oblique light until exhaustion and death (Palmer 1979; Knisley and Schultz 1997; R.A.G., personal observation). The photoperiod was a 14:10 (L:D) h timed to coincide with diurnal cycles from the locality and date of collection. All lights in a chamber turned on and off simultaneously. Temperature during the dark cycle was a constant 17–18°C. Warming began with the onset of light gaining \approx 1.5°C/h to a midphotoperiod high of 28–29°C and then cooled along the opposite time/temperature profile until reaching a low of 18–17°C. Ambient humidity was provided by pans of water inside the chamber as well as the soil moisture within each bin. Soil was moistened daily using tap water misted with a fine-spray wand as the porous native soils used for rearing were prone to rapid desiccation. When soil moisture was adequate, fungal growth sometimes developed but was inhibited with moderate air circulation or an occasional light mist of 3% hydrogen peroxide (Shelford 1908).

Food was provided ad libitum, as live crickets, *Acheta domesticus* (L.), sized appropriately for the beetle species (one-half size or less of beetles), and dead prey was removed daily. Crickets were maintained separately and fed on puppy chow and fresh fruits and vegetables. Water was provided ad libitum as polymer-gel placed on the substrate surface and replaced every 48 h.

Oviposition and Larval Development. Only newly collected soil from the site of origin was used for

oviposition. In all cases adults were left to continuously oviposit in a bin until the first appearance of a second instar at which time adults were transferred to a new bin. Numbers of oviposition holes per bin were recorded daily, and after each count the soil surface was flattened with a fine mist to erase holes. Larval feeding in adult bins was a "free range" method where prey (crickets), sized appropriately per instar, were always available. Instars were identified by the relative difference in hole diameter (Knisley and Schultz 1997).

Laboratory Experiments and Observations. We used five *Cicindela* species with spring-fall life histories for overwintering experiments. *C. repanda* were collected on 3 September 2005 from the Rainbow Beach Conservation Area, Northampton, Hampshire County, MA. *C. hirticollis* were collected on 2 October 2005 near Horseneck Beach, Westport, Bristol County, MA. *C. purpurea*, *C. scutellaris*, and *C. tranquebarica* were collected 10 September 2005 near the intersection of Route 72 and County Road 539, Ocean County, NJ. All adults were captured and held in the laboratory as described above.

We conducted a two by two factorial experiment manipulating overwintering conditions to examine the effects of different temperature and photoperiod treatments on proportional weight loss, mortality, and survival for a single species of adult tiger beetle, *C. repanda*. Males and females of *C. repanda* were subject to either a normal (12:12 [L:D] h) or short day (8:16 [L:D] h) and either a long cooldown (decreasing from 20 to 4°C by 1°C per day) or a short cooldown (decreasing from 20 to 4°C over 24 h), comprising four treatments. Replication for each treatment was as follows: normal day and long cooldown, 11 males (M) and 27 females (F); normal day and short cooldown, 16 M and 26 F; short day and long cooldown, 48 M and 33 F; and short day and short cooldown, 14 M and 25 F. In addition, two groups of 40 male and 40 female *C. repanda* were held at 20°C and exposed to each female without a cooldown.

Each treatment was conducted in separate environmental control chambers. Individual *C. repanda* were randomly assigned to treatments. Each beetle was sexed, assigned a unique number and placed in a sealed 3- by 8-cm glass vial filled with ≈6 cm of moist, sterilized native soil. Vials were sealed with clear plastic wrap to allow light penetration. Feeding was stopped 2 d before treatment. Beetles were weighed and treatments initiated 1 d after feeding stopped, on 28 by 2005. The long cooldown treatments reached the low temperature of 4°C on 13 November 2005. All treatments were warmed up 1°C per day to 20°C beginning on 24 January 2006. Long cooldown treatments spent 72 d at 4°C, and short cooldown treatments spent 88 d at 4°C. Treatments concluded and all beetles were weighed on 9 February 2006.

To examine how overwintering success varied among species, four other species: *C. hirticollis* (118 M and 124 F), *C. purpurea* (28 M and 44 F), *C. scutellaris* (6 M and 6 F), and *C. tranquebarica* (16 M and 15 F), were overwintered under the short day and long

cooldown conditions (see previous description). The overwintering and measurements of these species were concurrent with those of *C. repanda*, as described above.

Proportion of weight change was calculated for all surviving individuals, as the gain or loss of weight divided by preoverwintering weight. Before analysis, we tested normality of weight loss variables for species using Proc Univariate in SAS (SAS Institute 2009), which was used for all other statistical analyses. Most species results were normally distributed or nearly normal, and no distributions were improved by the use of log transformations, so data were used untransformed.

We used analysis of covariance (ANCOVA) to test whether proportion weight loss in *C. repanda* was affected by treatments. Daylength and cooldown were treated as fixed effects, with preoverwintering weight as a covariate. Similarly, we used ANCOVA to test whether proportion weight-loss varied among different species or sex within species. The four species listed above as well as *C. repanda* reared under short day and long cooldown conditions were included in this analysis, for five species in total. We tested the assumption of equal slopes by using Proc GLM (SAS Institute 2009), including interactions between the covariate and independent variables; none of these between species interactions were significant, and they were excluded from subsequent analysis. For significant factors, differences between groups were determined using a post hoc Tukey's test implemented with the least-squares means procedure. To examine variation in survival for both experiments, we used Proc CATMOD (SAS Institute 2009), a logistic regression procedure using maximum likelihood that allows the testing of complex models with binary variables as the response. For the factorial experiment, we treated cooldown and daylength as fixed effects and survival as a binary response. For species comparisons, we included species, sex, and their interaction as independent variables, with survival as the response.

Oviposition and Larval Abundance. We used four *Cicindela* species with summer life histories to observe oviposition, larval abundance and larval development in the laboratory. Adults of *C. punctulata* and *C. unipunctata* were collected on 29 July 2006 on the campus of the Richard Stockton College, Atlantic County, NJ. *C. abdominalis* adults were collected on 29 July 2006, Atlantic County, NJ, at Stafford Forge Wildlife Management Area. Adults were set up for rearing in the laboratory on 29 July 2006, and observations continued through 12 October 2006, 75 d in total. *C. puritana* adults were collected on 30 June 2007 near Calvert Beach-Long Beach, Calvert County, MD. Adults of *C. puritana* which became moribund or died during the rearing were frozen at -80°C and deposited in the Ambrose Monell Cryo-Collection (AMCC) of the American Museum of Natural History (accessions 167959-167988, males odd numbered, females even numbered; available upon request for morphological or genomic research).

Table 1. Peak number (mean \pm SE) of larvae per female for each larval instar, separated by whether bins experienced a desiccation event

Desiccation event	Species	No. females	First instar	Second instar	Third instar
Yes	<i>C. abdominalis</i>	3	7.3 \pm 5.3	0.7 \pm 0.7	0.0 \pm 0.0
No	<i>C. abdominalis</i>	1	9 ^a	4 ^a	4 ^a
Yes	<i>C. punctulata</i>	15	43 \pm 8.9	5.9 \pm 2.0	0.1 \pm 0.1
No	<i>C. punctulata</i>	12	26.3 \pm 5.6	18.9 \pm 3.8	16.0 \pm 3.6
Yes	<i>C. puritana</i>	9	2.1 \pm 0.5	1.3 \pm 0.6	0.1 \pm 0.1
No	<i>C. puritana</i>	5	15.8 \pm 4.9	7.8 \pm 2.6	2.4 \pm 0.7
Yes	<i>C. unipunctata</i>	4	2.8 \pm 1.2	1.5 \pm 1.5	0.0 \pm 0.0
No	<i>C. unipunctata</i>	6	13.5 \pm 5.3	12.8 \pm 5.8	2.8 \pm 0.5

^a *C. abdominalis* larvae are counts from only one female.

We observed two bouts of oviposition and rearing from field-collected *C. puritana* adults. In the first bout, adults oviposited from 1 July to 7 August 2007. On this date, adults were removed to new bins, and all first bout bins with first-instar larvae were transferred to Randolph-Macon College for a separate study. In the second bout, oviposition and development took place from 7 August 2007 to 4 January 2008, 150 d in total. The rearing data for *C. puritana* comprises only oviposition and larval development beginning at the second bout and under represents the true number of larvae produced from each female.

We compared oviposition and larval abundance between *C. punctulata*, *C. unipunctata*, *C. abdominalis*, and *C. puritana* by using surrogate measures of stage (instar) abundance over time. The nature of data collection (mass rearing in bins) precludes tracking individuals within bins (see Discussion). Individual counts would have allowed use of recruitment methods to estimate overall stage abundance. We considered the total number of oviposition holes as an estimate of egg number and peak numbers of each larval instar within each stage as a surrogate for total numbers. Here, peak numbers are the maximum number of per female oviposition holes or larvae per stage, observed on a single day within each bin. We calculated the mean peak numbers for each developmental stage within each species as the mean \pm SE across all females of that species. This method probably overestimates oviposition, because each oviposition hole may not contain an egg and underestimates the total number of larvae per female because larvae in bins cannot be tracked individually (see Discussion).

We used analysis of variance to compare estimated overall numbers within each stage across species. All measures of stage abundance were log transformed before analysis to meet assumptions of normality. However, for simplicity of interpretation untransformed means and SEs are presented in the figures. For significant analyses, differences between groups were determined using a post hoc Tukey's test implemented with the least-squared means procedure.

Some bins experienced a stochastic, accidental, one-time desiccation event (as the soil becoming dry for ≤ 24 h), resulting in mortality for many individuals within that bin. Offspring per female counts were calculated separately between bins that

did and did not experience a desiccation event to estimate the effects of desiccation on rearing success. Desiccation events occurred sporadically over all developmental stages and are not confined to any one species (Table 1).

Observation of *C. d. dorsalis* Oviposition and Development. Five male and five female *C. d. dorsalis* adults were collected on 31 July 2008 from the southern shore of Martha's Vineyard, Dukes County, MA. Only 10 individuals could be obtained due to the low population numbers and genetic distinctiveness of this population (Vogler et al. 1993, U.S. Fish and Wildlife Service 1994, Goldstein and Desalle 2003). Adults were set up in laboratory on 1 August 2008, and observations continued until 17 November 2008, at which time larvae were unintentionally destroyed due to failure of the environmental chamber. All adults and larvae were then frozen at -80°C and have been deposited in the AMCC of the American Museum of Natural History (for accessions, see Table 4). Daily instar counts were recorded in each bin until the appearance of the first second instar in a bin at which time individual larvae were transferred to tubes for more direct observations of larvae produced per female. *C. d. dorsalis* is the only species reported in this study to be reared in individual tubes. The tubes were 20- by 2.54-cm i.d. clear polystyrene that had been halved lengthwise, with several 0.158-cm (0.06-inch) drainage holes in the tube bottoms. The tubes were filled with ≈ 16.5 cm of moist compacted native soil.

Larvae were transferred to tubes by placing a Styrofoam rectangle on top of the surface within a rearing container (bin) to fill the space between the substrate surface and the top of the bin. The bin was then inverted to rest in a large shallow tray (100 by 50 by 8 cm), and the bin was pulled away. The soil was gently divided by hand to reveal larvae which were manipulated with featherweight forceps (BioQuip Products, Rancho Dominguez, CA) and moved using a laboratory spoonula. A short, 2.5-cm-deep, instar-specific-sized hole was made in the center of the soil surface in each tube. Larvae were placed in this hole and typically began excavating a new burrow within minutes. The hand-divided soil was replaced in the bin and rechecked in 24 h for the appearance of fresh burrows.

Tubes were held in a 16.5-cm square polyethylene bucket. The bucket sides were drilled to fit four par-

Table 2. Results of ANCOVA testing for effects of manipulation overwintering day length and cooldown time on proportion weight loss in *C. repanda* (A) and results of ANCOVA testing for effects of species and sex on proportion overwintering weight loss in different species of tiger beetles reared under short day and long cooldown conditions (B)

Source A	df	MS	F	P	Source B	df	MS	F	P
Model	4	0.034	8.73	<0.0001	Model	10	0.029	4.79	<0.0001
Day length	1	0.01	2.58	0.1104	Species	4	0.028	4.53	0.0015
Cooldown	1	0.052	13.33	0.0004	Sex	1	0.0015	0.26	0.613
Day length × cooldown	1	0.034	8.61	0.0039	Species × sex	4	0.011	1.82	0.126
Preoverwintering wt	1	0.0001	0.04	8.477	Preoverwintering wt	1	0.0005	0.09	0.78
Error	138	0.003			Error	248	0.0062		

allel dowels along each side, the bottom was then cut away and fitted with a 0.6-cm polyethylene grid. The dowels supported the tubes upright in a grid pattern such that each tube-bucket held 25 tubes. Buckets were held in environmental chambers under the same conditions as adults. Larval feeding in tubes was done by hand once per day. All instars were fed live crickets whose diameter was slightly smaller than the burrow. Food was chilled to reduce movement and was placed head first into an open burrow then sealed inside the burrow by dragging a finger across the soil surface. When the burrow reopened, the larva was again fed as above. Tubes were misted from the top after each feeding and were moistened from the bottom as necessary by immersing the bucket in 2.5 cm of water for several minutes or until moisture traveled halfway up the tubes.

Results

Overwintering. All *C. repanda* that did not have a cooldown perished and were excluded from further analysis. Proportion overwintering weight loss was significantly affected by cooldown ($P = 0.004$), but not daylength; however, there was a significant interaction between daylength and cooldown treatments ($P = 0.0039$). There was no effect of preoverwintering weight (Table 2, A). Beetles in the short day and long cooldown treatment lost significantly less weight than all other treatments where beetles lost similar proportions of weight (Fig. 1). Survival was not significantly affected by any factor (all $P > 0.05$).

Proportional overwintering weight loss differed significantly between species (Fig. 2) but did not differ significantly by sex or preoverwintering weight (Table 2, B). *C. purpurea* individuals lost proportionally less weight than other species (Fig. 2). Survival varied significantly between species, and sexes, and there was a significant sex × species interaction (Table 3). Overall, a higher proportion of females than males survived overwintering (62% survival for females, 56% survival for males). This was the case in all species, except for *C. hirticollis*, where females and males were similar in their survival (Fig. 3). Among species, survival was lowest in *C. scutellaris*, followed by *C. tranquebarica*, then *C. hirticollis*, whereas *C. repanda* and *C. purpurea* had similar and overall high overwintering survival (Fig. 3). Postoverwintering, all species from this study except *C. scutellaris* (no males survived), were placed in bins, mated normally and produced first-instar larvae (data not shown).

Oviposition and Development. Mean peak larval numbers were variable between *C. abdominalis*, *C. punctulata*, *C. puritana*, and *C. unipunctata* (Table 1). Larval survival at every stage was greater in nondesiccated bins. Mean peak instar counts for *C. d. dorsalis* reared in tubes were frequently higher than for other species (Table 4).

The number of oviposition holes did not vary between species ($F = 1.99$, $df = 3$, $P = 0.13$). The peak number of first instars differed significantly between species, with *C. punctulata* producing on average more than other species (first instars: $F = 8.51$, $df = 3$, $P = 0.0001$). The peak number of second instars also varied significantly between species, with *C. punctulata* producing the most followed by *C. unipunctata* and then *C. puritana* (second instars: $F = 3.36$, $df = 2$, $P = 0.043$). Finally, the peak number of third instars did not differ significantly among species (third instars: $F = 2.13$, $df = 2$, $P = 0.13$).

Discussion

Most species of *Cicindela* will readily mate and oviposit in the laboratory (Palmer 1979, Knisley and Schultz 1997), and our results suggest that laboratory rearing may be used as a tool for both experimentation and conservation. Our rearing methods are applicable to relatively common species such as *C. punctulata* as well as species considered rare or threatened such as *C. unipunctata* and *C. puritana* and *C. d. dorsalis* (Tables 1 and 4). Furthermore, our results highlight vari-

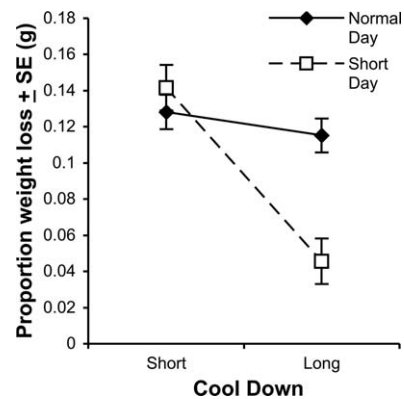


Fig. 1. Proportion weight loss of *C. repanda* individuals by overwintering treatment. The interaction of the treatments is presented as daylength, plotted by cooldown.

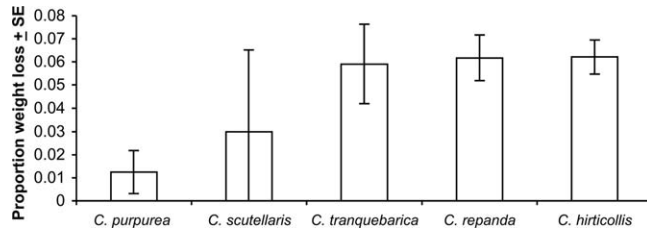


Fig. 2. Proportion weight loss of different tiger beetle species under short day and long cooldown conditions.

ation between species in overwintering survival and fecundity that may have predictive value when planning future rearing experiments.

Overwintering. The short day and long cooldown treatment seems to be adequate for overwintering adults of spring-fall species, because *C. repanda* individuals in this treatment had both the lowest overwintering weight loss and the highest amount of survival (Fig. 1). In the wild, mean overwintering weight loss of *C. repanda* has been measured as 8.5 mg (Knisley and Schultz 1997), and our results compare favorably with a mean loss of 5.3 mg in the short day and long cooldown treatment and a mean loss of 7.0 mg across all treatments. The proportion of weight lost by *C. repanda* was similar, regardless of sex, and a similar pattern has been observed in *C. japonica* overwintered in outdoor enclosures (Hori 1982).

Under the short day and long cooldown treatment, we successfully overwintered several other species (*C. hirticollis*, *C. purpurea*, *C. scutellaris*, and *C. tranquebarica*). Weight loss and survival varied among all species and there was also a species × sex interaction for survival (Figs. 2 and 3; Table 3). For most species, males were less likely to survive compared with females (Fig. 3; Table 3), with the notable exception of *C. hirticollis* that showed similar percentage of survival of both sexes. *C. hirticollis* males lost a proportion of weight similar to males of *C. repanda* and *C. tranquebarica* (Fig. 2), but they showed significantly greater survival than either species (Fig. 3; Table 3). *C. purpurea* showed significantly lower weight loss (Fig. 2) and higher percentage of survival (Fig. 3) than any other species. *C. purpurea* is considered a cold hardy species; adults emerge as early as 5 February in Kansas (Willis 1967). However, other species, such as *C. tranquebarica*, also are known to emerge early (Willis 1967) and it is possible multiple populations within a species may respond to this treatment differently (Brust et al. 2005). The percentage of survival of the remaining species is lower but comparable to the ≈80% survival of *C. japonica* experimentally overwintered outdoors (Hori 1982).

Table 3. Results of logistic regression examining differences between species and sexes in probability of overwintering survival

Source	df	χ^2	P
Intercept	1	0.28	0.598
Species	4	20.73	0.0004
Sex	1	5.71	0.0169
Species × sex	4	12.81	0.0122

Given these results, we propose the short day and long cooldown treatment as a provisional protocol to induce spring mating in adults of spring-fall species. The survival results presented in Fig. 3 also may guide collecting efforts to meet a target number for a post-overwintering population. Overall, it seems spring-fall species can be successfully overwintered in the laboratory. Larvae have been successfully overwintered by Shelford (1908); however, it remains to be explored whether our overwintering methods are suitable for larvae or for rearing multiple generations of adults in the laboratory.

Larval Production and Development. Overall, we successfully reared third-instar larvae from all species examined (Tables 1 and 4). Larval developmental time mirrored previous observations and suggests that larvae reared with our methods develop at a typical rate (Palmer 1978, 1979; Knisley and Schultz 1997). However, the number of third instars produced was highly variable between species and between bins within species, indicating that our methods could be refined to further improve rearing efficiency.

C. punctulata displayed significantly higher peak numbers of first and second instars compared with other species (Table 1.) This reproductive pattern accords with the general biology of *C. punctulata*. *C. punctulata* is considered to be a widespread, generalist species having one of the broadest distributions of any North American *Cicindela* (Pearson et al. 2006), where it occurs in suburban yards, sidewalks, agricultural areas, and at extreme altitudes (Knisley and Schultz 1997).

The variation in peak numbers between bins within species (Table 1) has several possible causes. Within bins, larvae will redig burrows, making it difficult to track any one individual and account for sources of individual mortality. In our study, larvae may have been lost to cannibalism due to their proximity (Willis 1967, Brust et al. 2006). Alternately, it is possible that eggs may be destroyed by crickets that are present in the bin as food for adults. Also, desiccation of bins has been a persistent problem for previous studies (Palmer 1979, Knisley and Schultz 1997) and has been for us as well. A bin undergoing a desiccation event can result in complete larval mortality, especially for first instars, which have the highest surface-to-volume ratio. Moisture levels in tubes, as used for *C. d. dorsalis*, seem more consistent than in bins, and they are less prone to saturation due to drainage holes. Short-term soil moisture meters seem to be sensitive to dissolved

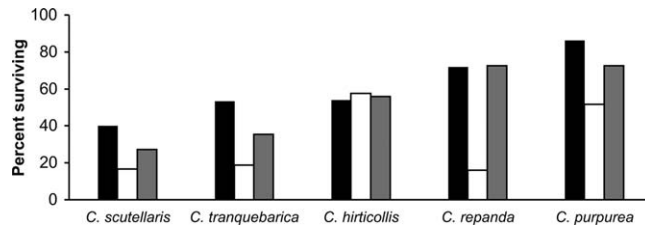


Fig. 3. Percentage of individuals surviving overwintering for sexes within different *Cicindela* species. Black bars, females; white bars, males; and gray bars, overall survival for each species.

solids (e.g., salts), and measurements vary widely between bins with equal moisture (data not presented), making a simple calibration for an optimum moisture level difficult.

A measure of female fecundity and the timing of larval development is important for its predictive value in rearing protocols. In the wild, development of North American *Cicindela* is highly influenced by food availability (Knisley and Juliano 1988) but typically takes 10 d from oviposition to egg hatch, 14–21 d for first instar, and 28–42 d for second instar (Knisley and Schultz 1997), suggesting a range of ≈52–73 d for reaching third instar. The third instar usually involves an overwintering phase and may take up to 3 yr before pupation (Willis 1967, Knisley and Schultz 1997). In this study, larval developmental time in the laboratory mirrored these general observations in *Cicindela* and suggests that larvae reared with our methods develop at a typical rate.

Rearing of Endangered Species. We successfully reared larvae to third instars for both *C. puritana* and the northern population of *C. d. dorsalis*; and under normal conditions, with no desiccation, larval production was relatively high. Larvae produced per female for these two species appear as an estimate for *C. puritana* (Table 1) and a direct count for *C. d. dorsalis* (Table 4). Although our sample size for *C. d. dorsalis* was low (5 females, 5 males), our rearing results suggest that the northern and southern population groups of *C. d. dorsalis* share similar measures of fecundity despite genetic (Vogler et al. 1993, Goldstein and Desalle 2003) and behavioral differences (U.S. Fish and Wildlife Service 1994). Knisley (1995; unpublished report), using rearing conditions similar to ours,

observed a mean number of *C. d. dorsalis* larvae per female of 10.9, which is similar to our mean of 9.6, and recorded a range of 2–23 larvae per female, which is similar to our range of 4–19 larvae. Our observations suggest that results of Knisley’s study may be used to guide rearing with the Martha’s Vineyard population.

Recommendations for Future Rearing. Rearing larvae in bins is a straightforward way to obtain very large numbers of first instars; however, bins seem suboptimal for rearing larvae through to third instar, as demonstrated by our low numbers of third instars (Table 4). Rearing in tubes is more labor-intensive, particularly for daily feeding; however, as evidenced by the *C. d. dorsalis* results, tubes may hold the possibility for obtaining a larger yield of larvae and higher quality of data than rearing in bins. Furthermore, disease could easily spread within a bin, and although we did not recognize any signs of disease, very little is known about *Cicindela* pathology (Braxton et al. 2003).

Oviposition behavior occurred readily for all species in this study, but our results may be influenced by female oviposition before our observation in the laboratory. Future studies may wish to collect females as early in the season as possible, before they have had the chance to oviposit as some species of *Cicindela* have high rates of early season oviposition (Cornelisse and Hafernik 2009). A direct count of all eggs and first instars per female is desirable, and some species of *Cicindela* will oviposit in petri dishes of soil (Hoback et al. 2000, Cornelisse and Hafernik 2009). These dishes could be changed daily to determine oviposition rates, with eggs immediately transferred to individual tubes to complete development (Hori 1982).

Table 4. *C. d. dorsalis* days to adult mortality and a direct count of fecundity per female

Adult AMCC no.	Sex	Days to mortality	First instar peak count	Instar of larvae at end of observation (second/third)		Larval AMCC no.
192384	Male	10				
192385	Female	10	7	4	0	192394–192397
192386	Male	11				
192387	Female	28	4	1	3	192398–192400
192388	Male	13				
192389	Female	18	6	2	3	192401–192403
192390	Male	15				
192391	Female	13	19	5	12	192404–192419
192392	Male	13				
192393	Female	10	12	4	8	192420–192431

Reintroduction efforts should seek to minimize the possibility for natural selection to affect captive populations by maintaining a minimum number of generations in captivity (Lewis and Thomas 2001, Schultz et al. 2009, Williams and Hoffman 2009). Given this guideline, our current methods, and tiger beetle life histories, we propose several options for rearing for reintroduction. Rearing could take place over 1 yr (or growing season), involve only one generation of larvae, and could be used for both summer and spring-fall species. Wild caught adults would oviposit in the laboratory, with larvae reared to late second or preferably late third instar and released into native habitat within the same year. No adults or larvae of this cohort would be held in the laboratory, and collection of adults could begin again in the spring or summer. Methods to maintain multiple generations will require additional experimentation to develop but could proceed as described above where the larvae would undergo laboratory overwintering and complete development to mate and produce a second cohort.

Our results show that for a group of insects with similar life histories, the general rearing method presented here can be used to create populations of laboratory reared insects of both common and endangered species. Despite their similar life histories, we observed species specific variation which suggests that further experimentation can make the general protocol more species specific. Lastly, it seems possible that established methods used to translocate and establish new populations by using wild larvae (Knisley and Hill 1999; Knisley et al. 2005; Davis 2007; P. Nothnagle and T. Simmons [1990], unpublished report) could now be used with laboratory-reared larvae to conduct large-scale reintroductions (Leonard and Bell 1999, Knisley et al. 2005).

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