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Hypoxia Tolerance in Adult and Larval *Cicindela* Tiger Beetles Varies by Life History but Not Habitat Association

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ABSTRACT   Flooding exposes terrestrial organisms to severe hypoxia. Among the best-studied insects that are frequently exposed to flooding are tiger beetle (Coleoptera: Cicindelidae) larvae. In previous studies with a limited number of species, a correlation was found between habitat and hypoxia tolerance. In the current study, we examined hypoxia tolerance among third instars of six *Cicindela* species that vary in habitat association; we also tested adult survival of these species. We found that larvae of the different species survived an average of between 60 and 120 h of submersion in severely hypoxic water at 20°C, with larvae that occur in dry sand having somewhat lower survival times. However, among larvae, there was no correlation between survival times and habitat risk of flooding. We found surprisingly high survival times among adult tiger beetles with a range of 10 h to 46 h. Adult *Cicindela formosa formosa* Say that are associated with dry sand and are active in the spring and fall survived submersion for >40 h, longer than any previously reported adult terrestrial insect exposed to hypoxia at moderate environmental temperatures. The lengthy survival times of adult tiger beetles are likely a result of adaptations to immersion during periods of inactivity. Our study suggests that additional insect species and life stages should be examined for hypoxia tolerance regardless of perceived threat of immersion.

KEY WORDS   tiger beetles, *Cicindela*, hypoxia, flooding, immersion

Tolerance of flooded conditions has been studied on a limited number of terrestrial insect taxa (Hoback and Stanley 2001). Among these taxa, tiger beetle (Coleoptera: Carabidae: Cicindelinae) species, which occur in habitats with bare soil and often rely on periodic disturbance associated with flooding to maintain their habitats, have received the most attention (Hoback et al. 1998, 2000; Zerm and Adis 2003; Zerm et al. 2004; Brust et al. 2005). Larvae form burrows in the substrate and typically spend their entire development in a single location, although exceptions are known. Among temperate cicindelid species most require 1 to 3 yr of development and are often exposed to extended immersion several times during their development.

Because standing water rapidly becomes hypoxic (Baumgärtl et al. 1994), flooded tiger beetles are exposed to severely hypoxic conditions. As an adaptation to hypoxic conditions, at least three tiger beetle species have larvae that switch to anaerobic metabolism during immersion (Hoback et al. 2000; Zerm and Adis 2003; Zerm et al. 2004a,b), and the capacity for anaerobic metabolism is likely to occur more widely in this taxa.

Hoback et al. (2000) found differences in hypoxia tolerance between tiger beetle species in different genera, potentially related to habitat occurrence and probability of hypoxia exposure. However, the authors only examined two species in total, each a member of a different genus. Brust et al. (2006) found differences in hypoxia tolerance among populations of *C. hirticollis* where seashore populations had low tolerance to hypoxia. These differences seemed to relate to the nature of immersion. The seashore population experiences immersion from daily tidal cycle and need faster recovery from immersion, whereas the riverine populations face seasonal prolonged immersion (Brust et al. 2006).

In contrast to the larvae, the adults of most temperate North American tiger beetles are agile and capable of flight (Pearson et al. 2006) and thus can likely escape flooding except during periods of inactivity when they often bury into the substrate. Temperate cicindelids follow seasonal patterns of activity, which differ by species and occasionally by geographic region (Knisley and Schultz 1997, Pearson et al. 2006). These patterns are defined by adult activity and are either summer, or spring-fall. Summer species have adults emerge from the pupa in summer and die by fall. Spring-fall species have adults emerge from the pupa in late summer or fall, often feed briefly, overwinter, and then resume activity in spring, and die by early summer. Adult spring-fall tiger beetles dig burrows in fall and spend the winter underground. They

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Table 1. Nebraska collection locations for larval and adult tiger beetles and number tested

Species	Location	Total no. tested	Control
C. denverensis larvae	5.0 km NW Sumner, Dawson Co.	40	5
C. denverensis adults	5.0 km NW Sumner, Dawson Co.	35	5
C. formosa larvae	9.0 km W Cairo, Hall Co.	28	4
C. Formosa adults	10 km NW Burwell, Garfield Co.	30	5
C. nevadica larvae	5.0 km S Antioch, Sheridan Co.	50	5
C. nevadica adults	5.0 km S Antioch, Sheridan Co.	50	5
C. punctulata larvae	Kearney, Buffalo Co.	50	5
C. punctulata adults	Kearney, Buffalo Co.	50	5
C. repanda larvae	5.0 km W Kearney, Buffalo Co.	40	5
C. repanda adults	3.5 km NW Grand Island, Hall Co.	27	3
C. tranquebarica larvae	Harlan Reservoir, Harlan Co.	35	5
C. tranquebarica adults	Harlan Reservoir, Harlan Co.	50	5

also burrow frequently during the warmer season, both at night and during inclement weather (Knisley and Schultz 1997, Pearson et al. 2006).

As a result of their life cycle, adult spring-fall tiger beetles may be exposed to prolonged flooding in saturated soil, particularly during spring snowmelt. Summer species either dig overnight burrows, or spend the night among vegetation (Knisley and Schultz 1997). Thus, summer-active adults are not as likely to be exposed to flooding except during heavy warm-season precipitation episodes.

In this study, we investigated two hypotheses: larvae of *Cicindela* species that differ in likelihood of immersion also differ in hypoxia tolerance, and spring-fall adult *Cicindela* species survive hypoxia longer than summer-active congeners. We examined both adult and third-instar stages of six *Cicindela* species from Nebraska that were chosen based on habitat and activity pattern.

Materials and Methods

Adults and third instars of six species of tiger beetles were used in this study: *Cicindela denverensis* Casey, *Cicindela formosa formosa* Say, *Cicindela nevadica knausii* Leng, *Cicindela punctulata punctulata* Olivier, *Cicindela repanda repanda* Dejean, and *Cicindela tranquebarica tranquebarica* Herbst. The collection locations for all adults and larvae used in this study are given in Table 1.

Larvae were collected by two different methods. All *C. repanda* and some *C. denverensis* were obtained by inserting a grass blade into the burrow and excavating an adjacent hole, from which the burrow was broken away to reveal the larva. Some *C. denverensis* and all larvae of *C. nevadica, C. punctulata,* and *C. tranquebarica* were obtained by "fishing" them out using a grass blade or stem. The grass blade was inserted into the burrow and quickly jerked out when the larva bit it. The larvae of *C. formosa* were obtained by digging them out or using the "stab and grab" method, which blocks larval retreat in the burrow (Knisley and Schultz 1997). Adults were collected with an aerial net. All were collected during the day. After collection, individuals were maintained with damp soil in the laboratory for no >72 h before testing.

Exposure to hypoxic water followed methods of Hoback et al. (1998). Spring water was bubbled with nitrogen gas for ≈5 min/liter to render it severely hypoxic (dissolved oxygen <0.3 ppm). Individual adults or larvae were placed in a 15-ml screw cap glass vial to which the deoxygenated water was added until full. In some cases, a piece of tissue was needed to keep individuals from floating to the top. Vials were then sealed and gently tapped to remove any air bubbles adhering to either the larva, the sides of the vial, or to the piece of tissue. The vials were then placed in an unlighted environmental chamber at 20°C. Three to five individual larvae or adults were individually placed in screw cap vials with a small piece of damp tissue to serve as controls. Controls were checked each time that immersed individuals were removed. No control individuals died during the course of the experiment.

Subsets of adults were removed at 2, 4, 6, 8, 10, 12, 24, 36, and in some cases 72 h, and larvae were removed at 24, 48, 72, 96, 120, 144, 168, and 192 h. After removal from immersion, the specimens were placed individually in small plastic containers with moist sand and returned to the environmental chamber. Adults unable to walk and larvae unable to turn themselves upright after 24 h were recorded as dead. Survivorship data were analyzed using Toxstat 3.4 (Western Ecosystems Technology, Inc., Cheyenne, WY), which calculates time to 50% mortality (LT_{50}) and 95% confidence intervals by probit analysis. Significant differences in survivorship were determined by nonoverlapping 95% confidence intervals (Hoback et al. 1998).

Results

On average, all larvae tested survived >56 h of immersion in hypoxic water (Fig. 1). Larvae of *C. tranquebarica* and *C. formosa* had the shortest survival times and were significantly different from *C. denverensis, C. nevadica,* and *C. repanda.* Larvae of *C. nevadica* and *C. repanda* had the longest average survival times, with LT_{50} values of 135.5 and 136.6 h, respectively.

In all cases, adults survived significantly shorter periods of hypoxia exposure (based on nonoverlapping confidence intervals) that their conspecific lar-
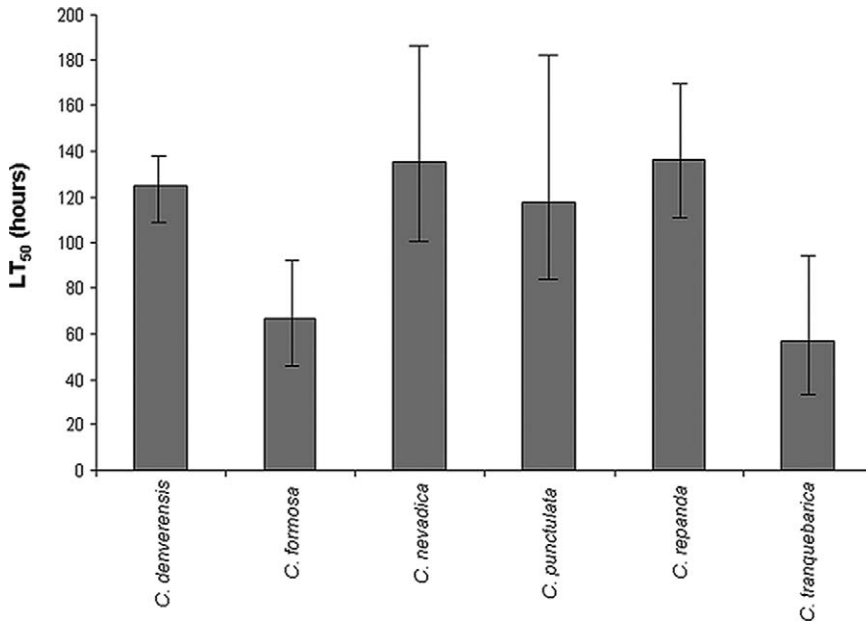
Fig. 1. Graph of LT_{50} and 95% confidence intervals for larval tiger beetles exposed to hypoxia at 20°C. Confidence intervals depicted by error bar.

vae. Adult *C. formosa* had significantly greater survival times than all other species (46.5 h), and in fact has the highest hypoxia tolerance recorded for any adult tiger beetle to date, including *Tetracha sobrina* Dejean (Adis and Messner 1997). The adults of most species tested here survived an average of 9–22 h of hypoxia exposure, and the only other significant difference was

found for *C. tranquebarica* adults that survived longer than *C. denverensis* (Fig. 2).

Discussion

This study tested survival of immersion in hypoxic water by a several species from a variety of common
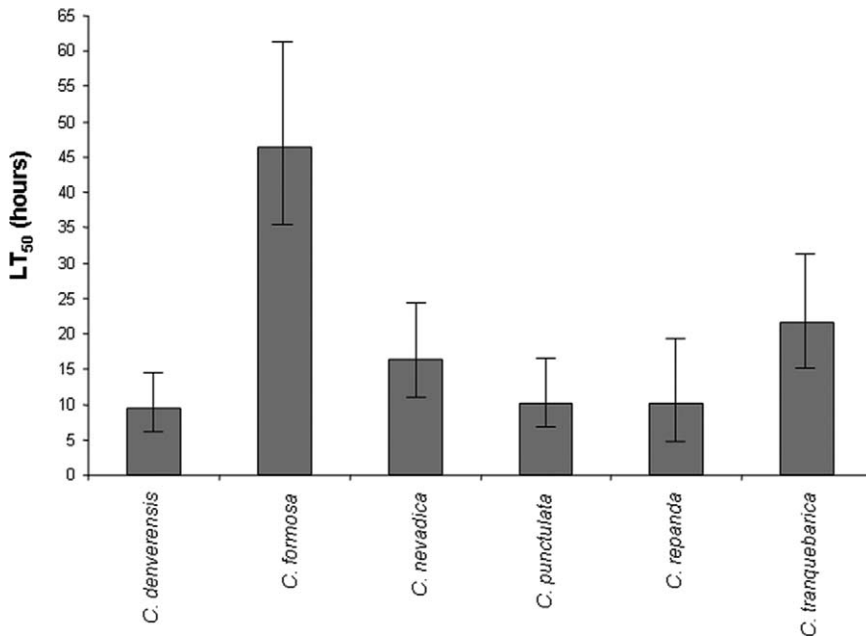

Fig. 2. Graph of LT_{50} and 95% confidence intervals for adult tiger beetles exposed to hypoxia at 20°C. Confidence intervals depicted by error bar.

tiger beetle habitats. *C. nevadica* inhabits inland saline environments subject to frequent flooding; *C. repanda* occurs on beaches and sandbars, which are also subject to frequent inundation, and *C. tranquebarica* occurs in areas where the water table is near the soil surface, such as upper beach areas. In contrast, *C. denverensis* occurs in steep, dry loess and clay banks; *C. formosa* occurs in upland blowouts and other dry sandy areas; and the larvae of *C. punctulata* occur in bare patches in a variety of upland habitats, including lawns. Thus, this study examined hypoxia tolerance in *Cicindela* larvae that occur in flood-prone areas and those that inhabit areas that rarely flood.

For larvae, we found no strong relationship between hypoxia tolerance and risk of flooding exposure. There were no substantial differences between these species, with the average hypoxia tolerance of *C. denverensis,* a species that lives in dry habitats, being approximately equal to that of *C. repanda,* a rivershore inhabiting species. Our results are similar to the hypoxia tolerance found by Hoback et al. (1998) for *Cicindela togata globicollis* Casey (LT_{50} = 131.9 h at 20°C), a saline wetland species.

All larval *Cicindela* and *Phaeoxantha* species tested to date differ from the primitive *Amblycheila cylindriformis* Say, which Hoback et al. (2000) found to only survive an average of 34 h of hypoxia at 25°C. Survival times of the larval *C. tranquebarica* and *C. formosa* overlap with this species based on 95% confidence intervals. Because these species live in well-drained dry sandy soil, the lower survival times may reflect selection to recover from inundation as was shown for larval *C. hirticolis populations* from seashore sites (Brust et al. 2005). Alternatively, lower survival times may reflect selection differences for larvae in these habitats. Previous work by Brust et al. (2006) on the sand-inhabiting *C. hirticollis* found substantial differences in hypoxia tolerance among populations. Those from riverine populations survived 108 h, whereas those from seashores survived only 78 h at 16.5°C. Because the metabolic activity of insects is closely tied to temperature and hypoxia tolerance among tiger beetle larvae decreases with increasing temperature (Hoback et al. 1998, 2000; Zerm and Adis 2003; Zerm et al. 2004; Brust et al. 2005) direct comparisons with this study are not possible.

The hypoxia tolerance observed for adult tiger beetles were much longer than those observed for adults of *C. togata* (Hoback et al. 1998). Moreover, the exceptional hypoxia tolerance noted in adults of *C. formosa* was surprising. This is especially interesting because this species occurs in upland sandy areas. The tolerance of adults of *C. formosa* may be related to their burrowing habits, or overwintering exposure to hypoxia, although frequent burrowing by adults also has been noted in *C. denverensis* and *C. repanda,* which also overwinter as adults (Knisley and Schultz 1997) and did not have similar tolerances.

It was somewhat surprising that all adult species other than *C. formosa* were similar in hypoxia tolerance. We expected that summer-active adult species (*C. nevadica* and *C. punctulata*) would have a lower tolerance of hypoxia because spring-fall species spend the entire winter burrowed underground and would be subject to hypoxia exposure, especially in early spring before they emerge. Adults of this group also tend to only be active in daylight, and dig burrows to avoid excessive heat, cool and cloudy weather, and for the night (Knisley and Schultz 1997). Summer species are only rarely known to burrow, and many species are active both day and night in the adult stage (Knisley and Schultz 1997). However, we found no differences in hypoxia tolerance between adults of the spring-fall life history and those of the summer life history.

Within species, comparison of adults and larvae also produced unusual differences. The adults of *C. formosa* and *C. tranquebarica* exhibited the highest tolerance to hypoxia, whereas their larvae exhibited the lowest levels of tolerance. In fact, the adults and larvae of *C. formosa* did not differ from one another in hypoxia tolerance based on overlapping 95% confidence intervals (Figs. 1 and 2).

Further studies of hypoxia tolerance in insects may reveal important morphological characteristics that may increase hypoxia tolerance. In this study, adults of larger species (*C. formosa* is the largest tested species) typically exhibited higher hypoxia tolerance. The same trend was noted in rangeland grasshoppers among both nymphs and adults (Brust et al. 2007). However, *A. cylindriformis* is the largest North American tiger beetle (Pearson et al. 2006), and yet adults of this species exhibited the lowest hypoxia tolerance among species tested (Hoback et al. 2000). Although larger insects are able to trap more air in the tracheae, potentially allowing them to survive longer periods of hypoxia, this trend toward larger size and higher hypoxia tolerance was not evident in larvae.

The occurrence of strong hypoxia tolerance among *Cicindela* tiger beetle adults and larvae suggests that it may be a conserved trait from an ancestral form that was subject to regular flooding. Alternatively, the ability to survive flooding, even if a species is only exposed once over many generations, might offer the species a selective advantage. Finally, work by Brust et al. (2006) showing apparent trade-offs between immersion survival and recovery time may indicate that multiple selection factors influence a population or species' tolerance of immersion.

To date, this work represents the most comprehensive study on hypoxia tolerance in terrestrial insect taxa. Using tiger beetles as model organisms, future studies may determine the mechanisms of hypoxia survival along with physiological responses among and between species as well as correlations among ecology, body size, and hypoxia tolerance.

Acknowledgments

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