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Linking energetics and overwintering in temperate insects.

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1 Mini-review

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3 **Linking energetics and overwintering in temperate insects**

4

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7

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12 **Abstract**

13 Overwintering insects cannot feed, and energy they take into winter must therefore fuel energy
14 demands during autumn, overwintering, warm periods prior to resumption of development in
15 spring, and subsequent activity. Insects primarily consume lipids during winter, but may also
16 use carbohydrate and proteins as fuel. Because they are ectotherms, the metabolic rate of insects
17 is temperature-dependent, and the curvilinear nature of the temperature-metabolic rate
18 relationship means that warm temperatures are disproportionately important to overwinter energy
19 use. This energy use may be reduced physiologically, by reducing the slope or elevation of the
20 temperature-metabolic rate relationship, or because of threshold changes, such as metabolic
21 suppression upon freezing. Insects may also choose microhabitats or life history stages that
22 reduce the impact of overwinter energy drain. There is considerable capacity for overwinter
23 energy drain to affect insect survival and performance both directly (via starvation) or indirectly
24 (for example, through a trade-off with cryoprotection), but this has not been well-explored.
25 Likewise, the impact of overwinter energy drain on growing-season performance is not well
26 understood. I conclude that overwinter energetics provides a useful lens through which to link
27 physiology and ecology and winter and summer in studies of insect responses to their
28 environment.

29
30 **Keywords:** Cold tolerance, Jensen's inequality, fat reserves, triglycerides, metabolic
31 suppression, diapause

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34 1. Introduction

35 Insects and other ectotherms that overwinter in temperate environments must overcome multiple
36 challenges including low temperatures, water deficit, and energetic drain (Williams et al., in
37 press). Because they are inactive and generally do not feed, the energy stores with which insects
38 enter dormancy must fuel the mechanisms that protect against cold and desiccation, as well as
39 provide for basal metabolism throughout the period when the insect is dormant – which may
40 include parts of autumn and spring, as well as winter (Fig. 1). In the case of insects that
41 metamorphose or develop prior to feeding in the spring, the energy stores remaining after winter
42 must also fuel that development. If the adult stage does not feed (or has restricted nutrition),
43 remaining energy stores may represent all, or the majority of, the energy available for
44 reproduction. Because ectotherm metabolism is temperature-dependent, the energy remaining at
45 the end of the dormant period is determined by the thermal conditions experienced during winter
46 (and the adjacent portions of autumn and spring), and energy conservation is thus a general
47 requirement of overwintering that transcends variation in microhabitat, cold tolerance strategy
48 and developmental stage. In this mini-review, I will explore the factors that affect overwinter
49 energy use and conservation by temperate insects, with a view to exploring how growing-season
50 fitness might be determined by dormant-season energy use and conservation.

52 2. Overwinter energy stores

53 The juvenile stages of many insects have been selected to maximise growth, energy acquisition,
54 and storage to maximise the success of the adult stage (Boggs, 2009). Indeed, pupal mass is
55 often used as a proxy for fitness because larger females are generally more fecund (Honek,

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4 56 1993). In preparation for winter dormancy, many insects utilise specific energy storage
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6 57 strategies, reviewed in detail elsewhere (Hahn and Denlinger, 2007, 2011). The stores used to
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8 58 fuel metabolism are generally divided between lipid (usually fats in the form of triglycerides)
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10 59 and carbohydrate (often as glycogen, but also as smaller molecules such as the disaccharide
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12 60 trehalose). Many insects also utilise storage proteins (Burmester, 1999); however, although
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14 61 storage proteins do have a role in diapause (Hahn and Denlinger, 2007), they do not appear to be
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16 62 a primary source of energy for overwinter metabolism, and may instead be a route for transfer of
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18 63 amino acids between larval and adult stages (e.g. O'Brien et al., 2002).
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27 65 Lipids offer the most energy-dense storage, and empirical measurements of body composition
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29 66 support the assumption that most non-feeding insects are consuming lipid (Sinclair et al., 2011).
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32 67 However, insects that are frozen, encased in ice, or in an otherwise hypoxic environment will
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34 68 likely rely on carbohydrate-fuelled anaerobic metabolism (Storey and Storey, 1986). Some
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36 69 species, such as the spruce budworm *Choristoneura fumiferana* rely on carbohydrates over the
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38 70 winter even when unfrozen (Han and Bause, 1993), while others switch from lipids to other
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40 71 energy sources mid-winter, possibly cued by diapause cessation (e.g. Adedokun and Denlinger,
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42 72 1985; Yocum et al., 2005). Insects may shift their fuel use for other reasons; overwintering
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44 73 insects are also water-stressed (Danks, 2000), and could therefore shift to carbohydrate
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46 74 metabolism to liberate water hydrogen-bound to glycogen, as is seen in desiccation-tolerant
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48 75 *Drosophila* (Marron et al., 2003).
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58 77 **3. How do winter conditions drive energy use?**
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78 Below the thermal optimum, there is usually a curvilinear relationship between enzyme-mediated
79 biological reactions and temperature (Schulte et al., 2011). Thus, warmer temperatures yield
80 higher rates of activity, development, growth, and metabolism (Fig. 2). During the winter, when
81 temperate insects generally are not actively growing, feeding, or developing, energy stores will
82 be consumed faster at higher temperatures. Consequently, winter energy use will be determined
83 by the mean temperature, the temperature variability, the length of winter, and the ability to
84 mitigate overwinter energy drain (Williams et al., in press).

85
86 Overwintering temperatures are modified by microhabitat. Persistent snow cover will usually
87 increase the mean temperature (potentially increasing energy use), but reduce the variability of
88 those temperatures (Pauli et al., 2013). Thermal variability is particularly important in
89 determining overwinter energy use because of the effect of Jensen's inequality (Fig. 3), whereby
90 an accelerating temperature-metabolic rate relationship results in an increased mean metabolic
91 rate (and energy consumption), because the high temperature portions of thermal cycles increase
92 metabolic rate more than the low temperature portions compensate by reducing energy
93 consumption (Ruel and Ayres, 1999; Williams et al., 2012b). Thus, thermal fluctuations,
94 especially to high temperatures, or about a warm mean, (e.g. during the autumn or spring) can
95 account for the majority of cumulative energy use during the dormant period (Sgolastra et al.,
96 2011; Williams et al., 2012b; Fig. 3).

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98 There are other potential forms of the relationship between temperature and energy consumption,
99 which may interact to produce a complex relationship (Fig. 2). In particular, there may be

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4 100 threshold changes in metabolic rate, as observed in freeze-tolerant caterpillars (Fig. 2B; Sinclair
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6 101 et al., 2004; discussed further in section 4.1), or transient cost of crossing thresholds, such as the
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9 102 increased metabolic rate associated with recovery from chill coma (Macmillan et al., 2012b; Fig.
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11 103 2C). These different forms can all be incorporated into models to estimate overwinter energy
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13 104 costs from microclimate temperature data (e.g. Sinclair et al., 2013). Costs associated with
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15 105 threshold-crossing, such as in Fig. 2C, could be a one-time cost, or accumulate, such that the
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17 106 energetic cost over a winter is a function of the number of threshold-crossing events, as is
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19 107 hypothesised for repeated cold exposure (Marshall and Sinclair, 2010; Fig. 2D).
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28 109 In the context of climate change, overwinter energetics can therefore be affected by changes in
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30 110 the mean temperature, in thermal variability, and by snow cover (which will determine thermal
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32 111 variability and exposure to thermal extremes). Current climate change predictions do a poor job
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34 112 of predicting extreme temperatures and variability, and the scale of climate documentation is
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36 113 inappropriate for insects (Potter et al., 2013). Furthermore, snow cover is a combination of local
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38 114 and regional patterns of precipitation (Henry, 2008; Kreyling and Henry, 2011), in combination
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40 115 with localised topography that influences snow drift formation and therefore both snow depth
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42 116 and persistence (e.g. Scott et al., 2008). These microtopographical influences are hard to predict
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44 117 (Scherrer and Körner, 2010) and may mean that small changes in microhabitat selection could
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46 118 have a big influence on exposure to overwinter energy drain, just as behaviour is a key
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48 119 determinant of climate change impacts in the summer (Sunday et al., 2014).
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58 121 **4. Mitigating overwinter energy drain**
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4 122 *4.1 Physiological mitigation of overwinter energy drain*

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7 123 Insects can reduce overwinter energy drain by altering their temperature-energy use relationship
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9 124 through modified thermal sensitivity and/or suppressed metabolic rate, either of which reduces
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11 125 the rate of energy consumption (Fig. 4). Metabolic suppression can result from a temperature-
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13 126 independent decrease in metabolic rate (decreasing the elevation of the temperature-metabolic
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15 127 rate relationship; Fig. 4B), or through temperature-dependent threshold decreases in metabolism,
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17 128 such as those observed upon freezing (Fig. 2B; Sinclair et al., 2004).
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25 130 Temperature-independent metabolic suppression is a defining feature of diapause (Hahn and
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27 131 Denlinger, 2011; Tauber et al., 1986). Metabolic suppression reduces overall energy use, and
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29 132 insects with extreme metabolic suppression and deep diapause may therefore have relatively
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31 133 little energy use over the duration of a winter, and be robust to increased winter temperatures
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33 134 (e.g. Fründ et al., 2013; Williams et al., 2012a). Indeed, diapause-associated metabolic
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35 135 suppression is such an effective energy conservation strategy that tropical insects can diapause at
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37 136 high temperatures during the dry season with almost no reduction in fat content (Nedvěd and
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39 137 Windsor, 1994). The mechanisms underlying metabolic suppression in diapausing insects are
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41 138 beginning to be understood from a genetic level (Denlinger and Armbruster, 2014; Hahn and
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43 139 Denlinger, 2011), but the metabolic biochemistry is not as well-explored as in some other taxa
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45 140 (see Storey and Storey, 2004; Storey and Storey, 2007).
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56 142 Temperature-metabolic rate relationships can change abruptly with temperature, such that
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58 143 metabolic suppression is temperature-dependent (Fig. 2B). There are many potential causes of
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4 144 such thresholds; for example, Sinclair et al. (2004) suggest that failure of transmembrane ion
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7 145 pumps at low temperature accounts for a threshold decrease in carbon dioxide emission when
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9 146 *Pringleophaga marioni* caterpillars are cooled. The best-understood low temperature threshold
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12 147 shift in insects is probably the very clear physiological and biological threshold associated with
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14 148 ice formation in freeze-tolerant insects. While ice formation is usually restricted to extracellular
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16 149 spaces (but see Sinclair and Renault, 2010), the dehydration and changes in pH and oxygen
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19 150 delivery associated with being frozen lead to metabolic rate suppression, and there is often a
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21 151 consequent switch to carbohydrate-fuelled anaerobic metabolism (Storey and Storey, 2013). By
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24 152 contrast, insects that remain unfrozen in the cold do not experience such a threshold change in
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26 153 metabolic rate. Voituron et al. (2002) argued that this metabolic suppression could therefore
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29 154 provide a selective advantage for freeze tolerance. In vertebrates, the energetic costs of active
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31 155 responses to freezing and thawing do not support energy conservation when frozen (Sinclair et
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34 156 al., 2013). However, the duration of time spent frozen does appear to account for reduced
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36 157 overwinter energy use by *Pyrrharctia isabella* larvae overwintering in exposed microhabitats
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38 158 compared to thermally-buffered subnivean microhabitats (Marshall and Sinclair, 2012). Thus,
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41 159 this temperature-dependent metabolic suppression could be important for energy conservation by
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43 160 overwintering insects more generally.

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49 162 Altered thermal sensitivity of metabolism changes the curvature of the temperature-metabolic
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52 163 rate relationship (Fig. 4). Both increased and decreased thermal sensitivity could be associated
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54 164 with overwintering. Increased thermal sensitivity would increase the energy savings at low
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57 165 temperatures, but carry the penalty of increased energy drain under variable conditions (Fig. 3).
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59 166 Increased thermal sensitivity could also be advantageous if it allows an overwintering insect to
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4 167 replenish energy stores during warm periods mid-winter, as is hypothesised for many insects in
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6 168 the Southern Hemisphere (Sinclair et al., 2003). To my knowledge, there are no reports of
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9 169 increased thermal sensitivity in overwintering insects (although few studies compare thermal
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11 170 sensitivity among seasons), but this hypothesis could be tested through seasonal comparisons of
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14 171 any species that exhibits winter activity and feeding, such as many New Zealand alpine insects
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16 172 (Wharton, 2011). By contrast, decreased thermal sensitivity reduces the impact of Jensen's
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19 173 inequality by decreasing the influence of high temperatures on metabolic rate (Fig. 3). For
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21 174 example, overwintering larvae of *Erynnis propertius* (Lepidoptera: HesperIIDae) reared under
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23 175 variable conditions decreased their thermal sensitivity of metabolic rate (Williams et al., 2012b).
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26 176 This suppressed thermal sensitivity was fixed in populations from variable environments, but
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29 177 phenotypically plastic in populations from stable environments, which suggests that thermal
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31 178 sensitivity is under selection in direct response to the thermal variability of the environment.
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33 179 However, modeling of energy consumption based on field temperatures showed that decreased
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36 180 thermal sensitivity did not sufficiently compensate for high thermal variability, and that
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38 181 populations from variable habitats had also accumulated more energy stores prior to
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41 182 overwintering.

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47 184 *4.2 Life history and behavioural mitigation of overwinter energy drain*
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50 185 Changes to the life cycle, behaviour, and phenology are key means by which animals avoid or
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52 186 mitigate exposure to abiotic challenges (Huey and Tewksbury, 2009; Sunday et al., 2014; Tauber
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55 187 et al., 1986). Overwintering at life stages that permit replenishment of energy reserves therefore
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58 188 reduces the fitness effects of energy drain. For example, an insect that overwinters as an egg or
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60 189 early-instar larva can compensate for energetic drain over the winter by feeding in the growing

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190 season, whereas insects that overwinter as pupae or that pupate at the end of winter may have
191 limited opportunity to offset energetic drain. Similarly, insects such as mosquitoes, which feed
192 as adults to provision eggs, have opportunity to compensate for energy drain.

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194 Overwinter phenology is an important determinant of overwinter energetics, particularly because
195 it determines exposure to warm temperatures in the autumn and spring. For example, models of
196 metabolic rate indicate that the bulk of energy used by diapausing *E. propertius* caterpillars is
197 consumed during high temperatures experienced in the autumn (Williams et al., 2012b). Thus,
198 shifting phenology such that diapause occurs later in the autumn could mitigate the effect of
199 warmer or more variable autumns. However, in this example, the larvae are apparently
200 constrained by the externalities of host plant quality and insufficient growing season to complete
201 another flight (Prior et al., 2009), such that compensation for autumn phenology is achieved
202 through a combination of decreased thermal sensitivity of metabolism and increased pre-winter
203 energy stores (Williams et al., 2012b). Nevertheless, autumn phenology is clearly under
204 selection: pitcher plant mosquitoes in North America have modified their autumn phenology in
205 response to climate change (Bradshaw and Holzapfel, 2001), and delayed entry into winter
206 diapause consumes energy reserves and decreases overwinter survival of *Osmia lignaria* bees
207 (Sgolastra et al., 2011). Longer winters would also be expected to lead to increased energetic
208 drain (Morris and Fulton, 1970).

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210 Finally, selection of colder or less-variable overwintering microhabitats can mitigate energy
211 drain associated with the thermal sensitivity of metabolic rate. For example, exposed habitats

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4 212 lead to energy savings in freeze-tolerant caterpillars (Marshall and Sinclair, 2012), and although
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6 213 being buried in the soil leads to buffered, warm temperatures (e.g. Clarke et al., 2013), it also
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9 214 prevents exposure to extreme high temperatures in autumn. However, insects cannot always
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11 215 control the microclimate to which they are exposed. For example, *Eurosta solidaginis* (Diptera:
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14 216 Tephritidae) larvae that overwinter above the snow use less energy than their below-snow
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16 217 counterparts (Irwin and Lee, 2003), but wintering above or below snow is dependent on
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19 218 stochastic events (whether or not the host plant falls over), rather than by any choice on the part
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21 219 of the insect. In addition, microclimate temperatures can be variable over a short distance, and
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24 220 variation in snow cover or snow drifting could modify the success of microhabitat selection. For
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26 221 example, low altitude populations of the montane beetle *Chrysomela aeneicollis* are extirpated in
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29 222 years with reduced snow cover, possibly due to extreme low temperatures (Rank and Dahloff,
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31 223 2002).

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35 36 37 225 **5. Do energetics determine insect overwinter survival?**

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40 226 Energetics takes a back seat to the abiotic stresses associated with exposure to low temperatures
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43 227 in most considerations of insect overwintering (e.g. Denlinger and Lee, 2010; Leather et al.,
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45 228 1993). However, energetics could determine overwinter survival directly, because long winters
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48 229 without resource depletion could lead to starvation, and indirectly, through a trade-off with
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50 230 cryoprotection or because of the costs of repair of winter-associated damage. Overwinter
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53 231 energetics could also affect development and reproduction at the conclusion of winter; these
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55 232 effects of winter on growing-season performance are discussed in Section 6.

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4 234 The extent of overwinter energy drain can be driven by autumn energy use, winter energy use,
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7 235 and also the duration of winter. In some cases, the duration of this winter energy drain does
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9 236 directly limit survival and fitness; insects starve to death when they run out of resources. For
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12 237 example, Morris and Fulton (1970) describe an ‘optimal’ length of winter for preserving energy
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14 238 reserves in pupae of the fall webworm *Hyphantria cunea*, and there is some evidence that
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16 239 overwintering *C. fumiferana* larvae die during extended winters when their carbohydrate reserves
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19 240 have been consumed (Han and Bauce, 1998; Regniere et al., 2012). In these cases, strategies that
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21 241 increase energy reserves at the start of overwintering, or that reduce depletion during winter, will
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24 242 have a direct impact on survival (see sections 4.1 and 4.2).
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30 244 The majority of insects accumulate low molecular weight cryoprotectants, usually polyols such
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33 245 as glycerol, prior to overwintering (Storey, 1997); thus, any trade-off between energy supply and
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35 246 cryoprotection may already have been decided at the beginning of winter. However, that trade-
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37 247 off has the potential to be important: reduced cryoprotection could reduce probability of survival.
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40 248 For example, populations of the goldenrod gall fly *E. solidaginis* with lower haemolymph
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42 249 glycerol concentrations are also less cold-hardy (Williams and Lee, 2008). Some insects modify
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45 250 cryoprotectants in response to winter conditions. For example, *E. solidaginis* accumulates a full
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47 251 complement of glycerol (its primary cryoprotectant) prior to winter, but accumulates further
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50 252 sorbitol (as a secondary cryoprotectant) in response to cold stress (Storey and Storey, 1983). In
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52 253 this case, freezing leads to both cryoprotectant synthesis and carbohydrate metabolism, meaning
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55 254 that thermal stress is placing a dual load on the energy reserve. This competition between
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57 255 cryoprotection and metabolic fuel has been best-explored in freeze-tolerant wood frogs. In frogs,
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59 256 carbohydrate stores fuel metabolism during freezing, thawing, and while frozen, but also
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257 (through conversion to glucose) constitute the main cryoprotectant (Storey and Storey, 1985).
258 Thus, metabolic depletion of the carbohydrate pool could potentially compromise cryoprotection,
259 leading to a trade-off in fuel use (Sinclair et al., 2013) that could also underlie geographic
260 variation in the quantity of glycogen accumulated pre-winter in these frogs (Costanzo et al.,
261 2013). Perhaps because insects usually rely on lipid reserves for the bulk of their overwinter
262 energy demands, this potential cryoprotection-metabolism trade-off has received little attention
263 in insects.

264
265 Overwinter mortality from extreme cold events is usually fairly clear-cut (Bentz et al., 2010;
266 Virtanen et al., 1998), and aside from cryoprotection (see above), is likely unrelated to
267 energetics. However, the mechanisms underlying mortality during long, milder, winters have not
268 been well-explored (Sømme, 1996). Under these conditions, longer winters not only lead to
269 energy store depletion (Sgolastra et al., 2011), but also to the accumulation of chilling injuries
270 (Košťál et al., 2006). It is not clear whether long winters lead to mortality solely because of
271 resource depletion, entirely through accumulation of cold injuries, or perhaps due to an
272 interaction. Interactions could be mediated in at least three ways. First, through a
273 cryoprotectant-fuel trade-off (see above). Second, because energy depletion reduces cold
274 tolerance at the end of winter via some other pathway, such as insulin signaling, which links
275 energetics and physiological responses over winter (Sim and Denlinger, 2008). Third, because
276 accumulated cold injuries demand additional energy for repair that is not available in energy-
277 depleted individuals. I suggest that these hypotheses could be readily tested in both field and
278 laboratory settings, and that they could apply to insects utilising any cold tolerance strategy.

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4 **280 6. How are winter energetics and summer performance linked?**

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7 281 With the exception of a few species that reproduce in the winter, the fitness consequences of
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9 282 winter are relevant only in the context of their impacts on summer performance (Williams et al.,
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11 283 in press). Throughout this review, I have assumed that more energy stores upon resumption of
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13 284 activity, growth and/or development equates with greater fitness, because larger individuals are
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15 285 more fecund (see also Honek, 1993). It is also possible that winter energetics may affect summer
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17 286 performance indirectly, and I develop an argument to this effect below.
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26 288 Metabolic rate and energy stores are useful proxies for fitness because they can be used to
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28 289 represent the costs associated with an organism's maintenance and development, and the energy
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30 290 available for fitness-related activities. Thus, the argument goes: overwinter energy use,
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32 291 especially in species that have limited ability to replenish reserves post-winter, is a proxy for
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34 292 energy available for reproduction (which is a determinant of fitness). In some species, the
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36 293 relationship between energy use and reproductive output is easy to establish. For example,
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38 294 adults of the goldenrod gallfly *E. solidaginis* do not feed, and their oocytes can be easily counted
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40 295 (= potential fecundity), allowing a clear link to be made between energy use and fecundity (Irwin
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42 296 and Lee, 2000). Similarly, overwinter fat body depletion in the solitary bee *O. lignaria* is
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44 297 associated with decreased post-emergence longevity, and likely delayed egg maturation and
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46 298 reduced provisioning of offspring (Bosch et al., 2010). In other species, the assumptions have
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48 299 been less well-justified. For example, in my own work, we often measure pupal mass as a fitness
49
50 300 proxy for Lepidoptera at the end of winter (e.g. Williams et al., 2012a; Williams et al., 2012b).
51
52 301 However, with some exceptions (e.g. spruce budworm; K.E. Marshall & B.J. Sinclair, in review)
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54 302 this proxy is based on relationships for other, distantly related species measured only during the
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303 growing season (e.g. Boggs and Freeman, 2005). Note that the majority of studies ignore the
304 impact of winter on male condition, although male reproductive performance is important, and
305 can be condition-dependent (e.g. Blanckenhorn and Hosken, 2003). Assuming a direct
306 relationship between resource availability and reproductive output, longer, warmer, and more
307 variable winters (all of which are potential consequences of climate change) will lead to
308 increased energy use, and thus decreased fitness.

309
310 For many species, however, energy reserves at the conclusion of dormancy are not directly
311 linked to fitness via resources because of the possibility of post-winter feeding. For example, *C.*
312 *fumiferana* overwinters as a pre-feeding larva (Royama, 1984), many species overwinter as eggs
313 (Danks, 2002), and Fründ et al. (2013) show that bees suffer more energy drain if they
314 overwinter as adults than as larvae. Any links between winter and summer are generally then
315 assumed to be driven by other sub-lethal impacts, for example via cold injury, perhaps to tissues
316 associate with feeding (such as the gut), or because of the costs of damage repair (e.g. Izumi et
317 al., 2005; Sinclair and Chown, 2005; Teets et al., 2011). However, because energy state is an
318 integrator of winter conditions (Fig. 1), and energy balance and cryoprotectant management are
319 closely associated with energy signaling pathways (Teets et al., 2012), one might also
320 hypothesise that energy state could provide information about the nature of the winter
321 experienced. This information could be transduced via insulin signaling, which can determine
322 behaviour (e.g. Wigby et al., 2011), and is involved with regulation of diapause in various insects
323 (e.g. Ragland et al., 2010). In addition, developmental conditions can determine adult metabolic
324 rate and performance. For example, Le Lann et al. (2011) show an increased fecundity and
325 metabolic rate (measured under common conditions) in parasitoid wasps reared at lower

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326 temperatures. This dependence of adult performance (and especially metabolism) on
327 developmental conditions provides another potential mechanism by which winter conditions
328 could drive summer performance, although the explicit role of overwinter energetics in
329 determining this relationship is not clear. In the context of closely connected ecosystems and
330 tight insect-host relationships, such information transfer between seasons could allow insects to
331 predict the performance of other species, and thus inform life-history decisions; to my
332 knowledge, this has not yet been investigated.

333

334 **7. Conclusions**

335 In conclusion, energy use is an important component of insect overwintering biology, and is
336 particularly relevant to understanding the potential impacts of changing thermal conditions with
337 climate change. There are a number of strategies insects can use to reduce overwinter energy
338 use, but little is known about the relative importance or phylogenetic distribution of these
339 strategies. Because the majority of overwinter energy consumption occurs in the warm periods
340 in autumn and spring when insects are dormant, this makes energy use particularly susceptible to
341 mismatches between (photoperiod-controlled) phenology and (temperature-controlled) winter
342 length. The links between winter energetics and mortality or summer performance have not been
343 well-established, and indirect relationships between energy use and fitness are particularly poorly
344 known. In sum, this speaks to a need to more rigorously involve physiology in ecological
345 studies, to do field studies on overwinter biology that can help to determine the proximate causes
346 of winter mortality, and to expand ecological studies to better understand links between winter
347 and the growing season.

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351 (University of British Columbia) for many discussions about overwinter energetics, and Lauren
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354 from the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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549 **Vita**

550 Brent Sinclair graduated with his undergraduate and PhD degrees in Zoology from the
551 University of Otago, New Zealand under the supervision of David Wharton. He was a postdoc
552 with Steven Chown at the University of Stellenbosch, and then with Steve Roberts at the
553 University of Nevada, Las Vegas, before beginning at the University of Western Ontario in
554 2006, where he is now an Associate Professor and Faculty Scholar. Sinclair has received early
555 career awards from the Canadian Society of Zoologists and the Entomological Society of
556 Canada, and is a member of the *Journal of Thermal Biology* editorial board.

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4 558 Figure Captions
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7 559 **Figure 1**
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10 560 Schematic of sources and sinks of energy before, during, and after wintering in insects, and how
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12 561 they might link to summer performance (which encompasses reproductive output and fitness).

13 562 Winter-specific processes are outlined with a dotted box; the dashed arrow from cryoprotection
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15 563 to energy remaining reflects the use of cryoprotectants as a fuel at the end of winter.
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21 564 **Figure 2**
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24 565 Potential forms of relationships between Metabolic rate (MR) and temperature in insects. For
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26 566 overwintering insects, metabolic rate determines energy consumption, so this is indicated as an
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28 567 alternative quantity on the vertical axis. A) A ‘normal’ exponential relationship between
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30 568 temperature and metabolic rate. B) A threshold (step) decrease in metabolic rate at a threshold
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32 569 temperature, similar to that expected when an insect freezes. C) A transient energetic cost of
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34 570 crossing a temperature threshold (T_{thresh}), in this case during rewarming. Note that this cost may
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36 571 be present in one direction but not the other; e.g. there is no energetic cost to cooling the fall
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38 572 field cricket *Gryllus veletis*, while recovery from chill coma carries an energetic cost (MacMillan
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40 573 et al., 2012a, b). D) A cumulative relationship between the number of threshold-crossing events
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42 574 and energy use.
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49 575 **Figure 3**
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52 576 Thermal sensitivity of the metabolic rate-temperature relationship determines instantaneous
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54 577 metabolic rate and daily energy use in ectotherms because of the disproportionate impact of
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56 578 variability at high temperatures (Jensen’s inequality). A) High (black), medium (grey) and low
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58 579 (dotted) thermal sensitivities of the temperature-metabolic rate relationship. μ indicates the mean
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580 temperature (also on B). B) A daily thermal cycle. C) Instantaneous metabolic rates for the
581 thermal sensitivities in A across the daily cycle depicted in B. μ indicates the metabolic rate for
582 a constant temperature. D) Cumulative daily energy use for the instantaneous metabolic rates
583 shown in C, with μ indicating the daily energy use from a constant temperature. The discrepancy
584 between the μ bar and the others indicates the impact of Jensen's inequality, while the
585 differences among the other bars indicates the impact of changing thermal sensitivity of
586 metabolic rate.

Figure 4

588 Modification of the metabolic rate (MR)-temperature relationship by insects. Metabolic rate
589 determines energy consumption in overwintering insects, so this is provided as an alternative
590 vertical axis. The normal metabolic rate-temperature relationship (black line, A) can be
591 suppressed by a simple change in elevation (grey line, B), be made more thermally sensitive (C),
592 which allows for a steeper decline in metabolic rate when temperature declines from the mean
593 (but increases cost of fluctuations to high temperatures), or be less thermally-sensitive (D),
594 reducing the impact of high temperatures on energy consumption.

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597 Picture to go with Vita



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Figure 1

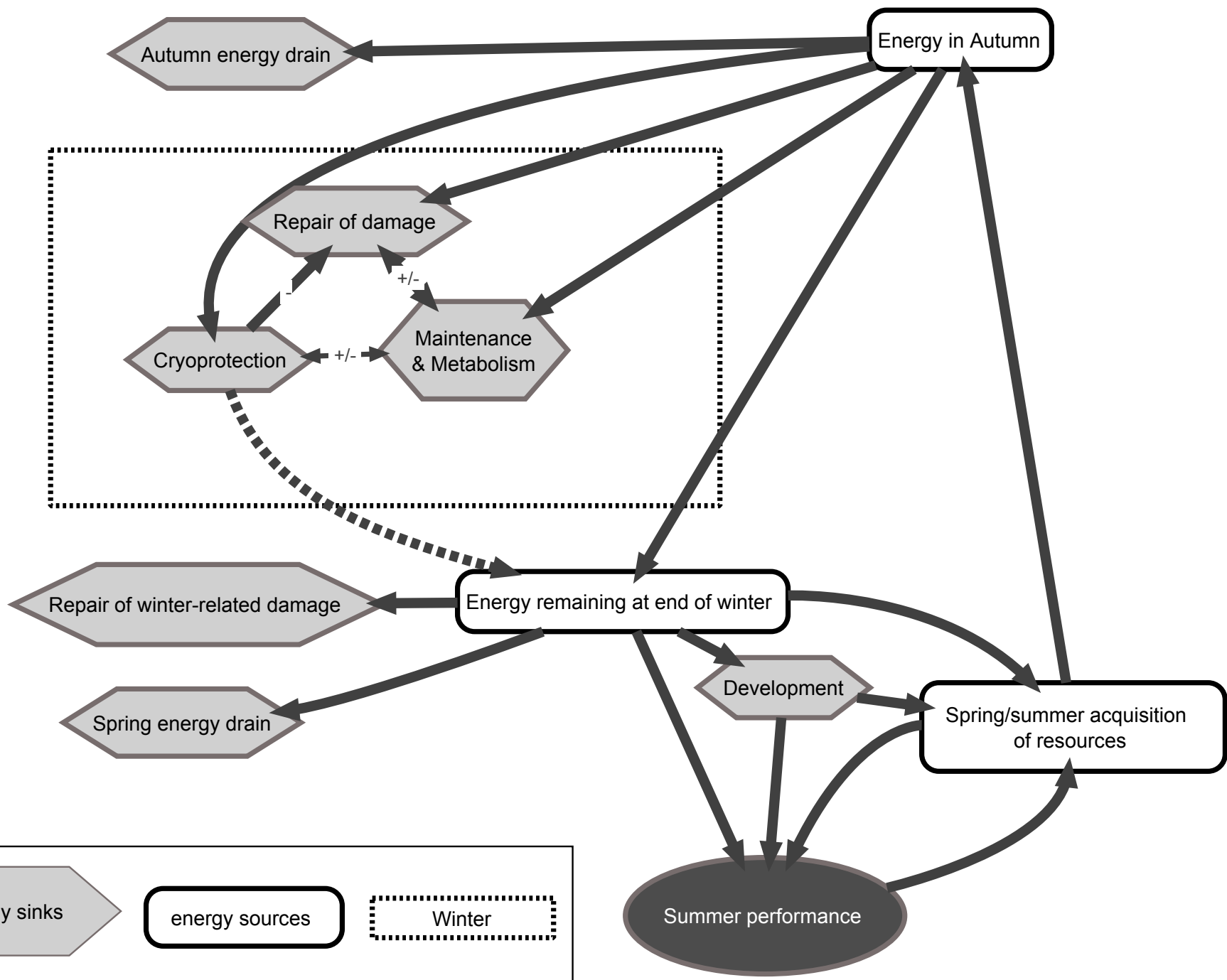


Figure 2

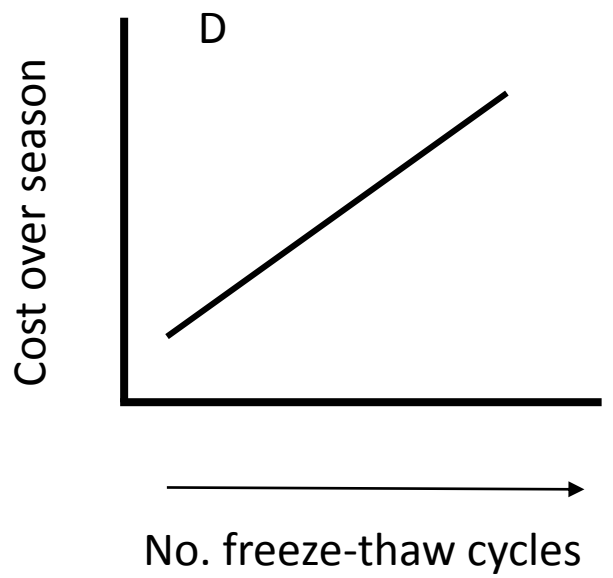
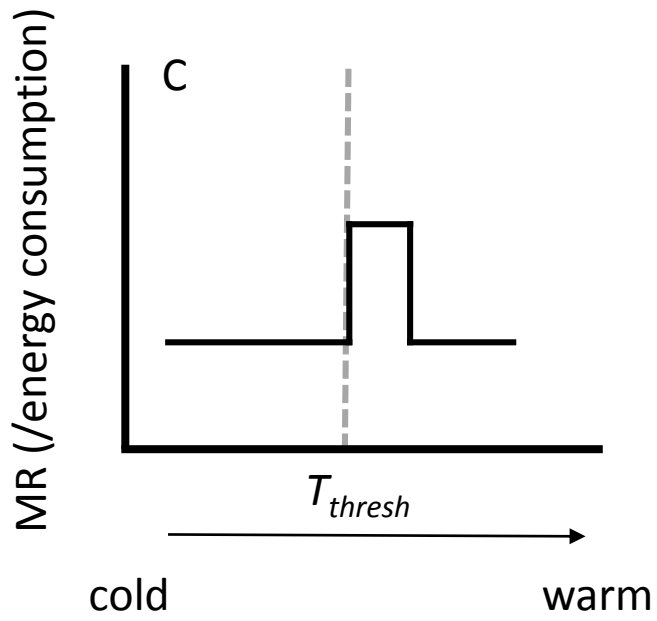
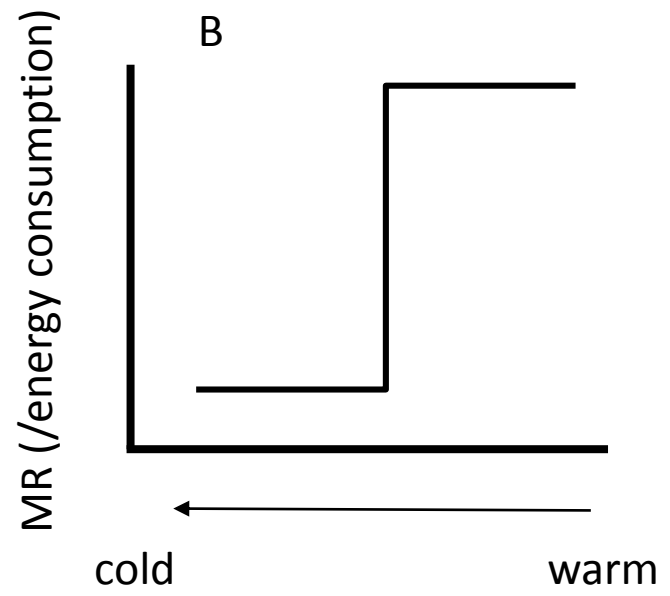
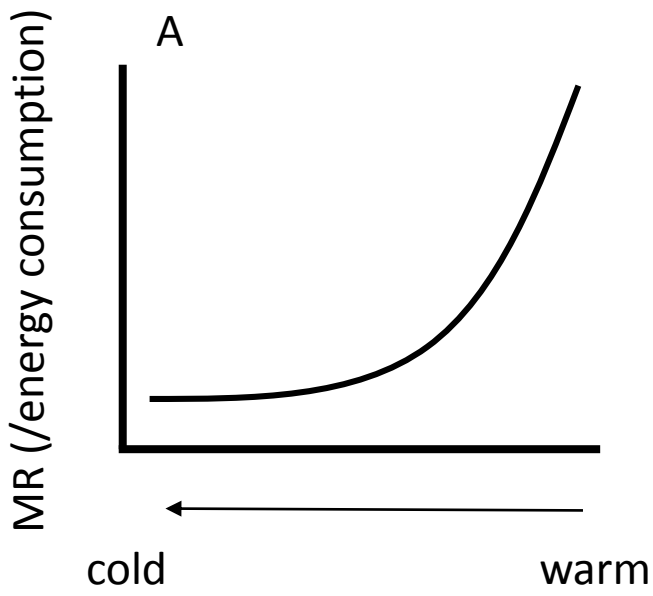


Figure 3

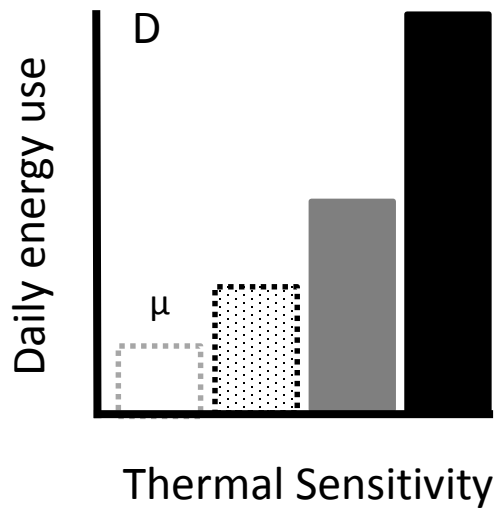
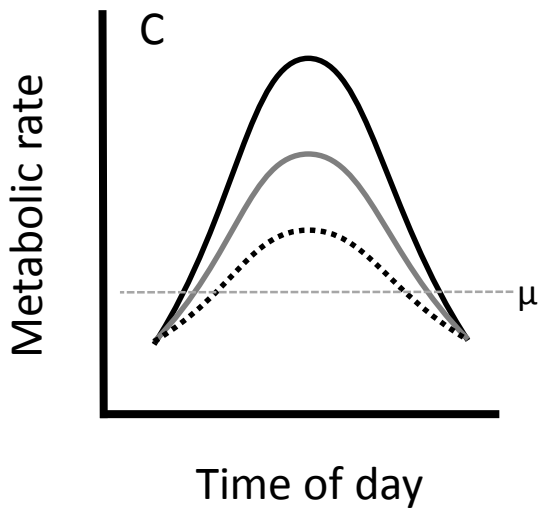
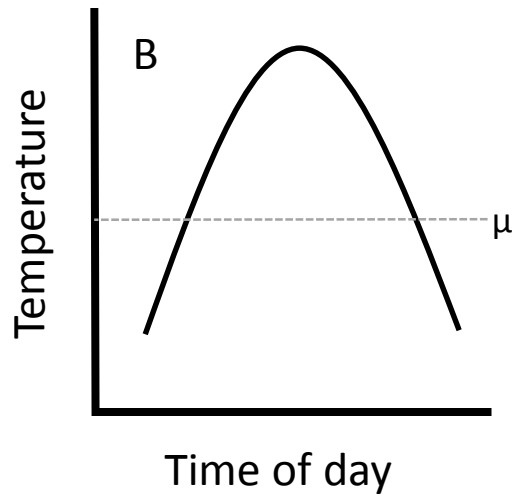
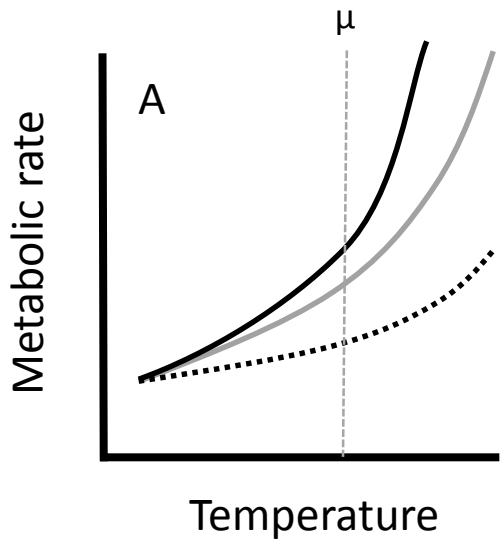


Figure 4

