

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF ENVIRONMENTAL SCIENCES



MASTER THESIS

Thermal stress in insects: a comparative study of low temperature performance of Central European grasshoppers

Caio Vitor Sales Lima de Oliveira

Supervisor: doc. Ing. Michal Knapp, Ph.D.

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Thesis title

Thermal stress in insects: a comparative study of low temperature performance of Central European grasshoppers

Objectives of thesis

The first goal of the thesis will be to complete the literature review focused on thermal tolerance of insects. The second goal of the thesis will be to conduct laboratory experiment investigating tolerance of Central European grasshoppers to low temperatures.

Methodology

The literature review will be written based on scientific papers searched in the Web of Science. Main focus will be on insect tolerance to extreme temperatures (both high and low). Specific attention will be paid to measurements of chill-coma recovery time in insects (including evaluation of relevance of this method for ecological studies).

The experimental part will consist of a comparative study across 14 grasshopper species with contrasting environmental requirements occurring in the Czech Republic. Chill-coma recovery times (two different measures) will be measured for both adult males and females. In addition, body mass of each individual will be recorded. Statistical models will evaluate sex, body mass, species xerothermophily and species phenology as main explanatory variables.

The proposed extent of the thesis

ca. 40-50 pages + supplements

Keywords

climate change, chill-coma recovery, stress resistance, lower thermal limits

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- Andersen, M. K., & Overgaard, J. (2019) The central nervous system and muscular system play different roles for chill coma onset and recovery in insects. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology* 233, 10–16.
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The Diploma Thesis Supervisor

doc. Ing. Michal Knapp, Ph.D.

Supervising department

Department of Ecology

Electronic approval: 26. 3. 2024

prof. Mgr. Bohumil Mandák, Ph.D.

Head of department

Electronic approval: 26. 3. 2024

prof. RNDr. Michael Komárek, Ph.D.

Dean

Prague on 26. 03. 2024

Declaration:

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

Prague, Date - 26/03/2024

Caio Tutor S. b. de Oliveira

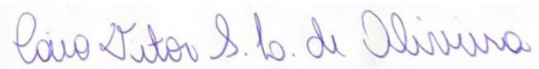
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Abstract

The study of heat tolerance mechanisms and adaptation strategies in grasshoppers, with particular emphasis on chill-coma recovery time (CCRT), serves as a crucial benchmark for understanding their physiological responses to temperature fluctuations. Field collections carried out in the summer months of August to September 2023 enabled the careful recording of 14 grasshopper species at different geographical locations in the Czech Republic. Contrary to prevailing assumptions, our results refute that habitat type, including xerothermophily, significantly influences CCRT. Instead, we provide compelling evidence for intrinsic physiological adaptations that allow locusts to mitigate temperature stress in different habitats. In particular, we observe significant sex-specific differences in recovery times, with females exhibiting a faster recovery rate. This observation highlights sex-specific physiological strategies to optimize survival and reproductive capacity amid fluctuating environmental temperatures.

Furthermore, our analysis reveals a compelling correlation between body mass and recovery durations, emphasizing the pivotal role of physical and metabolic characteristics in grasshopper thermal tolerance. This correlation further investigates the intricate interplay between body size and thermoregulation strategies among ectothermic organisms. Although our study was temporally constrained to a specific seasonal window, it underscores the critical significance of considering phenological and environmental diversity in evaluating ectotherm adaptation to climate dynamics.

By amalgamating ecological, physiological, and evolutionary insights, our research enriches the discourse on grasshopper thermal biology and adaptability in changing climates. Moreover, our findings carry broader implications, contributing to refining conservation tactics and enhancing predictions regarding biological responses to global climate shifts. This underscores the urgent imperative of integrating species-specific traits and environmental adaptations in assessing thermal tolerance and physiological resilience among grasshoppers, thereby informing more productive strategies for biodiversity conservation in an era of rapid ecological change.

Keywords: Climate change, chill-coma recovery, stress resistance, lower thermal limits

Abstrakt

Studium mechanismů tepelné tolerance a adaptačních strategií u kobylek, s důrazem na dobu zotavení se z chillové kómy (CCRT), slouží jako zásadní měřítko pro porozumění jejich fyziologickým reakcím na teplotní fluktuace. Terénní sběry prováděné v letních měsících srpnu a září 2023 umožnily pečlivé zaznamenání 14 druhů kobylek na různých geografických lokalitách v České republice. Na rozdíl od převládajících předpokladů naše výsledky vyvracejí, že typ prostředí, včetně xerothermofility, významně ovlivňuje CCRT. Namísto toho poskytujeme přesvědčivé důkazy o vnitřních fyziologických adaptacích, které umožňují kobylkám zmírňovat teplotní stres v různých habitatech. Konkrétně pozorujeme významné rozdíly mezi pohlavími v dobách zotavení, přičemž samice vykazují rychlejší tempo zotavení. Tento pozorovaný jev zdůrazňuje pohlavím specifické fyziologické strategie k optimalizaci přežití a reprodukční schopnosti při kolísání teplot v prostředí.

Dále naše analýza odhaluje přesvědčivou korelaci mezi hmotností těla a dobou zotavení, což zdůrazňuje zásadní roli fyzických a metabolických charakteristik v tepelné toleranci kobylek. Tato korelace dále zkoumá složité vzájemné působení mezi velikostí těla a strategiemi termoregulace u ektotermních organismů. I když byla naše studie časově omezena na konkrétní sezónní období, zdůrazňuje kritický význam zohledňování fenologické a environmentální rozmanitosti při hodnocení adaptace ektotermů na klimatické dynamiky.

Sloučením ekologických, fyziologických a evolučních poznatků náš výzkum obohacuje diskusi o termální biologii a adaptabilitě kobylek v měnících se klimatických podmínkách. Navíc naše zjištění nesou širší důsledky, přispívají k zdokonalení taktik ochrany přírody a zlepšení předpovědí týkajících se biologických reakcí na globální klimatické změny. To zdůrazňuje naléhavou nutnost integrovat druhově specifické vlastnosti a environmentální adaptace při posuzování tepelné tolerance a fyziologické odolnosti kobylek, čímž se informují produktivnější strategie pro ochranu biodiverzity v éře rychlé ekologické změny.

Klíčová slova: Klimatická změna, zotavení z chillové kómy, odolnost proti stresu, dolní teplotní limity

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1. Introduction

In recent decades, anthropogenic activities such as the burning of fossil fuels, deforestation, and industrial processes have significantly contributed to global warming, resulting in discernible increases in global temperatures (Barcellos et al., 2019; De Alcantara Silva & De Alcantara Ribeiro, 2012). This phenomenon has led to substantial alterations in climatic conditions, including heightened frequency and intensity of extreme weather events such as droughts, hurricanes, floods, and storms (Huang et al., 2020; Redlin & Gries, 2021), impacting various sectors, including agriculture, water resource management, and urban development (Angilletta et al., 2006; Bennett et al., 2018; IPCC, 2014).

Among the organisms affected by these climatic shifts, ectothermic organisms, notably insects, emerge as particularly vulnerable due to their reliance on environmental cues for thermoregulation (De Frenne, Graae, Rodríguez-Sánchez et al., 2013; García-Roger et al., 2013; Gaston et al., 2008). Fluctuating temperatures, exacerbated by ongoing alterations in global climate patterns, directly impact insect populations by inducing alterations in their physiology, behavior, and distribution patterns (Ahnesjö & Forsman, 2003; Chris J. Willott, 1997; Forsman, 1999). For instance, temperature increments may accelerate insect development and reproduction rates, influencing population dynamics and interspecies interactions (Kingsolver et al., 2011, 2012; Kingsolver & Huey, 2008).

Insects belonging to the Phylum Arthropoda, specifically the Class Insecta, fulfill indispensable ecological roles such as pollination, decomposition, and serving as prey for other organisms (Robert G. Foottit & Peter H. Adler, 2018). However, their survival and reproductive success hinge upon their capacity to navigate environmental fluctuations, particularly in temperature. As ectothermic organisms, insects are highly susceptible to climate change impacts due to their reliance on ambient temperatures for physiological processes (Gaitán-Espitia et al., 2013, 2013; Shah et al., 2017). Temperature variations influence their physiology and shape their life history traits, reproductive patterns, and distribution (Kingsolver & Huey, 2008; Mutamiswa et al., 2020). In response, insects have evolved diverse mechanisms encompassing physiological adaptations, phenotypic

plasticity, and morphological changes to adapt to changing thermal environments (Pawar et al., 2016; Ramadan et al., 2020; Weaving et al., 2022).

The significance of these adaptations for insect survival is underscored by their capacity to mitigate the impacts of extreme temperatures on fitness and population dynamics (Arthur et al., 2015; Noor-Ul-ane & Jung, 2021). Moreover, studies on taxa like Orthoptera, which includes locusts, emphasize the intricate nature of thermal adaptations, requiring a comprehensive understanding to forecast ecological and evolutionary trajectories (Ahnesjö & Forsman, 2003; Danks, 2006). Consequently, elucidating grasshoppers' thermal tolerance and performance is paramount in unraveling their responses to climatic shifts and upholding biodiversity and ecosystem stability (Awde et al., 2020; Chidawanyika & Terblanche, 2011).

This study aims to bridge this knowledge gap by examining the thermal tolerance and performance of grasshoppers native to Central Europe in response to temperature variations. By scrutinizing chill-coma recovery dynamics across multiple grasshopper species, we aim to elucidate the variability in recovery time and its implications for grasshoppers' resilience to temperature stress. This research seeks to advance our understanding of grasshopper ecology and refine predictive models of species responses to climate change through comparative analyses of recovery time metrics and integration of ecological observations with physiological data.

2. Literature review

2.1 Introduction to Global Temperature and Human Influence

The escalation of global temperatures attributed to human activities has emerged as a central theme in international discourse, reflecting concerted efforts to mitigate overproduction, pollution, and their adverse environmental and societal impacts. Insights from research, such as the seminal work by De Alcantara Silva and De Alcantara Ribeiro (2012), shed light on the trajectory of Earth's temperature rise. This surge is primarily attributed to two key factors: the excessive use of fossil fuels and agricultural expansion, leading to heightened concentrations of CO₂ and alterations in natural climatic cycles influenced by factors like solar activity and ocean dynamics (De Alcantara Silva & De Alcantara Ribeiro, 2012).

While natural processes inherently influence global temperature variations, the surge in human activities characterized by rampant production and pollution has substantially disrupted the planet's thermal equilibrium, manifesting in what is commonly referred to as the greenhouse effect (De Alcantara Silva & De Alcantara Ribeiro, 2012). The Intergovernmental Panel on Climate Change (IPCC), representing the consensus of the global scientific community, unequivocally asserts that human activities since the Industrial Revolution are the primary driver behind the observed global temperature increase (De Alcantara Silva & De Alcantara Ribeiro, 2012; IPCC, 2014). This assessment also highlights a consistent upward trend in temperature over the past 157 years, resulting in profound alterations in Earth's environment and atmosphere. Moreover, scholarly works underscore the potentially irreversible ramifications of this temperature surge, exemplified by phenomena such as polar ice cap melting and desertification (Barcellos et al., 2019; De Alcantara Silva & De Alcantara Ribeiro, 2012; IPCC, 2014; Shu, 2020).

The climatic modifications witnessed over time have fundamentally reshaped and influenced Earth's natural ecosystems, integral components of the global system (Shu, 2020). Researchers briefly address the profound repercussions of climate change, with a particular focus on the noteworthy deviation in average temperature, which has exceeded 1.14 °C since the consensus in January 2020, surpassing pre-industrial levels (IPCC, 2014;

Redlin & Gries, 2021; Shu, 2020). The escalating and intensifying temperatures have irregularly impacted various terrestrial events, such as snowstorms, hurricanes, precipitation, and storms, directly influencing fauna and flora (Shu, 2020). Moreover, persistent anthropogenic air pollution has also emerged as a significant contributor to global temperature change. This anthropogenic pollution releases emissions into the atmosphere, accumulating CO₂ and strengthening the greenhouse effect (De Alcantara Silva & De Alcantara Ribeiro, 2012; Shu, 2020).

Global warming occurs due to the increase in global average temperature, as emissions of greenhouse gases in the atmosphere (mainly CO₂) trap heat, resulting in phenomena ranging from unexpected rainfall to extreme heat waves due to the excessive burning of fossil fuels such as oil and coal by human industries (Barcellos et al., 2019; De Alcantara Silva & De Alcantara Ribeiro, 2012). Thus, the issue lies not in the natural mechanism itself but in human interference, characterized by overexploitation of natural resources and deforestation, leading to the extinction of existing organisms on Earth over time (Barcellos et al., 2019; De Alcantara Silva & De Alcantara Ribeiro, 2012; Shu, 2020).

Barcellos et al. (2019) underpin discussions about changes in global temperature triggered by external influences or the consequences of human activities. The impact of natural processes on temperature changes leads to climatic fluctuations within a natural timeframe. However, the influence of human activities has engendered adverse effects over an extended period (Barcellos et al., 2019; Shu, 2020). One of the causes of the temperature increase on Earth is climate change, with these climatic shifts primarily driven by global warming, inducing varied magnitudes of temperature changes in different regions (Barcellos et al., 2019; Shu, 2020).

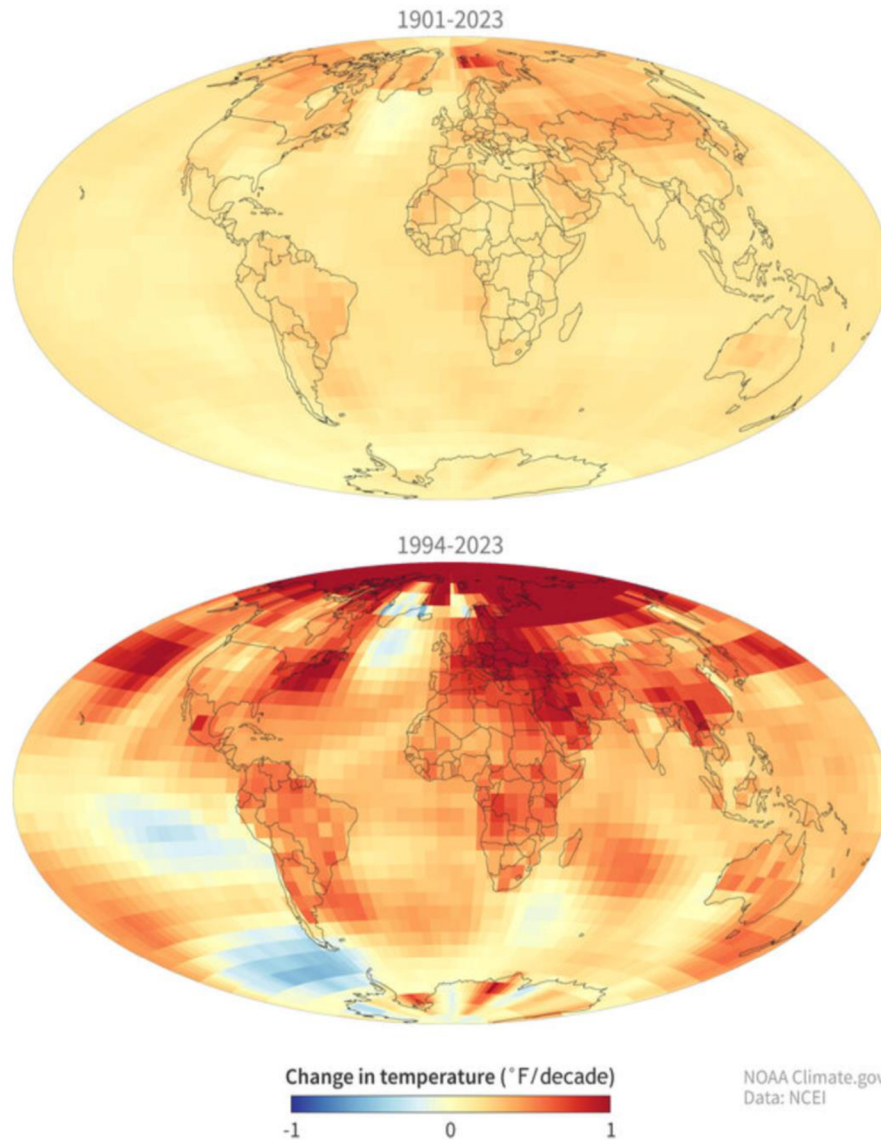


Fig 1. Trends in annual surface temperature in recent decades (1994-2023, top) compared to the trend since the beginning of the 20th century (1901-2023, bottom). Recent warming is much faster than the longer-term average, with some places warming by 1 degree Fahrenheit or more per decade. The differences are most dramatic in the Arctic, where the loss of reflective ice and snow amplifies the warming rate. NOAA Climate.gov, based on data from NOAA's National Centers for Environmental Information (2024).

Human endeavors have evolved to facilitate the use of natural resources for personal gain, posing a troubling challenge when these resources are overexploited, leading to the degradation of the critical natural environment for human habitation. Activities such as burning fossil fuels (coal and oil), cement manufacturing, land use change,

industrialization processes, and deforestation contribute to changes in the Earth's temperature, disrupting the susceptible climate system. This leads to an escalation in greenhouse gas concentrations and an increase in Earth's surface temperature (Barcellos et al., 2019; Huang et al., 2020; Li & Zhao, 2012; Redlin & Gries, 2021; Wu et al., 2022).

Evident shifts in temperature patterns across the years indicate a substantial rise, predominantly ascribed to human activities, extensively documented in publications like The IPCC (2018) reports. These reports underscore a noteworthy 45% surge in carbon dioxide concentration compared to pre-industrial levels (Redlin & Gries, 2021). Research indicates that anthropogenic developments involve industrial, heating, and electrical power generation activities such as coal, oil, or natural gas combustion in various sectors. Furthermore, modifications in land use linked to deforestation and land clearing exert an impact on the global carbon budget. These human-induced activities, encompassing pollution and the exploitation of natural land for agriculture, contribute to the escalation of Earth's temperature (Barcellos et al., 2019; De Alcantara Silva & De Alcantara Ribeiro, 2012; Huang et al., 2020; Li & Zhao, 2012).

While natural factors contribute to climate change, industrialization plays a crucial role in the recent and rapid surge in global warming (Huang et al., 2020; Redlin & Gries, 2021). The increasing needs of expanding populations lead to activities such as deforestation, the combustion of fossil fuels, and intensive agriculture, all of which release greenhouse gases such as CO₂, nitrous oxide, and methane into the atmosphere. Other studies, for instance, indicate a significant positive correlation between carbon emissions and temperature, validating previous findings related to carbon dioxide and temperature (Barcellos et al., 2019b; Li & Zhao, 2012; Wu et al., 2022).

Climate change, reflected in the increasing global temperature, poses diverse challenges, notably the melting ice caps and mountain glaciers. This contributes to heightened sea and river temperatures and rising water levels. The consequences of climate change extend to desertification, environmental degradation, and the profound impact on critical natural resources essential for the survival of both human populations and various organisms (Barcellos et al., 2019; Huang et al., 2020; Li & Zhao, 2012; Redlin & Gries, 2021; Shu, 2020). Furthermore, the elevated temperature on Earth gives rise to extreme weather events, including summers, colder-than-normal winters, heatwaves,

droughts, hurricanes, blizzards, and rainstorms. Thus, the increase in temperature leads to notable changes in the frequency, intensity, spatial extent, duration, and locations of these extreme events (Redlin & Gries, 2021; Shu, 2020).

Moreover, intensified climatic events, such as droughts, hurricanes, floods, and storms, contribute to rising ocean levels, altering rainfall patterns and consequently impacting agriculture, urban areas, water resource utilization, and the energy matrix. These changes have immeasurable consequences for both the natural environment and the organisms inhabiting it (Barcellos et al., 2019b; De Alcantara Silva & De Alcantara Ribeiro, 2012; Huang et al., 2020; Redlin & Gries, 2021; Shu, 2020). This applies to insects, whose significance in ecosystem development is pivotal, given that temperature is a crucial factor influencing species distribution and reproduction (Gaitán-Espitia et al., 2013).

Insects, integral to ecosystems, are experiencing substantial impacts from the global temperature increase due to climate change. This shift in climatic conditions demands adaptive strategies from various ecosystems and habitats to manage the erratic patterns of temperature changes. Scientific research extensively documents this impact, noting observable effects at both population and individual levels. A critical study by Montoya and Raffaelli (2010) highlights the influence of climate change on biotic interactions and the sustainability of ecosystem services. A primary concern arising from these environmental shifts is the degradation of ecosystems.

Additionally, the climate-induced alteration in species' geographic ranges can result in non-random biodiversity changes within local ecosystems, subsequently influencing the structure of food webs. These transformations significantly impact population and community dynamics, including their roles in cycling chemical elements such as CO₂ and nitrogen. Moreover, these ecological changes carry substantial implications for practices in pest management and efforts in biodiversity conservation, as discussed in the works of Grimm et al. (2013), Huey et al. (2012), Ma et al. (2020), O'Connor et al. (2011), and Weiskopf et al. (2020).

2.2 Temperature's Impact: From Natural Processes to Ecosystem Dynamics

Temperature plays a crucial role in shaping ecological processes and ecosystem dynamics, with high and low temperatures exerting profound influences on the environment. In colder climates, low temperatures initiate ecological responses, such as the formation of ice over water bodies, which affects nutrient cycling and alters habitats for aquatic organisms. These conditions also influence the metabolism, activity, and feeding behavior of ectothermic (cold-blooded) animals due to their reliance on external thermal conditions for physiological processes (Nagelkerken & Munday, 2016; O'Connor et al., 2011). In temperate regions, winter dormancy in plants and animals adjusts energy flow and trophic interactions, impacting ecosystem function (De Frenne, Graae, Rodríguez-Sánchez, et al., 2013; Montoya & Raffaelli, 2010). Extreme cold, characteristic of polar regions, dictate species distribution and abundance, favoring cold-adapted species while posing challenges for others. Furthermore, low temperatures slow soil processes, such as nutrient cycling and decomposition, critical for ecosystem health (Weiskopf et al., 2020).

Conversely, elevated temperatures in warmer climates lead to increased evaporation and transpiration, reducing water availability and exacerbating drought conditions. This can cause shifts in vegetation patterns and alter habitats, affecting a broad range of species. High temperatures also disrupt vital ecological processes, including pollination and seed dispersal, essential for sustaining plant populations and overall biodiversity (Brown et al., 2004; De Frenne, Graae, Rodríguez-Sánchez et al., 2013; Glazier, 2015). Thermal stress in animals can result in diminished reproductive success, altered behaviors, and heightened disease vulnerability. Coral reefs are notably sensitive to temperature changes; elevated temperatures cause bleaching events, leading to coral mortality and altered reef community structures (Parmesan, 2006).

The impact of temperature fluctuations, encompassing both highs and lows, is pivotal in defining the structure and functionality of ecosystems worldwide. This underscores the importance of addressing and mitigating climate change effects on natural systems,

emphasizing the need for comprehensive understanding and action (Litchman & Thomas, 2023; Nagelkerken & Munday, 2016; O'Connor et al., 2011).

The ongoing global temperature increase, driven by anthropogenic factors such as land-use changes, nitrogen fertilization, and elevated atmospheric CO₂ levels, has multifaceted impacts on ecosystems at various levels, from individual organisms to entire communities (Brown et al., 2004; Jiang & Morin, 2004; Litchman & Thomas, 2023). This warming trend is causing temperate regions to experience higher temperatures and colder regions to face even lower temperatures, affecting ecosystems that are particularly sensitive to these changes (Fontúrbel et al., 2021; Parmesan, 2006; Thompson et al., 2013).

Research highlights the profound impacts of climate change on marine ecosystems, mainly through factors like CO₂ levels and ocean acidification affecting coral reefs. Furthermore, warming trends in lower latitudes are causing tropical species to shift towards temperate regions, altering ecological dynamics and expanding water-limited areas, with significant implications for the interactions between herbivores and plants in these ecosystems (De Frenne, Graae, Rodríguez-Sánchez, et al., 2013; Deutsch et al., 2008; O'Connor et al., 2011).

Temperature changes in temperate and cold regions and other abiotic factors harm wildlife, especially ectothermic insects that depend on external thermal conditions for body temperature regulation. These climate fluctuations have important implications for various insect groups, affecting pollinators, pests, and disease vectors. Locusts, for example, are vital in transferring energy within food chains from plants to predators and contribute significantly to nutrient cycling, highlighting the complex interactions within ecosystems affected by temperature changes (Ahnesjö & Forsman, 2003; Chris J. Willott, 1997; R. Harris et al., 2012; Umbers et al., 2013).

2.3 Insect Performance of Temperature

Insects belonging to the Phylum Arthropoda and the Class Insecta occupy a pivotal ecological niche intricately linked with temperature dynamics, exerting influence from individual organisms to entire ecosystems. As environmental indicators, insects exhibit

dynamic responses to temperature variations, impacting their biology and ecosystem-level dynamics (Gaitán-Espitia et al., 2013; Robert G. Foottit & Peter H. Adler, 2018; Shah et al., 2017).

Temperature fluctuations pose a multifaceted challenge for insects, eliciting a spectrum of ecological responses. In colder climates, insects may enter diapause or adjust their metabolic rates to conserve energy during periods of low temperature, whereas warmer temperatures can accelerate insect development rates and alter phenological patterns, potentially disrupting trophic interactions and ecosystem services (De Frenne et al., 2013; Gaitán-Espitia et al., 2013; Glazier, 2015; Montoya & Raffaelli, 2010; Shah et al., 2017).

Furthermore, temperature alterations affect individual insects and reshape their interactions within communities and ecosystems. Shifts in temperature regimes can trigger changes in species composition, abundance, and distribution, thereby exerting cascading effects on ecosystem structure and function (Angilletta et al., 2004; Bale et al., 2002; Kingsolver et al., 2012; Mutamiswa et al., 2020; Pawar et al., 2016; Ramadan et al., 2020; Weaving et al., 2022). Understanding these temperature-mediated ecological responses is paramount for predicting ecosystem resilience amidst climate change and formulating effective conservation strategies (Chidawanyika & Terblanche, 2011; Pawar et al., 2016; Ramadan et al., 2020; Weaving et al., 2022).

Insects, as ectothermic organisms, demonstrate remarkable sensitivity to temperature fluctuations, critically influencing various facets of their biology, including reproduction, development, abundance, longevity, distribution, and ultimately, survival (Pawar et al., 2016; Ramadan et al., 2020; Scaccini et al., 2020; Weaving et al., 2022). Their adeptness at regulating body temperature is vital for adapting to shifting environmental conditions, relying on environmental cues to modulate their physiological, morphological, and behavioral traits. Through evolutionary processes and phenotypic plasticity, insects evolve mechanisms to withstand temperature stressors, ensuring their survival and ecological interactions across diverse ecosystems (Motswagole et al., 2019; Mousseau, 1997; Overgaard & Macmillan, 2017; Pawar et al., 2016; Ramadan et al., 2020; Scriven et al., 2016; Weaving et al., 2022)

Recent research, such as that by Weaving et al. (2022), underscores the role of thermal limit plasticity in the evolutionary adaptation of insect life history. Temperature fluctuations influence critical thermal maximum and minimum, shaping insect fitness (Pawar et al., 2016; Ramadan et al., 2020; Scaccini et al., 2020; Weaving et al., 2022). Certain temperature extremes, like frost events, can prove lethal for specific species, as observed in the case of adult *Halyomorpha halys* survival (Ramadan et al., 2020; Scaccini et al., 2020). Additionally, the plasticity of cold tolerance across different stages of *Aethina tumida* contributes significantly to winter survival (Noor-Ul-ane & Jung, 2021).

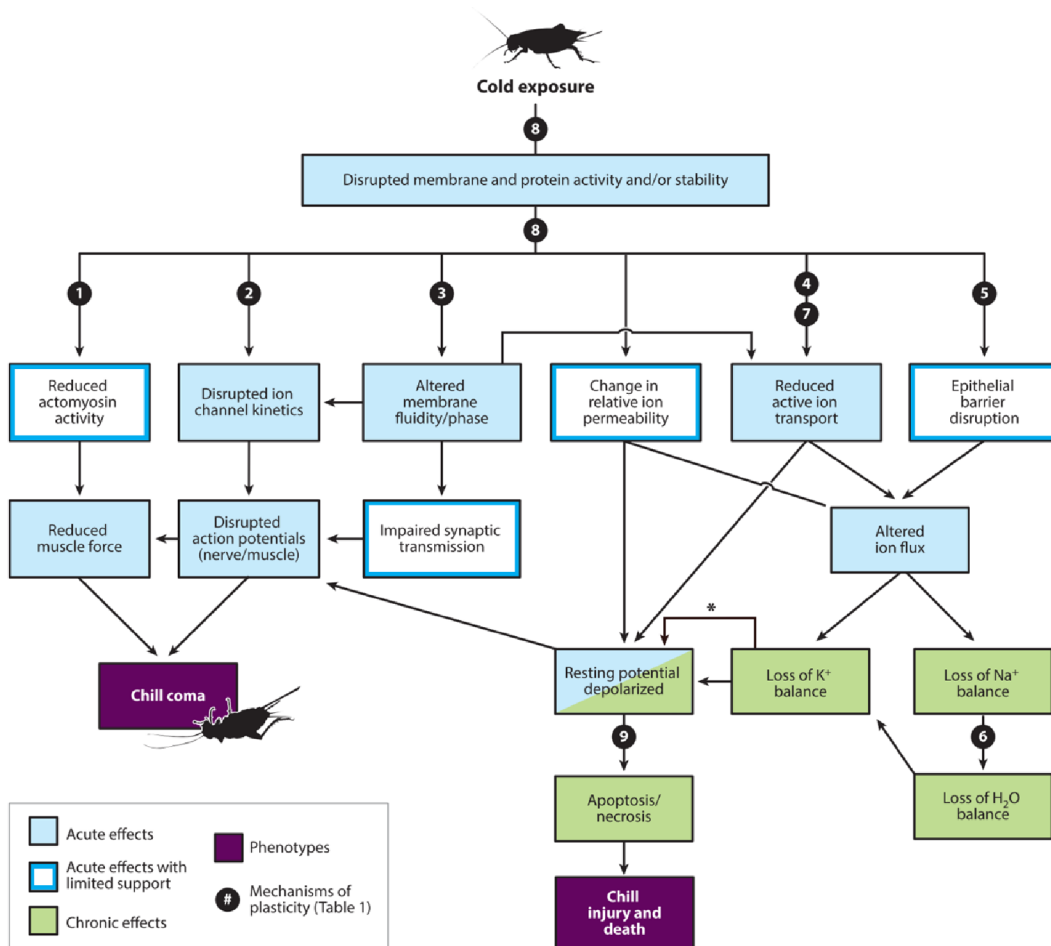


Fig 2. Insect Responses to Temperature Fluctuations - This diagram illustrates the adaptive responses of insects to varying thermal environments, highlighting the strategies employed during cold exposure, such as metabolic adjustments and diapause, alongside the physiological and ecological consequences of warmer temperatures on development rates and ecosystem interactions. These adaptations underscore the complex relationship between insect physiology and temperature, which is pivotal for understanding ecosystem dynamics amid climate change. Adapted from: Overgaard, J., & MacMillan, H. A. (2017). The Integrative Physiology of Insect Chill Tolerance. *Annual Review of Physiology*, 79, 187–208. <https://doi.org/10.1146/annurev-physiol-022516-03414>.

In addition to physiological adaptations, insects undergo changes related to body size in response to temperature variations. Body size decreases with decreasing average temperatures, impacting the number of generations per season (Kingsolver & Huey, 2008; Mousseau, 1997; Mutamiswa et al., 2020a, 2020b). Abiotic factors, particularly temperature, significantly influence insect life history, with mechanisms like plasticity and cold tolerance facilitating adaptation to changing environments (Mousseau, 1997; Mutamiswa et al., 2020a; Pawar et al., 2016; Scriven et al., 2016; Weaving et al., 2022).

Understanding thermal fitness, temperature tolerance, and phenotypic plasticity in insects is crucial for predicting their future distribution and ecological responses (Chidawanyika & Terblanche, 2011, 2011; Mutamiswa et al., 2020a; Pawar et al., 2016; Weaving et al., 2022), particularly amidst the rising frequency of extreme temperature events attributed to climate change (Pawar et al., 2016; Ramadan et al., 2020; Scaccini et al., 2020; Weaving et al., 2022).

2.4 Why Thermal Tolerance in Insects is Important

The capacity of insects to navigate temperature fluctuations, known as thermal tolerance, is crucial in dictating their ecological roles and influencing community dynamics (Angilletta et al., 2004, 2006; Scaccini et al., 2020; Wu et al., 2022). Thermal tolerance involves an array of physiological, morphological, and behavioral adaptations that empower insects to flourish across varied environmental landscapes (Bale et al., 2002;

Scriven et al., 2016). Insects possessing extensive thermal tolerance ranges are adept at harnessing diverse habitats and resources, bolstering their ecological prominence and adaptability to environmental shifts (Mutamiswa et al., 2020a; Weaving et al., 2022).

Grasping the ecological ramifications of thermal tolerance sheds light on ecosystem functionality and resilience. By unraveling the intricacies of thermal adaptation and its ecological fallout, researchers can more accurately foresee insect community reactions to climate change and devise strategies to safeguard biodiversity and ecosystem services (Chidawanyika & Terblanche, 2011; Mutamiswa et al., 2020; Pawar et al., 2016; Ramadan et al., 2020).

Adaptation to extreme thermal conditions epitomizes insect tenacity, where physiological, morphological, and behavioral modifications converge to sustain homeostasis. Thermal sensitivity impinges on pivotal life history traits such as reproduction, development, abundance, lifespan, distribution, and survival (M. K. Andersen & Overgaard, 2019; Motswagole et al., 2019; Mousseau, 1997; Overgaard & Macmillan, 2017; Pawar et al., 2016; Ramadan et al., 2020; Scaccini et al., 2020; Scriven et al., 2016; Weaving et al., 2022). Through evolutionary mechanisms and phenotypic plasticity, insects modulate their thermal thresholds to withstand temperature challenges, underpinning their ecological success and reinforcing ecosystem robustness (Overgaard & Macmillan, 2017; Ramadan et al., 2020; Weaving et al., 2022).

Moreover, thermal tolerance profoundly influences insect community structures and ecosystem processes. Species with superior thermal tolerance may eclipse less resilient counterparts, precipitating shifts in species composition and trophic relationships within ecosystems. Disparities in thermal tolerance among insect populations can also modify their vulnerability to environmental perturbations, such as climate-induced thermal variability, thereby affecting ecosystem resilience (Chidawanyika & Terblanche, 2011; Mutamiswa et al., 2020; Pawar et al., 2016; Weaving et al., 2022).

Acknowledging the criticality of thermal tolerance in insects is essential for understanding how microhabitat gradients and broader environmental conditions steer species distribution and ecological interactions (Angilletta et al., 2004; García-Roger et al., 2013; Kalkstein, 1991).

Thermal tolerance delineates insect adaptability to temperature variations within microhabitats, influencing their resource utilization and resilience to environmental shifts (Mutamiswa et al., 2020; Weaving et al., 2022). Furthermore, insights into thermal tolerance guide conservation and management efforts by elucidating insect population vulnerabilities to climate change-induced thermal stresses and informing initiatives to maintain biodiversity and ecosystem integrity (Chidawanyika & Terblanche, 2011; Pawar et al., 2016; Weaving et al., 2022).

2.5 Microhabitat Gradients and Environmental Conditions

Annual temperature fluctuation significantly influences habitat gradients, introducing substantial variability within microhabitats, each capable of supporting diverse species assemblages (Emery et al., 2019; García-Roger et al., 2013; Kalkstein, 1991). This variability in temperature across microhabitats plays a critical role in shaping insect community composition, as Emery et al. (2019) demonstrated in their study of vernal pool grasslands in California. The gradient of temperature variation can vary among microhabitat types, exhibiting either sharp or gradual transitions, which affect species distribution and ecological dynamics (García-Roger et al., 2013; Kalkstein, 1991).

Variations in temperature regulation within microhabitats are significantly influenced by the type and abundance of vegetation, which modulate environmental conditions and dictate species adaptation to their niches (García-Roger et al., 2013; Kalkstein, 1991). Each microhabitat, with its unique geomorphology and acclimation temperature, is subject to fluctuations resulting from numerous minor environmental perturbations (García-Roger et al., 2013; Honek, 1996). Species' spatial distribution and behavior across diverse microhabitats underscore the importance of understanding ecological patterns (Ellers & Van Alphen, 1997; R. Harris et al., 2012; R. S. Criddle et al., 2003).

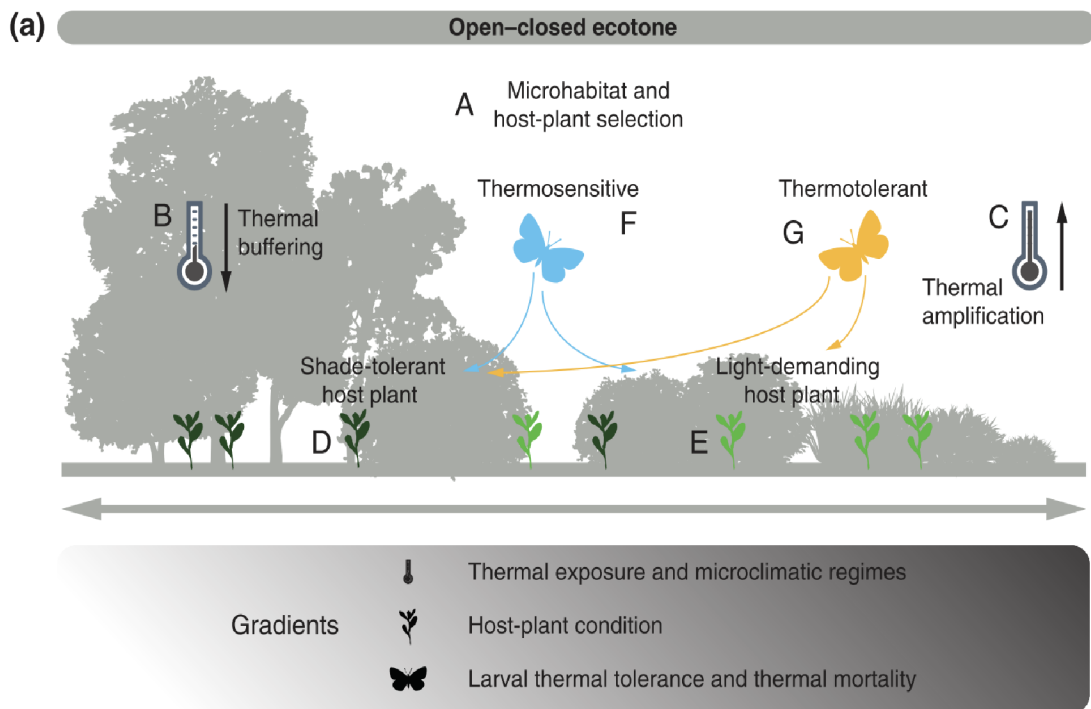


Fig 3. Insect Thermal Tolerance Across Microhabitat Gradients - This figure depicts how interspecific differences in microhabitat use expose insects to varying thermal conditions, influencing their survival. The microclimatic heterogeneity within ecotones, characterized by different vegetal structures and temperature regimes, impacts insect oviposition choices and larval performance. Adapted from Vives-Inгла et al. (2023).

Gaston et al. (2008) highlight the significance of distinct patterns within various eco-geographical regions that foster unique intraspecific and interspecific interactions, affecting biological traits and community structure. Microhabitat composition and temperature conditions influence species richness, further modulated by interacting environmental and geographical factors (R. S. Criddle et al., 2003). Given insects' propensity to inhabit a wide range of habitats, exceptionally distinct microhabitats characterized by varied gradients, it becomes evident that species distribution and richness follow unique patterns across latitudinal and elevational gradients (Gaston et al., 2008; Honek, 1996; Kalkstein, 1991; R. S. Criddle et al., 2003).

Temperature variation across spatial scales necessitates adaptive strategies for organisms to survive extreme conditions, with climate change exacerbating these challenges across microhabitats. Various adaptation mechanisms are likely at play at each spatial scale, reflecting the complexity of ecological responses to temperature fluctuations (Awde et al., 2020; Danks, 2006; Honek, 1996; Vives-Inglá et al., 2023). Insects adapt to the unique temporal variations of their microhabitats, employing strategies to mitigate climatic differences through a 'microhabitat effect.' These adaptations vary across latitudinal gradients and offer insights into the profound impact of temperature on insect ecology and survival strategies (Danks, 2006; Honek, 1996; Vives-Inglá et al., 2023).

The interplay between microhabitat and latitudinal gradients underscores the multifaceted nature of environmental influences on species distribution and ecological dynamics. Microhabitats, characterized by diverse environmental conditions and gradients, provide niche spaces where species exhibit intricate adaptations to local environmental factors (Emery et al., 2019; García-Roger et al., 2013; Kalkstein, 1991). These microhabitat variations are influenced by vegetation type and abundance, geomorphology, acclimation temperatures, shape species composition, and richness (García-Roger et al., 2013; Kalkstein, 1991). Moreover, microhabitat gradients often reflect broader latitudinal gradients, with temperature as a fundamental determinant of species distributions across spatial scales (Danks, 2006; Gaston et al., 2008; R. S. Criddle et al., 2003).

Studies highlight the role of temperature fluctuations along latitudinal gradients in driving changes in species' metabolic reactions, reproductive strategies, and body size adjustments (García-Roger et al., 2013; Gaston et al., 2008; Honek, 1996; R. S. Criddle et al., 2003). Furthermore, variations in thermal tolerance across latitudinal gradients indicate the adaptive responses of species to cope with temperature stressors, underscoring the intricate links between microhabitat conditions, latitudinal gradients, and species' ecological dynamics (Awde et al., 2020; Emery et al., 2019; Perez & Aron, 2020).

2.6 Latitudinal Gradients and Temperature Fluctuations

The intricate relationship between species distribution and environmental gradients across latitudinal zones highlights the adaptive capacity of organisms. Researchers emphasize that species exhibit localized adaptations to distinct distribution patterns, significantly shaped by latitudinal influences (Gaston et al., 2008). This adaptation extends across biological dimensions, including variations in clutch size among avians and parallels in body size adjustments within and among species, highlighting a universal response mechanism to latitudinal gradients (De Frenne et al., 2013; García-Roger et al., 2013; Gaston et al., 2008; Kalkstein, 1991).

Further investigations into latitudinal and elevational gradients reveal their profound impact on organisms' metabolic reactions related to growth, reproduction, defense mechanisms, and adjustments in body size, mass, or energy levels. These changes are responses to the diverse environmental pressures encountered across gradients, illustrating the complex interplay between organisms and their habitats (De Frenne et al., 2013; García-Roger et al., 2013; Gaston et al., 2008; Honek, 1996; R. S. Criddle et al., 2003; Vives-Inгла et al., 2023).

Temperature is critical in this dynamic, predominantly influencing species' spatial distribution and richness, particularly in insects. It is postulated that natural selection tailors metabolic phenotypes along these gradients to enhance survival under varying climatic conditions. This selection process underscores the direct impact of temperature fluctuations induced by climate change on species survival, with specific studies on snails demonstrating adherence to latitudinal patterns as a result of thermal adaptations (Blanckenhorn & Demont, 2004; Danks, 2006; Gaitán-Espitia et al., 2013; R. S. Criddle et al., 2003; Vives-Inгла et al., 2023).

As a form of abiotic stress, temperature fluctuations particularly challenge insects across latitudinal gradients. Investigations into ants reveal within-species variances in thermal tolerance, with populations at higher latitudes displaying enhanced cold tolerance. This phenomenon aligns with findings across altitudinal gradients, where specific ant species exhibit increased cold tolerance at higher elevations, pointing to a broad pattern of adaptation to temperature extremes (Awde et al., 2020a; De Frenne et al., 2013; Emery et al., 2019; R. Harris et al., 2012; Vives-Inгла et al., 2023).

Moreover, the latitudinal variation in cold hardiness is pivotal for forecasting shifts in the distribution of ectotherms in response to global warming. Nonetheless, these predictions must cautiously interpret the relationship between physiological tolerance and latitude, emphasizing the significance of microclimatic temperatures over broad geographical patterns (Awde et al., 2020; De Frenne, Graae, Rodríguez-Sánchez, et al., 2013; Deutsch et al., 2008; Pincebourde & Casas, 2015).

Insects' responses to latitudinal gradients and temperature fluctuations elucidate a spectrum of adaptations aimed at navigating the challenges posed by their environments. These adaptations, which impact reproduction, distribution, survival, and body mass, underscore the resilience of insects to environmental shifts, including those propelled by climate change (Awde et al., 2020; De Frenne et al., 2013; Pincebourde & Casas, 2015; Shah et al., 2017; Vives-Inгла et al., 2023). Through this lens, studying latitudinal gradients enriches our understanding of species distribution patterns and informs conservation strategies in the face of ongoing environmental alterations.

2.7 Influence of Body Mass and Sex on Chill Coma

Insects inhabiting icy conditions in temperate zones have evolved various adaptive mechanisms to ensure their survival and reproduction. These arthropods can swiftly enter a chill coma status as a rapid physiological response to extremely low temperatures, a phenomenon well-documented across various studies (Awde et al., 2020a, 2020b; García-Roger et al., 2013; Honek, 1996; Kalkstein, 1991; R. S. Criddle et al., 2003; Ramadan et al., 2020).

Bergmann's rule, proposed in 1847, suggests a positive correlation between thermal tolerance and body size, a prediction that supports a wide range of endotherms (Bergmann, 1847). More prominent individuals with greater body mass are predominantly observed in colder environments, illustrating an adaptation strategy within species and among closely related species (Blanckenhorn & Demont, 2004; Mousseau, 1997; Park, 1949; Pujol-Lereis et al., 2016). However, a reverse ecogeographic pattern in body size has been suggested for ectothermic invertebrates, where larger body sizes in colder environments may not apply universally. For instance, studies have identified differences

in body size and sex among cryptic bumblebee species and carabid beetles that correspond to Bergmann's rule, indicating a nuanced relationship between body size and cold adaptation in ectotherms (Awde et al., 2020; Danks, 2006; Honek, 1996; Scriven et al., 2016).

Amidst temperature fluctuations, a prevailing concern due to climate change, insects must develop robust adaptive mechanisms. The alteration in body mass demonstrates a tangible relationship with adaptation, enabling insects to withstand longer winters and recover efficiently from chill coma states. The specificity of these adaptive responses is contingent upon the region, microhabitat, and the intricate interplay of physiological and biochemical processes, highlighting the dynamic nature of insect adaptation to cold (Danks, 2006; Honek, 1996; Kingsolver et al., 2011; Vives-Inгла et al., 2023).

The term "chill coma" denotes the reversible paralysis induced by low-temperature shocks, a critical aspect of chill tolerance. This state occurs when temperatures plummet below the critical thermal limit for insect activity, showcasing a survival mechanism in the face of freezing temperatures (J. L. Andersen et al., 2013; Bayley et al., 2018; Gibert et al., 2001; Overgaard & Macmillan, 2017). For chill-susceptible insects, their ability to survive temperatures above freezing becomes a testament to their cold tolerance and highlights the importance of understanding and studying these mechanisms in detail (Gibert et al., 2001; MacMillan & Sinclair, 2011; Pujol-Lereis et al., 2016).

The study by Michal Knapp and Michal Řeřicha (2020) provides a pertinent exploration of winter temperature regimes on survival, body mass loss, and post-winter starvation resistance in laboratory-reared and field-collected ladybirds. Their findings, indicating that warmer winter conditions lead to increased survival rates but also more significant body mass loss and reduced post-winter starvation resistance, align with and extend upon the foundational research (Ahnesjö & Forsman, 2003; Knapp & Řeřicha, 2020; MacMillan & Sinclair, 2011; Xia et al., 2022). Moreover, this study complements and contrasts with the findings of the *Arma chinensis* study of Xia et al. (2022), which examined the impact of temperature fluctuations on insect survival strategies, providing a comprehensive understanding of the multifaceted nature of insect adaptation to cold.

Building on this, the research by Ramadan et al. (2020) underscores that limited stress may precipitate beneficial responses in insects, such as improved cold tolerance and enhanced starvation resistance. These adaptations, alongside increased body mass and other morphological changes, underscore the complex interplay between insects and their environments. These findings not only illuminate the pathways through which insects navigate thermal stress but also highlight the potential evolutionary implications of these adaptations (Chidawanyika & Terblanche, 2011; Danks, 2006; Kozłowski et al., 2004; Sinclair et al., 2003).

Insects' adaptation to cold temperatures in temperate zones involves a complex interplay of physiological changes, behavioral strategies, and genetic mechanisms. These adaptations underscore insects' resilience and adaptability to extreme environments and shed light on the evolutionary pressures shaping their survival mechanisms (R. M. B. Harris et al., 2015; Huey et al., 2012; Overgaard & Macmillan, 2017). Studies by Awde et al. (2020a), Chidawanyika & Terblanche (2011), and Overgaard & Macmillan (2017) highlight the diverse approaches insects employ to mitigate cold stress, from biochemical alterations to behavioral adjustments. Understanding these mechanisms becomes crucial for predicting future insect distributions, ecosystem impacts, and biodiversity in the face of shifting climate patterns. Insights from studies by Noor-Ul-ane and Jung (2021) and others provide a nuanced understanding of how insects adapt to changing climates, highlighting the dynamic interplay between life and the environment.

2.8 Low-Temperature Performance in Insects and Grasshoppers

In response to the pronounced temperature shifts attributed to global climate change, insects have exhibited varying performance and developed diverse strategies and mechanisms to cope with cold conditions over time. Several studies offer insights into these strategies, emphasizing the significance of genotypic effects, morphological adaptations, habitat selection, biochemical mechanisms (particularly in freeze-tolerant species), and the rapid evolution of traits related to thermal sensitivity (Awde et al., 2020; Chidawanyika & Terblanche, 2011; Danks, 2006; MacMillan & Sinclair, 2011; Noor-Ul-ane & Jung, 2021; Overgaard & Macmillan, 2017). Understanding these adaptations is

essential for comprehending insect species distribution, life cycles, population dynamics, migration patterns, potential extinctions, and their interactions with environmental factors (Awde et al., 2020; Danks, 2006; Perez & Aron, 2020).

For instance, a study by Noor-Ul-ane & Jung (2021) sheds light on the performance of the immature stages of the Small Hive Beetle (SHB) *Aethina tumida* Murray (Coleoptera: Nitidulidae) under cold conditions. The survival and adaptation capacity of SHB immature stages to tolerate cold environments are noteworthy, indicating a significant contribution to winter survival. Laboratory studies conducted by Chidawanyika and Terblanche (2011) on adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) revealed rapid thermal responses and significantly improved low-temperature survival. Additional studies explore various approaches to explain insect performance in low temperatures, considering physiological mechanisms, potential plasticity, or the rapid evolution of traits related to thermal sensitivity. These investigations encompass different insect orders, including Coleoptera, Dictyoptera, Diptera, Hemiptera, Orthoptera, Lepidoptera, and Hymenoptera (Awde et al., 2020; Chidawanyika & Terblanche, 2011; Danks 2006; Mutamiswa et al., 2020; Noor-Ul-ane & Jung, 2021; Overgaard & Macmillan, 2017).

Research conducted on the Orthoptera group has unveiled a range of adaptations that equip these insects to thrive in cold conditions, ensuring their survival and reproductive success even in harsh winter environments, where they can either tolerate or actively avoid freezing (Awde et al., 2020; Danks, 2006; Overgaard & Macmillan, 2017a; Sinclair et al., 2003). The order Orthoptera encompasses crickets, grasshoppers, and hoppers, characterized by their chewing mouthparts, tegmina forewings, and saltatory hind legs. These insects exhibit a segmented body with well-defined head, thorax, and abdomen regions, featuring three pairs of legs and a pair of antennae. Unlike the complete metamorphosis observed in butterflies, Orthoptera undergoes incomplete metamorphosis during their developmental stages (Chappell, 1983; Chris J. Willott, 1997; Robert G. Foottit & Peter H. Adler, 2018; Willott & Hassall, 1998).

These organisms inhabit various parts of the world, spanning tropical and temperate zones, and occupy different habitats with diverse climates, particularly within distinct microhabitats experiencing variations in temperature fluctuations, ranging from

the coldest to warmest locations (Awde et al., 2020; Danks, 2006; Honek, 1996; Sinclair et al., 2003). Studies such as Andersen et al. (2013) and Anderson et al. (1979) have shown that locust species possess an elaborate repertoire of thermoregulatory postures and shade-seeking behavior, indicating the evolution of a thermal generalist life history.

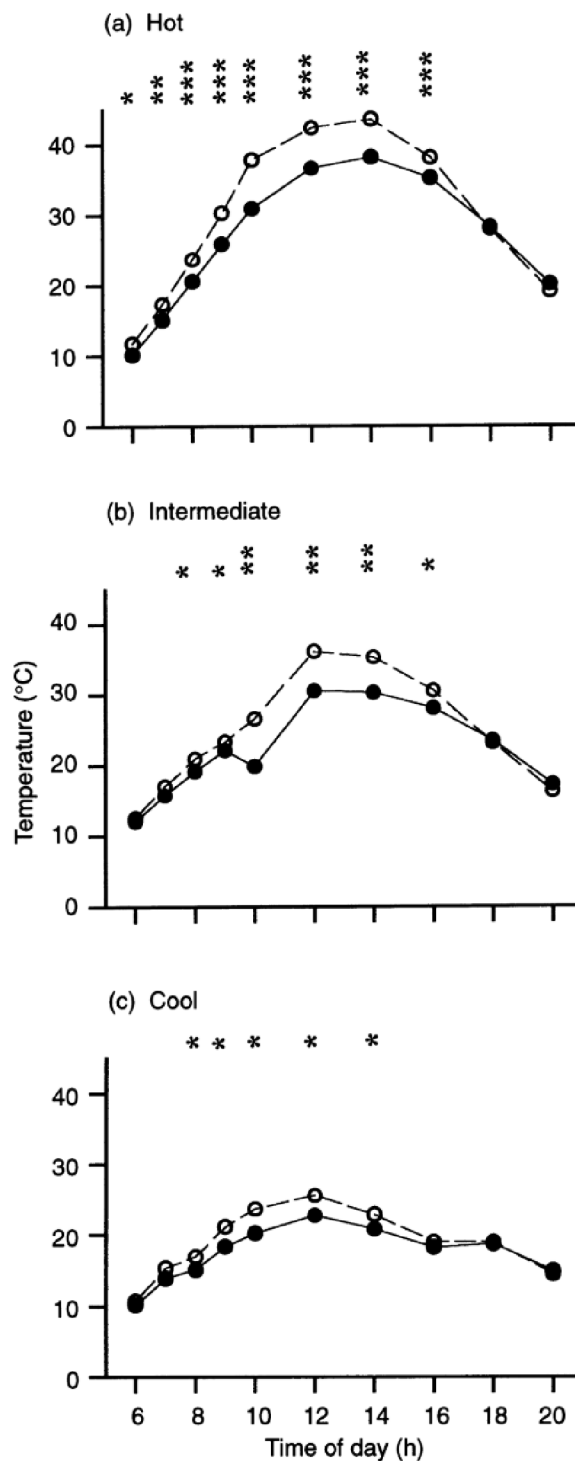


Fig 4. Comparison of grasshopper thermal regulatory capabilities across different environmental conditions. The figure illustrates the variability in thermal tolerance and adaptation strategies among grasshopper populations in response to temperature fluctuations. Male and female wingless grasshoppers exhibit distinct body modifications across seasons. Despite variations in thermal variability, no straightforward correlation is observed between thermal tolerance and environmental conditions within grasshopper populations. The data presented here provide insights into the complex interactions between grasshopper physiology and environmental factors. They were adapted from Willott (1997) with permission from *Functional Ecology*, 11(5), 705–713.

Various investigations have highlighted the thermal regulatory capabilities of grasshoppers, demonstrating their capacity to navigate extreme temperature conditions. Furthermore, research suggests that body modifications contribute to the selection for body size, with distinctions observed between male and female wingless grasshoppers across different seasons (Chidawanyika & Terblanche, 2011; Overgaard & Macmillan, 2017; Pujol-Lereis et al., 2016). Scientists have delved into the lack of a straightforward correlation between thermal variability and thermal tolerance within grasshopper populations. These populations exhibit variations in how sensitive their development, growth, and performance are to environmental conditions, especially temperature (Ahnesjö & Forsman, 2003; Chappell, 1983; Chris J. Willott, 1997; Preston & Johnson, 2020, 2022).

Understanding the adaptations of locusts is crucial for comprehending species movements, compositions, ecology, and evolution. As climate-induced temperature changes affect these animals, they must adapt to fluctuations in temperature. Investigating these adaptations is essential for understanding the evolutionary history and ecological aspects, particularly regarding thermal tolerance (Ahnesjö & Forsman, 2003; Chappell, 1983; Chris J. Willott, 1997; Preston & Johnson, 2020; Weldon et al., 2011).

2.9 Evolutionary History and Ecological Aspects of Thermal Tolerance

The thermal strategies employed by ectotherms can exhibit unpredictable variations, particularly in generally cool climates. This variability may preclude the adoption of low-temperature thermal specialist strategies in their life histories, which have evolved in thermally specialized insect species (Chappell, 1983; Weaving et al., 2022). Understanding these hidden traits in the life history of insects is crucial for comprehending developmental and reproductive responses to different rearing temperatures, encompassing aspects of behavioral thermoregulation, thermal physiology, and arthropod ecology (Angilletta et al., 2004; Belén Arias et al., 2011; Chris J. Willott, 1997; Willott & Hassall, 1998).

In the context of insects, gaining insights into life history variation proves valuable for understanding theories describing how temperature influences biochemical processes, exemplified by the observed temperature-size relationship (Angilletta et al., 2004, 2006; Belén Arias et al., 2011; Chris J. Willott, 1997; Pörtner et al., 2006). Furthermore, this understanding has broader implications, including the impact on the reproduction and survival of insects over time as temperatures change. This insight allows us to discern how plasticity and other mechanisms have been at play in orthopterans (Bennett et al., 2021; Carbonell et al., 2021; Ellers & Van Alphen, 1997; Kingsolver et al., 2012).

Studies, exemplified by Ellers & Van Alphen's (1997) work, illustrate that life history variation in species like *Asobara tabida* can arise from either genetic factors or environmental influences on the phenotype. Plasticity, which is pivotal for adaptation, is evidenced by differences in allocation, especially in the timing of production (Ellers & Van Alphen, 1997; Mitchell et al., 2013; Weaving et al., 2022). The theory of life history traits is crucial for comprehending the evolutionary processes that result in different optimal combination values in natural populations residing in diverse habitats (Carbonell et al., 2021; Ellers & Van Alphen, 1997; Motswagole et al., 2019; Pörtner et al., 2006). In insects like locusts, understanding the specialist strategies developed to avoid freezing, as well as the plasticity in developmental and reproductive aspects and efficient behavioral thermoregulation, is facilitated by insights from studies on life history traits (Angilletta et al., 2004; Belén Arias et al., 2011; Bennett et al., 2021; Willott & Hassall, 1998).

Prompt responses to thermal fluctuations can contribute positively to practical adaptations. Cold tolerance, along with various life histories, behavioral and physiological

traits, and their thermal plasticity, is linked to the ecological dynamics of insect life history (Awde et al., 2020; Carbonell et al., 2021; Danks, 2006; Pörtner et al., 2006). An illustrative example is found in the work of Carbonell et al. (2021), where the evolution of a faster life history was identified as enhancing the range-shifting ability of *Ischnura elegans*. Changes in these traits can be pivotal for ecological shifts in insect life history. It is increasingly evident that the evolution of trait alterations associated with plasticity, phenotypic changes, and variations in development and growth rates may significantly influence the ecological dimensions of cold tolerance (Belén Arias et al., 2011; Carbonell et al., 2021; Filho et al., 2021).

The thermal performance of insects varies significantly due to their ectothermic nature and their dependence on abiotic factors to regulate their temperature. For example, species with greater cold tolerance exhibit more remarkable plasticity and better life cycle performance, enabling effective development and reproduction (Filho et al., 2021; Vives-Inglá et al., 2023). Research by MacLean et al. (2019) has shown that *Drosophila* species exhibit evolutionary changes and plasticity in both heat and cold resistance, with variation observed between species depending on ambient temperature, particularly in colder habitats. Extreme thermal conditions may act as selection factors influencing the evolution of physiological abilities and cold resistance. This means that arthropods, such as insects, have developed mechanisms to recover from cold hibernation. Insects can be categorized into three groups based on their response to low temperatures: cold-sensitive insects, which die before freezing; cold-avoidant insects, which avoid lethal temperatures by lowering the freezing point of aqueous solutions in their bodies; and cold-tolerant insects, which can tolerate ice formation outside their cell bodies (Carbonell et al., 2021; Kingsolver et al., 2011; MacLean et al., 2019; Scaccini et al., 2020).

Exploring the evolutionary history and ecological aspects of thermal tolerance in insects unveils the intricate interplay between behavioral thermoregulation, thermal physiology, and arthropod ecology (Angilletta et al., 2004, 2006; Carbonell et al., 2021; Willott & Hassall, 1998). Understanding how insects respond to extreme cold temperatures, such as the induction of reversible comatose states known as chill coma, is crucial (Angilletta et al., 2006; Buckley & Huey, 2016; MacLean et al., 2019). Chill coma recovery time (CCRT) is a metric for assessing insect cold tolerance and predicting their

survival strategies in environmental stressors (J. L. Andersen et al., 2015; Awde et al., 2020).

By quantifying CCRT, researchers gain insights into species' sensitivity to climate change, influencing their survival strategies and evolutionary trajectories (J. L. Andersen et al., 2015; MacMillan et al., 2015). Understanding the mechanisms underlying chill coma recovery is fundamental for predicting the ecological dynamics of insect populations in response to shifting environmental conditions (Danks, 2006; R. Harris et al., 2012; Umbers et al., 2013).

2.10 Significance of Chill Coma Recovery Time Ecological Implications of Thermal Tolerance

The recovery from the chill, a phenomenon extensively studied across diverse insect species, holds significant implications for understanding the behavioral, physiological, ecological, and evolutionary aspects of these organisms in the face of adverse temperature changes (Angilletta et al., 2006; Buckley & Huey, 2016; Carbonell et al., 2021; MacLean et al., 2019). However, it is noteworthy that different insect species may employ distinct mechanisms to respond to environmental temperature variations, given the diverse abiotic stressors they encounter (Buckley & Huey, 2016; Kingsolver et al., 2011; MacLean et al., 2019; Pörtner et al., 2006).

The theory of thermal adaptation has emerged to elucidate the evolutionary processes shaping thermoregulation, thermal sensitivity, and thermal acclimation in insects (Awde et al., 2020; Emery et al., 2019; García-Roger et al., 2013; Kalkstein, 1991). Exposure to cold temperatures often induces a reversible comatose state known as chill coma, where neuromuscular transmission and movement halt. This phenomenon serves as a standard metric for assessing insect cold tolerance (Angilletta et al., 2006; Buckley & Huey, 2016; Carbonell et al., 2021; Kingsolver et al., 2011; MacLean et al., 2019; Pörtner et al., 2006).

Understanding how species respond to the chill coma reaction could lead to adaptations that alleviate stress for subsequent generations (R. S. Criddle et al., 2003; Ramadan et al., 2020; Weaving et al., 2022). Therefore, knowledge of chill recovery time, a measure of insect cold tolerance, is crucial for comprehending the performance of these

organisms in various environmental, evolutionary, ecological, behavioral, and physiological contexts. Additionally, understanding the movement of these arthropods, especially during migration, is essential (Motswagole et al., 2019; Mutamiswa et al., 2020; Noor-Ul-ane & Jung, 2021; Weldon et al., 2011).

Several studies emphasize the significance of investigating chill coma recovery in insects, as demonstrated by Ramadan et al. (2020) research. In their study involving the red flour beetle (*Tribolium castaneum*) and the rice weevil (*Sitophilus oryzae*), these insects were subjected to low-extreme cold shocks, inducing chill coma. Through the examination of chill-coma recovery time (CCRT), they identified a factor that delayed their development, signifying physiological costs and resulting in life-history consequences (Awde et al., 2020; Carbonell et al., 2021; MacLean et al., 2019; Pörtner et al., 2006; Vives-Inгла et al., 2023).

To quantify chill-coma recovery, researchers utilize the time taken to recover from a standardized exposure time at a chill-coma-inducing temperature, known as chill-coma recovery time (CCR). This metric was tested in the fall field cricket (*Gryllus pennsylvanicus*, Orthoptera: Gryllidae) by MacMillan et al. (2012). Beyond this specific study, research consistently reveals that understanding the mechanisms underlying this limitation enables predictions regarding species' sensitivity to climate change, influencing their survival and evolution (J. L. Andersen et al., 2015; MacMillan et al., 2015; MacMillan & Sinclair, 2011; Overgaard & Macmillan, 2017).

Therefore, comprehending chill coma recovery in insects is crucial for understanding species' survival and ecology (Danks, 2006; Pincebourde & Casas, 2015). Although some studies have been conducted on grasshoppers, an understanding of physiological ecology remains vital for predicting the impact of weather on insect populations, especially since the nature of the interaction between behavioral thermoregulation, thermal physiology, and the ecology of locusts is still poorly known (Ahnesjö & Forsman, 2003; Chris J. Willott, 1997; R. Harris et al., 2012; Umbers et al., 2013).

3. Methods

Grasshoppers were systematically collected from Prague and close surroundings, known for their rich biodiversity, during the late summer months of August to September 2023. Specimens were captured using an entomological net in the morning and placed in a large plastic box with fresh grass ad libitum and a source of heat and light from a 60w halogen bulb to achieve an acclimation and normal physiological response. After three hours in the box, they were subjected to a cooling regimen. Each grasshopper was individually housed in labeled pots and then transferred to distinct test tubes. A total of 14 species were selected for this study, including *Chorthippus albomarginatus*, *Chorthippus biguttulus*, *Chorthippus dorsatus*, *Chorthippus mollis*, *Chorthippus vagans*, *Euthystira brachyptera*, *Oedipoda caerulea*, *Omocestus haemorrhoidalis*, *Omocestus viridulus*, *Pseudochorthippus parallelus*, *Stenobothrus crassipes*, *Stenobothrus lineatus*, *Stenobothrus nigromaculatus*, and *Stethophyma grossum*, totaling 254 individuals. In all species except *S. lineatus*, a balanced sex ratio was achieved.



Fig 5. Illustrates the spatial allocation of animals and their corresponding labeling for subsequent weight measurements. Each animal was assigned a unique identification (ID) to facilitate control and future species identification. (Photograph by Caio Vitor Sales Lima de Oliveira, author of the thesis)

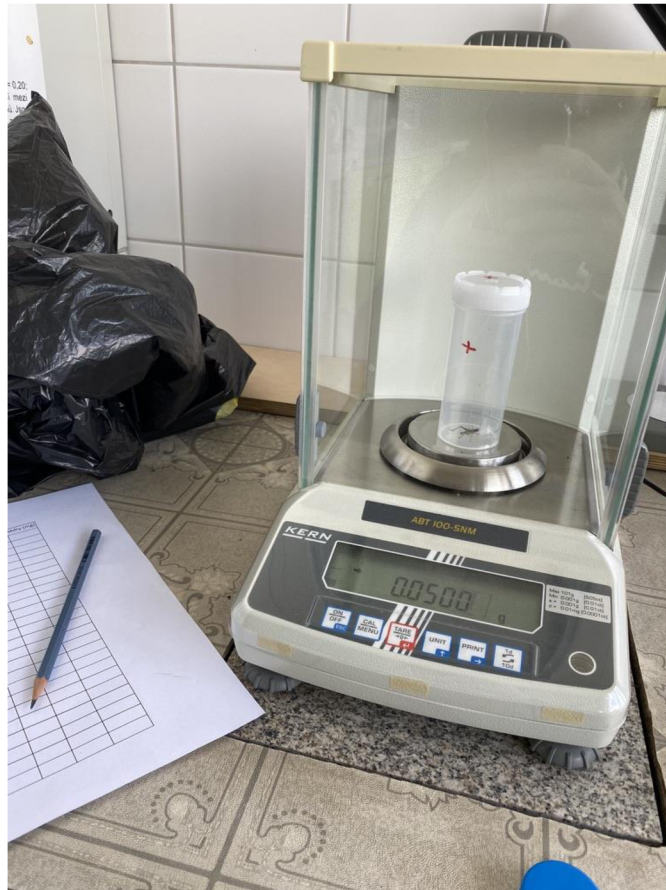


Fig 6. It depicts an animal being weighed using an electronic scale for precise mass determination. (Photograph by Caio Vitor Sales Lima de Oliveira, author of the thesis).

Upon capture, each grasshopper's weight was meticulously recorded as it is a crucial factor for subsequent analysis. After weighing, the animals were carefully returned to their respective containers and acclimated for the cold treatment. To minimize stress, the test tubes were filled with cotton to prevent excessive movement before immersion into a cooling bath. A cooling apparatus was then utilized to lower the temperature of the

bath surrounding the grasshoppers to -3°C . Temperature regulation during the experiment was meticulously maintained using LAUDA ECO water-cooled cooling thermostats, renowned for their exceptional precision and reliability in maintaining stable experimental conditions. These advanced instruments provided precise temperature control, ensuring uniform distribution throughout the setup, which is vital for accurate data collection.

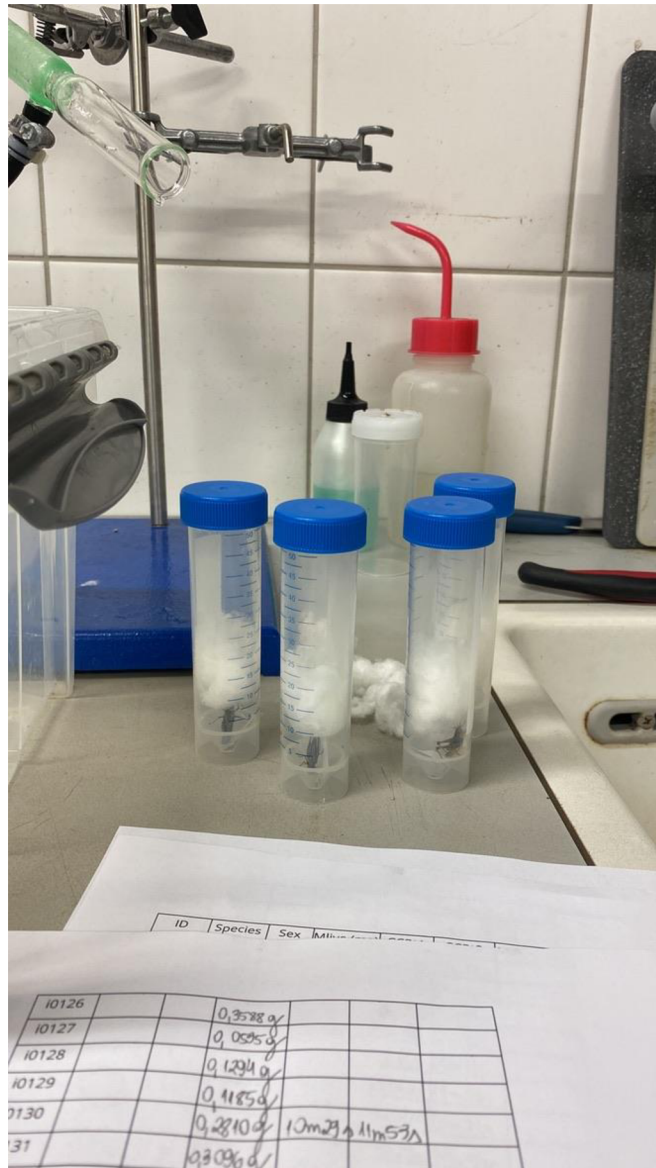


Fig 7. Illustrates the pre-submersion stage of the animals into a cold liquid at -3°C . Cotton was strategically employed to restrain the animals from jumping out of the container. Each vessel accommodated only one animal to minimize stress and ensure experimental precision.

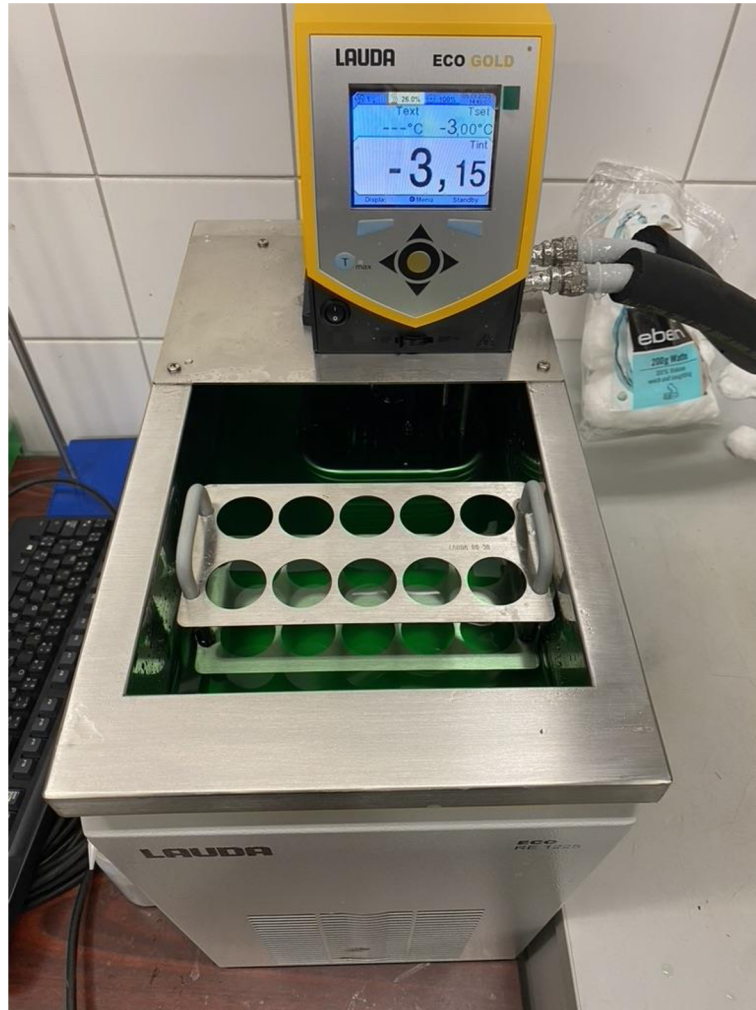


Fig 8. The LAUDA ECO water-cooled equipment features a green-colored refrigerant designed to sustain a predefined low temperature, set explicitly at -3°C . This apparatus is instrumental in furnishing meticulous and regulated cooling conditions, essential for experimental precision and control.

Grasshoppers, housed individually in labeled tubes, underwent a controlled cold treatment at -3°C for one hour to simulate natural exposure to freezing conditions. Subsequently, they were relocated to small Petri dishes and observed under laboratory conditions at an ambient temperature of 20°C . The recovery process from chill coma, characterized by the grasshopper assuming an upright position (CCRt1) and displaying jumping behavior (CCRt2), was closely monitored. All experiments were conducted

within one day of specimen collection to mitigate the stress associated with laboratory captivity.

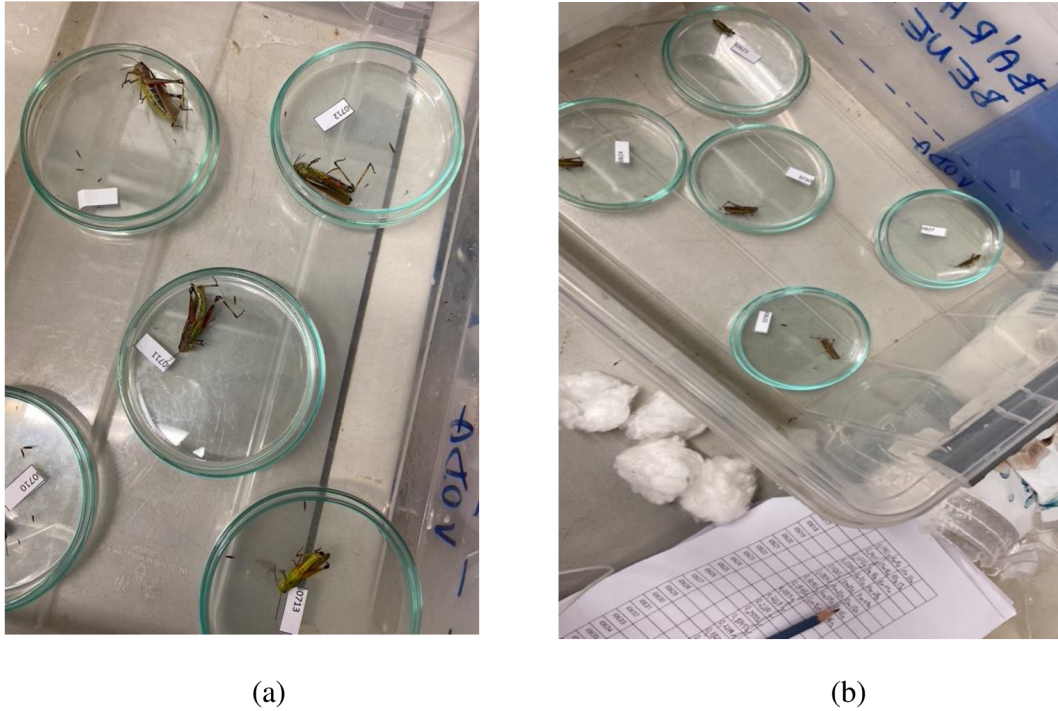


Fig 9. A and B illustrate the animals' relocation to the recovery dishes after being submerged in the cold liquid at -3°C for 1 hour. The animals were meticulously observed during this period, and their respective Chill-Coma Recovery Times (CCRT1 and CCRT2) were recorded for subsequent analysis.

Physiological traits, notably critical thermal limits, were assessed as standard indicators to gauge cold tolerance. Two distinct time points were meticulously recorded: CCRT1, representing the duration until recovery from chill coma in seconds, and CCRT2, marking the interval post-stimulation when grasshoppers exhibited jumping behavior upon prodding. Data analysis employed a linear mixed model, a cornerstone approach in ecological research, implemented through the R statistical software (R Core Team, 2022). Leveraging advanced packages such as lme4, modifications for evolvability in phylogenetically correlated data were applied. Each model incorporated interactions

between variables to unravel nuanced influences on chill-coma recovery times in locust species.

For each species under study, information regarding preference for local moisture and temperature (xerothermophily) was assigned. The Dvořák et al. (2022) study obtained these habitat preference data, where preferences for humidity, vegetation cover, vegetation height, sun exposure, and temperature were detailed. Given the high correlation among these variables, their scaled averages (ranging from 0 to 1) can be regarded as an index of xerothermophily, which we utilized in our study. We also assigned estimated peaks of adult occurrence during the season (phenology).

Data analysis was conducted using a linear mixed model, a foundational analytical approach in ecological research, facilitated by the R statistical software (R Core Team, 2022). Advanced packages such as lme4, which includes modifications for evolvability in phylogenetically correlated data, were employed. We tested the effects of xerothermophily, phenology, weight, and sex on chill coma recovery times while the model set species identity as a random effect. Furthermore, to assess relationships between species, the model was phylogenetically corrected. For further details on phylogeny creation, please refer to Dvořák & Knapp's work in 2023.

4. Results

4.1 Thermal Tolerance Mechanisms and Adaptive Strategies

Our investigation, which lasted from August to September, aimed to uncover the thermal tolerance mechanisms in grasshoppers by measuring chill-coma recovery times (CCRT). This period, encompassing the transition from late summer to early fall, is significant in deciphering physiological responses to environmental temperature fluctuations. We focused our study on species native to Central Europe, seeking to address critical knowledge gaps regarding grasshoppers' thermal adaptations and their ecological implications.

4.2 Habitat Preference and Recovery Dynamics

Exploring habitat preference, or Xerothermophilia, revealed unexpected results: habitat type had minimal influence on chill-coma recovery times. Grasshoppers from diverse environmental conditions, including arid, high-temperature habitats and cooler, moist regions, exhibited similar recovery times. This finding challenges the conventional view linking habitat-specific adaptations to thermal tolerance and recovery efficiency.

4.3 Sex-Based Variation in Thermal Tolerance

Our study uncovered significant sex-based differences in chill-coma recovery times, with females demonstrating notably faster recovery rates (CCRt1 average: X minutes, CCRt2 average: Y minutes) than males ($\chi^2 = 12.6623$, $df = 1$, $p = 0.0003731$ for CCRt1; $\chi^2 = 6.7350$, $df = 1$, $p = 0.009454$ for CCRt2) (Figure 10a and 10b). This disparity underscores the complexity of physiological adaptations to thermal stress, suggesting inherent advantages for female grasshoppers coping with temperature variations.

4.4 Body Mass Influence on Recovery

Investigating the influence of body mass (Mlive) on recovery rates in cold conditions provided convincing insights into the physiological dynamics of locust adaptation to thermal stress. Our analysis revealed a recognizable correlation between locust body mass and recovery time, with larger specimens showing a longer recovery time (Chisq = 6.7797, Df = 1, Pr(>Chisq) = 0.0092201) (Figure 11a and 11b). This correlation highlights the complex interplay between metabolic requirements and thermal adaptation strategies in ectothermic organisms.

4.5 Phenological Influence on Recovery

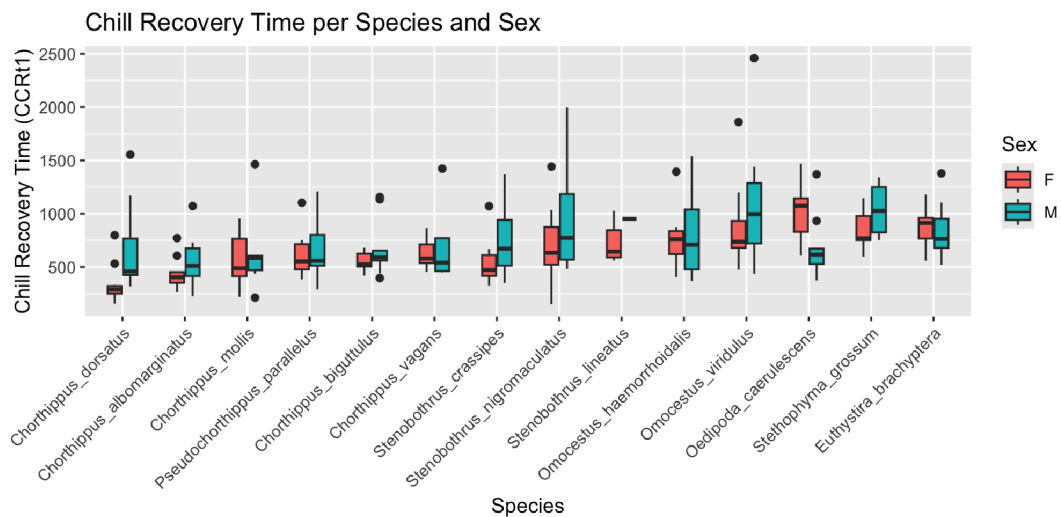
Investigating the influence of phenological timing on cold coma recovery rates revealed interesting nuances in grasshopper physiology and environmental adaptation. Although the statistical significance of the phenological recovery relationship for CCRt2

could not be demonstrated at a conventional level ($\text{Chisq} = 3.4896$, $\text{Df} = 1$, $\text{Pr}(>\text{Chisq}) = 0.061754$), the analysis indicated a complex interplay between locust life cycle stages and recovery dynamics (Figure 12a and 12b).

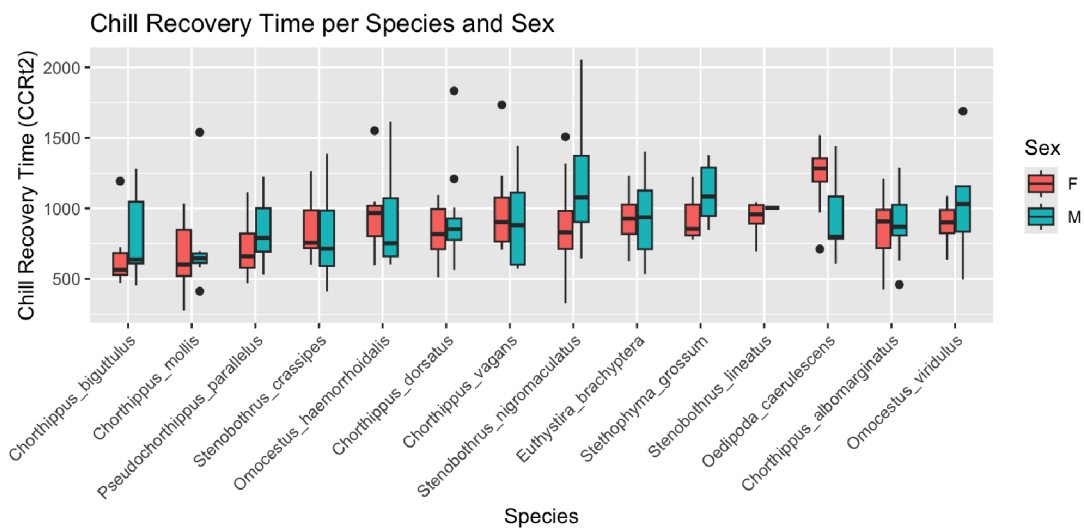
4.6 Environmental Conditions and Thermal Regimes

Examining various thermal regimes illuminated environmental influences on chill-coma recovery. Cold-acclimated locusts showed the most efficient recovery time, with an average recovery time of approximately 16.46 minutes (987.41 seconds), while the control group had a significantly longer recovery time, with an average recovery time of approximately 41.68 minutes (2500.74 seconds). These results highlight the critical role of thermal acclimation in improving locust resistance to cold stress. Analysis of the cold recovery dynamics of fourteen locust species provides valuable insights into the adaptive strategies locusts use to mitigate temperature-induced stress. This helps us understand locust ecology and informs conservation efforts in the face of ongoing environmental challenges.

Figures 10a and 10b - Sex Impact on Recovery in Different Species

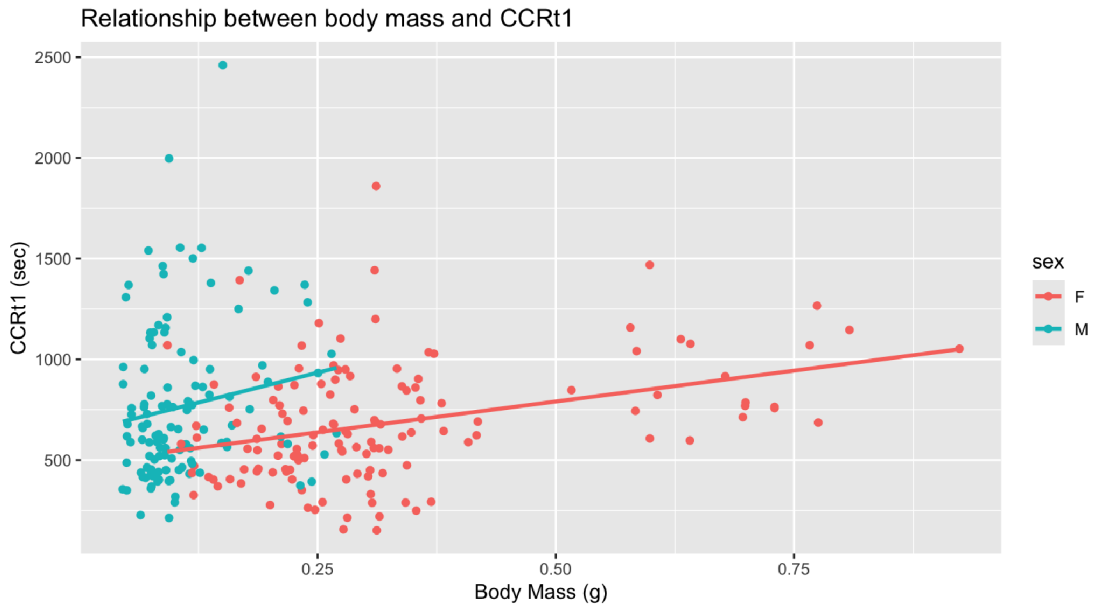


10a: Chill Recovery Time (CCRT1) per Species and Sex- This figure illustrates the time required for grasshoppers of different species and sexes to recover from a chill coma to a standing position, measured in seconds. Each bar represents the average recovery time, with separate bars for males and females within each species, highlighting sex-based differences in thermal tolerance.

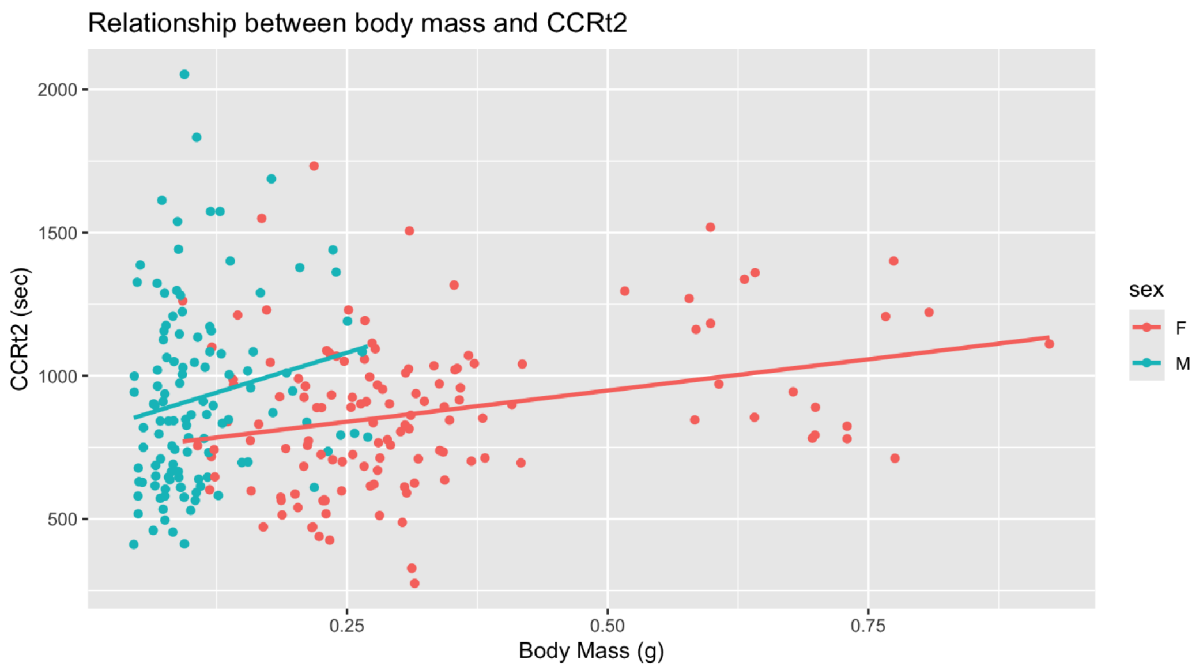


10b: Chill Recovery Time (CCRT2) per Species and Sex—This figure shows the time it takes grasshoppers of various species and sexes to progress from a chill coma to walking, measured in seconds. Like Figure 10a, the bars are divided by species and then further by sex, demonstrating the variance in recovery ability across different groups.

Figures 11a and 11b - Impact of Body Mass on Chill Recovery Time

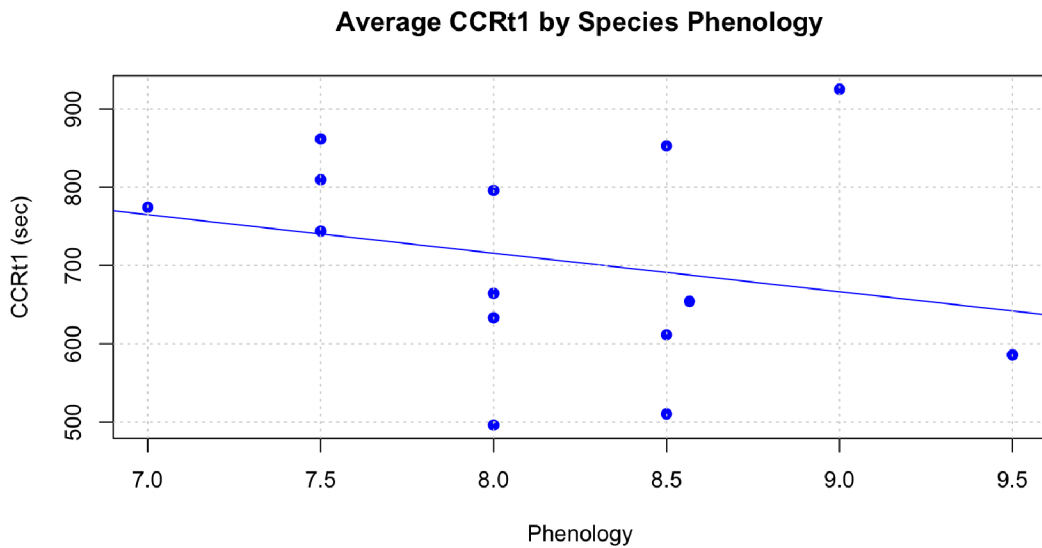


11 a: Relationship Between Body Mass and CCRt1 - Illustrates the relationship between body mass and Chill-Coma Recovery Time 1 (CCRt1), with differently colored dots representing males and females. Through a scatter plot, this figure depicts the correlation between body mass and the time grasshoppers recover to a standing position after a chill coma, measured in seconds. Each point represents an individual, with a trend line indicating the general relationship, suggesting that larger body mass is associated with longer recovery times. The pink regression line represents females, while the blue line represents males.



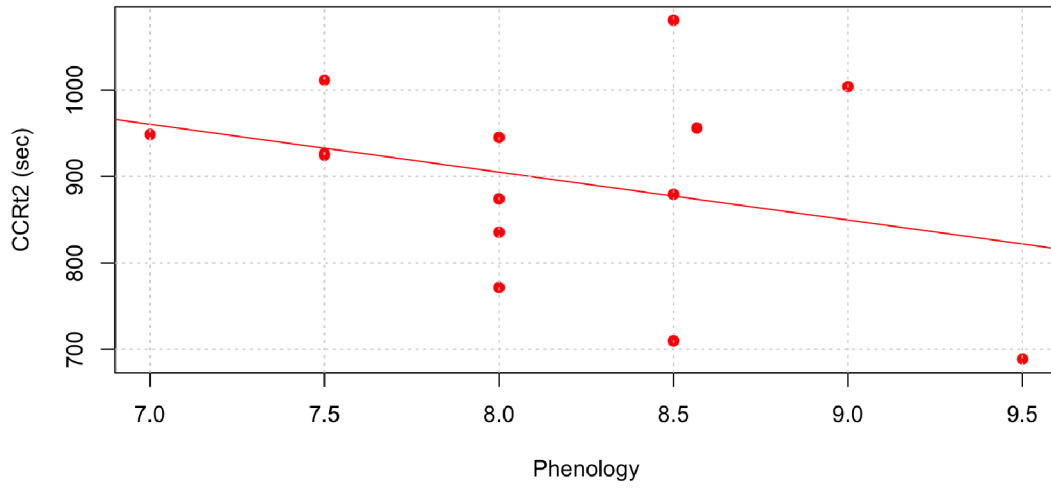
11b: Relationship Between Body Mass and CCRt2 - Similar to Figure 11a, this scatter plot explores the relationship between grasshopper body mass and the time required to recover from walking after a chill coma, measured in seconds. The trend line in this figure further supports the observation that increased body mass may negatively impact recovery speed.

Figures 12a and 12b - Phenological Impact on Chill Recovery Time/Average for Each Species



12a: Relationship Between Peak Occurrence (Phenology) and CCRt1 - This figure illustrates how the timing of peak grasshopper occurrence (phenology; in months number) correlates with CCRt1, the recovery time to a standing position. A significantly shorter recovery time for species peaking later in the season is visualized.

Average CCRt2 by Species Phenology



12b: Relationship Between Peak Occurrence (Phenology) and CCRt2 - Extending the analysis to CCRt2, this figure compares the recovery time to walking with the timing of peak occurrence. Here, the relationship was not significant, but it is still noticeable.

5. Discussion

The thermal tolerance mechanisms of grasshoppers are multifaceted, involving intricate interactions among physiological, ecological, and evolutionary dynamics that govern chill-coma recovery times. Contrary to previous assumptions, our study suggests that xerothermophilia is limited in influencing recovery times (Blackburn et al., 2014; Kellermann & van Heerwaarden, 2019; Santos et al., 2012). This finding challenges conventional perspectives and supports the concept of intrinsic physiological adaptations enabling temperature stress mitigation across diverse habitats, as indicated in prior research.

Our investigation into the thermal tolerance of grasshoppers, focusing on chill-coma recovery times (CCRT), suggests a nuanced relationship between habitat preference and thermal adaptation that diverges from the prevailing view that specific environmental conditions directly dictate recovery efficiency. For instance, despite our anticipation that grasshoppers from xerothermophilic environments would exhibit slower recovery due to presumed physiologic optimization for higher temperatures, our results indicated no significant difference in CCRT across habitats. This finding is particularly intriguing when juxtaposed with the research by Hoffmann & Sgrò (2011) and Macdonald et al. (2004), who demonstrated in *Drosophila* species that evolutionary adaptations to temperature stress could vary significantly within and between populations, suggesting a complex interplay of genetic and environmental factors in shaping thermal tolerance. Similarly, Huey et al. (2012) and Kearney et al. (2018) highlighted the role of behavioral adjustments and physiological plasticity in ectotherm thermal adaptation, proposing that organisms might not solely rely on evolutionary changes but also on immediate responses to environmental fluctuations.

Moreover, our study's suggestion that xerothermophilia has a limited impact on thermal tolerance aligns with the broader findings of Gaston & Chown (1999), who observed in dung beetles that climatic tolerance does not always correlate with elevation or habitat type, indicating that thermal adaptation mechanisms are more complex than previously understood. This complexity is further echoed by Angilletta et al. (2004) and Chown et al. (2010), who explored how thermal plasticity might enable organisms to cope

with varying thermal environments without necessitating long-term evolutionary changes. The apparent contradiction between our expectations based on habitat specificity and the observed uniformity in CCRT across different environmental contexts underscores the importance of considering both plastic and evolutionary responses to temperature stress.

Thus, while our results at first glance appear to challenge established theories by showing a lack of significant difference in recovery times based on habitat preference, they complement the growing body of evidence suggesting that thermal tolerance in ectotherms like grasshoppers involves a multifaceted suite of mechanisms beyond mere habitat adaptation. This realization calls for a deeper exploration into the roles of phenotypic plasticity, physiological resilience, and perhaps unconsidered aspects of behavior or microhabitat selection that contribute to these insects' overarching capacity to withstand temperature extremes.

The observed sex-based differences in recovery times, particularly the accelerated recovery rates in females, highlight the evolutionary implications of thermal tolerance as a selective force. This sexual dimorphism suggests that sex-specific physiological strategies may optimize survival and reproductive efficacy under fluctuating environmental temperatures, consistent with theories proposed by Bublly et al. (2012), Kingsolver & Huey (2008), and MacLean et al. (2019).

Several factors may contribute to the observed sex-based differences in recovery times and thermal tolerance among grasshoppers and other insects. One potential explanation is the differential investment in reproductive functions between males and females. Females often face greater metabolic demands due to egg production, leading to physiological adaptations that enhance their thermal tolerance and recovery rates (Bublly et al., 2012). Additionally, differing ecological roles and behaviors between males and females could contribute to these differences. For instance, male insects may prioritize activities such as mate searching or territory defense, influencing their physiological responses to temperature stress differently than females (Kingsolver et al., 2011, 2012; Kingsolver & Huey, 2008). Moreover, genetic and hormonal factors may shape sex-specific thermal adaptations, as evidenced by studies on the hormonal regulation of thermal tolerance in insects (Kingsolver et al., 2011; MacLean et al., 2019). The complex interplay between reproductive physiology, ecological roles, and genetic mechanisms

likely underlies the observed sex-based disparities in thermal tolerance and recovery times among grasshoppers and other insect species.

Furthermore, the correlation between body mass and recovery durations underscores the relevance of physical and metabolic characteristics in thermal tolerance. Extended recovery times in larger-bodied grasshoppers may reflect a nuanced balance between metabolic rate and thermal inertia, an area ripe for further investigation, especially considering the looming threats of climate change (Bennett et al., 2018; Deutsch et al., 2008; Kearney et al., 2018; Sunday et al., 2012)

Several scientific papers have explored the correlation between body mass and recovery durations concerning insect thermal tolerance (Hallsson & Björklund, 2012). Blackburn et al. (2014) found that larger-bodied insects generally exhibited longer recovery times from chill coma, suggesting a link between body size and thermal resilience. This pattern has also been observed in grasshoppers, as demonstrated by Kellermann and van Heerwaarden (2019), who reported extended recovery times in more prominent individuals. Factors such as surface area-to-volume ratios, metabolic rates, and thermal inertia likely contribute to this correlation (Bennett et al., 2021b; Huey et al., 2012; Kearney et al., 2018; Sunday et al., 2012).

Although our research was confined to a specific seasonal window, it highlights the importance of phenological and environmental diversity in evaluating thermal tolerance. This underscores the need for comprehensive studies over varied seasonal conditions to fully grasp ectotherm adaptation to climate dynamics (Hoffmann & Sgrò, 2011; Sgrò et al., 2016a). Results clearly show a better ability to cope with low temperatures in species occurring later in the season, when shorter days, cold weather, and first-light frosts can occur. In such conditions, quickly recovering from a cold period and effectively using the last warm days can be very beneficial. Such variation in the ability to recover between species with a different season occurrence was, e.g., shown for Wettlaufer et al. (2023), where species occurring early in spring and thus experiencing periods of colder weather can recover faster from chill coma than summer species.

Specifically, we observed a better ability to cope with low temperatures in grasshopper species occurring later in the season. This aligns with leveraging the last warm days after enduring shorter days, cold weather, and initial light frosts. This pattern

corresponds with the notion proposed by Bonamour et al., 2019, and Noh et al. (2017) that phenological cues and environmental heterogeneity influence species' responses to climate change city. While the statistical significance of the phenological recovery relationship for CCRT2 in our study could not be conclusively demonstrated, this finding resonates with the broader recognition of the complexities and uncertainties inherent in phenological studies, as discussed by Bell (2013), and Davis et al. (2021). Overall, our research contributes to the growing body of literature highlighting the intricate interplay between phenology, environmental diversity, and ectotherm adaptation to climate change, emphasizing the need for nuanced investigations and robust statistical analyses to unravel these complex ecological dynamics (Blackburn et al., 2014; Hoffmann & Sgrò, 2011; Sgrò et al., 2016b; Sunday et al., 2012).

Our study contributes to the discourse on grasshopper thermal biology and adaptability in changing climates. By integrating ecological, physiological, and evolutionary insights, we pave the way for nuanced explorations into the adaptive mechanisms employed by species to navigate temperature stress. Nonetheless, the scope of our investigation, limited to specific seasonal dynamics, underscores the critical need to integrate species-specific traits and environmental adaptations in assessing thermal tolerance and physiological resilience among grasshoppers and other insect taxa.

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