

Article

The effect of fasting and body reserves on cold tolerance in 2 pit-building insect predators

Inon SCHARF^{a,*}, Alma DANIEL^a, Heath Andrew MACMILLAN^b, and Noa KATZ^a

^aDepartment of Zoology, Faculty of Life Sciences, Tel Aviv University, POB 39040, 69978 Tel Aviv, Israel and

^bDepartment of Biology, York University, 4700 Keele St., Toronto, Ontario, Canada

*Address correspondence to Inon Scharf. E-mail: scharfi@post.tau.ac.il.

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Abstract

Pit-building antlions and wormlions are 2 distantly-related insect species, whose larvae construct pits in loose soil to trap small arthropod prey. This convergent evolution of natural histories has led to additional similarities in their natural history and ecology, and thus, these 2 species encounter similar abiotic stress (such as periodic starvation) in their natural habitat. Here, we measured the cold tolerance of the 2 species and examined whether recent feeding or food deprivation, as well as body composition (body mass and lipid content) and condition (quantified as mass-to-size residuals) affect their cold tolerance. In contrast to other insects, in which food deprivation either enhanced or impaired cold tolerance, prolonged fasting had no effect on the cold tolerance of either species, which had similar cold tolerance. The 2 species differed, however, in how cold tolerance related to body mass and lipid content: although body mass was positively correlated with the wormlion cold tolerance, lipid content was a more reliable predictor of cold tolerance in the antlions. Cold tolerance also underwent greater change with ontogeny in wormlions than in antlions. We discuss possible reasons for this lack of effect of food deprivation on both species' cold tolerance, such as their high starvation tolerance (being sit-and-wait predators).

Key words: chill coma, deprivation, ecophysiology, fat content, food, hunger, morphometrics, Myrmeleontidae, Vermileonidae.

When experiencing multiple stressors, the physiological response to 1 stress could enhance tolerance for another (cross-tolerance). For instance, mild starvation improves desiccation tolerance in fruit flies (Bubly et al. 2012), and there is often a cross-tolerance between cold and desiccation tolerance, which may be adaptive (Sinclair et al. 2013). Generally, mild (but not harsh) stress can have beneficial effects on various traits, such as the positive contribution of a mild cold stress to longevity, fungal resistance and climbing activity (Le Bourg 2009). In contrast, prior experience of certain stressors may impair resistance to a different stress type. One such example is an apparent trade-off between starvation and cold tolerance in both *Ceratitis* flies and flour beetles (Nyamukondiwa and Terblanche 2009; Scharf et al. 2016). Furthermore, if the stress is too high or several stress sources are combined, even a stress type that otherwise would induce cross-tolerance could turn destructive (Le Bourg 2015).

Starvation occurs if animals do not consume sufficient food to maintain their energy demands (McCue 2010). Animals respond to

starvation by either increasing activity, in order to locate new food resources, or decreasing activity, in order to conserve energy (Graf and Sokolowski 1989; Knoppin et al. 2000). When deprived of food, many animals limit behaviors that require energy, such as courting and mating (Travers and Sih 1991; Papadopoulos et al. 1998) and/or take higher risks in order to obtain food (Kohler and McPeck 1989; Reis and Miller 2011). Sit-and-wait predators do not actively search for their prey in contrast to widely foraging predators that actively search for prey. Instead, they choose an ambush site and wait for prey. Sit-and-wait predators are prone to long periods of time without food, owing to their low probability of encountering prey relative to their widely foraging relatives (Huey and Pianka 1981). Consequently, predators adopting the sit-and-wait foraging mode have developed a remarkable ability to lower their metabolic rate in the absence of prey (Anderson 1974; Nagy et al. 1984).

The effects of starvation on behavior are diverse and well studied, but its effects on thermal preference and tolerance are less

clear. Starving ectotherms resist starvation longer at lower temperatures (Da Lage and David 1989; Scharf et al. 2015) and actively seek habitats with lower temperature in order to reduce their metabolic rate (Lazzari 1991; Porter and Tschinkel 1993). Cold tolerance is influenced by environmental factors, such as habitat of origin, and growth and acclimation temperatures (Gibert and Huey 2001; Wilson and Franklin 2002; Sgrò et al. 2010). Relative to the environmental effects on thermal tolerance, the effects of physiological status and other abiotic stressors on cold tolerance have been largely neglected (Bowler and Terblanche 2008). One exception to this is the observed cross-tolerance between desiccation and cold tolerance, which may share similar underlying mechanisms, such as the homeoviscous adaptation of biological membranes, the production of compatible osmolytes, and the upregulation of heat shock proteins (Sinclair et al. 2013).

For some animals, such as tardigrades, there is no apparent link between starvation and cold tolerance (Hengherr et al. 2009). Other studies on a range of distantly related terrestrial arthropods (e.g., beetles, isopods and fruit flies), however, have reported a negative effect of starvation on cold tolerance (Lavy et al. 1997; Nyamukondiwa and Terblanche 2009; Scharf et al. 2016). This negative link is further supported by a trade-off between starvation and cold tolerance in fruit flies (at least for females; Hoffmann et al. 2005; but see MacMillan et al. 2009). This relationship is suggested to be driven by either an energetic trade-off between these 2 stressors (i.e., mediated through lipid reserves; Hoffmann et al. 2005), or an effect of starvation on the hemolymph osmotic balance, leading to slower recovery from cold stress (MacMillan et al. 2012, 2015; Scharf et al. 2016). Other studies, on freeze-avoiding insects, in contrast, found a positive effect of starvation on cold tolerance. Emptying of the gut through fasting or mild starvation may prevent ice formation and thereby lower the supercooling point of a freeze-avoiding insect (Somme 1982; Salin 2000). For chill-susceptible insects that die from effects of temperature, unrelated to ice formation, fasting may have different effects on low temperature survival. For example, fasting locusts recover their osmotic balance faster following chilling stress (Andersen et al. 2013) and mildly fasting fruit flies survive cold stress better than fed flies (Le Bourg 2013, 2015). Thus, whether starvation and cold tolerance are linked, whether such a link is positive or negative, and what physiological and molecular mechanisms might produce such a link, all remain unclear.

The uncertainty as to whether and how starvation impacts cold tolerance might be partly related to variance both in how starvation is induced and in how cold tolerance is measured in the laboratory. Cold tolerance can be measured, for example, as the temperature that induces a loss of coordination followed by neuromuscular paralysis (critical thermal minimum [CT_{min}] or chill coma onset temperature), the time required to recover from chill coma after being removed to a warmer temperature (chill coma recovery time [hereafter, CCRT]), the lower lethal time or temperature (Lt_{50} or LT_{50} , respectively), or the supercooling point (Andersen et al. 2015; Sinclair et al. 2015). It could be that freeze-avoidant insects improve their cold tolerance by means of starvation, whereas starving chill-susceptible insects leads to changes in their ion and water balance or lipid composition that consequently impair chill tolerance. However, even when using the same cold tolerance proxy of CCRT in 2 chill-susceptible insects (migratory locusts and flour beetles), the effect of starvation is opposite (cf. Andersen et al. 2013; Scharf et al. 2016). One clear difference between the 2 latter studies is that the locusts were fed immediately before the cold tolerance test, whereas the beetles are “continuous feeders” that live in their food,

and may or may not have consumed a large quantity of food immediately before the test. Thus, both the nature of the starvation or fasting stress, as well as the manner in which cold tolerance is measured, could lead to confusion regarding the effects of starvation on cold tolerance.

Here, we measured the cold tolerance (CCRT) on the larvae of 2 pit-building insect predators, antlions and wormlions. These species are members of 2 different insect orders (Neuroptera and Diptera, respectively), but present a unique example of convergent evolution, owing to similar natural history and foraging mode (Dor et al. 2014). We compared the cold tolerance of both antlions and wormlions following food deprivation, when fed immediately before the cold tolerance test, and when fed 2 days prior to the test. Our goals were: (1) to understand the effect of mild starvation and recent feeding on cold tolerance, which can affect cold tolerance in different directions; (2) to investigate the effect of body mass, condition, and morphology on cold tolerance; and (3) to explore an aspect of the convergent evolution between antlions and wormlions. We predicted that either long fasting or feeding immediately before the test would impair cold tolerance, whereas feeding 2 days before the test would lead to the best cold tolerance (a trade-off between fasting and cold tolerance). Alternatively, fasting, especially when not too harsh, might also enhance cold tolerance (a cross-tolerance induced by one stress type against another one). We further predicted a positive effect of body mass and condition on cold tolerance (e.g., Terblanche et al. 2008), and expected a similar response by antlions and wormlions.

Materials and Methods

Studied insects and collection

Both antlions and wormlions occur in loose soil, sometimes in the same habitat. Larvae of both species are ambush predators, digging pits that serve to trap insect prey (Wheeler 1930). Although the morphology of each species is strikingly different (see Figure 1 in the Supplementary Material), their pits are almost indistinguishable. Other similarities include a high starvation tolerance (typical of any sit-and-wait predator; Scharf and Ovadia 2006; Scharf and Dor

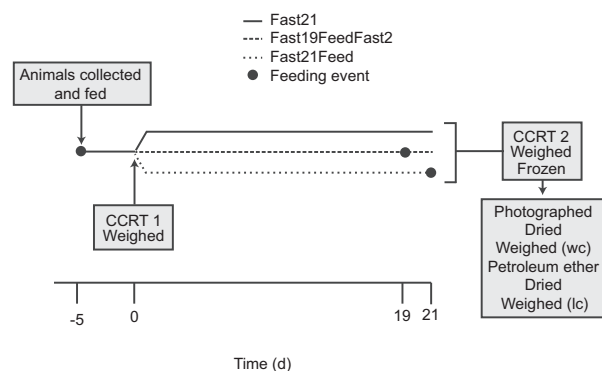


Figure 1. Overview of the experimental design (identical for both species). Antlions and wormlions were fed on the day they were collected and CCRT and body mass were recorded 5 days later (first CCRT). The animals were divided equally into 3 treatment groups: (1) fasting for 21 days (Fast21), (2) fasting for 21 days, with feeding on Day 21 (Fast21Feed), and (3) fasting for 19 days, followed by feeding, and 2 additional days of fasting (Fast19FeedFast2). On Day 21, CCRT was measured again, and was followed by weighing, freezing, photographing (for morphometrics), and sample analysis to determine body composition. wc: water content, lc: lipid content.

2015) and an active process of habitat choice based on abiotic factors, such as substrate type (Devetak and Arnett 2015). However, whereas antlions are cannibals (Barkae et al. 2014), wormlions are not, and occur at much higher densities (Dor et al. 2014). Thermal tolerance of pit-building predators has been rarely studied. Two exceptions studied the thermal activity range of an antlion and its common ant prey (Marsh 1987) and another one demonstrated that antlions of shaded habitats have lower heat tolerance than those of sun-exposed habitats (Rotkopf et al. 2012).

The studied antlion, *Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae), is the most common pit-building antlion in Israel (Simon 1988) and occurs in sand in the Israeli coastal and desert regions (Scharf et al. 2008; Alcalay et al. 2015). The larvae are found under trees and bushes, and the diet of this and related antlions mainly comprises ants (Marsh 1987; Simon 1988). Antlion larvae were collected in August 2015 from a coastal sand dune near Caesarea (N32.4819, E34.8988). The studied wormlion, an undescribed *Vermileo* sp., is the only wormlion known in Israel (Friedberg A, personal communication). Wormlions were collected in October 2015 from Tel Aviv University grounds and adjacent streets (N32.1140, E34.8018). This insect is very common in cities, inhabiting the thin loose soil layer below buildings (Dor et al. 2014; Scharf and Dor 2015). The larval stage in both species is long (at least a year), and the adults are weak-flying and short-lived. Antlion larvae have 3 instar stages, whereas the number of instars of the studied *Vermileo* sp. is probably 6 but not known for certain (Wheeler 1930). Although heat probably poses higher danger for both species in their Mediterranean habitats, winter can be cool, with minimal temperatures of about 3°C (BioGIS 2012). Measuring cold tolerance is therefore important to understand the effect of cold winter days on antlion/wormlion prey capture success, as prey is active also during winter.

Fasting and cold tolerance

After collection, antlions and wormlions were fed once with similar-sized larvae of red flour beetles *Tribolium castaneum*. Flour beetles are not the common prey in the field, but are used in order to standardize prey mass and its growth conditions, as common in previous studies (Scharf et al. 2008, 2010; Dor et al. 2014; Alcalay et al. 2015). Five days after feeding, we placed the insects on icy water (~0°C) in individual petri dishes, whereupon both species ceased movement entirely within a few seconds. After 90 min, the insects were removed from the ice to room temperature and observed continuously until they righted. Both species have a typical behavior when recovering from chill coma: wormlions try digging with their head down, whereas antlions move backwards looking for sand to bury themselves below. This procedure (CCRT) is a common, non-lethal proxy of cold tolerance for insect systems (MacMillan and Sinclair 2011; MacMillan et al. 2012). For example, CCRT is heritable in *Drosophila*, differs among related species and populations from geographical regions with different local climates, and correlates well with the minimal temperature in the habitat of origin (Chown and Nicolson 2004, ch. 5; MacMillan and Sinclair 2011; Andersen et al. 2015). Insects were weighed using a microbalance (accuracy of 0.01 mg) and evenly distributed based on body mass and CCRT to one of the following 3 treatments: (1) fasting for 21 days (Fast21), (2) fasting for 21 days followed by a single feeding event (Fast21Feed), and (3) fasting for 19 days followed by a single feeding event and then 2 additional days of fasting (Fast19FeedFast2; $N_{\text{antlions}} = 32, 34, 33$ and $N_{\text{wormlions}} = 29, 31, 30$ for the 3 treatments, respectively). On Day 21 after the first feeding

event, we again measured CCRT of all individuals to evaluate their cold tolerance, weighed them again and froze them at -20°C (Figure 1). During the 2 CCRT measurements room temperature was kept as constant as possible ($25.5 \pm 1^\circ\text{C}$). Before and after CCRT measurements, insects were kept in climate cabinets at 26°C, with a 12:12h light:dark photoperiod regime. We expected to find a positive correlation between the 2 measurements. A starvation period of 21 days was chosen because it induces behavioral changes in antlions (such as pit relocation and response time to prey; Hauber 1999; Scharf et al. 2010; reviewed in Scharf and Ovadia 2006) and significant mass loss in wormlions (Scharf and Dor 2015). On the other hand, it is not a too harsh stress, which would lead to drastic physiological changes or mortality.

Water, lipid, and morphological measurements

All individuals were photographed under a stereomicroscope (magnification of 10×). For antlion larvae, we measured head width and length, mandible length, and abdomen width and length (similar to Scharf et al. 2008; Alcalay et al. 2015). For wormlion larvae, we measured only body length and width of the broadest segment, on the posterior side (10th of its 11 segments; Wheeler 1930). All photo measurements were taken using ImageJ version 1.46 (Abramoff et al. 2004). Water content was measured by drying the insects in an oven (60°C) for 3 days and weighing them again. The difference between the fresh body mass and the dry mass provided the water content. Non-polar lipids were extracted by placing each individual in 1 ml petroleum ether for 5 days, which is a recommended method for avoiding co-extraction of polar lipids (e.g., phospholipids; Williams et al. 2011). The petroleum ether was topped up 3 times during this period to compensate for evaporation. The insects were then dried again for 3 days at 60°C and reweighed. The difference between the dry mass and the lipid-free mass provided the lipid content (Figure 1).

Statistical analysis

Because antlions and wormlions showed distinctly different trends, they were analyzed separately. First, we checked for collinearity of all potential explanatory variables (dry mass, water, lipids and all morphological measurements), using Pearson correlations. Due to the tight correlations among variables (see Results), it was problematic to include all explanatory variables in multiple regression tests, and we therefore sought to reduce their number. For antlions, we used Principal Component Analyses (hereafter, PCA) for the morphological traits, after using a Z-score transformation. The only significant PC was the first one, with an eigenvalue larger than one (see Results). We refer to this PC1 as a proxy of body size, because all coefficients had positive loadings. We measured for wormlions only length and width. Due to the high correlation between the two, we included only body length in further analyses. We created an index of body condition by calculating the residuals of the regression of the dry body mass on PC1 for antlions and body length for wormlions (similar to Schulte-Hostedde 2005; Elimelech and Pinshow 2008; the latter used this index for another antlion species). Square-root body mass was used, because it provided higher R^2 values than body mass for both species (antlions: $R^2 = 0.682$ vs. $R^2 = 0.653$; wormlions: $R^2 = 0.879$ vs. $R^2 = 0.845$, for square-root and non-transformed body mass, respectively).

We then examined to what extent body mass had changed following the fasting/feeding treatments. We expected only a small difference, because in the 2 feeding treatments individuals were fed

only once (compared with complete fasting). We calculated the proportion of mass change $[(\text{Mass2} - \text{Mass1}) / \text{Mass1}]$, with values above or below zero indicating that mass had been gained or lost, respectively. We then used a 1-way ANOVA, separately for each species, with treatment as an explanatory variable.

Next, we correlated between the first CCRT, before applying the treatments, and the second CCRT. If the correlation was strong, then paired analyses would be required for understanding the possible treatment effect, whereas if the correlation was weak, the first CCRT could be ignored. Since the correlation was weak for antlions and not significant for wormlions (see Results), we did not use paired analyses.

We then used linear models with either antlion or wormlion second CCRT as response variables, and treatment, body condition, dry mass, and lipid content as explanatory variables. Water was not included in the analyses due to its tight correlation with dry mass (see Results). We included all 2-way interactions of dry mass, lipid content and body condition with treatments. These interactions were gradually removed according to F-ratio values, if non-significant, but main effects were not removed. The most significant explanatory variable was then regressed as a single factor against the second CCRT in order to better characterize its relationship with CCRT. Finally, we examined whether antlions and wormlions generally differed in their CCRTs using a *t*-test. As most variables did not affect CCRT in both species, such a comparison is possible. CCRT was always \log_{10} -transformed because it was right skewed and deviated from a normal distribution.

Results

All explanatory variables were tightly correlated for the wormlion dataset ($r > 0.35$ for all pairwise combinations), and also for antlions ($r > 0.35$), except those for lipid content versus head length and width and mandible length (Tables 1 and 2). For antlions, PC1 and PC2 explained 79.3% and 13.1% of the variance, respectively (Table 3). All loadings on PC1 were positive, as is common in morphological analyses.

Regarding antlions, the proportion of mass change differed based on treatment ($F_{2,96} = 9.95$, $P = 0.0001$; Figure 2), with antlions belonging to the Fast21Feed treatment losing less mass than those of the Fast21 treatment (Tukey post hoc test: $P < 0.0001$) and the Fast19FeedFast2 treatment ($P = 0.038$). The effect of fasting was weaker for wormlions ($F_{2,87} = 3.51$, $P = 0.036$; Figure 2). Here, the only difference was between the Fast21Feed and Fast19FeedFast2 treatments ($P = 0.039$).

For antlions, there was a weak positive correlation between the first and second CCRTs, with several outliers, which we did not

Table 1. Correlation coefficients (*r*) of measured traits for antlions (*N* = 99)

Mass	Mass	Water	Lipids	HW	HL	ML	AW
Water	0.875						
Lipid	0.509	0.372					
HW	0.669	0.666	0.066				
HL	0.637	0.640	0.074	0.947			
ML	0.637	0.640	0.025	0.948	0.937		
AW	0.861	0.827	0.300	0.670	0.646	0.647	
AL	0.844	0.803	0.373	0.608	0.604	0.608	0.724

Mass stands for the Dry Mass. HW: head width, HL: head length, ML: mandible length, AW: abdomen width, AL: abdomen length.

remove ($r = 0.306$, $P = 0.0021$; Figure 3A). For wormlions, the same correlation was marginally not significant ($r = 0.198$, $P = 0.061$; Figure 3B). Because of the weak link between the 2 CCRTs, we did not use paired analyses and referred, from this point onward, only to the second CCRT, after the 3 treatments were applied.

For the antlions, CCRT was weakly negatively correlated to lipid content, meaning that individuals with greater lipid stores recovered from chill faster and thus demonstrated a better cold tolerance ($F_{1,91} = 5.08$, $P = 0.027$). Treatment, dry mass, and body condition had no effect on CCRT ($F_{2,91} = 0.57$, $P = 0.57$; $F_{1,91} = 0.25$, $P = 0.62$; $F_{1,91} = 1.09$, $P = 0.30$, respectively). However, the treatment \times body condition interaction was significant ($F_{2,91} = 3.23$, $P = 0.044$), indicating that body condition was negatively correlated with CCRT, but only for antlions that were starved for the entire 21-day period (the Fast21 treatment; Figure 4A). The treatment \times dry mass and treatment \times lipid content were not significant and were thus removed ($P > 0.14$ for both). When regressing antlion

Table 2. Correlation coefficients (*r*) of measured traits for wormlions (*N* = 90)

Mass	Mass	Water	Lipids	Length
Water	0.968			
Lipid	0.454	0.374		
Length	0.919	0.917	0.376	
Width	0.874	0.876	0.388	0.882

Mass: dry mass

Table 3. PCA on morphological traits for antlions

Antlions	PC1	PC2
Eigenvalue	3.96	0.65
% variance	79.27	13.06
HW	0.4771	-0.3166
HL	0.4730	-0.3332
ML	0.4732	-0.3321
AW	0.4118	0.5196
AL	0.3939	0.6391

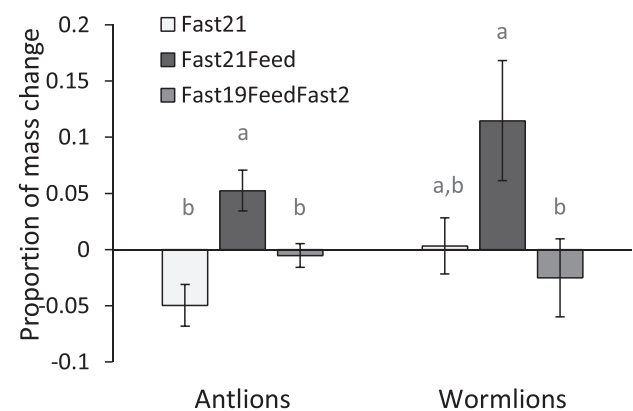


Figure 2. The proportion of change in body mass before and after applying the fasting/feeding treatments. Values above or below zero mean mass gained or lost, respectively. Fast21, Fast21Feed, and Fast19FeedFast2 stand for fasting for 21 days, fasting for 21 days followed by feeding, and fasting for 19 days, feeding and then 2 additional days of fasting. Letters denote significant differences based on a Tukey post hoc test.

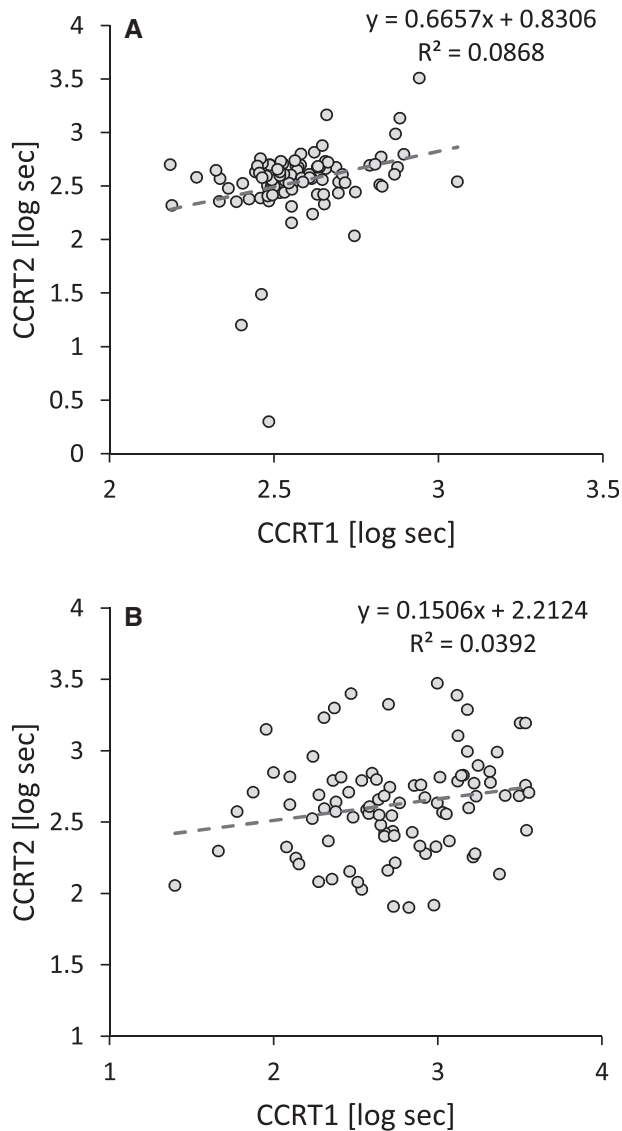


Figure 3. The (weak) correlation between the first and second CCRT measurements, before and after applying the fasting/feeding treatments, for (A) antlions, and (B) wormlions.

CCRT on lipids alone, the negative correlation remained significant ($F_{1,96} = 6.36$, $t = -2.52$, $P = 0.013$; Figure 4B).

For the wormlions, CCRT tended to correlate with dry mass (albeit not quite significantly; $F_{1,84} = 3.64$, $P = 0.060$). Treatment, lipid content and body condition all had no effect on wormlion CCRT ($F_{2,84} = 2.11$, $P = 0.13$; $F_{1,84} = 0.56$, $P = 0.46$; $F_{1,84} = 0.39$, $P = 0.54$, respectively). All 2-way interactions with treatment were not significant and were removed ($P > 0.14$ for all). When regressing wormlion CCRT on dry mass alone, the effect was significant ($F_{1,88} = 7.77$, $t = -2.79$, $P = 0.0065$; Figure 4C). Finally, there was no difference in the CCRT of antlions and wormlions among any of the treatment groups ($t = -1.37$, $df = 187$, $P = 0.17$).

Discussion

We expected that either starving the antlions and wormlions for 3 weeks or feeding them immediately prior to chill coma would impair

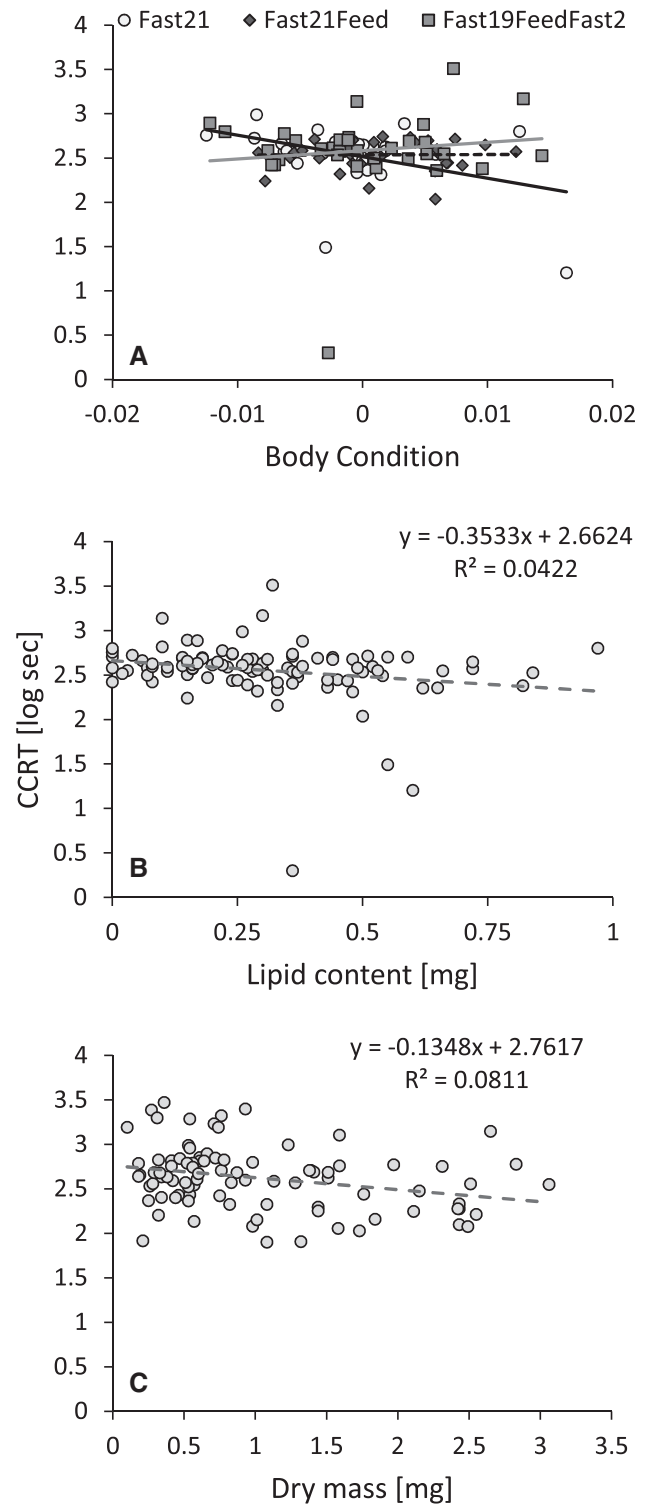


Figure 4. (A) The interaction between the effect of treatment and body condition on CCRT for antlions. Fast21 (a continuous black line), Fast21Feed (a segmented black line) and Fast19FeedFast2 (a grey line) stand for the 3 treatments. (B) The negative effect of lipid content on CCRT for antlions, and (C) the negative effect of dry mass on CCRT for wormlions (both 1-way linear regressions).

their cold tolerance. However, none of the treatments affected the cold tolerance of either species. Antlions were weakly affected by an interaction of body condition (body mass-to-size residuals) with treatment, suggesting that when starved, body condition positively

contributes to cold tolerance. Antlions and wormlions differed in the effects of dry mass, lipid content, and body condition on cold tolerance. There was a positive correlation between the dry mass and cold tolerance of wormlions (i.e., bigger wormlions were more cold tolerant), whereas antlion cold tolerance was similarly related to lipid content (i.e., fatter antlions were more cold tolerant).

No effect of fasting on cold tolerance

Our food deprivation and feeding treatments did not affect cold tolerance in both antlions and wormlions. Therefore, our results support neither our prediction for a trade-off between fasting and cold tolerance nor the alternative prediction of cross-tolerance between the 2 stress types. A possible reason for this is that the starvation period was not long enough, and it is possible that both insect species did not experience much stress. This possibility is supported for wormlions by the weak non-significant difference found in mass change between the complete fasting and the feeding treatments (Fast21 vs. Fast21Feed). The effect for antlions, even though significant, was also quite weak, and was independent of the length of fasting (2 vs. 21 days). This suggests that the individuals merely experienced fasting, or at the most mild starvation. Generally, all pit-building predators are able to withstand long periods of starvation (up to 4 months; [Matsura and Murao 1994](#)). However, milder responses to starvation, such as increased movement and pit relocation, are evident even after 2–3 weeks in different pit-building antlion species ([Eltz 1997](#); [Hauber 1999](#); [Scharf et al. 2010](#)). It is also possible that the difference between treatments was not strong enough, and that a single feeding event is not enough to compensate for starvation. This suggestion could have been supported had we found a significant interaction between body mass and treatment, indicating that larger and smaller individuals had been differently affected by fasting and feeding. However, we did not find such a relationship. It is possible that fasting has a species-specific effect on cold tolerance and CCRT in particular. The effect could be related to the timing of feeding and to whether insects are continuous feeders or not, but might be also related to mechanisms of reestablishing ion and water balance and/or energy allocation for different physiological processes ([MacMillan et al. 2012](#); [Andresen et al. 2013](#)).

Sit-and-wait insect predators, owing to their low metabolic rate, may have some physiological parallels with dormant insects regarding their thermal tolerance. Studies of thermal tolerance in dormant insects (e.g., [Danks 2000](#)) could therefore serve as a basis for understanding better sit-and-wait predators. In conclusion, it could be that longer starvation would have an effect of cold tolerance, or that another measure of cold tolerance, such as response to prey at different temperatures, would be more affected by the feeding regime.

Body mass and lipid content predict cold tolerance

Antlions and wormlions differed in the effect of mass, body condition and lipid content on cold tolerance. Dry mass had a contributing effect, but only in wormlions. The effect of body mass on cold tolerance is inconsistent among species and study methods (cf., e.g., [Angilletta et al. 2007](#); [Colinet et al. 2007](#)). It even differs based on physiological state, as the effect of body mass on CCRT of hungry individuals may be stronger than on satiated ones ([Halle et al. 2015](#)). Wormlion larvae are fly maggots, and due to their shape, dry mass is more closely related to true body reserves than it is for antlions, which have a larger structural mass to which their large head and strong mandibles contribute. Lipid content contributed to

cold tolerance in antlions but not in wormlions, perhaps because lipid content in antlions is a better representation of body reserves. Indeed, whereas dry mass is correlated with head length and width, which do not reflect any body reserves, lipid content is mainly correlated with abdomen length and width, reflecting body reserves, and it shows no correlation with head traits. Lipid content has been found to affect cold tolerance in several other systems. For instance, aphids raised under a lower temperature were heavier, fatter, and resisted cold temperatures better than those raised under a higher temperature ([Colinet et al. 2007](#)). Growth temperature and even thermal acclimation as adults affect membrane lipid composition consequently influencing cold tolerance ([Kořtal and Šimek 1998](#); [Overgaard et al. 2008](#)). Body condition, as measured here, had little if any effect on cold tolerance. However, different measures of body condition exist, and we might not have chosen the 1 best fitting the studied animals (e.g., [Peig and Green 2009](#)). However, the choice of such a body condition index should be made *a priori*, and has been previously found relevant for antlion movement activity ([Elimelech and Pinshow 2008](#)). Because the two studied insects differ greatly in their body structure, we chose a general and simple index that intended to fit both.

Our data also shed light on the effect of ontogeny on cold tolerance of antlions and wormlions. Larger, older antlion larvae were not more cold tolerant than the smaller larvae. This result holds true both in regard to the non-significant effect of body mass on CCRT and when considering the effect of instar stage on CCRT (not shown). Wormlion cold tolerance improved with increasing body mass and consequently also by instar stage. Although older, larger larvae are often more cold tolerant than younger, smaller ones ([Lee and Denlinger 1985](#); [Chauvin and Vannier 1997](#); this study), this is not always the case ([Carrillo and Cannon 2005](#); [Jensen et al. 2007](#); reviewed in [Bowler and Terblanche 2008](#)). Repeatability of CCRT was low for antlions and non-existent for wormlions. Between-individual repeatability measurements are often neglected in ecophysiology, with the exception of metabolic rates ([Chown and Nicolson 2004](#), ch. 1). Because repeatability is important in order for natural selection to affect a trait, this lack of repeatability (especially for wormlions) suggests that CCRT may be of limited importance from an evolutionary ecological perspective. In conclusion, our dataset indicates that either body mass or lipid content could affect cold tolerance, but the exact impact of each physiological trait is probably species-specific.

Comparison of antlion and wormlion cold tolerance

Although antlions and wormlions were collected in different months, and despite the different contribution of body mass and lipid content for each species, they showed similar CCRTs. They were also similarly unaffected by fasting. We interpret this similarity with caution as a product of their similar natural history and ecology, as they both occur in shaded areas, and both were collected from a coastal Mediterranean region. Nonetheless, one might assume that the wormlions would demonstrate a weaker cold tolerance, because they were collected in a city, which experiences higher annual temperatures than the nearby natural sites ([Grimm et al. 2008](#)). Because both species showed similar cold tolerance, we suggest it is caused by the resembling abiotic environmental conditions.

Conclusions and Future Directions

Fasting did not affect the cold tolerance of antlions and wormlions, in contrast to our expectation, and previous results with other

insects, such as flour beetles, migratory locusts, and fruit flies. Body mass and lipid content had, nevertheless, a positive effect on cold tolerance. A better comparison of the thermal tolerance of the 2 studied species, including different populations along climatic and urban-natural gradients, should contribute to understanding how ecological factors determine their thermal tolerance. Furthermore, the ecological consequences of thermal tolerance, such as longer activity times under unfavorable conditions, and repeatability of thermal tolerance measurements are of interest in order to better understand the way by which natural selection operates on thermal tolerance.

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