Comparative Biochemistry and Physiology, Part A 260 (2021) 111037

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Editorial on combatting the cold: Comparative physiology of low temperature and related stressors in arthropods

1. Introduction

In this Special Issue of *Comparative Biochemistry and Physiology - Part A*, we bring together 16 articles that advance our understanding of the low temperature physiology of insects and other arthropods. These studies cover many levels of biological organization, including molecular responses to temperature stress, organ and tissue systems affected by cold, whole organism physiology, and population-level responses. This issue not only reflects the breadth of integrative approaches being applied to this topic, but also the diversity of species studied (27 different arthropod species are featured in the 16 articles) and the diverse international community engaged in this work (with authors from 10 countries across five continents). In addition, the Special Issue features the stellar work being done by early career researchers in our discipline – for 13 articles, the lead author was either a student, postdoc, or recent graduate.

As small-bodied ectotherms, the biology and distribution of insects and other arthropods is tightly linked to environmental temperature (Chown et al., 2004). Several high-profile papers have demonstrated that latitudinal distributions and population dynamics are tightly linked to thermal tolerance, and consequently climate change will lead to range shifts, increased risks of extinction, and changes in insect pest pressure (e.g., Deutsch et al., 2008; Deutsch et al., 2018; Parratt et al., 2021; Sunday et al., 2011; van Heerwaarden and Sgrò, 2021; Vasseur et al., 2014). Much of this work has focused on responses to high temperatures, and yet arthropods at temperate and polar latitudes often have to spend more than half the year in the overwintering phase to combat the seasonal challenge of cold (Leather et al., 1995). Thus, a thorough understanding of ectotherm responses to variable environments and climate change requires extensive knowledge of seasonal responses and associated cold tolerance (Bale and Hayward, 2010; Williams et al., 2015). Further, low temperatures cause widespread physiological disruptions, so integrative studies of physiological systems in the cold can provide fundamental insights into ectotherm function and the processes that determine survival in extreme conditions (Overgaard and MacMillan, 2017).

Our goal in this special issue was to highlight the diversity of research on arthropod cold physiology, and so submissions weren't confined to specific topics. However, some core thematic elements emerge from this collection of papers, which we summarize below. Specifically, the articles in this issue highlight 1) mechanisms of cold injury that limit survival, 2) the importance of considering multiple challenges beyond low temperature when investigating overwintering physiology, including the influence of diet, metabolism and energy budgets, 3) the role of phenotypic plasticity, 4) inter- and intraspecific variation in cold tolerance physiology, and finally, across all of these studies 5) the importance of comparative work using both model and non-model species for capturing diverse adaptations to low temperature.

2. Mechanisms of cold injury

Low temperature causes chilling injury through several mechanisms. An initial decrease in membrane potential impairs neuromuscular function, and prolonged chilling leads to disrupted ion and water balance in tissues, which can then trigger cell death (Overgaard and MacMillan, 2017). Overgaard's group is at the forefront of research on membrane responses and associated ion transport under cold stress, and they provide two new important studies here. Gerber et al. (2021) identify how cGMP and CHA-dependent ion transport in the rectum are normally blocked during cold exposure but are preserved by cold acclimation. In addition, Bayley et al. (2021) employ a mathematical "charge difference" model to investigate membrane processes that explain membrane potential (E_m) in insect muscle, confirming the importance of both Na^+/K^+ ATPase and selective ion channels in maintaining E_m stability in the cold. Together, these articles add important details to our understanding of ion transport in the cold and generate intriguing hypotheses for follow-up studies.

Despite recent progress in our understanding of cold injury, there are still many unknowns, including whether different types of cold exposure (i.e., short vs. long-term, mild vs. severe) cause similar or distinct physiological injury, and how fluctuating thermal regimes (FTR), with brief recovery periods, can help reduce cold injury. In addition, we have a limited understanding of the different molecular processes that determine sensitivity to cold injury (Hayward, 2014), especially the processes that might contribute to reduced cold injury under FTR. In this issue, Cambron et al. (2021) investigate how genes in the insulin signaling pathway vary throughout winter diapause in the alfalfa leafcutting bee, *Megachile rotundata,* and determined that gene expression underpinning this pathway responds differently to FTR vs. static thermal regimes. Further studies are now needed to identify any causal link between the expression of these insulin signaling genes and protection from cold, and how these results may relate to overwintering energetics (see discussion below).

3. Combined stressors during winter

While low temperature is the most conspicuous feature of winter environments, overwintering arthropods must often cope with multiple

https://doi.org/10.1016/j.cbpa.2021.111037

Available online 15 July 2021

stressors simultaneously. In addition to cold injury, overwintering arthropods are at risk of energy drain. Reduced metabolism in the cold often prevents active foraging and feeding, and many food sources for arthropods are unavailable from late autumn and throughout the winter. As a result, most terrestrial arthropods enter a state of dormancy (i.e., diapause or quiescence), often well in advance of winter cold, and rely on bulk energy storage to sustain them during the winter months (Hahn and Denlinger, 2011). Increasingly warmer autumn and/or winter conditions are putting many temperate and polar species at higher risk of energy drain, which can reduce fitness in the spring or lead to death (Williams et al., 2015). Thus, characterizing the physiological adaptations that allow overwintering arthropods to store and conserve energy is critical for predicting arthropod responses to environmental change. In recent years, studies ranging from ecophysiological (e.g., Marshall and Sinclair, 2018; Potts et al., 2020; Williams et al., 2012) to molecular (e.g., Poelchau et al., 2013; Sim and Denlinger, 2008; Sim and Denlinger, 2013) have investigated how arthropods allocate energy to different functions in the winter.

In this special issue, Rozsypal et al. (2021) report how female *Culex pipiens* mosquitoes arrive at protected winter microhabitats with sufficient lipid reserves to persist for potentially two winter periods (~500 days). However, if females are disturbed or initiate flight to seek alternative overwintering sites, then fast oxidation of lipid reserves occurs, which could limit winter survival. In contrast, the apple maggot *Rhagoletis pomonella* remains inactive throughout winter and can survive low sub-zero temperatures, whether in winter diapause or a cold-induced quiescence (Toxopeus et al., 2021). However, the inherently lower metabolic rates of diapausing larvae, compared to quiescent individuals, make them better able to retain sufficient energy reserves under prolonged chilling. Oxidative stress is another challenge related to overwintering metabolism, as prolonged periods of cold disrupt metabolism and can lead to oxidative imbalance (Lalouette et al., 2011). In this issue, King et al. (2021) show that the antioxidant gene *oxidor* is upregulated during diapause in the mosquito *C. pipiens,* and that knockdown of this transcript increases degradation of ovarian follicles and reduces longevity in diapausing females.

Beyond the role of diet in sustaining energy levels, specific dietary components can also directly contribute to cold tolerance. For example, Koštál et al. (2011) provided clear evidence of how certain free amino acids, such as proline, can enhance freeze tolerance. Dietary lipids and their direct influence of membrane fatty acid composition are also known to influence cold tolerance phenotypes (Murray et al., 2007). Within this special issues, Littler et al. (2021) determined that composition of artificial diets can influence cold tolerance in *Drosophila melanogaster,* but that it does so in a genotype-specific manner. This study is particularly important because it emphasizes how insect diets must be carefully considered to ensure accurate comparisons across studies.

While many studies of overwintering stress address one type of stressor at a time (e.g., cold stress, energy stress, dehydration, etc.), in nature organisms likely experience multiple stressors simultaneously. The interactions between these distinct stressors can be complex, sometimes causing cumulative effects that are greater than might be predicted from individual stressors, while other times leading to cross-tolerance (Sinclair et al., 2013). When distinct stressors converge on the same injury mechanisms (ion imbalance, protein denaturation, membrane damage, etc.), multiple stressors can increase risk of mortality. However, exposure to one stressor can sometimes increase tolerance to another stressor, and cross-protection to distinct stressors can occur through either cross-talk (i.e., shared signaling pathways) or cross-tolerance (i.e., shared downstream protective mechanisms), or a combination of both (Everatt et al., 2015). For example, freezing and desiccation stress both lead to internal osmotic challenges and cell shrinkage (Toxopeus and Sinclair, 2018), and indeed, prior exposure to desiccation can improve freezing tolerance (Hayward et al., 2007; Kawarasaki et al., 2019). Yoshida et al. (2021) extend our understanding of combined cold and desiccation tolerance mechanisms in this special issue. Their study documents how two aquaporins in the Antarctic midge, *Belgica antarctica,* appear to play a key role in cryoprotective dehydration and desiccation stress in dry air, but respond differently to hyperosmotic stress in solution. Thus, even though desiccation in air and hyperosmotic stress are predicted to result in similar dehydration at the cellular level, the molecular response appears to be distinct.

4. Phenotypic plasticity at low temperature

To cope with cold injury and energetic challenges of winter, arthropod physiology is shaped by both evolutionary adaptation and phenotypic plasticity. For many arthropods, environmental cues trigger transition to a distinct overwintering state, which is often characterized by suppressed metabolism, arrested development, and increased stress tolerance (i.e., diapause; Denlinger et al., 2005). In addition to distinct developmental transitions like diapause, insects can increase resistance to cold injury through seasonal cold acclimatization and short-term cold hardening (Teets and Denlinger, 2013; Teets et al., 2020). Rapid cold hardening (RCH) can occur within minutes to hours of cold exposure (Lee and Denlinger, 2010) and is a crucial adaptation in environments with potentially rapid day to night-time fluctuations in temperature, such as alpine, polar, and continental interior climates. Accounting for these plastic responses to temperature is critical for predicting adaptability to temperature change in natural environments (Sgro et al., 2016), and there have been an increasing number of studies that address the mechanisms of cold acclimation. As predicted, cold acclimation influences many of the physiological processes negatively impacted by cold, including ionoregulatory processes (e.g., Des Marteaux et al., 2018; MacMillan et al., 2017), membrane fluidity and composition (e.g., Koštál et al., 2003; Michaud and Denlinger, 2006), and protein homeostasis (Colinet and Hoffmann, 2012).

While recent studies have identified important physiological changes that accompany cold acclimation, many molecular correlates of short- and long-term cold acclimation have unclear functional relevance (Des Marteaux et al., 2017; Lecheta et al., 2020; MacMillan et al., 2016; Poupardin et al., 2015). Thus, additional research is needed to comprehensively assess the myriad physiological processes that influence cold tolerance plasticity. Ongoing areas of investigation include the precise organ systems and molecular processes that are responsible for cold acclimation observed at the organismal level, the extent to which plasticity in the cold preserves essential ecological functions following sublethal stress (in addition to its more well-defined role in extending thermal limits), and the extent to which different species and populations rely on plasticity vs. adaptation to shape their overwintering physiology. Together, characterizing cold tolerance plasticity at the molecular, physiological, and population-level will improve understanding of the evolutionary physiology of cold tolerance and inform predictions on species distributions and temporal dynamics of arthropod populations.

In this special issue, Cheslock et al. (2021) present evidence that cold acclimation alters Na^{+}/K^{+}-ATPase pump activity in a tissue-specific manner in *D. melanogaster,* and that increased activity in certain tissues may be important for maintaining K^{+} homeostasis in the cold. These results again point to the critical role of membrane function and ion balance in determining cold injury, as discussed above. Benoit et al. (2021) report how RCH in several tick species reduces chill coma temperature, as well as increasing survival and questing behavior at low temperatures. Concomitant increases in oxygen consumption at low temperatures, however, and the associated metabolic expense are predicted to reduce longevity. In contrast, Anthony et al. (2021) report that cold tolerance of sub-Arctic wolf spiders is largely non-plastic, at least in response to short-term cold acclimation, and it remains a mystery how these species survive extended winter cold at high latitudes. Mensch et al. (2021) bring together many themes within this special issue and show that tropical and temperate *Drosophila* species differ in lower thermal limits and cold tolerance plasticity, and that phenotypic plasticity in cold tolerance is tightly linked to reproductive status. Metabolic signatures underpinning this plasticity suggest that cold acclimated females of temperate species accumulate energy-related and protective metabolites (e.g., glycerol and proline) while delaying reproduction, and that these metabolites are relevant to cold tolerance even at modest concentrations.

5. Inter- and intraspecific variation in cold tolerance

Physiological studies can also help clarify the evolutionary processes that shaped cold tolerance across the arthropod phylogeny. Most lineages of organisms originated in the tropics (Brown, 2014), necessitating the evolution of cold tolerance as arthropods moved poleward. While large-scale assessments of interspecific variation in cold tolerance are challenging, studies within select taxa have revealed important insights into the evolution of cold tolerance. Several cold tolerance parameters align closely with latitude (Andersen et al., 2015), and basal tolerance to cold appears to constrain plasticity (Nyamukondiwa et al., 2011), suggesting potential tradeoffs between investment in basal cold tolerance and the ability to shift cold tolerance in response to environmental signals. As discussed above, plastic responses like cold acclimation and rapid cold hardening are pervasive across the arthropod phylogeny (Teets et al., 2020), suggesting an early evolutionary origin, while more specialized adaptations like freeze tolerance appear to have evolved multiple times (Sinclair et al., 2003). Given that cold stress affects multiple physiological systems simultaneously, the evolution of cold tolerance across arthropods likely involves gradual, incremental transitions, but additional research in understudied taxa is needed to more accurately reconstruct the evolution of cold tolerance.

While interspecific variation in cold tolerance sets potential species boundaries, characterizing intraspecific variation in cold tolerance is important for predicting a species' ability to invade new environments in response to environmental change. Classic latitudinal clines exist for some cold tolerance traits (Hoffmann et al., 2002), indicating that local adaptation can shape cold tolerance of geographically separated populations. There can also be substantial variation in cold tolerance within a single population (e.g., Teets and Hahn, 2018), such that cold tolerance has high heritability and can likely evolve in response to rapid environmental change. Indeed, recent field cage studies indicate that cold tolerance can shift across generations over the course of the year to track seasonal changes in temperature (Rudman et al., 2021). Epigenetic/trans-generational influences on seasonal strategies and associated cold tolerance therefore also need to be considered (Coleman et al., 2014). As an added layer of complexity, distinct cold tolerance traits (e.g., cold shock tolerance, thermal limits, chill coma recovery time) are genetically distinct, at least in *D. melanogaster* (Garcia et al., 2020), and likely have the capacity to evolve independently. There can also be intraspecific variation in cold tolerance plasticity traits (Gerken et al., 2015; Ørsted et al., 2019), such that acclimation capacity has the potential to evolve to changes in thermal variability. Thus, multiple aspects of cold tolerance can vary both between and within species, and ongoing efforts seek to understand the physiological mechanisms that underly this variation.

In their study of six species of ixodid ticks in this special issue, Fieler et al. (2021) noted significant interspecific differences in cold and heat tolerance, despite most species having a similar thermal preference and metabolic rate adjustments across a temperature gradient. Hidalgo-Galiana et al. (2021) report here on geographic variation in the acclimation responses of both high and low thermal tolerance in South African diving beetles (Coleoptera: Dytiscidae). As observed in previous studies, upper thermal limits demonstrated limited acclimation-induced plasticity, while lower thermal limits demonstrated far greater plasticity with interspecific variation that could be explained by both phylogeny and ecoregion. Butterson et al. (2021) also report population differences in the plasticity of cold tolerance phenotypes in the eastern spruce budworm, *Choristoneura fumiferana.* While there is no evidence for transgenerational plasticity in cold tolerance in this highly destructive boreal forest pest, there is local adaptation in both seasonal and short-term plasticity of cold hardiness, with more northerly populations demonstrating higher degrees of plasticity.

6. Insights from non-model species

Like many disciplines, a disproportionate number of studies on cold tolerance have been conducted in select model species. Terrestrial arthropods are the most diverse animals on the planet, and the physiological and molecular mechanisms that underly overwintering survival appear to be similarly diverse. For example, insects can undergo diapause in any life stage, but molecular studies of diapause are biased towards dipteran pupal diapause (Ragland and Keep, 2017). Furthermore, even within Diptera, transcriptional responses to diapause are incredibly diverse, such that only a handful of molecular mechanisms appear to be shared across distinct diapause programs. Organismal studies of cold tolerance are also biased to certain taxa; for example, most studies on intra- and interspecific variation in cold tolerance (see above) were conducted in *Drosophila,* a short-lived, cosmopolitan genus that primarily overwinters as adults. While huge insight can come from studies of highly tractable species such as *D. melanogaster,* as evidenced by this special issue (see Cheslock et al., 2021; Littler et al., 2021), comparative studies are needed in other taxa to determine the extent to which evolutionary patterns of cold tolerance are consistent across the diversity of arthropods.

Many unique overwintering adaptations are found outside of classic model species. For example, none of the well-studied insect models (e.g, *Drosophila, Tribolium, Bombyx,* etc.) are capable of freeze tolerance, which has made it challenging to investigate the molecular basis of this important adaptation. Recent work is developing tractable laboratory models for freeze tolerance (Toxopeus et al., 2019), which when coupled with high-throughput sequencing technologies, will make it possible to study these adaptations at a level that was previously impossible. In addition to the general utility of studying unique adaptations in non-model species, information from these studies can inform hypotheses for species living in less extreme conditions. As an analogy, research on high altitude adaptation in humans and animals (Witt and Huerta-Sánchez, 2019) has informed some general principles on oxygen transport and cardiovascular disease states, and similarly, research on extreme-adapted insects may reveal the key process that limit survival in more typical environments.

As set out at the start of this editorial, this special issue presents research papers on 27 different arthropod species. Several of these are important vectors of diseases, such as the mosquito *C. pipiens* (King et al., 2021; Rozsypal et al., 2021) or tick species (Benoit et al., 2021; Fieler et al., 2021), while the eastern spruce budworm *C. fumiferana* is an important pest of forest stands. Another emerging forest pest, the Asian longhorned beetle *Anoplophora glabripennis,* is a relatively new target for comparative stress physiology research and is featured in this issue by Torson et al. (2021). Understanding the adaptability of this species to different climate zones and changing temperature regimes has thus taken on increasing urgency, and Torson et al. (2021) provide the first systematic characterization of its cold tolerance strategies and lower lethal limits. Eggs and larvae both have very low supercooling points (ca. -25 °C), but eggs are freeze-avoidant while larvae are freeze-tolerant. Pupae are notably less cold tolerant and exhibit symptoms of sublethal chilling injury well before freezing. Such information about the capacity of different life stages to tolerate cold is key to better understanding the potential distribution and establishment risk for this, and many other, pest insects.

7. Conclusions

The above introduction just scratches the surface regarding the exciting work being conducted on arthropod cold tolerance. Beyond its fundamental importance, knowledge of cold tolerance and its mechanisms has several important applications, including predicting distributions of pest species, documenting spread and establishment of invasive species, and modeling population dynamics. Further, biomedical applications like cryopreservation can benefit from extensive knowledge of cold tolerance mechanisms (Brockbank et al., 2011). The articles in this issue make several important advances in our understanding of cold injury, phenotypic plasticity, variation in cold tolerance, and molecular mechanisms of overwintering survival. Arthropods are the most diverse group of metazoans on the planet, and fittingly, the articles in this issue highlight that diversity.

Declaration of Competing Interest

None.

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