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High water-loss rates and rapid dehydration in the burying beetle, *Nicrophorus marginatus*

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Abstract. During the summer months, there is a high mortality of burying beetle (Coleoptera: Silphidae) species in pitfall traps containing dry soil. The present study investigated the possibility that the burying beetle *Nicrophorus marginatus* is highly susceptible to death from desiccation. In the laboratory, adult beetles lose 1–5% body mass per hour in low humidity conditions (25–30% relative humidity), depending on temperature. This rate of water-loss results in a 50% mortality within 7–16 h at temperatures between 16 and 28 °C. *Nicrophorus marginatus* produces oral and anal defensive secretions when disturbed but these secretions do not significantly contribute to the high rate of water loss. Beetles readily drink and thus beetles with access to water or in high humidity conditions suffer near zero mortality. For comparison, the similarly sized mesic bess beetle (Coleoptera: Passalidae), *Odontotaenius disjunctus*, and a large nocturnal tiger beetle (Coleoptera: Cicindelidae), *Amblychila cylindriformis*, are exposed to dehydrating conditions. *Nicrophorus marginatus* have higher rates of water-loss (by 26% and 79%, respectively). It is predicted that the diurnal *N. marginatus* uses behavioural mechanisms to avoid lethal water loss. In the field, *N. marginatus* displays a strong bimodal activity pattern, avoiding the hottest hours of the day. The significance of these findings is that *N. marginatus* defies normal predictions of association between water-loss rates and habitat type, and has extended its range into apparently unfavourable habitats despite high water-loss rates. In addition, the results suggest that researchers should provide moisture when studying burying beetles, including the endangered American burying beetle (*Nicrophorus americanus*).

Key words. American burying beetle, bess beetle, desiccation, *Nicrophorus americanus*, tiger beetle, water-loss.

Introduction

Because of their small size and unfavourable surface area to volume relationships, terrestrial insects face enormous challenges in maintaining their water balance (Hadley, 1994). Despite many behavioural, physiological and structural adaptations that work to conserve body water, insects can lose substantial proportions of their water content through two routes: (i) transpiration and (ii) excretion (Flemister, 1964). Transpiration refers to water loss across the cuticle and through respiration, which varies tremendously among insect species (Edney, 1977; Cloudsey-Thompson, 1988; Hadley, 1994) and by habitat types (Hadley, 1994; Chown, 2001). For example, soft-bodied insects, such as collembolans, are virtually unable to restrict transpiratory water loss and these insects uniformly are associated with cool, moist habitats. On the other hand, insects with low transpiration rates, such as the desert-dwelling tenebrionid beetles, *Onymacris plana*, are associated with very dry habitats (Edney, 1971; Nicolson *et al.*, 1984).

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Excretion is the second route of water-loss. For example, the locust, *Locusta migratoria*, can lose up to 75% of its body water in faeces (Loveridge, 1975). Excretory behaviours also include spitting and secreting defensive fluids, which are expected to result in substantial water losses. However, water loss through these behaviours remains understudied, and the relative influences of transpiration and excretion in overall body water-loss rates are not well understood.

Both processes probably contribute to water loss by the federally endangered American burying beetle, *Nicrophorus americanus*, as observed when investigating the distribution and behaviour patterns of an American burying beetle population in south-central Nebraska (Bedick *et al.*, 1999). In accordance with United States Fish and Wildlife Service protocols, carrion baited pitfall traps were used to catch burying beetles and, during the study, up to seven species of *Nicrophorus* beetles were collected. In August, substantial mortality was observed in pitfall traps left unserviced for more than 12 h. Despite the fact that these beetles are found in the hot and relatively arid habitat of western Nebraska, burying beetles also possess a combination of behavioural and morphological characters that may render them particularly susceptible to desiccation. Specifically, many burying beetles are active during the day, they bury into the substrate during periods of inactivity and, when agitated, they expel defensive secretions. Morphologically, burying beetles have truncate elytra, leaving approximately 25% of their abdomens exposed (Anderson and peck, 1985). Relative to other beetles of similar size, the exposed body surface may subject these beetles to high rates of transpiration. Behaviourally, burying beetles secrete copious amounts of oral and anal secretions when disturbed, including when the beetles are trapped in pitfalls or handled (Rana *et al.*, 1997).

Shortened elytra and a propensity to secrete defensive fluids appear to be unusual features for animals living in a hot, arid environment. If these beetles are subject to high transpiration rates and high excretory water losses, they would represent a sharp departure from the general wisdom of a strong relationship between habitat aridity and transpiratory water-loss rates (Hadley, 1994; Chown, 2001). To investigate this departure, water-loss rates are examined in the burying beetle, *Nicrophorus marginatus* Fabr. For comparison, two other large beetle species that are expected to have relatively high water loss rates because of habitat association are also tested. Relative to other insect species, *N. marginatus* expresses high water-loss rates in low humidity environments. In the absence of compensating mechanisms, the high water-loss rates must exclude *N. marginatus*, and perhaps other burying beetles, from these habitats. One compensatory strategy is to behaviourally avoid periods when water loss is highest. Although *N. marginatus* is a diurnal species, its daily activity pattern has not been examined in detail (Ratcliffe, 1996). If water-loss is a significant factor in the biology of *N. marginatus*, then their activity should be periodic, with avoidance of high temperature periods when water-loss is likely to be greatest. The significance of these findings provides a more detailed understanding of the general relationship between transpiratory water-loss rates and habitat associations. In addition, the results suggest that adding moist soil to pitfall traps will substantially reduce beetle mortality, but still generally conform to the existing trapping protocol (Bedick *et al.*, 2004).

Materials and methods

Study site

The research focused on burying beetle populations in Lincoln County, south central Nebraska. The landscape of this area features loess hills, a series of sharply rising hills and associated narrow canyons and valleys formed by erosion of an ancient prairie. The environment is arid due to the unique properties of loess and the resulting topography. Loess is a highly erodable mixture dominated by small grained particles of quartz silt and calcium carbonate sand (Mutel, 1989). The steep slopes of the hills cause rain to run off rather than soaking into the soil and the high permeability of loess causes water to quickly drain away from the flat areas. Contributing to the aridity, the loess hills receive an average of 37.5 cm of rain per year and often suffer drought (Barry, 1983).

Insects

Burying beetles were trapped using baited pitfall traps in accordance with the protocol of the U.S. Fish and Wildlife Service (1991). The traps consisted of 18.9-L white plastic buckets buried in the ground. Exhumed soil was placed in the bottom of the bucket to a depth of 2–4 cm. Salvaged roadkill (various mammals, birds, reptiles and amphibians) was placed in a screened container located at the bottom of the bucket; the container excluded beetles from contacting the bait. The bucket was covered with an elevated board, which acted as a rain shield. Exhumed soil was placed on top of the board and helped shade the bucket. Each morning, the traps were checked and beetles were identified to species.

Adults of the most commonly trapped beetle, *N. marginatus*, were maintained in 5-L plastic containers provided with paper towel substrate and water-soaked cotton. Beetles were held for 1–2 weeks before the experiments and were fed ground beef *ad libitum*.

The effects of low humidity were also tested on water-loss rates for the damp-wood inhabiting bess beetle, *Odontotaenius disjunctus* (Illiger) and for the giant nocturnal tiger beetle, *Amblychila cylindriformis* Say. *Odontotaenius disjunctus* were purchased from the Carolina Biological Supply Company (Burlington, North Carolina) and *A. cylindriformis* were collected in Wallace County, Kansas.

Desiccation induced mortality

To determine survival times of *N. marginatus* exposed to low humidity conditions, sets of 10 beetles were placed individually into 60-mL plastic containers with cotton-covered $CaSO_4$. For high humidity controls, sets of 10 beetles were placed individually into plastic containers containing water-soaked cotton balls. The containers were fitted with perforated lids and were placed into pairs of darkened environmental chambers at 12, 16, 20, 24 and 28 °C (total 40 beetles per treatment; four replications). Beetles were visually examined every hour. Beetles that did not move in response to probing were recorded as dead, removed to air temperature, and rechecked. No beetle initially counted as dead subsequently recovered. Survivability was plotted verses time and lethal time to 50% mortality (LT_{50}) was calculated for each temperature.

Rates of water loss

The effects of exposure to desiccant were tested on transpiratory water-loss rates because, in their daily movement and in the pitfall traps, burying beetles are in contact with loess soil and burrow into it both for refuge during inactive periods and to rear young (Ratcliffe, 1996). Contact with dry, abrasive or water-absorbing materials can significantly increase water-loss rates of insects (Hadley, 1994; Appel *et al.*, 1999).

Water-loss rates of *N. marginatus* were determined by measuring weight loss. Individual beetles were randomly placed into one of four treatments. Two treatments were designed to distinguish rates of water loss during periods of exposure to dry air from rates of water loss during periods of direct contact with drying agents. Two additional treatments were designed to determine the influence of higher humidity and access to water on water-loss rates and survivorship. All experiments were conducted in sets of growth chambers set at 12, 20 or 28 °C and began with weighing individual beetles. Water-loss rate was assessed at 2-h intervals for 12 h. At each interval, each beetle was removed from its chamber, survivorship was determined based on movement and the beetle was cleaned briefly with a dry paper towel and then weighed. The relative humidity (RH) in each container was determined at the beginning and end of each experiment using a pen-type thermo-hydrometer (DeltaTrak Inc., China). After all experiments, beetles were dried at 60 °C for 3 days and then the dry mass of each individual was recorded.

Nicrophorus marginatus in low and high humidity conditions. In treatment one, the influence of low humidity (approximately 25–30% RH) and direct contact with $CaSO_4$ (W. A. Hammond Drierite Company, Xenia, Ohio) on water-loss rate was determined by placing three sets of seven beetles individually into 60-mL plastic containers with perforated lids. Before adding the beetles, the containers were prepared with approximately 12.5 g of $CaSO_4$, which was covered with a thin layer of cotton. The beetles quickly burrowed into the cotton and $CaSO_4$. The RH in these chambers was between 25 and 30%.

In treatment two, the effect of low humidity was determined without contact with $CaSO_4$ on water-loss rates by placing beetles individually into nested containers. The outer chamber was a 60-mL plastic container charged with approximately 12.5 g of $CaSO_4$. Each beetle was placed into an identical container that was perforated in the bottom and sides. The containers were sealed together using parafilm. The RH in these chambers was between 28 and 31%.

In treatment three, similar nested containers were used to assess water-loss rates in beetles exposed to high humidity (approximately 83–87% RH). In this experiment, the outer chamber was charged with water-soaked cotton balls, and the beetles were placed into the perforated container sealed to the outer container with parafilm.

In treatment four, water-loss rates were determined in beetles exposed to high humidity (approximately 85–89% RH) with direct access to water. Beetles were placed individually into 60-mL plastic containers with a water-soaked cotton ball.

Excretory water-loss rates and mechanism of water gain. Individual burying beetles frequently spat and defecated when in the desiccation chambers, and the possibility that these defensive behaviours contributed to the observed high rates of water-loss was considered. The beetles exposed to high humidity lost no weight. To determine the source of water gain, the possibility that *N. marginatus* could passively gain water from near saturated air was considered, as reported in a few other insect species (Edney, 1966; Hadley, 1994). Five treatments were conducted at 12, 20 and 28 °C using five *N. marginatus*. Each beetle was weighed once every 2 h for 8 h. A new set of beetles was used for each temperature.

In treatment one, beetles were killed by exposure to cyanide and placed in contact with $CaSO_4$. In treatment two, killed beetles were exposed to water-soaked cotton ball. In treatment three, the mouth and anus of living beetles were sealed with paraffin wax and they were placed in contact with $CaSO_4$. In treatment four, wax-sealed beetles were placed with a water-soaked cotton ball. As a control in treatment five, living, unmanipulated beetles were placed in contact with $CaSO_4$.

Water-loss rates compared. For comparison, water-loss rates in two other beetle species was studied, *A. cylindriformis* ($n = 10$ individuals) and *O. disjunctus* ($n = 24$ individuals). These beetles were exposed to the most challenging conditions, in direct contact with $CaSO_4$ at 28 °C, and the rate of water-loss was determined as previously described at 2-h intervals for 10 h. After the experiment, the beetles were dried at 60 °C and re-weighed to determine dry mass.

Daily activity patterns. The daily activity pattern for *N. marginatus* was determined using three pitfall traps baited with mammalian roadkill. Three pitfall traps were monitored at hourly intervals from 06.00–22.00 h on 8, 11 and 12 July and, to minimize recaptures, beetles were released more than 200 m from the traps. Trapped burying beetles were identified to species.

Statistical analysis

TOXSTAT 3.4 Probit analysis (WEST, Inc., Cheyenne, Wyoming) was used to calculate LT_{50} mortality for *N. marginatus*. Statistical significance was judged as a non-overlap of 95% confidence intervals. For each temperature, an analysis of covariance was performed. The starting mass was compared with the end mass of each individual, with humidity as the covariant. Mean slopes of the curves were then compared for each experimental condition using JMP 5.1 (SAS Institute, 2003).

Results

Desiccation induced mortality

Nicrophorus marginatus died when body mass decreased by approximately 30%. Beetles died in a relatively short period when exposed to $CaSO_4$ and low humidity (Fig. 1). Warmer temperatures resulted in faster beetle mortality with over half the beetles dead after 6–8 h in the temperature range between 20 and 28 °C. By contrast, access to water in the high humidity condition drastically reduced beetle mortality in all experiments (only two of 40 beetles died after 36 h in the 28 °C condition with access to water).

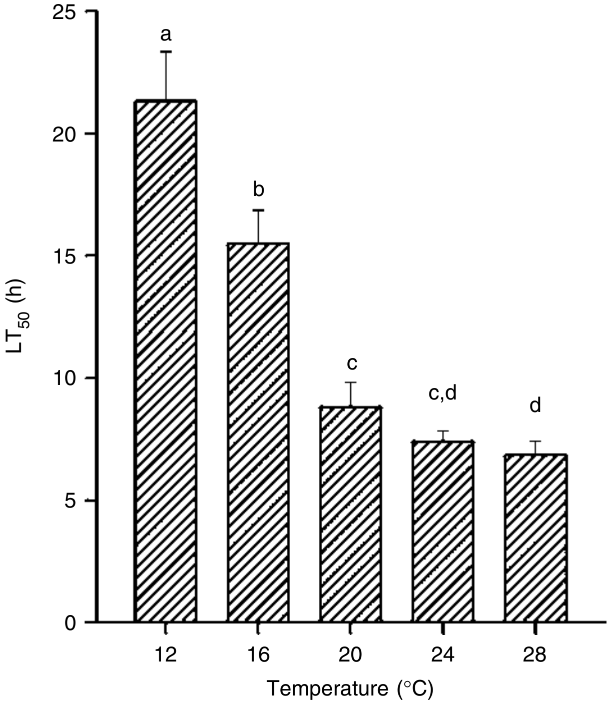

Fig. 1. Time to 50% mortality (LT_{50}) vs. temperature for *Nicrophorus marginatus* placed in low humidity conditions. Bars indicate calculated LT_{50} ± 95% confidence interval for 40 individuals in each condition. Letters above the columns indicate a significant difference based on nonoverlap of confidence intervals.

Rates of water-loss

Nicrophorus marginatus in low and high humidity conditions. In all experiments, beetle starting mass and experimental condition were significant predictors of final mass ($P < 0.001$); however, the interaction effect was not significant in any case ($P > 0.05$). Water-loss rates, judged by loss of mass, significantly ($P < 0.05$) increased with increasing temperature and exposure time for all beetles in low humidity conditions (Fig. 2). Contact with $CaSO_4$ increased water-loss rates and mortality at higher temperatures. Water-loss rates were identical at 12 °C (approximately 1% loss of body mass per hour). At 20 °C, beetles in contact with $CaSO_4$ lost mass at approximately 2.5% per hour whereas excluded beetles lost approximately 1.8% body mass per hour ($P < 0.05$). At 28 °C, beetles in contact with $CaSO_4$ lost approximately 3.3% body mass per hour compared with 2.1% per hour by the excluded beetles ($P < 0.05$). During the 12-h time course of the experiment, beetles at the higher temperatures suffered increased mortality. At 28 °C, beetles exposed to $CaSO_4$ suffered greater mortality (85%) compared with beetles in the same low humidity conditions but separated from the $CaSO_4$ (57%). Mortality rates were 29 and 16%, respectively, at 20 °C. All beetles survived at 12 °C.

In all temperatures, the mass of *N. marginatus* with access to water did not significantly change, although there was a weak correlation between mass change and exposure time at higher temperatures (Table 1). All beetles with access to water survived the experiment.

Excretory water-loss rates and mechanism of water gain. Water-loss rates at all temperatures were similar among untreated beetles and freshly killed or living beetles with wax sealed mouth and anus (Fig. 3). At 12

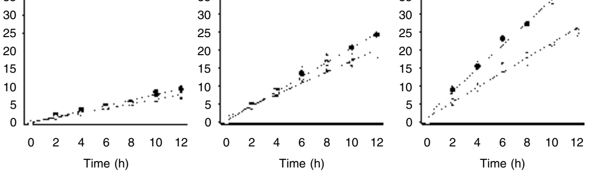

Fig. 2. Comparison of water loss of *Nicrophorus marginatus* vs. time at 12, 20 and 28 °C. Data indicate the mean ± SE of seven individuals per treatment. Solid symbols indicate beetles in contact with $CaSO_4$ and open symbols indicate beetles excluded from contact with $CaSO_4$.

Table 1. Regression equations of water-loss rates as a function of mass vs. time for *Nicrophorus marginatus* for adult beetles in contact with $CaSO_4$ (low) or with access to water (high).

Regression equation	Temperature (°C)	Humidity	r^2
%loss = −1.61 − 0.349 h	12	High	0.22
%loss = 0.778 + 0.683 h	12	Low	0.58
%loss = 0.902 + 0.474 h	20	High	0.18
%loss = 0.92 + 02.34 h	20	Low	0.83
%loss = −1.37 + 0.684 h	28	High	0.17
%loss = 2.35 + 03.16 h	28	Low	0.89

and 20 °C, rates of mass loss were nearly identical. At 28 °C, live untreated animals had slightly higher water-loss rates ($P = 0.13$) compared with wax-treated and dead individuals (Fig. 3). Thus, it appears that defensive secretions have little influence on water-loss rates in this species.

Nicrophorus marginatus did not absorb water at approximately 85% RH. When beetles were prevented from drinking (dead, or sealed with wax), water-loss occurred at a rate that was approximately one-third compared with that of animals exposed to a desiccating environment. For example, at 28 °C, sealed animals lost 1.2% body mass per hour and killed animals lost 0.9% body mass per hour whereas live untreated animals did not lose mass when able to drink from the water-soaked cotton ball. Beetles in all treatments at lower temperatures lost less than 0.4–0.5% body mass per hour.

Water-loss rates compared. By comparison to the large beetle species tested, the water-loss rates of *N. marginatus* were much higher. At 28 °C for 10 h, *N. marginatus* lost an average of 26% more mass than the similarly sized, mesic-dwelling *O. disjunctus*, and *N. marginatus* lost 89% more mass than the nocturnal *A. cylindriformis* (Fig. 4).

Daily activity patterns. The activity pattern of *N. marginatus*, inferred by pitfall trap captures, was strongly bimodal and skewed toward evening, fitting the predicted pattern based on the assumption that beetles avoid high temperatures (Fig. 5). Activity was generally low during the morning (approximately 20 captured beetles per hour) and declined to near zero from 13.00–15.00 h, which corresponded to the hottest part of the day. Activity peaked (approximately 80 captured beetles per hour) during twilight hours but ceased entirely after sunset (approximately 21.50 h).

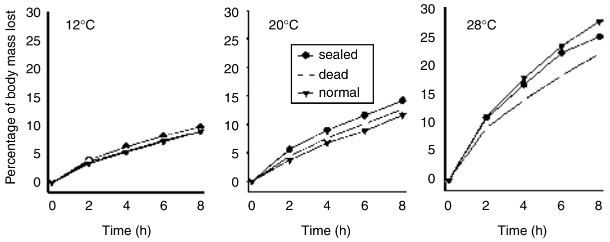

Fig. 3. Comparison of mean ± SE water loss as percentage of initial body mass between *Nicrophorus marginatus* in contact with $CaSO_4$. Beetles were either unmanipulated (normal), killed with cyanide gas just before the experiment or alive with their mouth and anus sealed before the experiment.

Discussion

Nicrophorus marginatus is unable to survive low humidity conditions for long without access to water. In desiccating conditions, the beetles die after losing approximately 30% of body mass, which occurred in as little as 8 h at 28 °C, and survival was correlated with temperature. In the field, beetles exhibit a strong bimodal activity pattern with near zero activity during the hottest part of the day, supporting the idea that beetles avoid the most challenging conditions.

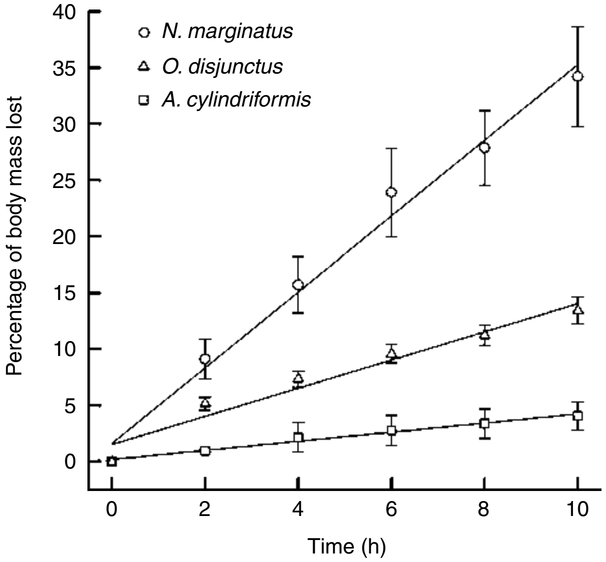

Fig. 4. Comparison of water loss as percentage of initial body mass for *Nicrophorus marginatus* ($n = 7$), *Odontotaenius disjunctus* ($n = 10$) and *Amblychila cylindriformis* ($n = 5$) exposed to $CaSO_4$ at 28 °C. Symbols represent means ± 95% confidence interval for each species.

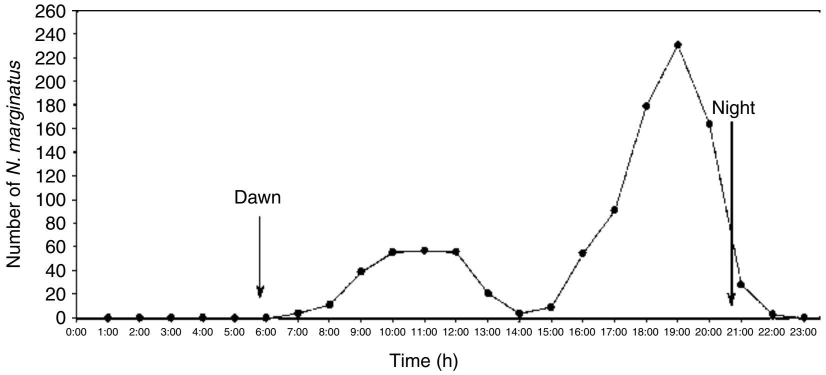

Fig. 5. Daily activity pattern of *Nicrophorus marginatus*. Total number of individuals caught over 3 days in July. Time 0 represents dawn (approximately 06.00 h). No *N. marginatus* were captured between 23.00 and 06.00 h.

A loss of 3–5% per hour and a 30% loss of body mass resulting in death is comparable with the results reported in other mesic-adapted insects (Mack & Appel, 1986). Direct comparison with *O. disjunctus* and *A. cylindriformis* also shows that *N. marginatus* is not well adapted to prevent water loss. Although it is nocturnally active, which lowers the need for water conservation, *A. cylindriformis* is flightless and inhabits arid environments, two characteristics that are often correlated with low water loss rates (Cloudsey-Thompson, 2001). Despite contact with desiccant at 28 °C, *A. cylindriformis* lost less than 2% of its body mass over 10 h. The moist wood inhabiting *O. disjunctus* is expected to have a high rate of water loss (Mack & Appel, 1986). Although higher than the water loss rate of the tiger beetle, *O. disjuntus* has a significantly lower rate of water loss than *N. marginatus*, losing only approximately 12% of its body mass over the 10-h trial (Fig. 4).

The high sensitivity to desiccation of *N. marginatus* may be partially explained by a number of mechanisms, including contact with desiccating material or defensive secretions. Higher rates of water loss are expected when the beetles come into contact with the $CaSO_4$, which abrades the cuticle and coats it with hygroscopic dust. The presence of dust increases the permeability of other insect cuticles by between two- to eight-fold (Wigglesworth, 1944; Beament, 1945; Arthur, 2000) and up to 17-fold in cowpea weevils (Appel *et al.*, 1999). However, contact with $CaSO_4$ only slightly increases *N. marginatus* water loss rates and mortality at 28 °C and does not have significant affects at lower temperatures, suggesting that water-loss rates are naturally high for *N. marginatus*. Moreover, the other species tested do not appear to be substantially affected by contact with the dust. The water-loss appears to be primarily via transpiratory loss because cyanide-killed individuals do not have significantly different water-loss rates compared with those seen in other species. For example, *Solenopsis invicta* water loss increases by 1.5-fold after death (Appel *et al.*, 1991). Although relatively high for a terrestrial insect, water-loss rates of *N. marginatus* are less than those reported for a large palm-dwelling weevil, *Rhynchophorus cruentatus*, which loses over 10% body mass per hour at lower humidities (Weissling & Giblin-Davis, 1993). However, unlike *N. marginatus*, the weevil burrows into the palm and should rarely encounter desiccating conditions.

A second factor in *N. marginatus* sensitivity to desiccation may be the defensive behaviour of the beetle. When disturbed, *Nicrophorus* species stridulate and excrete copious amounts of saliva and faeces (Rana *et al.*, 1997). This defensive mechanism occurs both in pitfall traps and in laboratory chambers, and presumably increases the rate of water loss beyond the normal transpiratory rates. However, similar water-loss rates are observed for active animals, dead animals and animals that have their mouth and anus sealed with paraffin wax. These data suggest that defensive secretions play a small role in water-loss rates and are only likely to factor in the early stages of exposure to desiccation.

From the available data, it appears that *N. marginatus* avoids lethal desiccation through behavioural mechanisms, including reduced activity during the hottest part of the day. It is also likely that burying beetles seek moist areas in the field and burrow into these areas during inactive periods. *Nicrophorus marginatus* is more generalized in habitat association than most other *Nicrophorus* species; however, it occurs most often in sandy soils (Anderson and Peck, 1985). Other silphid species (e.g. *N. orbicollis*) are found in moister areas, such as forests near water, and are active at night; thus, they may have even higher water-loss rates (Bishop *et al.*, 2002).

The sensitivity of *N. marginatus* and the deaths of *N. americanus* observed in the field suggest that RH and soil moisture may be limiting factors in the range and distribution of the species. Correlations of habitat and water-loss rates are well documented in other species (Weissling & Giblin-Davis, 1993; Hadley, 1994). Potential correlations of *Nicrophorus* distribution with soil moisture should be investigated further because environmental moisture may be a factor in the distribution of *N. americanus*, which has never been collected in areas with less than 50 cm annual precipitation (J. Bedick, personal observation).

Implications for trapping

In trapping, access to damp soil drastically reduced mortality of *Nicrophorus* species during August 1996. Before maintaining soil dampness, trap mortality approached 20% or more in some traps on some days (J. Bedick, personal observation); subsequently, few beetles died even when ambient temperatures were above 27 °C. During field sampling, traps often were re-checked after 14–16 h. The predictive regression equations (Table 1) produce values that are comparable with observed trap mortality, assuming that a 30% loss of mass is fatal in field situations. In addition to providing humidity and a source of water, dampness may mitigate the effects of abrasion and spiracle clogging by dust. If these results are similar for congeners of *N. marginatus*, damp soil will reduce trap mortality during surveys for the endangered American burying beetle.

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