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# **Title: Insects in Fluctuating Thermal Environments**

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Running title: Effects of Fluctuating Temperatures

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## **Abstract**

All climate change scenarios predict an increase in both global temperature means and the magnitude of seasonal and diel temperature variations. The nonlinear relationship between temperature and biological processes means that fluctuating temperatures lead to physiological, life history and ecological consequences for ectothermic insects that diverge from those predicted from constant temperatures. Fluctuating temperatures that remain within permissive temperature ranges generally improve performance. By contrast, those which extend to stressful temperatures may have either positive impacts, allowing repair of damage accrued during exposure to thermal extremes, or negative impacts from cumulative damage during successive exposures. We discuss the mechanisms underlying these differing impacts. Fluctuating temperatures could be used to enhance or weaken insects in applied rearing programs, and any prediction of insect performance in the field – including models of climate change or population performance – must account for the effect of fluctuating temperatures.

**Keywords:** Temperature variations, Jensen's inequality, life-history traits, thermal tolerance, climate change

## 1. Introduction

Insects drive terrestrial ecosystems, and – as small ectotherms – their biology is closely linked to environmental temperature. Temperature therefore determines insect survival, population dynamics, and distribution (1, 23, 24), and thus their responses to climate change (4, 22, 38). Temperature in the field fluctuates, and the impact of these variations has been recognized in areas as diverse as forensic entomology (18, 53), thermal tolerance physiology (9, 80, 96), biocontrol (13, 28), insect-mediated pollination (98, 124), disease vector biology (73, 87) and simulated climate warming studies (4, 10, 56, 116, 126).

Researchers in the early 1900s reported that insects grow faster under fluctuating temperatures (FTs) compared to constant temperatures (CTs), (34, 100), and early reviews (25, 93) acknowledged that FTs reflected natural conditions better than CTs. In the context of development, these early reviews already pointed out that the “non-linear temperature-velocity relationship” means that FT treatments should be “the norm” while CTs were essentially conducting insect development studies under “abnormal” conditions (25, 93). In the 1970s, it became apparent that FTs improved thermal tolerance of insects over those exposed to CTs (17, 82), and that fitness could be greater in FTs (6). Research on FTs resurged in the early 2000s, particularly in the context of insect cold tolerance (75, 83, 96). Presently, FTs are under extensive investigation in the context of climate change and the extrapolation of laboratory studies to the field, with the goal of incorporating thermal variability and extreme events in ecological and physiological studies (4, 109, 116).

Here, we synthesize the disparate work on the impact of FTs on insects, emphasizing the need for particular care when interpreting results derived from static designs. We give an overview of the methods and approaches that have been used to explore the differences between insect

responses to FTs and CTs, and focus on general principles and responses, rather than specific organisms.

## **2. The biological impacts of temperature**

### **Thermal variability in the environment**

The environmental temperature in terrestrial habitats fluctuates on multiple time scales (36, 80). The amplitude of daily thermal fluctuations varies by season and habitat (92), and can be over 30°C (102). At high latitudes and altitudes, these fluctuations may cross a species' freezing threshold at any time of year (80, 112). Likewise, temperatures fluctuate above thresholds for heat shock year-round in hot climates (47). Weather patterns that occur over multiday periods can modulate the amplitude of diel temperature cycles within a season (80). The occurrence and amplitude of daily FTs can also be modulated by habitat (45), and microhabitat (119). For example, the insulating effect of snow cover or thermal inertia from soil, trees or litter (36); but these microclimate temperatures are generally not well-captured by global-scale weather datasets. Thus, individual insects may experience FTs on a scale that fits within the developmental period and lifespan of even short-lived species. As a consequence, they must constantly adjust their physiology to changing thermal conditions.

### **Temperature effects in biology**

In ectothermic animals like insects, thermal performance curves (TPCs) are nonlinear and asymmetric (1) (**Figure 1**). Temperature shifts will thus result in uneven effects depending on whether the temperature varies above or below the optimal temperature (99). Even at permissive temperatures, an animal can pass physiological thresholds during a thermal cycle, reaching critical temperatures such as the critical thermal minimum ( $CT_{min}$ ) or maximum

( $CT_{max}$ ). At extreme temperatures, the temperature-process relationship can change abruptly; for example, proteins are denatured by heat, and water freezes at low temperatures (24, 74). The asymmetry of TPCs places the maximum rates of TPCs close to the upper thermal limits (1, 81, 99); thus, small increases in temperature may push insects over the  $CT_{max}$  (**Figure 1**). At low temperatures, the changes in rates are slower, and therefore there is less chance of hitting abrupt limits. In the concave (accelerating) part of the TPC, the total ‘output’ of a rate process in FT-exposed insects will exceed that predicted for CT-exposed insects with an equivalent mean (45, 57, 81). This disproportionate effect is exacerbated by FTs with greater amplitude (**Figure 1**). The opposite will be observed in the convex (decelerating) part of TPC. This phenomenon, known as Jensen’s inequality (57), explains many of the discrepancies between FT and CT experiments. The physiological response to FTs, such as metabolic rate changes, are asymmetrical (119), with limited effects of decreasing temperatures and larger effects of increasing temperatures (57, 81) (**Figure 2**). The discrepancies between FT and CT experiments will also depend on the degree of thermal sensitivity of the process, with smaller effects of FTs when thermal sensitivity is lower (i.e. lower degree of curvature), and the amplitude of the thermal cycle: larger amplitudes will have a greater impact (**Figure 2**) (45, 99). Although this means that development should be faster under FTs than CTs, the energetic costs incurred by a fasting ectotherm will be greater in the warming part of a daily cycle than the energetic savings resulting from the cooling part, especially in thermally-sensitive species (**Figure 2**) (119); thus, fluctuating environments are more energy-demanding than static environments.

### **3. Design and interpretation of experiments incorporating FTs**

The term “fluctuating temperatures” covers a range of timescales and temperature transitions. Insects can respond to these fluctuations in ways stretching from hardening responses (on the

scale of minutes) to evolutionary responses over geological time. Here, we focus on FTs that recur more than once within a single developmental stage, although FT experiments may apply those fluctuations throughout development. The FT literature contains almost as many exposure regimes as it does experiments, from the simple use of two alternating temperatures to more sophisticated simulations of the daily temperature patterns. A glimpse of the diversity of these approaches is summarized in **Figure 3** and in **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at <http://www.annualreviews.org>).

The temperatures included in an FT experiment will be dictated by the purpose of the study and by the tolerances of the insect. An initial decision is whether the fluctuations should be within the permissive range – appropriate if the goal is to understand diel thermal cycles (62, 87) – or include extreme temperatures – appropriate if the goal is to understand the consequences of crossing physiological thresholds (80, 83). Although it may be sufficient to have simple step-function transfers from one temperature to another, ramped temperature changes, or even curvilinear temperature regimes, will better reflect the natural environment (**Figure 3**). These temperature regimes will differ in the amount of time spent outside the permissive temperature range.

Many FT experiments use a CT equivalent to the mean of the FT as a control. However, controls must account for the amount of time spent at high or low temperatures, taking into account the non-linear effects of FTs on physiological rates. Sinclair & Marshall (80) suggested a ‘matched cold’ design (also adaptable to heat experiments) which includes a control for the effect of a single exposure equivalent to one cycle of the regime, and a control which exposes the insect to the low temperature for an amount of time that is equivalent to the total cumulative amount of time exposed to cold. This design limits the choice of



temperatures to those that the insect can survive for a long period. Because FT experiments are often conducted over multiple cycles, experimental animals are aging: an insect that is exposed to ten daily cycles is not only responding to the repeated cycles, but is also ten days older than an animal exposed on the first day. Simple preliminary experiments should be carried out to rule out any putative ageing effect. Finally, variables other than temperature fluctuate in the wild, and these may provide important cues for physiological responses. For example, photoperiod and humidity cycles may be as important as temperature (8, 64). Fortunately, these cues are often synchronized with temperature cycles, so laboratory procedures can fairly easily reproduce this synchronicity.

#### **4. Effects of FTs on life-history traits and fitness**

##### **Development**

Fluctuating temperatures that extend to deleterious high or low temperatures can allow development outside the temperatures where it would normally occur (37, 46, 76, 90). However, FTs using deleterious temperatures generally delay development compared to development at optimal CTs (46, 63). These delays are likely a consequence of direct cold or heat injuries and of the costs of subsequent physiological and biochemical repair (23, 43). By contrast, FTs that remain within the permissive thermal range can result in diverse responses, including accelerated development (2, 13, 44, 65, 66), slower development (25, 42, 66), or no change in developmental rate (65). One explanation of this variation in responses is that the effect of FTs on the development may depend on the thermal mean that is used and its proximity to developmental thresholds (65). Accelerated development appears to be the norm if the lower temperature of the FT is not injurious, but falls below a species' thermal threshold for development (93). Finally, the effect of FTs on development time also depends on the

amplitude of the variation (14, 42, 46, 66), likely because of Jensen's inequality. For example, *Aedes aegypti* mosquitoes reached pupation four days faster when reared under large (18.6°C), rather than small (7.6°C), daily FTs (14).

## **Morphology**

The body size, shape, and symmetry of imagoes integrate the stresses experienced during development, and can thus provide a measure of developmental stability. Perhaps the most subtle morphological impact of developmental stress is fluctuating asymmetry (FA) (7). Early studies comparing FA of CT- vs. FT-reared *Drosophila* were contradictory: FTs led to both reduced (5) and increased (11) asymmetry. Temperature cycles that included a cold stress during development reduced FA in the noctuid moth *Helicoverpa punctigera*, although the experimental design did not allow for the effects of FTs and low temperatures *per se* to be teased apart (54). FTs that approach thermal limits can also result in increased variability of morphological traits (89). Thus, FTs that encompass deleterious temperatures appear to increase phenotypic variation and developmental instability.

The temperature-size rule predicts that development at higher temperatures should result in small-sized insects (3). If, like other rate processes, the temperature-final size relationship is curvilinear, Jensen's inequality would predict a disproportionate influence of high temperatures under FTs (81, 99). Indeed, FTs with large thermal amplitudes reduced the pupal size of *Manduca sexta* (65), and thorax size, wing size, and body weight (35, 42, 89, 90) of drosophilids. Reduced size is likely mediated by an energy use-structural allocation tradeoff (more energy is diverted to metabolism and maintenance at higher temperatures), and earlier maturity possibly because elevated temperatures affect the differentiation rate of the cells

more than their growth rate (114). However, the effects of FTs on cell differentiation is scarce, but see (71).

## **Lifespan**

Fluctuating temperatures have been reported to increase (33, 42), decrease (13, 16), or have no effect (66) on lifespan. These discrepancies likely arise from the diversity of species and approaches, and a systematic comparative approach (e.g. (84)) could yield a more meaningful signal. If injury is incurred during the high or low temperature portions of FTs, a straightforward tradeoff between damage repair and somatic maintenance could reduce longevity. However, *Alphitobius diaperinus* exposed to 5°C alternating with 20°C showed a large overshoot in oxygen consumption associated with increased reactive oxygen species (ROS) production during the warm period (72), which is consistent with the theoretical role of ROS production as an underlying mechanism of ageing (104). From this, we predict that FTs that do not lead to lifespan reduction would not increase ROS production. Because metabolic rate (and presumably ageing) fluctuates in a curvilinear fashion throughout the FT (10), FTs may decouple physiological age from chronological age, yielding a complexity of results consistent with the observed discrepancies in FT effects on longevity.

## **Fecundity**

Reproductive output is a central component of fitness, and can thus be used as part of a measure of the fitness consequences of FTs (79, 95). FTs increased reproductive output in some studies (95), but this effect appears to be dose-dependent. For example, FTs within the optimal thermal zone lead to a positive relationship between amplitude of FTs and egg production in *Ceratitidis capitata* (107), whereas FTs that encompass stressful temperatures reduce fecundity (16, 79). Increasing number of cold exposures (0°C) decreased reproductive

output of female *D. melanogaster* (79), as did a single, 1-3 day exposure to a sub-optimal temperature (39). Similarly, fewer eggs were produced by *Zeiraphera canadensis* moths in a 10-25°C FT regime compared to controls at 20°C (25°C being supra-optimal for this species) (16).

Stressful temperatures impair oocyte development (48), and decrease mating success (27), sperm production, and sperm viability (97), so the mechanisms underlying a decrease in reproductive output after a stressful temperatures are easy to envisage. However, it is unclear whether these different processes have differing thresholds or responses to FTs, and this should be a topic for future research. The mechanisms underlying increased reproductive investment under FTs (107) may be as simple as an effect of Jensen's inequality on reproductive physiology, or may involve more complex signaling pathways; these have not been investigated. Similarly, the duration of the FT effect has not been well-characterized: it is as yet unclear whether FTs lead to a lifetime change in reproductive investment, or a transient change which can be modified with repair and recovery.

## **5. Effects of FTs on thermal tolerance**

### **Effects of FTs during acclimation**

Most laboratory acclimation experiments on insects use CTs, even though the studied organism would typically experience thermally-variable environments. Egg-to-adult development under fluctuating acclimation regimes (FARs) increased *D. melanogaster* cold tolerance (85), and also the heat tolerance of drosophilids (9, 101) and lycaenid butterflies (44), compared to development under constant acclimation regimes (CARs). Acclimation of adult stages under FTs also improves thermal tolerance of *D. melanogaster* (62) and the

tephritid *Dacus tryoni* (82). The response to FARs is dependent on the mean (44) and on the amplitude (107) in a species- and stress-specific manner: small FT amplitudes increased cold tolerance of *Ceratitis capitata*, but heat tolerance was highest under high amplitude FTs (107). Antarctic springtails (*Cryptopygus antarcticus*) had greater cold tolerance and plasticity in thermally-variable compared to buffered microcosms, which suggests that FTs may also drive thermal tolerance in the field (50). By contrast, acclimation of fall field crickets, *Gryllus pennsylvanicus*, was unaffected by the amplitude or predictability of FARs (84), suggesting that FARs are not uniformly effective at increasing thermal tolerance.

### **Fluctuating temperatures can mitigate prolonged low temperature stress**

Many insects are killed by chilling at temperatures not associated with ice formation (24, 74), and these injuries can be reduced or avoided if the cold period is interrupted with brief exposures to warmer temperatures. For example, pharate adults of the flesh fly *Sarcophaga crassipalpis* were killed by 20 days at 0°C, but survival increased to 53% when the cold exposure was interrupted by a single 6 h pulse at 15°C on day ten. This was among the first reports of a “recharge” process under FTs (19). The beneficial effect of interrupting prolonged cold exposure with warm periods has since been reported in Hemiptera (68), Orthoptera (58), Diptera (19, 75, 79), Coleoptera (96), Hymenoptera (28, 32, 124), Lepidoptera (8, 64, 113) and Collembola (83), suggesting that the response is highly conserved across taxa. Warm periods as short as five minutes can improve cold survival (124), and increased duration of the warm phase usually results in improved survival (58, 83, 124), to a point where any effect of chilling becomes negligible (30). The effect of warm interruption is temperature-dependent, although the warmest temperatures do not necessarily yield the best survival gains (83). Increased frequency of warming pulses also promotes longer survival (32, 58, 83, 124).

Reduced cold mortality under FTs is probably not due to a reduction of cumulative chill injury, as the effects persist even when comparing strictly equivalent cold doses (8, 30, 70, 96). Alternatively, it seems likely that chilling injury is repaired during the warming intervals (32, 68, 96) (see section 6). Interestingly, the benefits of FTs appear to apply only to freeze-avoiding and chill-susceptible species: repeated freeze-thaw is damaging to freeze tolerant species (80). In addition, long warming interruptions can lead to deacclimation and loss of cold tolerance (103, 113). For example, overwintering emerald ash borer (*Agrilus planipennis*) prepupae irreversibly lost their cold tolerance after exposure to +10°C for more than a week, reducing survival of subsequent cold exposures (103).

### **Fluctuating temperatures during heat stress**

Insects generally have a well-developed heat shock response (43) and this is clearly relevant to FTs that extend above the optimum range. Although upper thermal limits are in dangerous proximity to optima because of Jensen's inequality (81), there is capacity for this threshold to shift with return to permissive temperatures, as well as for repair to occur. As with low temperatures, FTs allow development and survival under conditions that include high temperatures that might otherwise be lethal (37, 46, 76, 108). For example, *D. melanogaster* cannot develop at 33°C, but can develop and survive if the temperature fluctuates from 33 to 13°C (42). Thus, FTs can increase thermal range when recovery is possible.

Prior exposure to high temperatures also improves survival of insects on hot days in the field (21, 86) which implies that insects may be able to survive in the field at higher temperatures than predicted from laboratory experiments conducted under CTs. Because of the asymmetrical shape of TPC (1) (**Figure 1**), there is also more chance of hitting abrupt limits and irreversible thresholds at high temperatures. Thus, heat damages may not be as easily

repaired as cold damages. Until recently, much less was known about the impacts of repeated heat stress and FTs that span high temperatures compared to repeated cold stress and cold FTs. However, recent data support the notion that extreme heat events, even of short duration or when occurring only once, are highly detrimental for species' performance and survival, and that averaging daily temperature will not capture these effects (4, 56, 88, 116, 126).

## **6. Mechanisms underlying the response to fluctuating temperatures**

### **Physiological correlates of FARs**

Fluctuating acclimation regimes generally promote cold tolerance compared to cold CARs (see section 5), possibly because the warm intervals allow physiological changes that are not otherwise possible. Membrane lipid composition shifts after the first temperature cycle between 5 and 20°C in *Orchesella cincta* springtails (115), but these changes were not consistent with homeoviscous adaptation (51). In most cases, chaperone proteins appear to be upregulated more under FARs than CARs, potentially allowing increased protection of proteins against thermal shock (2, 118). However, lycaenid butterflies exposed to multi-step FT regimes (daily means of 17.7 or 23.7°C) showed the opposite response: a decrease in HSP70 expression in insects exposed to FTs (44). These discrepancies may reflect variations in the degree to which the thermal conditions are physiologically stressful, and may also arise from a focus on basal heat shock protein (Hsp) expression, which may be a poor reflection of the real (stress-induced) capacity for protection from thermal stress (43).

Cold tolerance of insects is usually associated with the accumulation of polyols and sugars (74), so it may be expected that the improvement in cold tolerance under FARs would be accompanied by increased concentrations of these cryoprotectants. This has been observed in

Hemiptera (67) and Orthoptera (118). Collembola submitted to FARs (8 to 32°C) were more heat-tolerant than control counterparts (at 20°C), and had also dissimilar metabolomic profiles (117). Similarly, *Dendroides canadensis* beetles accumulate antifreeze proteins under short-day thermoperiods in the absence of any light-dark cycle (55). This may reflect a role of FARs in stimulating acclimation responses for organisms that do not receive reliable photoperiodic cues because they are under bark (in this case) or in the soil. Finally, increased thermal variance during FAR treatment reduced maximum metabolic rate in *Tenebrio molitor* (10), suggesting that FARs may drive metabolic depression, as reported in overwintering lepidopteran larvae (119). Even if various physiological correlates of FARs have been described, the molecular mechanisms underlying the responses to FARs have not yet been investigated.

### **Physiological responses to FTs: repair and protection during the warm phase?**

Fluctuating temperatures could improve cold survival by allowing physiological preparation for subsequent cold exposures in a similar manner to the rapid cold hardening (RCH) response (105). However, increased survival of prolonged cold is most likely improved by FTs because damages accrued during the cold phase of the temperature cycles are then repaired during warming episodes (31, 32, 68, 83, 96). Chilling in insects is accompanied by a loss of ion homeostasis (69, 78). When rewarmed, insects must therefore re-establish ion balance – which is energetically expensive (78) – and repair damages caused by osmotic and ionic stress. Under FTs, chill-susceptible *Pyrrhocoris apterus* and *A. diaperinus* re-established ion balance during each warm spell, which likely increased the duration of survival over counterparts exposed to constant cold (68).



Protein unfolding or misfolding is another stress associated with thermal extremes, and one that is managed in cells by the upregulation of Hsps, particularly those in the 70 kDa family (43). The *hsp70* gene and/or its protein concentration were upregulated under FTs compare to CTs in Coleoptera (122), Hymenoptera (31) and Lepidoptera (8). However, although *hsp70* mRNA abundance increased 1000-fold during warming phases in *P. apterus*, no significant change was found at the protein level (110), presumably because the 2 h warming intervals were too short for translation to occur. Other Hsp families are also upregulated in response to FTs (125) and might thus also contribute to FT response. Whether Hsp expression during the FTs is a reaction to stress or an adaptive protective effect is not yet clear, but experiments using RNA interference or transgenic overexpression could help determine the role of Hsp in phenotypes associated with FTs. Hsps would also be expected to play an important role in repair of (and protection against) damage in FTs that span high temperatures but, to our knowledge, this has not yet been examined.

Compatible solutes, such as sugars, polyols or free amino acids (FAA), have a range of protective properties, such as detoxification or stabilization of proteins and membranes (121). These molecules might therefore play a role in repair of damages (or protection from future damages) during FTs. Increased glucose concentration is a common feature of insects exposed to repeated cold (106), and polyols (especially glycerol) accumulate in response to FTs in Diptera and Coleoptera (70, 91). Although the cryoprotectant role of polyols and sugars is well-established (24, 74), the role of amino acids is less well-understood. Most FAAs are accumulated by *Aphidius colemani* in response to cold CTs (29), while the FAA pool decreased during the warm periods of FTs in *A. diaperinus* (70) and *A. colemani* (29), suggesting that warming intervals reactivate the utilization of amino acids for protein synthesis and energetic purpose. Energy metabolism is an alternative role for many

putatively-cryoprotective solutes. Although ATP supply does not always decline in the cold (26, 77), any depletion can certainly be regenerated during the warm spells in FTs (40) but see (26), and may be required for energy-demanding repair and recovery processes. Several studies have reported increased metabolic rates during the warm period of FTs (8, 72, 123), and proteins related to energy metabolism are upregulated during the warming periods in *A. colemani* (31). Such increased metabolism might be associated with generation of free radicals (ROS) and oxidative stress. However, cold-induced oxidative stress (measured as the ratio of reduced to oxidized glutathione) actually decreased during the warm phase of FTs in *A. diaperinus* (72), likely because of increased function of antioxidant enzymes at warmer temperatures. Together, these data suggest an active regulation of ion homeostasis, chaperone machinery, energy metabolism and respiration during FTs, particularly during warm periods.

## **7. Applications and implications of FTs**

### **Exploiting the protective effects of FTs**

Because FTs that re-warm insects for brief periods during cold exposure mitigate many of the negative impacts of low temperatures, they have obvious applications in the context of cold storage of beneficial insects (28). For example, storage at constant 2°C for 20 days reduced survival to less than 40% in several aphidine parasitoids, while storage at FTs (2°C interrupted by spells at 20°C) allows maintenance of a high survival (equal to untreated control), thus improving stockpiling and mass-rearing efficiency (28). Storage under FTs also holds applications for mass-rearing of insects for pollination (98, 124) and potential applications for sterile insect technique (21). FTs could also be exploited to deacclimate insects (103), and thus to reduce cold tolerance of pests as part of a thermal quarantine

procedure. Similarly, the negative impacts of repeated cold or heat stress (80) could be leveraged to control stored product pests (59), but this has not been well-explored.

### **Implications of FTs for prediction and management**

It is clear that the survival, development, and performance of insects under FTs are poorly predicted by CTs. Nevertheless, many approaches for predicting the performance and survival of insects are dependent on data gathered using CTs, or expect simple linear relationships between biological rate processes and temperature. For example, degree-day models underpin estimates of development time used to forecast population dynamics in agriculture (120). However, such models could be flawed, as they do not account for Jensen's inequality, and therefore underestimate the contribution of temperatures above the mean on development and fecundity. This implication of FTs for degree-day models has been well recognized in forensic entomology (18, 53). Just as FTs affect development, they also affect overwinter survival and thus predictability of agricultural pests outbreak (111). In addition, because FTs lead to changes in thermal tolerance over time (62, 118), predictions of winter mortality derived from static estimates from CTs may be flawed. Régnière & Bentz (94) developed a model for mountain pine beetle mortality that recognized the dynamic nature of thermal tolerance, and such an approach could likely be utilized to reflect the cumulative impact of FTs on survival and performance. Fluctuating temperatures modify life-history traits, population dynamics, and immunocompetence of disease-vector insects (73, 87) and large FTs (18°C swings around 20°C) accelerate virus transmission by mosquitoes (15). Thus, it is clear that the performance effects of FTs on insects can have a broad societal significance.

## **8. Fluctuating temperatures and insect responses to climate change**

Ongoing global climate change is predicted to lead to increases in mean temperature, increased variance around that mean, and an increased incidence of transient extreme temperatures (41, 49, 116). The distribution and phenology of insects have been responding apace to this changing climate (20, 22). However, the role of thermal variability has not been regularly included in experimental studies and predictive models (52, 109), despite the relevance of variability to model outputs (60, 88, 119). The asymmetric nature of TPCs means that increased temperature places ectotherms closer to their upper thermal limits (38, 61), and an increase in variability exacerbates this risk (88) such that the impacts of climate change are best described by the interaction between mean temperature and variability (109, 116). We have shown that, for insects, this mean  $\times$  variance interaction is even more complex. Fluctuating temperatures alter fitness components including fecundity, longevity, and body size, while affecting thermal plasticity, stress survival, recovery and response to transient extremes. Importantly, these traits vary with FTs in a curvilinear, or even threshold fashion that is not accounted for by bioclimatic envelope models, and is captured poorly in the current generation of mechanistic models (12, 116). Thus, models of insect responses to climate change that are parameterized from datasets gathered under CTs (or that assume no change in the variance of thermal regimes) may contain systematic errors when compared to the real world (52). Although there is much to learn about how insects respond to FTs, we suggest that initial models that incorporate thermal variance (109, 116) could provide some guidance for the exploration of insect responses to FTs in a context directly usable to drive policy and management.

## **9. Conclusions**

Insect responses to FTs contrast with responses to CTs at multiple levels of organization, from physiology and stress tolerance to life-history traits and fitness. This divergence has important implications not only for the design and interpretation of thermal biology studies, but also for predicting responses to climate change. We conclude that CTs are an unrealistic approach for studying thermal response of insects that typically occur in thermally variable environments. As a result, FTs should be incorporated into predictive models of growth, performance, survival and climate change responses, and play a central role in the design of all laboratory studies in insect thermal biology.

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## 10. Literature cited

1. Angilletta MJ Jr. 2009. *Thermal Adaptation. - A Theoretical and Empirical Synthesis*. Oxford, UK: Oxford Univ. Press. 320 pp
2. Arias MB, Poupin MJ, Lardies MA. 2011. Plasticity of life-cycle, physiological thermal traits and Hsp70 gene expression in an insect along the ontogeny: Effect of temperature variability. *J. Therm. Biol.* 36:355-62
3. Atkinson D. 1994. Temperature and organism size - a biological law for ectotherms? *Adv. Ecol. Res.* 25:1-58
4. Bauerfeind SS, Fischer K. 2014. Simulating climate change: temperature extremes but not means diminish performance in a widespread butterfly. *Popul. Ecol.* 56:239-50
5. Beardmore JA. 1960. Developmental stability in constant and fluctuating temperatures. *Heredity* 14:411-22
6. Beardmore JA, Levine L. 1963. Fitness and environmental variation. I. A study of some polymorphic populations of *Drosophila pseudoobscura*. *Evolution* 17:121-29
7. Beasley DE, Bonisoli-Alquati A, Mousseau TA. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. *Ecol. Indic.* 30: 218-26
8. Boardman L, Sørensen JG, Terblanche JS. 2013. Physiological responses to fluctuating thermal and hydration regimes in the chill susceptible insect, *Thaumatotibia leucotreta*. *J. Insect Physiol.* 59:781-94
9. Bozinovic F, Bastías DA, Boher F, Clavijo-Baquet S, Estay SA, Angilletta MJ Jr. 2011. The mean and variance of environmental temperature interact to determine physiological tolerance and fitness. *Physiol. Biochem. Zool.* 84:543-52
10. Bozinovic F, Catalan TP, Estay SA, Sabat P. 2013. Acclimation to daily thermal variability drives the metabolic performance curve. *Evol. Ecol. Res.* 15:579-87
11. Bradley BP. 1980. Developmental stability of *Drosophila melanogaster* under artificial and natural selection in constant and fluctuating environments. *Genetics* 95:1033-42.

12. Buckley LB, Urban MC, Angilletta MJ, Crozier LG, Rissler LJ, Sears MW 2010. Can mechanism inform species' distribution models? *Ecol. Lett.* 13:1041-54
13. Butler CD, Trumble JT. 2010. Predicting population dynamics of the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae) resulting from novel interactions of temperature and selenium. *Biocontrol Sci. Techn.* 20:391-406
14. Carrington LB, Armijos MV, Lambrechts L, Barker CM, Scott TW. 2013. Effects of fluctuating daily temperatures at critical thermal extremes on *Aedes aegypti* life-history traits. *PLoS One* 8(3):e58824
15. Carrington LB, Armijos MV, Lambrechts L, Scott TW. 2013. Fluctuations at a low mean temperature accelerate dengue virus transmission by *Aedes aegypti*. *PLoS Negl. Trop. Dis.* 7(4):e2190
16. Carroll AL, Quiring DT. 1993. Interactions between size and temperature influence fecundity and longevity of a tortricid moth, *Zeiraphera canadensis*. *Oecologia* 93:233-41
17. Casagrande RA, Haynes DL. 1976. A predictive model for cereal leaf beetle mortality from sub-freezing temperatures. *Environ. Entomol.* 5:761-69
18. Catts EP, Goff ML. 1992. Forensic entomology in criminal investigations. *Annu. Rev. Entomol.* 37:253-72.
19. Chen CP, Denlinger DL. 1992. Reduction of cold injury in flies using an intermittent pulse of high-temperature. *Cryobiology* 29:138-43
20. Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333(6045):1024-26
21. Chidawanyika F, Terblanche JS. 2011. Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. *Evol. Appl.* 4:534-44
22. Chown SL, Hoffmann AA, Kristensen TN, Angilletta MJ, Stenseth NC, Pertoldi C. 2010. Adapting to climate change: a perspective from evolutionary physiology. *Climate Res.* 43:3-15
23. Chown SL, Terblanche JS, Simpson SJ. 2006. Physiological diversity in insects: ecological and evolutionary contexts. *Adv. Insect Physiol.* 33:50-152

24. Chown SL, Nicolson SW. 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford, UK: Oxford Univ. Press. 254pp
25. Cloudsley-Thompson JL. 1953. The significance of fluctuating temperatures on the physiology and ecology of insects. *Entomologist* 86:183-89
26. Colinet H. 2011. Disruption of ATP homeostasis during chronic cold stress and recovery in the chill susceptible beetle (*Alphitobius diaperinus*). *Comp. Biochem. Physiol. A* 160:63-67
27. Colinet H, Hance T. 2009. Male reproductive potential of *Aphidius colemani* (Hymenoptera: Aphidiinae) exposed to constant or fluctuating thermal regimes. *Environ. Entomol.* 38:242-49
28. Colinet H, Hance T. 2010. Interspecific variation in the response to low temperature storage in different aphid parasitoids. *Ann. Appl. Biol.* 156:147-56
29. Colinet H, Hance T, Vernon P, Bouchereau A, Renault D. 2007. Does fluctuating thermal regime trigger free amino acid production in the parasitic wasp *Aphidius colemani* (Hymenoptera: Aphidiinae)? *Comp. Biochem. Physiol. A* 147:484-92
30. Colinet H, Lalouette L, Renault D. 2011. A model for the time-temperature-mortality relationship in the chill-susceptible beetle, *Alphitobius diaperinus*, exposed to fluctuating thermal regimes. *J. Therm. Biol.* 36:403-08
31. Colinet H, Nguyen TTA, Cloutier C, Michaud D, Hance T. 2007. Proteomic profiling of a parasitic wasp exposed to constant and fluctuating cold exposure. *Insect Biochem. Mol. Biol.* 37:1177-88
32. Colinet H, Renault D, Hance T, Vernon P. 2006. The impact of fluctuating thermal regimes on the survival of a cold-exposed parasitic wasp, *Aphidius colemani*. *Physiol. Entomol.* 31:234-40
33. Cônsoli FL, Parra JRP. 1995. Effects of constant and alternating temperatures on *Trichogramma galloi* Zucchi (Hym., Trichogrammatidae) biology II.– parasitism capacity and longevity. *J. Appl. Entomol.* 119: 667-70
34. Cook WC. 1927. Some effects of alternating temperatures on the growth and metabolism of cutworm larvae. *J. Econ. Entomol.* 20:769-82



35. Czarnoleski M, Cooper BS, Kierat J, Angilletta MJ Jr. 2013. Flies developed small bodies and small cells in warm and in thermally fluctuating environments. *J. Exp. Biol.* 216:2896-901
36. Danks H.V. 2006. Insect adaptations to cold and changing environments. *Can. Entomol.* 138:1-23
37. Davis JA, Radcliffe EB, Ragsdale DW. 2006. Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environ. Entomol.* 35:1461-68
38. Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, et al. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* 105:6668-72
39. Dillon ME, Cahn LR, Huey RB. 2007. Life history consequences of temperature transients in *Drosophila melanogaster*. *J. Exp. Biol.* 210:2897-904
40. Dollo VH, Yi SX, Lee RE Jr., 2010. High temperature pulses decrease indirect chilling injury and elevate ATP levels in the flesh fly, *Sarcophaga crassipalpis*. *Cryobiology* 60:351-53
41. Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO. 2000. Climate extremes: observations, modeling, and impacts. *Science* 289:2068-74
42. Economos AC, Lints FA. 1986. Developmental temperature and life span in *Drosophila melanogaster*. II. Oscillating temperature. *Gerontology* 32:28-36
43. Feder ME, Hofmann GE. 1999. Heat-shock proteins, molecular chaperones and the stress response: evolutionary and ecological physiology. *Annu. Rev. Physiol.* 61:243-82
44. Fischer K, Kölzow N, Hölzje H, Karl I. 2011. Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia* 166:23-33
45. Foray V, Desouhant E, Gibert P. 2014. The impact of thermal fluctuations on reaction norms in specialist and generalist parasitic wasps. *Funct. Ecol.* 28:411-23
46. García-Ruiz E, Marco V, Pérez-Moreno I. 2011. Effects of variable and constant temperatures on the embryonic development and survival of a new grape pest, *Xylotrechus arvicola* (Coleoptera: Cerambycidae). *Environ. Entomol.* 40:939-47

47. Gibbs AG, Perkins MC, Markow TA. 2003. No place to hide: microclimates of Sonoran Desert *Drosophila*. *J. Therm. Biol.* 28:353-62
48. Gruntenko NE, Bownes M, Terashima J, Sukhanova MZ, Raushenbach IY. 2003. Heat stress affects oogenesis differently in wild-type *Drosophila virilis* and a mutant with altered juvenile hormone and 20-hydroxyecdysone levels. *Insect Mol. Biol.* 12: 393-404
49. Hansen J, Sato M, Ruedy R. 2012. Perception of climate change. *Proc. Natl. Acad. Sci. USA* 109:14726-14727, E2415-23
50. Hawes TC, Bale JS, Worland MR, Convey P. 2008. Trade-offs between microhabitat selection and physiological plasticity in the Antarctic springtail, *Cryptopygus antarcticus* (Willem). *Polar Biol.* 31:681-89
51. Hazel JR. 1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* 57:19-42
52. Helmuth B, Broitman BR, Yamane L, Gilman SE, Mach K, Mislan KAS, et al. 2010. Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. *J. Exp. Biol.* 213:995-1003
53. Higley LG, Haskell NH, Byrd JH, Castner JL. 2001. Insect development and forensic entomology. In *Forensic Entomology: the utility of arthropods in legal investigations*, ed. JH Byrd, JL Castner, pp. 287-302. Boca Raton, FL: CRC Press
54. Hoffmann AA, Collins E, Woods R. 2002. Wing shape and wing size changes as indicators of environmental stress in *Helicoverpa punctigera* (Lepidoptera: Noctuidae) moths: comparing shifts in means, variances, and asymmetries. *Environ. Entomol.* 31:965-71
55. Horwath KL, Duman JG. 1986. Thermoperiodic involvement in antifreeze protein production in the cold hardy beetle *Dendroides canadensis*: implications for photoperiodic time measurement. *J. Insect Physiol.* 32:799-806
56. Jeffs CT, Leather SR. 2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid, *Sitobion avenae*. *Entomol. Exp. Appl.* 150:240-49
57. Jensen JLWV. 1906. Sur les fonctions convexes et les inégalités entre les valeurs moyennes. *Acta Math.* 30:175-193

58. Jing XH, Wang XH, Kang L. 2005. Chill injury in the eggs of the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae): the time-temperature relationship with high-temperature interruption. *Insect Sci.* 12:171-78
59. Kaliyan N, Carrillo MA, Morey RV, Wilcke WF, Kells S. 2007. Mortality of Indianmeal moth (Lepidoptera: Pyralidae) populations under fluctuating low temperatures: model development and validation. *Environ. Entomol.* 36:1318-27
60. Kearney MR, Matzelle A, Helmuth B. 2012. Biomechanics meets the ecological niche: the importance of temporal data resolution. *J. Exp. Biol.* 215:922-33
61. Kellermann V, Overgaard J, Hoffmann AA, Fløjgaard C, Svenning J, Loeschcke V. 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proc. Natl. Acad. Sci. USA* 109:16228-33
62. Keltly JD, Lee Jr RE. 2001. Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophilidae) during ecologically based thermoperiodic cycles. *J. Exp. Biol.* 204:1659-66
63. Kersting S, Satar U, Uygun N. 1999. Effect of temperature on development rate and fecundity of apterous *Aphis gossypii* Glover (Hom., Aphididae) reared on *Gossypium hirsutum* L. *J. Appl. Entomol.* 123:23-27
64. Kim Y, Song W. 2000. Effect of thermoperiod and photoperiod on cold tolerance of *Spodoptera exigua* (Lepidoptera: Noctuidae). *Environ. Entomol.* 29:868-73
65. Kingsolver JG, Ragland GJ, Diamond SE. 2009. Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. *Evolution* 63:537-41
66. Kjærsgaard A, Pertoldi C, Loeschcke V, Blanckenhorn WU. 2013. The effect of fluctuating temperatures during development on fitness-related traits of *Scatophaga stercoraria* (Diptera: Scathophagidae). *Environ. Entomol.* 42:1069-78
67. Kostál V, Slachta M, Simek P. 2001. Cryoprotective role of polyols independent of the increase in supercooling capacity in diapausing adults of *Pyrrhocoris apterus* (Heteroptera: Insecta). *Comp. Biochem. Physiol. B* 130:365-74

68. Kostál V, Renault D, Mehrabianová A, Bastl J. 2007. Insect cold tolerance and repair of chill-injury at fluctuating thermal regimes: role of ion homeostasis. *Comp. Biochem. Physiol. A* 147:231-38
69. Kostál V, Yanagimoto M, Bastl J. 2006. Chilling-injury and disturbance of ion homeostasis in the coxal muscle of the tropical cockroach (*Nauphoeta cinerea*). *Comp. Biochem. Physiol. B* 143:171-79
70. Lalouette L, Kostál V, Colinet H, Gagneul D, Renault D. 2007. Cold exposure and associated metabolic changes in adult tropical beetles exposed to fluctuating thermal regimes. *FEBS J.* 274:1759-67
71. Lalouette L, Renault D, Ravaux J, Siaussat D. 2010. Effects of cold-exposure and subsequent recovery on cellular proliferation with influence of 20-hydroxyecdysone in a lepidopteran cell line (IAL-PID2). *Comp. Biochem. Physiol. A* 155:407-14
72. Lalouette L, Williams CM, Hervant F, Sinclair BJ, Renault D. 2011. Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comp. Biochem. Physiol. A* 158:229-34
73. Lambrechts L, Paaijmans KP, Fansiri T, Carrington LB, Kramer LD, Thomas MB, et al. 2011. Impact of daily temperature fluctuations on dengue virus transmission by *Aedes aegypti*. *Proc. Natl. Acad. Sci. USA* 108:7460-65
74. Lee RE Jr. 2010. A primer on insect cold-tolerance. In *Low Temperature Biology of Insects*, ed. DL Denlinger, RE Jr Lee, pp. 3-34. Cambridge: Cambridge University Press
75. Leopold RA, Rojas RR, Atkinson PW. 1998. Post pupariation cold storage of three species of flies: increasing chilling tolerance by acclimation and recurrent recovery periods. *Cryobiology* 36:213-24
76. Luz C, Fargues J, Grunewald J. 1999. Development of *Rhodnius prolixus* (Hemiptera: Reduviidae) under constant and cyclic conditions of temperature and humidity. *Mem. Inst. Oswaldo Cruz* 94:403-09
77. MacMillan HA, Williams CM, Staples JF, Sinclair BJ. 2012. Metabolism and energy supply below the critical thermal minimum of a chill-susceptible insect. *J. Exp. Biol.* 215:1366-72

78. MacMillan HA, Williams CM, Staples JF, Sinclair BJ. 2012. Reestablishment of ion homeostasis during chill-coma recovery in the cricket *Gryllus pennsylvanicus*. *Proc. Natl. Acad. Sci. USA* 109:20750-55
79. Marshall KE, Sinclair BJ. 2010. Repeated stress exposure results in a survival-reproduction trade-off in *Drosophila melanogaster*. *Proc. R. Soc. London Sci. Ser. B* 277:963-69
80. Marshall KE, Sinclair BJ. 2012. The impacts of repeated cold exposure on insects. *J. Exp. Biol.* 215:1607-13
81. Martin TL, Huey RB. 2008. Why “suboptimal” is optimal: Jensen’s inequality and ectotherm thermal preferences. *Am. Nat.* 171:E102-18
82. Meats A. 1976. Developmental and long-term acclimation to cold by the Queensland fruit-fly (*Dacus tryoni*) at constant and fluctuating temperatures. *J. Insect Physiol.* 22:1013-19
83. Nedved O, Lavy D, Verhoef HA. 1998. Modelling the time–temperature relationship in cold injury and effect of high-temperature interruptions on survival in a chill-sensitive collembolan. *Funct. Ecol.* 12:816-24
84. Niehaus AC, Wilson RS, Storm JJ, Angilletta MJ. 2012. Fall field crickets did not acclimate to simulated seasonal changes in temperature. *J. Comp. Physiol. B* 182:199-207
85. Overgaard J, Hoffmann AA, Kristensen TN. 2011. Assessing population and environmental effects on thermal resistance in *Drosophila melanogaster* using ecologically relevant assays. *J. Therm. Biol.* 36:409-16
86. Overgaard J, Sørensen JG. 2008. Rapid thermal adaptation during field temperature variations in *Drosophila melanogaster*. *Cryobiology* 56:159-62
87. Paaijmans KP, Blanford S, Bell AS, Blanford JI, Read AF, Thomas MB. 2010. Influence of climate on malaria transmission depends on daily temperature variation. *Proc. Natl. Acad. Sci. USA* 107:15135-39
88. Paaijmans KP, Heinig RL, Seliga RA, Blanford JI, Blanford S, Murdock CC, et al. 2013. Temperature variation makes ectotherms more sensitive to climate change. *Glob. Change Biol.* 19:2373-80

89. Pétavy G, David JR, Debat V, Gibert P, Moreteau B. 2004. Specific effects of cycling stressful temperatures upon phenotypic and genetic variability of size traits in *Drosophila melanogaster*. *Evol. Ecol. Res.* 6:873-90
90. Pétavy G, David JR, Gibert P, Moreteau B. 2001. Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *J. Therm. Biol.* 26:29-39
91. Pio CJ, Baust JG. 1988. Effects of temperature cycling on cryoprotectant profiles in the goldenrod gall fly, *Eurosta solidaginis* (Fitch). *J. Insect Physiol.* 34:767-71
92. Ragland GJ, Kingsolver JG. 2008. The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*. *Evol. Ecol. Res.* 10:29-44
93. Ratte HT. 1985. Temperature and insect development. In *Environmental Physiology and Biochemistry of Insects*, ed. KH Hoffmann, pp. 33-66. Berlin: Springer-Verlag
94. Régnière J, Bentz B. 2007. Modeling cold tolerance in the mountain pine beetle, *Dendroctonus ponderosae*. *J. Insect Physiol.* 53:559-72
95. Renault D. 2011. Long-term after-effects of cold exposure in adult *Alphitobius diaperinus* (Tenebrionidae): the need to link survival ability with subsequent reproductive success. *Ecol. Entomol.* 36:36-42
96. Renault D, Nedved O, Hervant F, Vernon P. 2004. The importance of fluctuating thermal regimes for repairing chill injuries in the tropical beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during exposure to low temperature. *Physiol. Entomol.* 29:139-45
97. Rinehart JP, Yocum GD, Denlinger DL. 2000. Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the flesh fly, *Sarcophaga crassipalpis*. *Physiol. Entomol.* 25:330-36
98. Rinehart JP, Yocum GD, West M, Kemp WP. 2011. A fluctuating thermal regime improves survival of cold-mediated delayed emergence in developing *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 104:1162-66
99. Ruel JJ, Ayres MP. 1999. Jensen's inequality predicts effects of environmental variation. *Trends Ecol. Evol.* 14:361-66

100. Sanderson ED. 1910. The relation of temperature to the growth of insects. *J. Econ. Entomol.* 3:113-39
101. Sarup P, Loeschcke V. 2010. Developmental acclimation affects clinal variation in stress resistance traits in *Drosophila buzzatii*. *J. Evol. Biol.* 23:957-65
102. Sinclair BJ, Terblanche JS, Scott MB, Blatch GL, Klok CJ, Chown SL. 2006. Environmental physiology of three species of Collembola at Cape Hallett, North Victoria Land, Antarctica. *J. Insect Physiol.* 52:29-50
103. Sobek-Swant S, Crosthwaite J, Lyons DB, Sinclair BJ. 2012. Could phenotypic plasticity limit an invasive species? Incomplete reversibility of mid-winter deacclimation in emerald ash borer. *Biol. Invasions* 14:115-25
104. Sohal RS, Orr WC. 2012. The redox stress hypothesis of aging. *Free Radic. Biol. Med.* 52:539-55
105. Teets NM, Denlinger DL. 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. *Physiol. Entomol.* 38:105-16
106. Teets NM, Kawarasaki Y, Lee RE, Denlinger DL. 2011. Survival and energetic costs of repeated cold exposure in the Antarctic midge, *Belgica antarctica*: a comparison between frozen and supercooled larvae. *J. Exp. Biol.* 214:806-14
107. Terblanche JS, Nyamukondiwa C, Kleynhans E. 2010. Phenotypic plasticity of thermal tolerance contributes to the invasion potential of mediterranean fruit flies (*Ceratitidis capitata*). *Ecol. Entomol.* 35:304-15
108. Thoeys C, van der Linden A, Bernaerts F, Blust R, Declerck W. 1987. The effect of diurnal temperature cycles on survival of *Artemia* from different geographical origins. In *Artemia Research and its Applications, volume 1, Morphology, Genetics, Strain characterization, Toxicology*, ed. P Sorgeloos, DA Bengston, W Declerck, E Jaspers, pp. 233-39. Wetteren, Belgium: Universa Press
109. Thompson RM, Beardall J, Beringer J, Grace M, Sardina P. 2013. Means and extremes: building variability into community-level climate change experiments. *Ecol. Lett.* 16:799-806

110. Tollarová-Borovanská M, Lalouette L, Kostál V. 2009. Insect cold tolerance and repair of chill-injury at fluctuating thermal regimes: role of 70 kda heat shock protein expression. *CryoLetters* 30:312-19
111. Turnock WJ, Lamb RJ, Bodnaryk RP. 1983. Effects of cold stress during pupal diapause on the survival and development of *Mamestra configurata* (Lepidoptera: Noctuidae). *Oecologia* 56:185-92
112. Turnock WJ, Fields PG. 2005. Winter climates and cold hardiness in terrestrial insects. *Eur. J. Entomol.* 102:-561-76
113. Turnock WJ, Bodnaryk RP. 1993. The reversal of cold injury and its effect on the response to subsequent cold exposures. *CryoLetters* 12:251-56.
114. Van der Have TM, de Jong G. 1996. Adult size in ectotherms: temperature effects on growth and differentiation. *J. Theor. Biol.* 183:329-0
115. Van Dooremalen C, Suring W, Ellers J. 2011. Fatty acid composition and extreme temperature tolerance following exposure to fluctuating temperatures in a soil arthropod. *J. Insect Physiol.* 57:1267-73
116. Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS, et al. 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc. R. Soc. Sci. Ser. B* 281:20132612
117. Waagner D, Heckmann LH, Malmendal A, Nielsen NC, Holmstrup M, Bayley M. 2010. Hsp70 expression and metabolite composition in response to short-term thermal changes in *Folsomia candida* (Collembola). *Comp. Biochem. Physiol. A* 157:177-83
118. Wang HS, Zhou CS, Guo W, Kang L. 2006. Thermoperiodic acclimations enhance cold hardiness of the eggs of the migratory locust. *Cryobiology* 53:206-17
119. Williams CM, Marshall KE, MacMillan HA, Dzurisin JDK, Hellmann JJ, Sinclair BJ. 2012. Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. *PLoS One* 7(3):e34470
120. Worner S. 1992. Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environ. Entomol.* 21:689-99



121. Yancey PH. 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* 208:2819-30
122. Yocum GD. 2001. Differential expression of two Hsp70 transcripts in response to cold shock, thermoperiod, and adult diapause in the Colorado potato beetle. *J. Insect Physiol.* 47:1139-45
123. Yocum GD, Greenlee KJ, Rinehart JP, Bennett MM, Kemp WP. 2011. Cyclic CO<sub>2</sub> emissions during the high temperature pulse of fluctuating thermal regime in eye-pigmented pupae of *Megachile rotundata*. *Comp. Biochem. Physiol. A* 160:480-85
124. Yocum GD, Rinehart JP, Kemp WP. 2012. Duration and frequency of a high temperature pulse affect survival of emergence-ready *Megachile rotundata* (Hymenoptera: Megachilidae) during low-temperature incubation. *J. Econ. Entomol.* 105:14-19
125. Zhang J, Marshall KE, Westwood JT, Clark MS, Sinclair BJ. 2011. Divergent transcriptomic responses to repeated and single cold exposures in *Drosophila melanogaster*. *J. Exp. Biol.* 214:4021-29
126. Zhao F, Zhang W, Hoffmann AA, Ma CS. 2013. A single hot event that does not affect survival but decreases reproduction in the diamondback moth, *Plutella xylostella*. *PLoS One* 8(10):e75923

## Figure captions

**Figure 1:** Relationship between performance and temperature of an insect. (a) Thermal performance curve, showing accelerating temperature-performance relationship below an optimal temperature ( $T_{opt}$ ) and decelerating relationship above the  $T_{opt}$ . Horizontal lines indicate the spans of three symmetrical fluctuating temperature regimes (between time 0 and time 1) with the same mean (indicated by grey dotted line). Note that the regime depicted in blue (bottom) spans temperatures above the  $T_{opt}$ . (b) Change in performance trait shown in (a) over the course of a single cycle (from time 0 at the minimum of the cycle, to time 1 at the top of the cycle, and back to the minimum of the cycle at time 0') of the three temperature regimes shown in (a), with the means displayed as dotted lines (grey = constant temperature). Note that average performance (dotted lines with the corresponding colors) declines if the temperature spans temperatures above the  $T_{opt}$  (blue).

**Figure 2:** The effect of Jensen's inequality, thermal sensitivity, and cycle amplitude on the relationship between temperature and metabolic rate under fluctuating temperatures. (a) Representative curvilinear relationships between metabolic rate and temperature for species with high (brown) and low (blue) thermal sensitivity. Dotted lines indicate a standard shift in temperature above and below a mean (grey), arrows indicate the magnitude of the shift in metabolic rate. (b) Energy decreases (savings) or increases (costs) in response to a standard shift in temperature up or down from a mean (grey) for the curves in (a). (c) Hypothetical daily temperature cycle (black) or constant temperature (grey). (d) Instantaneous metabolic rate of thermally-sensitive (brown) and thermally-insensitive phenotypes from (a) under the temperature regimes shown in (c). Dotted lines indicate mean rate across the day, compared to the constant temperature (grey). (e) Hypothetical thermal cycles of large (pink) and small

(green) amplitude, or constant temperature (grey). (f) Instantaneous metabolic rates of a single phenotype under the temperature regimes shown in (e), compared to the constant temperature (grey). Note that although metabolic rate is used for this example, any curvilinear process will follow a similar pattern if temperature fluctuations are within the accelerating portion of the curve shown in Figure 1; see (99, 119).

**Figure 3:** The diversity of FT treatments. (a) Two temperatures alternating around a constant mean (continuous green line). These protocols use rapid step transitions. (b) Interruption of a prolonged cold stress (blue part) by repeated bouts at optimal temperature (green part). (c) Repeated exposures to damaging temperatures simulating the effect of heat (red part) and cold (blue part) waves. In (b) and (c), the dotted green line represents the optimal temperature. (d) Multiple step-transitions regime around a mean (green line) used to simulate complex diel cycles or nature-mimicking thermoperiods. (e) Fluctuating temperatures with controlled gradual transitions (ramp) around a mean (green line). (f) Sine-like wave thermal cycles (night-days) – can be symmetric or asymmetric around a mean (green line). (g) Stochastic sinusoidal or diel thermal variations. (h) Field temperature variations. For all these treatments (a to g), different amplitudes (dotted black lines), durations and frequencies of temperature breaks can be applied.

## Optional element: Acronyms and Definitions

1. **Fluctuating temperatures (FTs)** [page 4]: a generic term that refers to any discontinuous thermal regime that occurs on a short-term (intra-generational)
2. **Thermal performance curves (TPCs)** [page 5]: the (usually asymmetric) relationship between temperature and performance of an ectotherm
3. **Critical temperatures  $CT_{\min}$  or  $CT_{\max}$**  [page 5]: low and high temperatures at which motor function stops and coordination is lost
4. **Fluctuating asymmetry (FA)** [page 9]: a pattern of deviation from bilateral morphological symmetry
5. **Thermal acclimation** [page 11]: physiological plasticity that enhances thermal tolerance following pre-exposure to mild and sub-lethal temperatures
6. **Fluctuating acclimation regime (FAR)** [page 11]: a thermal acclimation treatment that uses fluctuating pre-exposures for conditioning individuals
7. **Constant acclimation regime (CAR)** [page 11]: a thermal acclimation treatment that uses constant pre-exposures for conditioning individuals
8. **Heat shock response** [page 13]: the physiological and molecular responses to a brief exposure to high temperatures; usually includes the synthesis of heat shock proteins
9. **Rapid cold hardening (RCH)** [page 15]: a type of acclimation response where brief (minutes to hours) exposure to nonlethal low temperatures enhances subsequent cold tolerance

## Optional element: Annotated References

9. Bozinovic F, Bastías DA, Boher F, Clavijo-Baquet S, Estay SA, Angilletta MJ Jr. 2011. The mean and variance of environmental temperature interact to determine physiological tolerance and fitness. *Physiol. Biochem. Zool.* 84:543-52

*Demonstrates, using acclimation protocol, that the mean and magnitude of FTs are equally important*

14. Carrington LB, Armijos MV, Lambrechts L, Barker CM, Scott TW. 2013. Effects of fluctuating daily temperatures at critical thermal extremes on *Aedes aegypti* life-history traits. *PLoS One*8(3):e58824

*Demonstrates the discrepancy of results in CTs vs. FTs experiments, highlighting that CTs under- or over-estimate values for life-history traits*

44. Fischer K, Kölzow N, Höltje H, Karl I. 2011. Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia* 166:23-33

*Eight out of the nine traits examined were affected by thermal regimes. Overall FTs are more beneficial compared to CTs.*

45. Foray V, Desouhant E, Gibert P. 2014. The impact of thermal fluctuations on reaction norms in specialist and generalist parasitic wasps. *Funct. Ecol.* 28:411-23

*Rearing experiments show how Jensen's inequality mediates the effect of FTs on reaction norms*

**65. Kingsolver JG, Ragland GJ, Diamond SE. 2009. Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. *Evolution* 63:537-41**

*Selection experiments show that both mean and extreme temperatures may constitute strong selective forces on reaction norms*

**83. Nedved O, Lavy D, Verhoef HA. 1998. Modelling the time–temperature relationship in cold injury and effect of high-temperature interruptions on survival in a chill-sensitive collembolan. *Funct. Ecol.* 12:816-24**

*A detailed study showing that repeated warming spells increase cold survival, including a modelling approach for cold survival and recovery*

**88. Paaijmans KP, Heinig RL, Seliga RA, Blanford JI, Blanford S, et al. 2013. Temperature variation makes ectotherms more sensitive to climate change. *Glob. Change Biol.* 19:2373-80**

*Suggest that FTs alter the sensitivity to climate warming by reducing ‘thermal safety margins’*

**93. Ratte HT. 1985. Temperature and insect development. In *Environmental physiology and biochemistry of insects*, ed. KH Hoffmann, pp. 33-66. Berlin: Springer-Verlag**

*An early synthesis on the role of CTs and FTs in insect development studies*

**98. Rinehart JP, Yocum GD, West M, Kemp WP. 2011. A fluctuating thermal regime improves survival of cold-mediated delayed emergence in developing *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 104:1162-66**

*Documents that high temperature pulses promote bee’s tolerance to cold storage, and thus can be useful in applied entomology*

**116. Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, et al. 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc. R. Soc. London Sci. Ser. B* 281:In press**

*Illustrates, using a modeling approach, how FTs have disproportionate effects on species performances (owing to Jensen's inequality)*

Figure 1

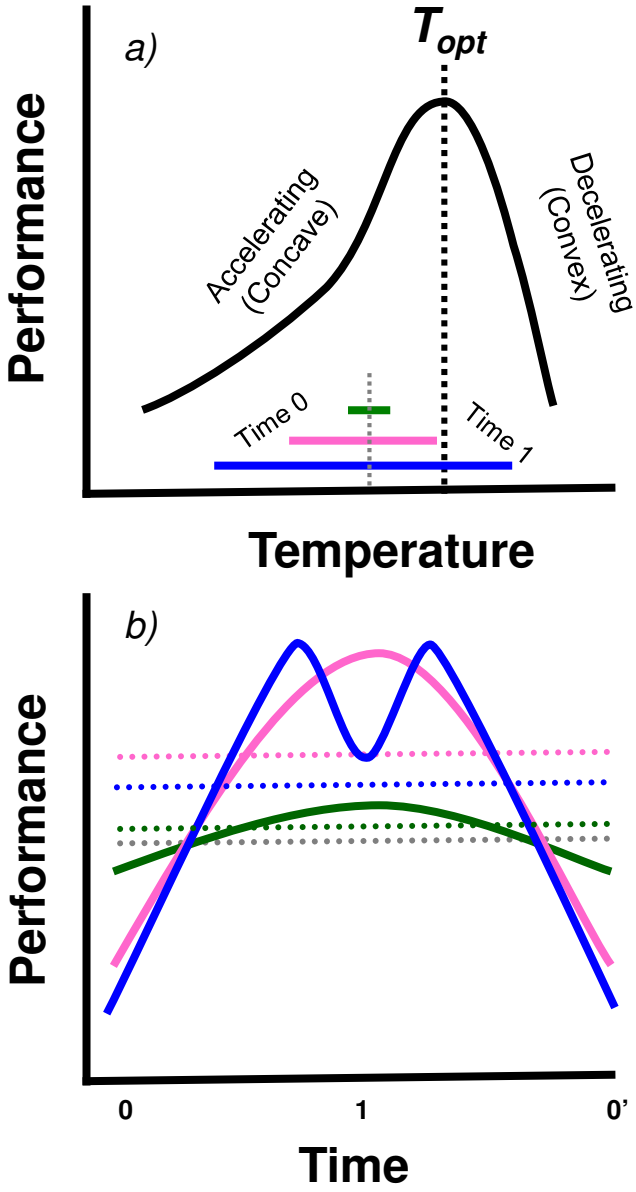




Figure 2

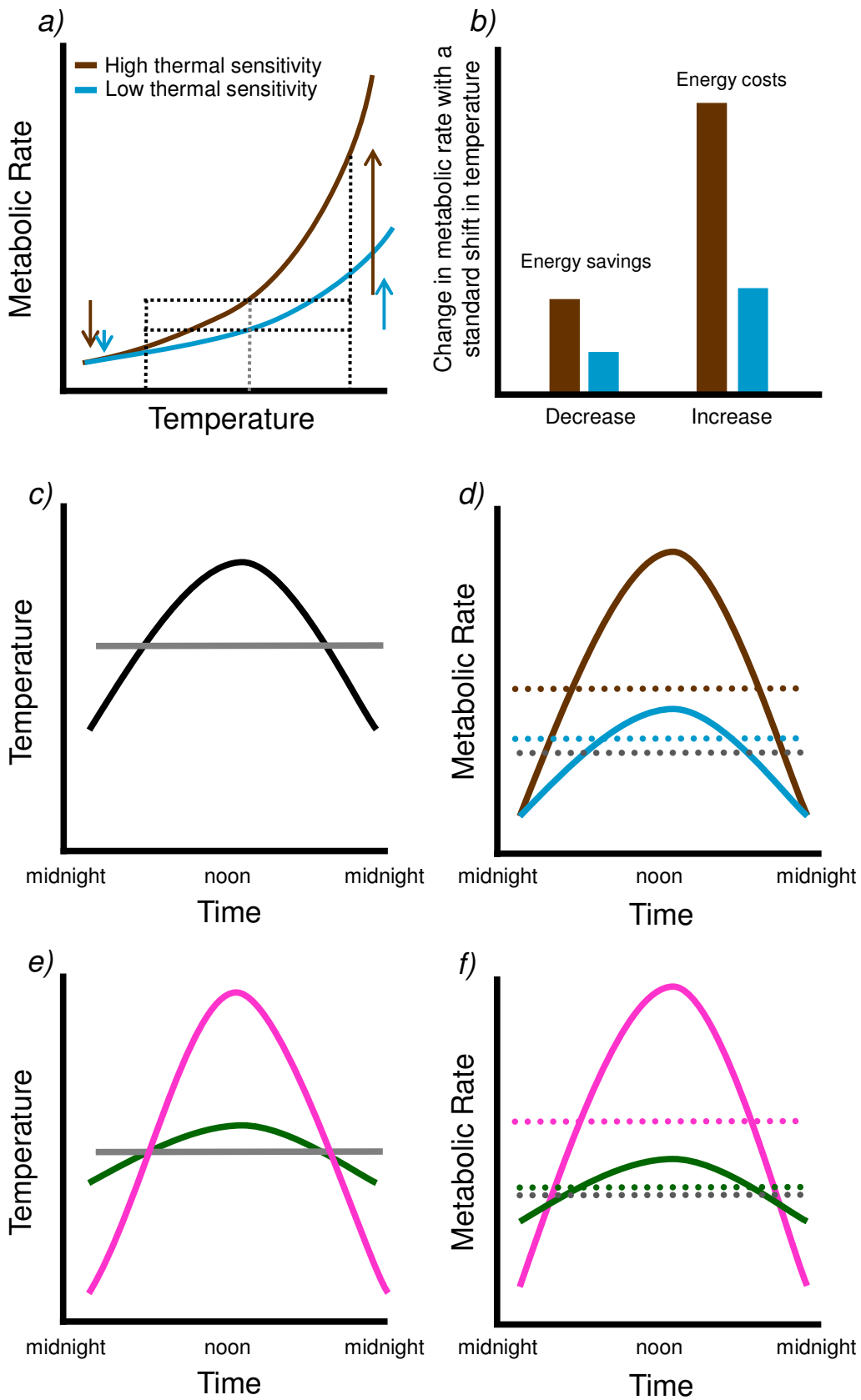


Figure 3

