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Mathew Louis Brust  
*University of Nebraska at Kearney*, mbrust@csc.edu

William Wyatt Hoback  
*University of Nebraska at Kearney*, whoback@okstate.edu

Kerri Farnsworth Skinner  
*University of Nebraska at Kearney*

Charles Barry Kinsley  
*University of Nebraska at Kearney*

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Physiology, Biochemistry, and Toxicology

Differential Immersion Survival by Populations of Cicindela hirticollis (Coleoptera: Cicindelidae)

MATHEW LOUIS BRUST, WILLIAM WYATT HOBACK, KERRI FARNSWORTH SKINNER, AND CHARLES BARRY KNISLEY

Department of Biology, University of Nebraska at Kearney, Kearney, NE 68849


ABSTRACT In the past 20 yr, populations of Cicindela hirticollis Say (Coleoptera: Cicindelidae) that inhabit river shorelines have declined dramatically. These habitats have routinely flooded in winter and spring historically, but they have been altered by damming and controlled water releases for irrigation and power generation. We tested the ability of C. hirticollis larvae from two river and one bayshore population to survive immersion in severely hypoxic water. This is the first report of population-level differences among insects in immersion survival and likely relates to exposure to different flooding regimes. The larvae from the Chesapeake Bay population survived ~3 d of immersion, and those from river populations survived about a day longer. Despite survival differences between riverine and seashore populations, recovery times after exposure to severe hypoxia were comparable. Second and third instars from the seashore population had similar survival at 9.0 and 16.5°C. Survival times of larvae more than doubled under aerated conditions. Adults survived ~30 h of immersion in severely hypoxic water, substantially longer than reported for other tested tiger beetle species. Although riverine populations survive longer periods of immersion, dams cause habitats used by riverine populations of this species to be inundated for weeks at a time, far longer than larvae were able to survive under hypoxic or aerated conditions in the laboratory. Thus, alteration of flooding regimes and subsequent larval habitat immersion is probably a major cause of the observed decline of riverine populations of C. hirticollis. Moreover, these data represent the first report of significant physiological differences among populations of an insect species exposed to different frequencies of immersion and thus have both important experimental and evolutionary implications.

KEY WORDS Cicindelidae, tiger beetle, anoxia, soil moisture, flooding.

Natural rivers undergo fluctuations in water level, the extremes of which vary by geography and seasonal precipitation. Seasonal floods maintain heterogeneity within riparian floodplains, creating distinct regeneration zones among plants (Naiman and Decamps 1997) and leaving many areas without vegetation. Over the past century, human alterations to rivers, such as damming and channelization, have significantly affected river flow, flooding cycles, and habitat immersion periods. The negative impact of dams on North American fish species is well documented (Moyle and Nichols 1974, Bain et al. 1988, Moyle and Williams 1990, Frissell 1993, Ricciardi and Rasmussen 1999). Changes to terrestrial systems adjacent to dammed rivers include prolonged periods of high and low water levels, siltation, and vegetative encroachment of downstream areas. Many terrestrial species such as Cicindela hirticollis Say use these riparian areas, having life cycles adapted to annual flooding. Although these specialized habitats have become increasingly altered by human activities, relatively few studies have examined the potential impacts of habitat changes on terrestrial floodplain inhabitants. The careful documentation of carabid species may be useful in understanding historic flooding regimes (Ellis et al. 2001); however, despite the importance of terrestrial invertebrates in ecosystem function, there is little knowledge of the impacts of flooding on them.

Tiger beetles in the genus Cicindela (Coleoptera: Cicindelidae) occupy a broad range of habitats, usually occurring in areas with exposed soil such as stream and pond edges, dunes and blowouts, seashores, salt flats, and open patches in grasslands (Pearson 1988, Larochelle and Lariviére 2001). The larval stages for most tiger beetle species are soil-dwelling, long-lived, and sedentary (Knisley and Hill 1992, Pearson and Vogler 2001). Many of the areas inhabited by tiger beetles are subject to occasional flooding (Willis 1967, Wilson 1974, Knisley and Schultz 1997, Pearson 1988), which maintains open soil areas by scouring or drowning vegetation and depositing sediment (Naiman and Decamps 1997). The ability of tiger beetle larvae to survive prolonged periods of immersion has been

1 Department of Biology, Randolph-Macon College, Ashland, VA 23005.
known for more than a century since Hamilton (1885) reported active *Cicindela repanda* Dejean larvae in burrows that had been submerged under \( \approx 1.7 \) m of water for 1 wk. Willis (1967) and Wilson (1974) recorded larval activity in habitats submerged for periods exceeding 2 wk.

Because of their adaptations to specific environments, many tiger beetle species are sensitive to habitat changes and may be especially vulnerable to the effects of hydrologic change. In fact, several "water-edge" tiger beetle species have experienced sharp declines in both abundance and distribution over the past century, including *Cicindela columbica* Hatch, *Cicindela dorsalis dorsalis* Say, *Cicindela gabbii* Horn, *Cicindela marginipennis* Dejean, *Cicindela puritana* Horn, and several subspecies of *C. hirticollis* (Nagano 1980, Shook 1981, Knisley and Schultz 1997, Larochelle and Lariviere 2001).

*C. hirticollis* Say inhabits moist, sandy substrates such as river sandbars, lake and ocean beaches, and areas between dunes (Larochelle and Lariviere 2001). It prefers clean moist sand and does not occur in areas with heavy silt (Graves et al. 1988). The distribution of *C. hirticollis* extends across much of the continent from central Canada to Mexico, including both the Pacific and Atlantic Coasts of North America (Graves et al. 1988). Eleven subspecies are currently recognized (Graves et al. 1988), and at least three of these have declined seriously in the past 30 yr (Nagano 1980, Dunn 1981, Larochelle and Lariviere 2001, Knisley 2003). *Cicindela hirticollis rhodensis* Calder is threatened in much of its former range in New England (Dunn 1981, Larochelle and Lariviere 2001) and has declined severely along the Great Lakes (M.L.B., unpublished data). In addition, *Cicindela hirticollis gravida* LeConte is extinct over most of its former range in the southern half of California (Nagano 1980; Larochelle and Lariviere 2001). *Cicindela hirticollis abrupta* Casey once occurred in five limited areas within \( \approx 8,000 \) km\(^2\) on the Sacramento and Feather rivers in central California (Knisley and Schultz 1997), and its recent dramatic decline in distribution and abundance is thought to be the result of prolonged inundation or other habitat changes associated with the Oroville and Shasta dams (Knisley 2003).

The greatest population declines of *C. hirticollis* (sensu latu [s.l.]) have been observed among inland populations, particularly in riverine habitats, whereas populations of *C. hirticollis* (s.l.) in the Chesapeake Bay region have persisted in good numbers (C.B.K., personal observation). Under natural conditions, larval habitats along rivers may be inundated for several days at a time and usually only several times per year. Because of the decline of this species across a geographically broad area, and because dams have altered water flow and shoreline inundation, we designed experiments to test immersion tolerance of *C. hirticollis* (s.l.) larvae from two riverine populations. Because of easy access to large numbers of larvae and their distinct pattern of inundation, we also tested larvae from a Chesapeake Bay population. In addition, adults from this population were tested for immersion tolerance. Because this species is frequently exposed to immersion, we hypothesized that *C. hirticollis* (s.l.) larvae would be more resistant to immersion and hypoxia than previously tested North American species (*Cicindela togata globicollis* Casey and *Amblycheila cylindriformis* Say). Despite our expectations that tiger beetle larvae from different populations of the same species would respond similarly to immersion and hypoxia, we found significant differences in survival. This observation serves as an important reminder that experimental assumptions should be tested (Riggins and Hoback 2003) because populations of any species exposed to different selection pressures may vary physiologically.

**Materials and Methods**

**Organisms.** *C. hirticollis* (s.l.) adults and larvae are active from April to September (Knisley and Schultz 1997). Third instars of *C. hirticollis hirticollis* Say \((n = 36)\) for our experiment were collected from the Rappahannock River \(\approx 18\) km upstream from the Chesapeake Bay, Middlesex County, 11 km west northwest of Deltaville, VA. Hereafter, these larvae are referred to as VA riverine. Third instars of *C. hirticollis shelfordi* Graves \((n = 45)\) were collected from Calamus Reservoir, \(\approx 12\) km northwest of Burwell, Loup County, Nebraska, and Harlan County Reservoir, Harlan County, Nebraska, \(\approx 6\) km south of Republican City. These larvae were pooled and are hereafter referred to as NE riverine. Second instars, third instars, and adults of *C. hirticollis hirticollis* were obtained from Virginia on the shorelines of the Chesapeake Bay in Northumberland County, 6 km north northeast of Reedville. Although this population occurs along a bay, they will be referred to as the seashore population for convenience. Larvae were collected in July, August, and September 2003 and 2004, and adults were collected in September 2003. Larvae were collected by using a trowel to block their retreat into the burrow. Adults were collected with an aerial net. Animals were maintained in the lab in containers with wet sand from their habitats and were fed apterous fruit flies. All experiments took place within 2 wk of collection of larvae and adults. After the experiments, voucher specimens of adults and second and third instars of *C. hirticollis hirticollis* and *C. h. shelfordi* were deposited in the insect collection at the University of Nebraska at Kearney.

**Larval Immersion.** To simulate flooding, larvae were immersed individually in 15-ml screwcap vials following the methods of Hoback et al. (1998). The vials were filled with dechlorinated tap water, which was bubbled with nitrogen to produce severely hypoxic water \((<0.1\) ppm dissolved oxygen). It was necessary in many cases to force the larvae to the bottom of the vial with a small piece of wet tissue because they floated readily. The vials were then gently tapped to dislodge any trapped air bubbles. The vials were placed in unlit environmental chambers at 9.0 and 16.5°C. In all experiments, the 16.5°C temperature was selected to simulate typical water temperatures under
flood ing conditions after summer rains. The cooler temperature was selected to simulate temperatures of bottom waters (hypolimnetic waters) of dammed lakes, which are released for irrigation or for power generation. For example, in the range occupied by C. h. abrupta, Sacramento River water is 8–10°C when released from the Shasta Dam (Bettelheim 2001).

At 24-h intervals, we removed subsets of five or 10 larvae from each group immersed in hypoxic water. These larvae were individually placed on moist sand in small containers. Larvae were checked for recovery after 24 h. Larvae that did not resume movement and dig a burrow in the sand were recorded as dead. Third instars of the two riverine populations (36 from Virginia and 45 from Nebraska) were tested for immersion tolerance at 16.5°C. From the seashore population, a total of 64 second and 93 third instars were tested. Second (n = 25) and third instars (n = 65) were tested at 16.5°C. In addition, second (n = 35) and third (n = 28) instars were tested at 9°C. Subsets of five larvae were removed at ~24-h intervals and allowed to recover following the methodology of the previous tests. As controls, a set of second (n = 5) and third (n = 5) instars from the seashore population and third instars from each of the riverine populations (n = 5) were placed individually in empty vials with a small piece of moist paper towel (to prevent desiccation) at each experimental temperature. These larvae were checked each time that immersion survival was checked. In all cases, they suffered zero mortality over the course of the experiments.

All immersion data were analyzed using Toxstat 3.4 (Western Ecosystems Technology, Inc., Cheyenne, WY.) to calculate lethal time to 50% mortality (LT50) and 95% confidence intervals (CI). Because of the difficulty of obtaining large numbers of larvae of the inland populations of C. hirticollis standard statistical analyses were not applied. Rather, significant differences in survivorship were judged by nonoverlapping confidence intervals (Hoback et al. 1998, 2000).

**Immersion in Aerated Water.** Because flowing water in natural habitats may replenish dissolved oxygen and Zerm et al. (2004) showed oxygen uptake by immersed tiger beetle larvae, we tested larval survival in aerated water. Third instars (n = 30) from the seashore population were submerged in individual 5-ml vials covered with pantyhose (to prevent escape, but allow water movement) and placed in ~4 liters of water, which was bubbled with an aquarium pump. The container was placed in the 16.5°C environmental chamber, and the water was close to air saturation (9.2 ppm dissolved oxygen as measured with an YSI model 30 dissolved oxygen meter). Sets of five larvae were removed at 24, 48, 72, 120, 168, and 192 h, and those that did not resume activity after 24 h were recorded as dead.

**Postimmersion Recovery.** Because survival times differed markedly between populations, a second set of experiments was conducted to determine whether populations of C. hirticollis (s.l.) differed in postimmersion recovery times. Third instars from each population were immersed in severely hypoxic water by the same methods as the immersion survival experiments, but at 20°C. Subsets of five larvae from each of the three populations were removed at 2, 6, 16, and 24 h. The larvae were placed on their sides or backs in the plastic containers with moist sand as before, except that the containers were not closed, and the larvae were observed throughout the recovery period. Larval recovery was determined as the time to the nearest minute when larvae were able to immediately right themselves again when flipped over with a wire. Recovered larvae usually began to burrow within 10 min. A two-way analysis of variance (ANOVA) was used to test for differences in recovery time among populations.

**Adult Immersion Tolerance.** Because adult tiger beetles dig burrows before periods of inactivity (Knisley and Juliano 1988) and could become immersed by increases in water level, we also tested adult survival of immersion. Thirty adults from the seashore population were immersed in severely hypoxic water and placed in a 16.5°C environmental chamber. Sets of five adults were removed at ~8-h intervals and placed on moist sand; those that did not resume activity after 24 h were recorded as dead.

**Results**

**Larval Immersion.** Under severely hypoxic conditions at 16.5°C, third instars from the NE riverine populations survived significantly longer periods than larvae from the seashore population (Fig. 1). All NE riverine larvae survived 4 d of immersion at 16.5°C; LT50 and 95% CI for third instars was 108.42 ± 6.8 h. The LT50 and 95% CI for the seashore population was 79.10 ± 15.8 h. Neither was significantly different from the VA riverine population, which had a LT50 and 95% CI of 101.70 ± 16.5 h (Fig. 2).

Mean survival times were similar at both 9 and 16.5°C (Fig. 3) for second and third instars from the seashore population. The LT50 and 95% CI was 77.96 ± 20 h for third instars and 85.72 ± 19.8 h for second instars at 9°C. At 16.5°C the LT50 and 95% CI for second instars from the seashore population was 59.84 ± 20 h.

**Immersion in Aerated Water.** Third instars placed in aerated water survived much longer than those in hypoxic water (Fig. 4). The LT50 for third instars under aerated conditions at 16.5°C was 177.01 ± 26 h and was significantly greater than the 79.10 ± 15.8 h LT50 in severely hypoxic water.

**Postimmersion Recovery.** The recovery times after immersion did not differ between the populations tested. The ratio of recovery time to immersion time was between 0.068 and 0.081 min of the immersion time for all trials (Fig. 5). A two-way ANOVA showed no differences in recovery times between populations (P = 0.72).

**Adult Immersion Tolerance.** As the adults were immersed, they struggled for 1 to 3 min and then stopped moving and seemed dead. However, adults survived up to 72 h of immersion (Fig. 6), which was
significantly less than larvae. Adults had an LT_{50} of 34.96 ± 6.9 h at 16.5°C.

**Discussion**

Although larvae of *C. hirticollis* (s.l.) survived 1–5 d of immersion, the substantial difference in immersion survival of different populations was an unexpected result of this study (Fig. 2). Larvae of the NE riverine population survived hypoxia significantly longer than the seashore population. Survival of larvae from the VA riverine population was intermediate between the other two populations but more similar to the NE river population (Fig. 2). The differences in immersion survival between populations of the same species are most likely the result of different selection pressures within their habitats. The majority of the *C. h. hirticollis* larvae along the shoreline of the Chesapeake Bay is found above the normal high tides and rarely inundated, whereas those within the intertidal zone are only submerged for about an hour or less during high tide periods (C.B.K., unpublished data). Riverine populations are often exposed to flooding that can last for several days, and this has likely led to populations dominated by individuals with higher immersion tolerance.

Before this study, it was assumed that differences in anoxia tolerance among tiger beetles were a species-level trait that reflected adaptations to specific selection pressures (Hoback et al. 2000, Hoback and Stanley 2001). However, based on our results, abiotic factors can select differences in physiological response at the population level that are not species specific. These differences have been previously dem-
onstrated experimentally with populations of *Drosophila melanogaster* (Meigen) (Gibbs et al. 2003). Future genetic testing of *C. hirticollis* (s.l.) populations could reveal the genetic mechanism that confers hypoxia resistance in tiger beetle larvae. Previous studies found remarkable resistance to flood-induced hypoxia among different species of tiger beetle larvae (Table 1). Moreover, *Phaeoxantha klugii* Chaudoir larvae slowly flooded in their burrows and then placed in a nitrogen environment survived an average of 26 d at 29°C (Zerm and Adis 2003). The differences in survival by *A. cylindriformis*, which inhabits dry clay banks away from water, and *P. klugii*, an inhabitant of floodplains of the Amazon River, are expected. However, the substantial differences we found in immersion tolerance among *C. h. hirticollis* larvae from seashore and riverine populations were surprising. In fact, the two populations of *C. h. hirticollis* from Virginia where larvae were collected were only ~20 km apart and yet exhibited different (although not significant) immersion tolerances. The NE riverine population was similar in immersion tolerance to the VA riverine population despite being a different subspecies, *C. hirticollis shelfordi*.

Hoback et al. (1998) found no difference in survival between third instars of *C. togata*, a North American salt flat species, exposed to aerated or severely hypoxic water. However, *C. h. hirticollis* larvae from the seashore habitat survived ~3 times longer in aerated water than larvae from the same population in hypoxic water (Fig. 4). Unfortunately, larvae from riverine populations were unavailable to test for survival in aerated water. In riverine habitats, larvae could remain in burrows and continue to extract dissolved oxygen from flowing water. Aquatic respiration also may help to explain the differential survival between the seashore and riverine populations, because wave action along seashores might maintain aerated conditions and prevent larvae from being exposed to severe hypoxia. Zerm et al. (2004) found *P. klugii* larvae to extract dissolved oxygen from water and maintain metabolic rates between 22 and 4% of the rates in air.

Table 1. Comparison of published tiger beetle anoxia tolerance (in hours)

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th><em>C. hirticollis</em></th>
<th><em>C. togata</em></th>
<th><em>A. cylindriformis</em></th>
<th><em>P. klugii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>77.96</td>
<td>&gt;240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>127.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.5</td>
<td>78.10 (seashore)</td>
<td>101.70 (riverine)</td>
<td>108.42 (riverine)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>131.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>102.2</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td>136.8</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td>25.7</td>
<td></td>
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</tr>
</tbody>
</table>

*Present study.*

*Hoback et al. (1998).*

*Hoback et al. (2000).*

*Zerm and Adis (2003).*
Dissolved oxygen levels were found to be reduced with time in areas adjacent to the larval spiracles and the authors suggest that larvae maintain a small bubble of air at the spiracular opening and obtain oxygen by diffusion through a plastron.

Because we observed survival differences among populations of C. hirticollis (s.l.), we hypothesized that inland populations were suppressing their metabolic depression further than seashore populations as an adaptation to longer flooding duration. We anticipated riverine populations would recover more slowly from immersion. However, in all three populations tested, the relationship between recovery time and anoxia exposure was found to be linear (Fig. 4). These results suggest that differences in metabolic depression are unlikely. Another mechanism, such as different anaerobic end products (Hoback and Stanley 2001), might be the cause of the differences observed between populations.

In addition to the unexpected results for larval C. hirticollis (s.l.), adult C. h. hirticollis are substantially more tolerant of immersion in anoxic water than other species examined. Zerd and Adis (2003) found that adults of P. kluigii died after <6 h in a nitrogen environment, and Hoback et al. (1998) found C. togata adults survived <3 h of immersion. In comparison, adult C. h. hirticollis survived 35 h in anoxic water, and one adult recovered after 72 h (Fig. 6). This was similar to adults of another tiger beetle, Megacephala (Tetrachra) sobrina Dejean, that survived 24–30 h of immersion (Adis and Messner 1997). These authors suggested that adults survive by trapping a bubble of air beneath the elytra. We suggest that adults of C. hirticollis (s.l.) are able to survive immersion by similar means. Although the reason for adults having high tolerance to drowning is unknown, the life cycle of C. hirticollis (s.l.) may offer an explanation. C. hirticollis (s.l.) is a member of a large group of spring–fall active U.S. species that most often overwinter as adults and would be exposed to winter submergence along river edge habitats, unlike C. togata, which has a summer life cycle and overwinters only as larvae (Pearson and Vogler 2001).

Human-induced changes to aquatic ecosystems, including damming, channelization, and bank stabilization, have had negative impacts not only on aquatic organisms but also on terrestrial organisms in adjacent areas (Layzer et al. 1989). Because C. hirticollis (s.l.) seems to be adapted to regular flooding cycles and specific habitat requirements, dam construction and intensive recreational use of sandy shorelines likely contribute to the decline of this species across much of its range. Although such human activities have likely affected other terrestrial insect species, some water-edge tiger beetle species such as C. repanda and Cicindela oregona LeConte seem less affected. Continuing investigations into the mechanisms that allow immersion survival and the effects of dams on terrestrial invertebrates are critical to understanding ecosystem function in these water-edge ecosystems.

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