Journal of Thermal Biology 127 (2025) 104063

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

The role of life stage and season in critical thermal limits of carrion beetles

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ARTICLE INFO

Keywords:
Elevational gradients
Global change
Life stages
Seasonal variation
Winter
Silphinae

ABSTRACT

Larval and winter thermal limits may be vital for understanding responses to climate variability, but many studies of insect critical thermal limits focus on adults reared in benign conditions (lab or summer field conditions). For insects generally, temperature variability and thermal tolerance breadth are correlated. Thus, we predict broader thermal limits in adults compared to less-mobile larvae developing within a restricted microclimate. We also predict lower cold limits in winter adults compared to summer adults. To test for this thermal variability across life stages and seasons, we used a recirculating bath to determine critical thermal limits in two species of Colorado carrion beetles (Coleoptera: Staphylinidae: Silphinae) in which larvae develop within a carcass microclimate. For larval and adult comparisons, we used summer *Thanatophilus lapponicus* ($n = 111$) and *Thanatophilus coloradensis* ($n = 46$). For winter and summer comparisons, we used adult *T. lapponicus* ($n = 103$). We detected no difference between larvae and adults in *T. lapponicus* for either upper thermal limits (CTmax) or lower thermal limits (CTmin) for wild caught adults, bred larvae, and bred adults. In contrast, wild caught adults of *T. coloradensis* had a significantly lower CTmin (-5.7 ± 0.5 °C) compared to wild caught larvae (-3.0 ± 1.3 °C) and bred larvae (-3.5 ± 0.8 °C) with no difference in CTmax. Winter *T. lapponicus* adults displayed a nearly one-degree lower CTmin (-2.8 ± 1.6 °C) than summer adults (-1.9 ± 1.9 °C) with no difference in CTmax. These results demonstrate that even closely related, co-occurring species can have distinct strategies for coping with cold temperatures. And, in some cases, particularly for high-elevation specialists, larvae may benefit from a temperature-buffered microclimate. Heat tolerance was broad and less variable across life stages and seasons, emphasizing that variation in cold temperatures will be critical for responses to climate change, for example, changes in snow levels impacting insulation.

1. Introduction

Insect responses to climate change can involve multiple strategies to cope with global temperature changes (Parmesan, 2006; Sgrò et al., 2016; Halsch et al., 2021). Common insect strategies focus on avoiding suboptimal temperatures by shifting range limits (Parmesan and Yohe, 2003; Valladares et al., 2014; McCain and Garfinkel, 2021), shifting phenology to track resources or to avoid temperature extremes (Nufio et al., 2010; Yang and Rudolf, 2010; Scranton and Amarasekare, 2017), or using behavioral modification or selection of microclimates (Jones and Oldroyd, 2006; Pincebourde and Woods, 2020). Shifting range limits or phenology requires significant biological changes and can introduce issues with concurrent changes in biotic interactions (Parmesan, 2006; Visser and Gienapp, 2019). Changes to behavior or microclimate usage are often less complex because they are transient and operate on smaller spatial scales. For example, species can shift daily activity patterns to avoid the hottest part of the day (Cook et al., 2019) or increase thermoregulatory behavior frequency or onset time (MacLean et al., 2016). In cases where temperature avoidance is impossible or less practical, thermal limits, the minimum and maximum temperatures that can be tolerated, may broaden through plasticity or acclimation (Huey and Kingsolver, 1989; Schulte et al., 2011; Diamond, 2017). While these smaller scale responses are likely more common, they are often more difficult to detect and understand (Sunday et al. 2011, 2019; Fey et al., 2021). Temperature tolerance, broadly referring to the ability to withstand a span of temperatures, can be assessed in many ways, including using critical thermal limits (temperature at loss of function or mobility), survival assays (temperatures that result in a specific percentage of mortality), and lethal thermal limits (temperature at mortality; Terblanche et al., 2007; Bennett et al., 2018; Sunday et al., 2019).

Insect thermal limits commonly vary across taxa and within taxa

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https://doi.org/10.1016/j.jtherbio.2025.104063
Received 19 March 2024; Received in revised form 8 January 2025; Accepted 20 January 2025
Available online 27 January 2025

depending on abiotic and biotic conditions (Bowler and Terblanche, 2008; Sunday et al. 2011, 2012, 2019; Oyen et al., 2016; Bennett et al., 2018; Truebano et al., 2018; Smith et al., 2021). Despite the variation in thermal limits, research on insect thermal limits typically focuses on specific mechanisms underlying thermal limit variation such as ion and water homeostasis (Koštál et al., 2007; Overgaard et al., 2008; MacMillan et al., 2015), or on thermal limits during only a single season, usually summer, or developmental stage, mostly adults (Hoffmann et al., 2013; García-Robledo et al., 2016; Shah et al., 2017). Furthermore, research on developmental stage and season is often limited to the effect of temperature on emergence dates (Scranton and Amarasekare, 2017) and studies assessing the effect of senescence on thermal tolerance focus on a limited diversity of taxa (Service et al., 1985; Bowler and Terblanche, 2008; Feng et al., 2016, 2017; Zajitschek et al., 2020). Thermal tolerance studies that do not address the role of context (biotic and abiotic conditions) in setting thermal limits may fail to identify temperature as an important driver of climate change responses (Bowler and Terblanche, 2008; Chown et al., 2009; Kingsolver and Buckley, 2020). An understanding of contextualized thermal limits will enable improved predictions of insect responses to anthropogenic temperature change, and ultimately improve conservation of at-risk species (Bale and Hayward, 2010; Kingsolver et al., 2011; Kingsolver and Buckley, 2020).

Life stage is an important context across which thermal tolerances may vary. For a particular life stage, thermal tolerance breadth is expected to correlate with the range of environmental temperatures experienced (Sunday et al. 2011, 2012, 2019; Oyen et al., 2016; Bennett et al., 2018; Truebano et al., 2018). These experienced temperatures can depend heavily on mobility, which influences the ability to select and avoid temperatures (Chown, 2001; Bowler and Terblanche, 2008; Pincebourde and Woods, 2020). More immobile life stages like eggs and pupae are fully limited to temperatures chosen by parents or late-stage larval forms (Leather et al., 1993; MacLean et al., 2016). While both larvae and adults are mobile and thus able to behaviorally thermoregulate, adults often travel faster and over farther distances, especially if they are flighted. This has led some authors (e.g. Bowler and Terblanche, 2008, Weaving et al., 2022) to suggest that larvae may require broader thermal tolerances than adults to compensate for lower mobility (Nyamukondiwa and Terblanche, 2009; Lockwood et al., 2018; Truebano et al., 2018; Bretzlaff et al., 2023). Alternatively, for species whose larvae develop in a restricted microclimate like a carcass and the adults experience more temperature variability (e.g., carrion beetles (Silphinae) or skin beetles (Dermestidae)), adults would be predicted to have broader thermal tolerance. Thus, depending on larval thermal environment and strategies for temperature tolerance, this life stage could be particularly important for responses to a rapidly changing climate.

Season is another important context across which thermal tolerances may vary. In cold and snowy environments, most insects survive winter by employing a suite of behavioral and physiological strategies (e.g., burrowing, hardening, diapause, cryoprotectants; Leather et al., 1993; Rinehart et al., 2007; Bale and Hayward, 2010; Khanmohamadi et al., 2016; Hasanvand et al., 2020; Zajitschek et al., 2020). Some of these strategies may involve reducing their lower critical thermal limit (Leather et al., 1993; Chown, 2001; Rinehart et al., 2007). In addition to improved minimum thermal limits during winter months, winter physiological strategies have the potential to lead to reduced upper thermal limits (Houghton and Shoup, 2014; Harada et al., 2018; Bujan et al., 2020). The winter life stage (adult, pupa or late stage larva) varies across taxa (Leather et al., 1993; Bale and Hayward, 2010). For insects with lifespans of a year or more, the overwintering stage will experience both summer and winter temperatures, which in some cases may lead to broader thermal tolerances matching the larger range of experienced temperatures (Rinehart et al., 2007; Colinet et al., 2015; Shah et al., 2017). Specifically, the overwintering stage may in some cases retain a broad and relatively constant thermal range across the entire year rather than two different season-specific thermal ranges (Hu and Appel, 2004; Colinet et al., 2015; Harada et al., 2018; Teets et al., 2020; Huey and Buckley, 2022). Thus, while this winter stage can be uniquely vulnerable, it can also be uniquely tolerant of extreme conditions depending on the specific life-history strategy employed.

For carrion beetles (Coleoptera: Staphylinidae: Silphinae, formerly family Silphidae, see Sikes et al., 2024), a unique group of insects that use vertebrate carrion as a food and a reproductive resource, no studies exist on their thermal limits across life stages or seasons (for other studies of thermal tolerance in Silphinae see Merrick and Smith, 2004; Sheldon and Tewksbury, 2014; Keller et al., 2021; Wettlaufer et al., 2023). While general predictions of contextual variation in thermal limits may serve as a starting point, they may apply differently to carrion beetles given their unusual life history and behavior (Scott, 1998; Smith, 2002). In Colorado, there are 15 montane species with highly overlapping elevational ranges despite specialization on the same food and reproductive resources (McCain et al., 2018, McCain, 2021). Further, there is large overlap in thermal tolerance (i.e., critical thermal range) of summer adults across ten species in both tribes (Nicrophorini and Silphini; Peck and Anderson, 1982; McCain, 2021, Garfinkel and McCain *In Prep.*). Two species, *Thanatophilus coloradensis* and *Thanatophilus lapponicus*, are both present at the upper elevational limit of carrion beetle habitat in the alpine. Contrastingly, *T. coloradensis* is limited to this high elevation alpine habitat near and above tree line (3,371 m to 3,638 m in the Front Range, Colorado), while *T. lapponicus* populations occur across a broad variety of habitats from the plains to the alpine (~1,500 m to 3,638 m in the Front Range, Colorado).

Life stages of both focal species differ in mobility, with adults flying and walking large distances to find mates, food, and breeding resources, while wingless larvae are typically restricted to the carcass selected by their parents (Anderson and Peck, 1985; Scott, 1998). Both adults and larvae have access to and make use of air, soil, and carcass microclimates, though larvae may be more buffered from environmental temperatures by remaining primarily within the carcass (Scott, 1998, personal observation). As a result, we predict the less-mobile larvae will have a narrower thermal range than adults (McCain et al., 2018). Across seasons, we may expect differences between these two species based on their contrasting elevational ranges and experienced temperatures (Houghton and Shoup, 2014; Harada et al., 2018; McCain et al., 2018, *In Prep.*). Carrion beetles in general are freeze-avoidant insects that burrow below the frostline in the soil and enter diapause to survive winter (Hoback and Conley, 2014). As a result, for both species we expect lower minimum thermal limits during winter due to their various behavioral and physiological winter strategies, including hardening and diapause (Teets and Denlinger, 2013; Teets et al., 2020). *Thanatophilus lapponicus* winters as an adult, whereas the winter stage of *T. coloradensis* is unknown but is likely an adult as its congener (Peck and Anderson, 1982; Anderson and Peck, 1985). In Colorado, fluctuating seasonal and daily temperatures may lead to retention of cold tolerance year-round, especially for the specialist *T. coloradensis* restricted to colder temperatures above tree line (McCain et al., 2018). For *T. lapponicus,* which has a broad elevational range and varied span of experienced temperatures, enhanced winter cold tolerance may be a seasonal strategy.

In this work, we provide a case study for variability in beetle thermal tolerance across life stage and seasonal contexts, investigating how existing thermal tolerance theory applies to an understudied taxon with a unique life history strategy. To assess changes in carrion beetle thermal tolerance across contexts, we compared both lower critical thermal limits (CTmin) and upper critical thermal limits (CTmax) of adults and larvae of both species, and summer and winter adults of one species. Due to the importance of summer behavioral thermoregulation of larvae in the carcass and the various winter survival strategies, we expected 1) a smaller range of thermal critical limits in larvae than the more mobile and exposed adults (adults: lower CTmin and higher CTmax) in the summer and 2) lower CTmin in winter life stages and during winter.

2. Methods

2.1. Carrion beetle trapping

Six sites (1,780 m, 2,230 m, 2,450 m, 2,780 m, 3,000 m, and 3,450 m; Fig. 1) were established along an elevational gradient from near Boulder, CO to Niwot Ridge in Nederland, CO, spanning the majority of carrion beetle habitat in the Front Range Mountains (1,718 m to 3,638 m, McCain et al., 2018; McCain, 2021). The large-ranged species *Thanatophilus lapponicus* (<1,718 m to 3,638 m) was collected across the entire gradient, while the high elevation specialist *Thanatophilus coloradensis* (3,371 m to 3,638 m) was collected only at the highest site (McCain et al., 2018, McCain, 2021). At all sites except the highest, adult carrion beetles were collected using four to six baited pitfall traps in the summers of 2021 and 2022 (Bedick et al., 2004, see Appendix S1 for more detail). At the highest (3,450 m) site, an alternative trapping method was necessary due to high disturbance of pitfall traps by marmots (McCain, 2021). Larger, above-ground traps consisted of a staked, wire trap which was baited with a rabbit carcass from which both larval and adult carrion beetles were collected directly off the rabbit carcasses in the summer of 2022. Beetles collected at all sites were transported back to the lab (McCain Lab at University of Colorado, Boulder) in a cooler with ice packs within 6 h of collection. In the lab, larval and adult carrion beetles were identified to species (using Anderson and Peck, 1985; McCain, 2021), housed in fish tanks with sand and mulch, given water every day, and fed raw chicken or turkey every other day.

2.2. Lab rearing

Carrion beetle breeding pairs were selected from wild-caught individuals from the same site and collection date. Each male and female pair were placed into a plastic container (4″L x 5-1/4″W x 8-1/8″H) with a mulch and sand substrate, a defrosted mouse carcass (∼15–20 g), and a synthetic sponge soaked in water. Containers were stored at 22–25 °C with a 14:10 light-dark cycle. Larvae emerged between two and five days after placement of parents into breeding containers and were given raw chicken as needed to supplement depleted carcass resources. All larvae were reared to the third instar, which was visually confirmed by size comparisons over time and among larvae.

The species *T. lapponicus* was reared to both larval and adult stages, allowing for comparison of wild adults, bred larvae (as wild larvae could not be collected), and bred adults. *Thanatophilus coloradensis* was reared only to the larval stage as wild larvae could be collected for comparison,

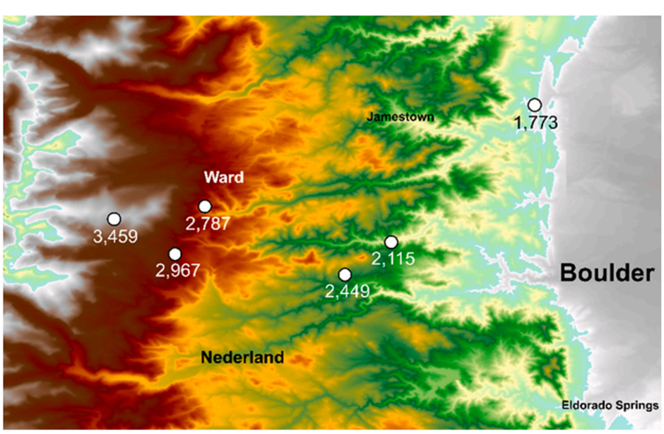

Fig. 1. Map of carrion beetle sampling sites in the Front Range Mountains, Colorado, USA. Elevational variation is represented in color from grey and light green at low elevations to red, brown, and white at high elevations. Sampling sites are denoted with white circles with the site elevation in meters listed directly below. The distance between the lowest site (1773 m) and highest site (3459 m) is roughly 25 km or 15.5 miles.

and wild adults, wild larvae, and bred larvae were compared. We included an additional lab bred stage in thermal limit testing to identify if lab rearing itself affected thermal critical limits, which would make comparisons of lab-bred larvae and wild adults inappropriate. Large divergences in thermal limits between bred and wild individuals would suggest a highly plastic trait. While this would need to be confirmed more robustly with common garden or reciprocal transplant experiments, it would identify an issue with using lab reared individuals for thermal comparisons as we conducted here (Schulte et al., 2011; Chown et al., 2009, Sgrò et al., 2016). We did not detect statistical differences in thermal limits between bred and wild individuals (see results) or across mating pairs (see Appendix S2).

2.3. Winter thermal limits

To assess changes in critical thermal limits across seasons, researchers use three main experimental methods: 1) collecting wild insects across seasons, 2) collecting wild insects in a single season, placing them in semi-natural enclosures, and periodically extracting them, and 3) using laboratory conditions to mimic seasonal changes (Huey and Buckley, 2022). In Colorado, where carrion beetles winter underground at depth in the soil and are insulated below the snow pack, winter carrion beetles cannot be located and collected from their natural habitat. Since such winter conditions would be unlikely to be well-improvised in the lab, we chose to release summer-collected adults into semi-natural, buried enclosures to experience winter at our collection sites for more realistic conditions. Based on existing protocols for wintering burying beetles (Staphylinidae: Silphinae: tribe Nicrophorini), we used cylindrical containers with drainage holes that were filled with substrate to allow for individual selection of burying depths within the container (Smith, 2002; Hoback and Conley, 2014). Winter protocols used *T. lapponicus* adults collected from the three lowest sites (1,780 m, 2,230 m, and 2,450 m) from mid-July to mid-August. These individuals were housed in the lab until mid-September, when carrion beetles were no longer active (assessed through trapping), and night temperatures dropped below 10 °C. Roughly 25 beetles were placed in each 0.6 gallon cylindrical plastic container with drainage holes on the top and bottom, which was filled with a sand-mulch substrate and included a synthetic sponge soaked in water for humidity. Eight containers were buried at each of the two lowest trapping sites (1,780 m and 2,230 m) in mid-September so that the lid of each container was roughly 6-inches underground and the base at roughly 12-inches underground to achieve a depth range of 6–12 inches (Smith, 2002; Hoback and Conley, 2014). Overwintering containers were thin and uninsulated to allow temperatures inside containers to match that of the surrounding soil. As a result, we did not monitor temperatures within the overwintering containers as they sufficiently mirrored the natural winter conditions. Containers were retrieved in batches over a three-week period (late--February to mid-March) when snow was still present at each site but not so deep that the containers were completely inaccessible. Retrieved containers were kept cool with ice packs during transportation to the lab. Beetles were sifted from the container substrate and allowed to recover (regain movement) at room temperature for 5–20 min before immediate thermal limit testing. We did not attempt winter protocols for *T. coloradensis* due to the depth of snow and general inaccessibility of the alpine during the winter months.

Live, winter adults ($n = 21$) were retrieved from five of the sixteen winter containers: three containers buried at the 2,230 m site ($n = 1, 9, 9$) and two containers buried at the 1,780 m site ($n = 1, 1$). Winter containers included a mix of several local species, but only individuals of *T. lapponicus* ($n = 19$) and *Nicrophorus guttula* ($n = 2$) were recovered alive. Given the low survival rate of wintered individuals, we are unable to rule out the possibility that we selected for cold-tolerant individuals. However, if we had unintentionally selected for cold-tolerant individuals, we would have expected to recover a few individuals from each container, but this was not the case since individuals primarily survived in groups based on container. Containers were buried in varying locations and as a result were exposed to different soil temperatures, snow depths, and moisture levels both across and within sites. Most beetles were from the 2,230 m site, which had notably sandier, drier soil as well as deeper snow than the lower site, which may have been favorable for winter survival. Additionally, the high variation in lower and upper critical thermal limits among the recovered individuals suggests a lack of selection for cold-tolerance in our sample. Variation in lower thermal limits for winter *T. lapponicus* (standard deviation: 2.92, $n = 19$) exceeded that of summer *T. lapponicus* from the same sites (1.59, $n = 84$), even when randomly subsampled to match winter sample sizes (1.38, $n = 19$). However, variation in upper thermal limits for winter *T. lapponicus* (standard deviation: 0.67, $n = 17$) was smaller than that of summer *T. lapponicus* from the same lowest sites (1.85, $n = 67$) and also when randomly subsampled to match winter sample sizes (2.19, $n = 19$). Lastly, there is no reason to suspect winter carrion beetles would be moving within the soil or coming to the surface of the snow as carcasses and other potential food sources would be extraordinarily rare and breeding occurs in the summer.

2.4. Thermal limit testing

Except for winter beetles, beetles were acclimated in the lab at 22–25 °C for a minimum of 24 h and a maximum of 96 h before thermal limit testing (Sheldon and Tewksbury, 2014). Carrion beetles were tested for both lower and upper thermal limits consecutively, with time for recovery at room temperature after cold testing and before heat testing, as is common practice (Gaston and Chown, 1999; Terblanche et al., 2007; Sunday et al., 2011; Oyen et al., 2016). Air temperatures were controlled with an advanced programmable recirculating bath (PolyScience 7-L Refrigerated Circulator, −20 °C) filled with a water and the propylene glycol-based bath fluid PolyScience polycool PG -20 (Grant and Lamp, 2017). Beetles were individually placed into 12 six-dram glass vials attached with marine epoxy to the top of an aluminum block, which was lowered into the bath so that vials were submerged up to the cap threads. Original vial lids were replaced with clear lids cast with epoxy, treated with an anti-fog coating, and drilled to create a small opening for a thermocouple wire. Type-K thermocouple wires were suspended mid-way into each vial to allow for evaluation of insect response to touch (through manual manipulation of wires) and attached to a thermocouple reader to continuously monitor air temperatures (Oyen et al., 2016). Air temperatures were measured rather than internal body temperatures to allow for 12 beetles to be tested simultaneously without disrupting the testing apparatus. Additionally, air temperatures can be more directly linked to environmental temperatures than internal body temperatures, which also reflect how body size, shape, and weight influence heating of the internal body cavity (Merrick and Smith, 2004).

We used dynamic, ramping temperatures to more closely mimic temperature changes insects may experience naturally (Terblanche et al., 2007; Oyen and Dillon, 2018). Specific test temperatures and ramping rates followed Oyen et al., (2016), which assessed elevational trends in bumblebee thermal limits using testing temperatures common in the insect thermal limit literature. For each experiment, beetles were placed into the bath and first held at 22 °C for 15 min before starting lower thermal limit trials. Temperature was then ramped to −5 °C at a rate of 0.25 °C/min, with the exception of tests of the high elevation specialist *T. coloradensis,* for which temperature was ramped to −7 °C at a rate of 0.25 °C/min based on preliminary testing. Lower thermal limits (critical thermal minima, often referred to as CTmin) were measured as the air temperature in degrees Celsius when each beetle entered chill coma, which was visually identified as the complete cessation of movement and response to touch. All beetles were taken to the minimum testing temperature after reaching chill coma to ensure a consistent minimum temperature across experiments. The bath was then returned to room temperature at a rate of 0.5 °C/min, and beetles were held at 22 °C for 10 min. Upper thermal limit trials began immediately after this recovery period, and the bath was ramped to 45 °C at a rate of 0.5 °C/min. Upper thermal limits (critical thermal maxima, often referred to as CTmax) were measured as the air temperature in degrees Celsius when each beetle experienced loss of the righting reflex, which was visually identified as a loss of coordination, twitching, and the inability to turn over when upturned. After testing, beetles were individually weighed to the nearest 0.1 mg (Mettler AE 100 scale with 0.1 mg minimum readability).

Both CTmin and CTmax are sensitive to methodological differences, and could be influenced by transportation methods, holding time before testing, and irregular heating of the bath (Terblanche et al., 2007). To assess if transportation at low temperatures influenced thermal limit determinations, a rapid cold hardening experiment was conducted using beetles that had been returned to the lab and acclimated for between 5 and 6 days (Teets and Denlinger, 2013; Teets et al., 2020). Fed and watered beetles of *T. lapponicus* from the same site and collection date ($n = 24$) were randomly selected, divided into two groups, and placed in containers within cardboard boxes. One box was left at room temperature (22–22.4 °C) while the other was placed into the fridge (6.7–6.9 °C) for 1 h, after which six of the room-temperature beetles and six of the cold-challenged beetles were tested for thermal limits. The other six beetles in each group were held at room temperature (22–22.4 °C) for an additional 24 h before thermal limit testing. Neither CTmin nor CTmax differed between cold-challenged beetles and those held at room-temperature (t-tests; CTmin: $t_{(20.62)} = -0.19$, $p = 0.85$, CTmax: $t_{(20.68)} = 1.67$, $p = 0.11$), or within those groups between the immediate testing and 24-h time points (t-tests; CTmin, cold-challenged: $t_{(7.85)} = -1.19$, $p = 0.26$, CTmin, room-temperature: $t_{(6.77)} = -2.16$, $p = 0.07$, CTmax, cold-challenged: $t_{(8.20)} = -0.70$, $p = 0.50$, CTmax, room-temperature: $t_{(9.97)} = 0.70$, $p = 0.50$). Nonetheless, we continued to wait a minimum of 24 h after collection to ensure their thermal limits were not influenced by transportation at low temperatures. To ensure beetle holding time in the lab before testing was appropriate, a subset of beetles ($n = 11$) of *T. lapponicus* were acclimated in the lab for either one or three weeks before thermal limit testing to serve as a comparison (Sunday et al., 2011). There was no difference in either CTmin or CTmax between individuals tested within 96 h of collection, those acclimated for one week, and those acclimated for three weeks (ANOVAs, CTmin: $F_{(2,151)} = 1.71$, $p = 0.19$, CTmax: $F_{(2,126)} = 0.17$, $p = 0.84$). Additionally, there was no difference in either CTmin or CTmax between individuals tested within 96 h of collection and those acclimated for either 1 or 3 weeks (t-tests, CTmin: equal variance, $t_{(152)} = -1.85$, $p = 0.07$, CTmax: unequal variance, $t_{(19.49)} = -0.52$, $p = 0.61$). Despite a lack of evidence of an effect of transport methods or holding time on thermal limits, all individuals used in this analysis were tested between 24 and 96 h of collection for consistency. Finally, neither CTmin nor CTmax were strongly correlated with location within the testing apparatus (CTmin: $r = -0.04$, CTmax: $r = 0.16$), room temperature during thermal limit testing (CTmin: $r = -0.10$, CTmax: $r = 0.03$), or testing date (Julian dates, CTmin: $r = -0.18$, CTmax: $r = 0.05$). Removal of the high-elevation specialist *T. coloradensis,* which has a relatively expanded CTmin and was only collected in late July and August, resulted in weaker correlations between lower thermal limits and testing date (CTmin: $r = -0.11$).

2.5. Statistical analysis

To assess the effects of life stage on thermal limits, we compared average upper and lower thermal limits across life stages within each of our two focal species. Separate one-way, parametric ANOVAs with Tukey post-hoc tests were used to compare average upper and lower thermal limits of wild adults, bred larvae, and bred adults in *T. lapponicus* and of wild adults, wild larvae, and bred larvae in *T. coloradensis.* Parent generations of *T. lapponicus* bred larvae and bred adults were sourced from the two lowest sites (1,780 m, 2,230 m), and were only compared with summer adults from the same two elevations (see Figure S1 for data plotted by source elevation). To evaluate changes in thermal limits between seasons, we used t-tests to separately compare average upper and lower thermal limits of *T. lapponicus* in summer and winter. Winter *T. lapponicus adults* were sourced from the three lowest sites (1,780 m, 2,230 m, 2,450 m), and were similarly only compared with summer adults from the same three elevations (see Figure S2 for data plotted by source elevation). All analyses were conducted in *R* after testing for acceptable normality and homogeneity of variances (version 3.6.2, R Core Team, 2021).

3. Results

3.1. Life stage

Validation of thermal limit testing found no effect of transport methods, holding time, location within the testing apparatus, room temperature during testing, or testing date on critical thermal limits and these factors were not further assessed in the analyses (section 2.4). For *Thanatophilus lapponicus,* we compared lower (CTmin) and upper (CTmax) critical thermal limits of wild adults, bred larvae, and bred adults (CTmin: $n = 69$, 23, and 19 respectively; CTmax: $n = 52$, 19, 18 respectively). We found no significant differences in either average CTmin ($F_{(2,108)} = 0.84$, $p = 0.43$, Fig. 2A) or average CTmax ($F_{(2,86)} = 0.69$, $p = 0.50$, Fig. 2C) across these three life stages (Table 1). As there was no difference in thermal limits between wild adults and reared adults, we did not identify any methodological issues with using lab reared individuals for comparison.

For *Thanatophilus coloradensis,* we compared lower and upper thermal limits of wild adults, wild larvae, and bred larvae (CTmin: $n = 18$, 11, and 12 respectively, CTmax: $n = 23$, 10, 11 respectively). While we found no differences in average CTmax across these three life stages ($F_{(2,41)} = 0.28$, $p = 0.76$, Fig. 2D), we found a significant difference in average CTmin ($F_{(2,38)} = 43.84$, $p < 0.0001$, Fig. 2B). CTmin of wild larvae (mean ± standard deviation: 3.0 ± 1.3 °C) and bred larvae (−3.5 ± 0.8 °C) did not differ ($p = 0.48$), but wild adults had a significantly lower average CTmin (−5.7 ± 0.5 °C, $p < 0.0001$): 2.2–2.7 °C lower than larvae (Fig. 2B–Table 1). Similar thermal limits in wild larvae and bred larvae again suggested no methodological issues with using lab reared individuals for comparison. Variation in thermal limits within wild larvae and adult testing groups ranged from 0.5 °C to 2.0 °C, and variation in thermal limits within bred larvae and adult testing groups ranged from 0.7 °C to 1.7 °C.

Table 1
Average CTmin and CTmax for each testing group of *Thanatophilus*. Shown are summary statistics for both life stage and season (indicated on the left) for each species, written as means plus or minus one standard deviation and standard error (mean ± sd/se°C). Stars (*) indicate significantly distinct critical thermal limits.

	Species	Testing Group	CTmin (mean ± sd/se°C)	CTmax (mean ± sd/se°C)
Life Stage	*T. lapponicus*	Wild Adults	−1.8 ± 1.6/ 0.2 °C	41.2 ± 2.0/ 0.3 °C
		Bred Larvae	−1.9 ± 1.7/ 0.4 °C	41.7 ± 1.0/ 0.2 °C
		Bred Adults	−2.3 ± 0.8/ 0.2 °C	41.4 ± 0.7/ 0.2 °C
	T. coloradensis	Wild Adults	−5.7 ± 0.5/ 0.1 °C*	41.6 ± 0.9/ 0.2 °C
		Wild Larvae	−3.0 ± 1.3/ 0.4 °C	41.6 ± 0.7/ 0.2 °C
		Bred Larvae	−3.5 ± 0.8/ 0.2 °C	41.8 ± 0.7/ 0.2 °C
Season	*T. lapponicus*	Summer Adults	−1.9 ± 1.9/ 0.2 °C	41.7 ± 1.2/ 0.2 °C
		Overwintered Adults	−2.8 ± 1.6/ 0.4 °C*	41.4 ± 1.9/ 0.3 °C

3.2. Season

We compared summer and winter thermal limits of adults in *T. lapponicus* (CTmin: $n = 84$ summer adults, 19 winter adults; CTmax: $n = 67$ summer adults, 17 winter adults). There was a significant difference in average CTmin ($t_{(101)} = -2.12$, $p = 0.04$) between seasons, but no difference in average CTmax ($t_{(82)} = 0.35$, $p = 0.72$) between seasons (Fig. 3, Table 1). Winter adults had a significantly lower average CTmin (mean ± standard deviation: 2.8 ± 1.6 °C) than summer adults (−1.9 ± 1.9 °C).

4. Discussion

Critical thermal limit studies in beetles typically focus on a single season or life stage, usually adults in summer, limiting the detectability of important temperature effects across an organism's lifespan (Feng et al., 2016, 2017). Winter and juvenile stages may be particularly critical for responses to a rapidly changing climate (e.g., Bale and

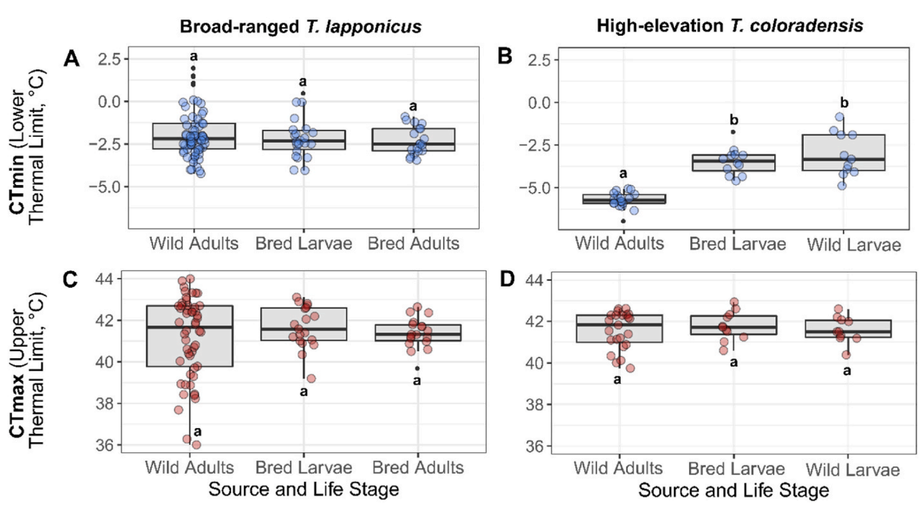

Fig. 2. Thermal limits across life stages in the two species (*Thanatophilus lapponicus*: left, A and C, *T. coloradensis:* right, B and D). Plotted are raw data for CTmin (blue points, top panels) and CTmax (red points, bottom panels) overlaid on box plots, with different letters within a panel indicating groups with significantly different thermal limits (ANOVA, $p < 0.5$). Life stages for *T. lapponicus* include wild adults, bred larvae, and bred adults, while life stages for *T. coloradensis* include wild adults, bred larvae, and wild larvae.

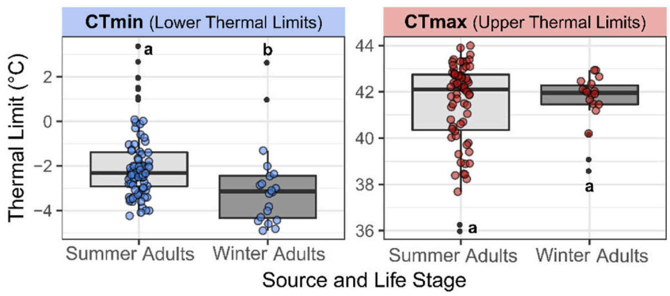

Fig. 3. Thermal limits across seasons in the species *Thanatophilus lapponicus*. Plotted are raw data for CTmin (blue points, left panel) and CTmax (red points, right panel) overlaid on box plots, with different letters within panels indicating groups with significantly different thermal limits (*t*-test, $p < 0.5$). Boxplots are in light grey for summer adults and in dark grey for winter adults.

Hayward, 2010; Kingsolver and Buckley, 2020; Huey and Buckley, 2022). Here, we used carrion beetles as a study system to assess the effect of context, specifically life stage and season, on both lower (CTmin) and upper (CTmax) critical thermal limits. We found an effect of life stage (larvae versus adults) on thermal limits in *Thanatophilus coloradensis* but not *Thanatophilus lapponicus* (Fig. 2). Aligning with our predictions, *T. coloradensis* had lower CTmin only in adults, with larval thermal limits more similar to all life stages of *T. lapponicus.* Thus, *T. coloradensis* larvae appear to benefit from a buffered microclimate within the carcass while the more mobile adults exhibit a greater thermal breadth. We also detected an effect of season in *T. lapponicus,* which had lower CTmin in winter compared to during summer months as predicted (Fig. 3). Individual variation in carrion beetle thermal limits was relatively high, even in lab-bred individuals, although it was particularly notable among *T. lapponicus* adults (Figs. 2 and 3). In line with global patterns showing lower variability in upper thermal limits, we detected similar CTmax across species, life stages, and seasons (Figs. 2 and 3) that was quite high (41–43 °C), emphasizing that cold-tolerance, particularly during changing overwintering conditions, may be critical for future climate change responses (Sunday et al., 2011, 2012; Hoffmann et al., 2013; Diamond, 2017; Sunday et al., 2019).

Across life stages, the general expectation in the insect literature is for differences in both CTmin and CTmax to reflect differences in mobility, as more mobile stages typically experience a broader range of temperatures (Chown, 2001; Truebano et al., 2018). Specifically for carrion beetles, we predicted broader thermal tolerance (lower CTmin and higher CTmax) in adults because larvae occupy a restricted microclimate in the carcass. In *T. lapponicus*, we detected no difference in average thermal tolerance for wild adults, bred adults, and bred larvae. Given that these wild adults came from three sites with distinct temperature regimes, this may indicate low plasticity in thermal tolerance of this species (Schulte et al., 2011; Sgrò et al., 2016). In contrast, *T. coloradensis* displayed a relatively large difference (−2.5 °C) in lower thermal limits across life stages, with the adult stage displaying a lower CTmin as predicted. This may indicate that for less mobile carrion beetle larvae, the carcass plays an important role in buffering environmental temperature as expected (Scott, 1998; Merrick and Smith, 2004; Truebano et al., 2018). Direct comparisons of critical thermal limits across life stages in flies found differences in heat and cold tolerances across life stages of a similar magnitude, typically ranging between 2.5 °C and 15 °C (Bowler and Terblanche, 2008; Nyamukondiwa and Terblanche, 2009). Some fly studies also show evidence of reduced cold and heat tolerance with aging, contrasting with the carrion beetle results herein (Chown, 2001; Bowler and Terblanche, 2008). Nonetheless, the two congeners tested in our study clearly display divergent thermal tolerance strategies across life stages. Similar variability among other tested insects emphasizes that thermal tolerance strategies differ among life stages and among various insect species, even closely-related species like our congeners, thus making these differences important for variable future responses to climate change (Nyamukondiwa and Terblanche, 2009; Oliveira et al., 2021).

Across seasons, we expected differences between *T. lapponicus* winter and summer thermal tolerances, with lower CTmin during the winter. While fewer studies test critical thermal limits in winter insects, there are many known physiological changes associated with winter preparation that may enhance cold thermal limits, including changes in protein expression, membrane permeability, and among others physiological effects (Leather et al., 1993; Rinehart et al., 2007). Indeed, we detected that winter *T. lapponicus* adults had critical thermal minimum temperatures 0.9 °C colder than summer adults, showing an effect of winter survival strategies (e.g., cold hardening, diapause, cryoprotectants, and/or cold acclimation). This is in accordance with global patterns showing upper thermal limits are generally constrained while cold thermal limits are more likely to change across space and time (Chown, 2001; Diamond, 2017). However, this seasonal difference in thermal limits was smaller than those found in other systems. In Oklahoma ants, September heat thermal limits were on average 6 °C higher than in December and March among four of five species (including *Crematogaster laeviuscula, Forelius pruinosus, Pheidole bicarinata,* and *Solenopsis invicta*, Bujan et al., 2020). In Florida mosquitoes, both summer heat thermal limits and cold thermal limits were reduced by roughly 5 °C among ten species (including 2 *Aedes* species and 4 *Culex* species, Oliveira et al., 2021). While these patterns are not consistent, both studies show seasonal differences in both heat and cold thermal limits of a greater magnitude than we detected for carrion beetles. However, carrion beetles are large-bodied insects with lower surface area-to-volume ratios than flies and ants, such that beetles lose and gain heat more slowly (Huey and Kingsolver, 1989). Given their size, shape, and elytral covering, which make them more buffered from environmental temperatures, we might expect less variation in thermal tolerance. Alternatively, critical thermal limits may vary less than in fly and ant systems because their span of temperature limits are sufficiently broad to tolerate the greater range of environmental temperatures. Carrion beetle thermal limits are not expected to be regularly exceeded by sub-soil winter temperatures or average and maximum summer temperatures (Morse & Niwot Ridge LTER, 2023), based on our results at these sites. This may temper the need for greater plasticity or tolerance.

We also expected the life stage that experiences winter (adults in this case) to display lower critical limits based on their suite of winter survival strategies, and that they could potentially retain this enhanced cold tolerance year-round (Leather et al., 1993; Rinehart et al., 2007). Lower thermal critical limits of adult *T. lapponicus* were different between summer and winter, thus supporting a two-season strategy for temperature regulation. *Thanatophilus coloradensis* adults had the lowest CTmin across all tested species, which may be an effect of enhanced cold tolerance in the winter life stage lasting into the summer, but the winter stage of this species is unknown (Peck and Anderson, 1982, Garfinkel and McCain *In Prep.*). If *T. coloradensis* winters as an adult like its congener *T. lapponicus*, a lower CTmin may allow the species to tolerate low underground temperatures during winter in addition to low air temperatures during early spring emergence or at the end of the summer. Given air temperatures at the highest elevation site and the uniquely low CTmin, *T. coloradensis* adults could emerge earlier and remain active longer than *T. lapponicus* adults (Morse & Niwot Ridge LTER, 2023).

In both wild and lab-bred populations, we found a relatively high level of variation in thermal tolerance among individuals. High variation in thermal limits among individuals observed here could reflect variability in experienced temperatures, hydration status, food resource availability, solar intensity, and parental care among others (Scott, 1998; Chown, 2001; Merrick and Smith, 2004; Wettlaufer et al., 2023). Variation in thermal limits of bred individuals was slightly lower overall, which may suggest an effect of lab acclimation to rearing temperatures (Terblanche and Hoffmann, 2020; Weaving et al., 2022). However, there remains a significant amount of variation in thermal limits among individuals that cannot be explained by rearing conditions alone. And interestingly, the variation in the widely distributed species (*T. lapponicus*) exceeds that of the alpine-restricted species (*T. coloradensis*). Such variation may be important for evolutionary adaption to novel climates by representing multiple possible trajectories that could be selected for depending on the directionality of change (Diamond, 2017; Marshall et al., 2020).

Critical thermal limits are also affected by factors like desiccation and behavioral thermoregulation, but we were not able to assess the contribution of either of these factors here. Given our winter study design, carrion beetle movement was limited to within the six-inch height and roughly 140 cubic inches of the buckets. Therefore, we potentially modified normal behavioral thermoregulation by not allowing downward movement in the soil during colder periods or onto the surface during warm days. Nonetheless, we do not think such movements are common during the winter months. Behavioral thermoregulation is undoubtedly important, especially during spring emergence and late fall activity when thermal tolerances could be exceeded by environmental temperatures (Fey et al., 2019; Pincebourde and Woods, 2020). Indeed, in the thermal limit data alone, we found evidence of physiological changes (winter reductions in CTmin) that indicate that carrion beetles use a combination of physiological adaptation and behavioral thermoregulation. Thermal tolerance across life stages in *T. coloradensis* tracked microclimate exposure, and broader cold limits in the more exposed adults suggests temperature avoidance may not be sufficient. Thus, physiological changes reflect that behavioral temperature avoidance is unlikely to be the sole strategy.

4.1. Conclusions

In one of few studies of critical thermal limits in beetles across life stage and season, we found differences in lower thermal limits between adult and juvenile life stages in one high-elevation specialist, and between winter and summer thermal limits in one broadly distributed species. Our results provide support for larval microclimate buffering leading to narrower thermal tolerances in comparison with more mobile adults with broader thermal tolerances often a result of a lower CTmin. For the species we were able to assess for cold tolerance during the winter, we indeed detected a lower winter CTmin. In contrast, we detected high averages and lower variation in CTmax across species, life stages, and seasons. Thus, the interplay of cold temperatures, microclimate, and mobility across life stages can be critical for differential susceptibility to temperature change even between closely related species. Since the coldest temperatures are buffered in the soil by snow, as winter temperatures warm and snow depths decline, insects overwintering in the soil like beetles may be more susceptible to extreme cold temperature in a warming world. Therefore, additional study of contextualized thermal limits in other beetles and insects in general, especially in combination with other types of temperature responses that affect breadth of thermal limits, will broaden our understanding of which species' thermal life-history strategies will make them more at risk in future climate regimes.

CRediT authorship contribution statement

Chloe F. Garfinkel: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Christy M. McCain:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Conceptualization.

Funding sources

This work was supported by the Department of Ecology and Evolutionary Biology and the Museum of Natural History at the University of Colorado Boulder in Boulder, Colorado.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Alexander Han for his significant contributions to carrion beetle collection, care, and curation for this project. We also thank Deane M. Bowers, Michael E. Dillon, Nancy Emery, Dan Doak, and members of the McCain lab, including Grant Vagle, Cameron Pittman, and Garett Jolma, for helpful comments and suggestions that greatly improved the quality of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2025.104063.

Data availability

Other (please explain: e.g. 'I have shared the link to my data as an attachment').

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