Title

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Overwintering energetics and life-history trade-offs in insects:

the interplay between stochastic and seasonal variation

By

Andre Szejner Sigal

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Caroline M. Williams, Chair

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Abstract

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Seasonality shapes major life-history strategies and adaptations across the tree of life. The alternating nature of seasons for growth and reproduction and seasons of adverse conditions can put conflicting selective pressures on energy use. Growing seasons often drive evolution of fast-paced processes and activity, while adverse seasons like winter drive evolution of dormancy and energy conservation. Thus, energy use strategies in seasonal environments have profound impacts on fitness. Integrating fitness across seasons and organismal responses to environmental change is key to understanding how populations will respond to ongoing global climate change. Energetics can play two essential roles in mediating organismal fitness in seasonal environments: energy reserves can determine winter survival, and energy at the end of winter can determine reproductive success. Survival and reproduction are both critical components of fitness, and thus energy allocation strategies may favor one over the other, revealing potential trade-offs when energy is limited. However, we do not know how environmental variation and predictability affect energy allocation strategies. Understanding the impacts of environmental change in seasonal environments will help us make better predictions of population responses and vulnerability to climate change.

My dissertation aims to expand our understanding of overwintering energy use strategies, then explore how variable environments affect energy allocation strategies under a trade-off between maintenance and future reproduction. Finally, I test my predictions of energy allocation strategies under realistic winter conditions. Winter performance can determine summer performance, linking life cycles in seasonal environments. This work reveals the extent to which stochastic and predictable variation in seasonal environments can affect selective pressures and thus fitness in overwintering organisms. The carry-over effects of winter fitness into the growing season and vice versa expand our understanding of population responses to variable and changing environments.

Chapter 1 explores the role of group behavior on energy conservation in overwintering aggregations, a potential energy use strategy that remains widely underexplored. The convergent ladybeetle (*Hippodamia convergens*) overwinters in massive aggregations, making it an ideal system for testing the effect of aggregation size on metabolic rates in overwintering insects. I measured energy use and thermal sensitivity of beetle aggregations across two ecologically relevant temperatures, and measured locomotor activity as one possible driver of group effects on energy use. Metabolic rates per beetle decreased with increasing aggregation size and scaled

hypometrically with mass at both temperatures tested, with responses more pronounced at low temperatures. Activity decreased with aggregation size, but only at low temperatures. These results suggest that individuals within aggregations enter a deeper metabolically inactive state that single individual beetles cannot achieve, which is partly but not completely explained by a reduction in locomotor activity. This behavioral strategy for energy conservation may provide an additional selective advantage for the evolution of large overwintering aggregations.

Chapter 2 explores the impact that variation in predictable and stochastic environments has on an energy allocation trade-off between somatic maintenance and future reproduction. Seasonality can modulate selection of energy use strategies, but we currently lack a theoretical framework to generate predictions on energy allocation strategies under changing stochastic conditions. Stochastic extreme events, like heat waves or cold snaps, are increasing in frequency and can have major impacts on population fitness. Overwintering organisms can prepare for harsh conditions by investing energy into stress tolerance (somatic maintenance). However, energy reserves at the end of winter can also influence future reproductive fitness, depending on the relative importance of stored versus recently acquired reserves for reproduction across the capital to income breeding continuum. In this chapter, I develop a general theoretical model that assesses fitness under increasing probability of stochastic lethal events, temporal variation in probability of stochastic events through winter, and how a stochastic end of winter affects optimal allocation strategies in income and capital breeders. Increasing probability of extreme events modulates allocation strategies in capital breeders. Temporal variation in extreme events and stochastic end of the season can alter optimal timing of investments, offering insights on the mismatch costs that changing environments may impose on overwintering organisms. These results highlight the role of end reserves in seasonal transitions and identify capital breeders to be especially susceptible to climate change.

Chapter 3 tests the predictions from Chapter 2 by applying it to a case study of a capital breeder, the beetle *Chrysomela aeneicollis*, and answering how variation in snow cover can affect a trade-off between winter survival and future reproduction in realistic scenarios. Variation in snow cover modulates stochastic cold events and baseline energetic costs for organisms overwintering underground. Winters with deep snow cover have high baseline winter costs while having a low risk of lethal cold events. On the other hand, winters with little or no snow cover have low baseline winter costs but organisms are exposed to a high risk of lethal cold. In this chapter, I build on Chapter 2 by incorporating organism-specific parameters for energy reserves, rates of energy use, and microclimate conditions. I develop a model to systematically explore how cold risk, baseline winter costs, and their interaction under realistic winter scenarios affect energy use, survival, and allocation strategies of the willow leaf beetle (*Chrysomela aeneicollis*). My results support conclusions from Chapter 2, as increased cold risk and baseline costs can drive allocation strategies, but the response is strongly dependent on starting reserves. Increased cold risk affects future reproduction of beetles starting winter with high reserves, while increased baseline costs affect both reproduction and survival for lean beetles. Under realistic winter conditions, snow cover can modulate the trade-off between winter survival and future reproduction. Overwintering organisms under deep snow cover show high survival at the cost of reproduction, while winters with low snow favored reproduction at the cost of survival. These results show that both starting reserves and winter conditions, in the form of cold risk and baseline costs, drive distinct energy allocation strategies, and offer new insights into population dynamics and predictions under climate change.

A mi mamá Marybel y a mi papá Ricardo.
Gracias por todo el apoyo que me han dado toda mi vida.
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Introduction

   Winter poses one of the strongest and most widespread selective pressures organisms face in temperate latitudes (Williams et al., 2017). Selective pressures include lack of available food, and harsh abiotic conditions such as low temperatures. These selective pressures have shaped major life-history adaptations across the tree of life including dormancy. The interannual predictability of seasonality has allowed natural selection to consistently pick overwintering traits like increased energy reserves and stress hardiness, that prepare organisms for winter. However, seasonal environments also have a stochastic, or unpredictable component that organisms must cope with in order to survive. Climate change is increasing the frequency of extreme events, albeit remaining unpredictable. Additionally, climate change is also changing the seasonal timings between environmental cues and population responses. These increases in environmental stochasticity are likely to strengthen the selective pressures in already harsh winters. Thus, we need to better understand how organisms respond to both predictable and unpredictable environments in order predict how climate change will impact organisms and identify which populations are most at risk.

   Most overwintering organisms share energy use strategies that allow them to survive long periods of time with limited or no food available. There are several ways in which organisms shift their energy use that span from the molecular level to the population level. Some strategies to decrease energy demands include metabolic suppression, tissue histolysis, and reduced locomotor activity. Other strategies include increasing the energy stores prior to winter in order to sustain long periods of no food availability. Importantly, none of these strategies are mutually exclusive, and organisms show any combination of these. Insects in particular show an outstanding level of diversity in overwintering strategies, even in closely related species. Regardless of strategy used, energy use during winter is tightly tied to fitness. Even though there are increasing number of studies on seasonal adaptations, there is still a large gap in our knowledge on how organisms respond to a changing environment. More specifically, what tools do organisms have to cope with unpredictable conditions, and how do these conditions affect cross-seasonal fitness. In this dissertation, I address the central hypothesis that energy allocation must be tightly regulated to meet energetic demands under both predictable and unpredictable environments. When energy is limited, we may reveal a potential trade-off between winter survival and reproduction in the growing season.

   In the following chapters I explore three main energy use strategies for overwintering organisms to test my central hypothesis. In Chapter 1, I focus on how behavior can impact energy use in overwintering insects. Many overwintering organisms form aggregations during winter. Overwintering aggregations can modify their internal environment by buffering thermal fluctuations and conserve water, but little is known about their role on energy use. The convergent ladybeetle (*Hippodamia convergens*) forms aggregations of up to several million individuals in the wild while maintaining a relatively active state, making it the perfect system to study the role of aggregations on energy use. In Chapter 2, I take a theoretical approach to disentangling energy allocation under predictable and unpredictable environments. I develop a general model to test how stochastic events in seasonal environments, such as cold snaps and unpredictable end of the season, affect energy allocation between maintenance and future reproduction. The model sets the stage to test life history strategies that vary in the relative importance of the reserves left at the end of winter. The model results will help us identify how changing environments may affect fitness and which organisms may be the most at risk. Finally, in Chapter 3 I take a closer look at the model

developed in Chapter 2 and apply real ecophysiological parameters to test how snow cover affects energy allocation strategies between survival and reproduction during the growing season. Snow cover acts as a natural buffer from thermal fluctuations for organisms overwintering underground. Interannual variation in snow cover results in organisms facing sometimes very stable winters, while some years have larger and colder thermal fluctuations. Organisms tend to invest energy into cryoprotection in order to survive cold spells, while also carefully saving energy to survive long periods of no food during winter. This is particularly important for capital breeders, as energy left at the end of winter is used for reproduction during the growing season. In this chapter I take the winter physiology of the willow leaf beetle *Chrysomela aeneicollis*, a capital breeder, to better understand the role of natural stochastic variation on energy allocation strategies and the potential trade-off between survival and future reproduction. Together, these chapters will assess the role of energy use strategies on overwintering fitness, and the importance of the life history context on shaping allocations strategies under unpredictable environments.

Chapter 1

Aggregations reduce winter metabolic rates in the diapausing ladybeetle *Hippodamia convergens*

1.1 Introduction

Many overwintering animals seek sheltered hibernacula, in which they are buffered from abiotic extremes. In some cases, overwintering animals seek shelter by aggregating in large groups of conspecifics (Brower et al., 2008; Hagen, 1962). Aggregating behavior in insects is associated with a wide range of benefits, including increased mating success (Susset et al., 2018), amplified aposematic signaling (Beatty et al., 2005; Riipi et al., 2001), and reduced water loss (Benoit et al., 2007; Yoder and Smith, 1997). For overwintering organisms, aggregations can buffer external conditions such as temperature fluctuations and low humidity, reducing desiccation stress and improving overwintering success (Brower et al., 2008; Yoder et al., 1992; Yoder and Smith, 1997). Despite evidence that aggregations reduce rates of water loss, the impact of overwintering aggregations on energetics has not been well explored, despite the important contribution of energetics to fitness for overwintering insects (Hahn and Denlinger, 2011, 2007; Sinclair, 2015, but see Tojo et al. 2005).

Aggregations may impact fitness through their effects on energetics. Overwintering insects have a set amount of energy stores to survive winter (Hahn and Denlinger, 2007; Sinclair, 2015). The rate of depletion of these energy stores is set by metabolic rate, and any factors that increase metabolic costs during winter can decrease fitness via increased mortality or decreased reproductive success (Irwin and Lee, 2003; Williams et al., 2012; reviewed in Sinclair, 2015). Metabolic rates of insects within some aggregations decrease with increasing group size, suggesting that aggregations may provide energetic benefits (Su et al., 2007, Tanaka et al., 1988; Tojo et al., 2005; Waters et al., 2017, but see Schoombie et al., 2013). Tanaka et al., 1988 first documented the group effects in estivating tropical fungus beetles *(Stenotarsus subtilis)*, showing that *per capita* metabolic rates decrease with increasing group size. Since then, Tojo et al., 2005 and Su et al., 2007 have reported metabolic suppression in overwintering aggregations of Hemipterans (*Parastrachia japonensis* and *Pyrrhocoris apterus*). Mass-specific metabolic rates of whole *Pogonomyrmex californicus* ant colonies are lower than experimentally reduced-size colonies (Waters et al., 2017). Even groups of *Drosophila melanogaster*, a non-aggregating insect, show decreased *per capita* metabolic rates relative to flies individually housed (Burggren et al., 2017), suggesting that group metabolic savings may be widespread. Despite this, energetic benefits of aggregations are not widely recognized as a component of insect overwintering, despite the

potential importance of this phenomenon for determining performance and fitness in the face of climate change (Sinclair, 2015).

Many species of ladybeetles (Coleoptera: Coccinellidae) aggregate during winter (Hagen, 1962; Hodek, 2012). Ladybeetle aggregations provide benefits including mate-finding (Susset et al., 2018), reduced water loss (Yoder and Smith, 1997), and improved survival (Murakami et al., 2019; Turnock and Wise, 2004), as well as costs including increased risk of infection (Güven et al., 2015) and predation (Chapman et al., 1955). The convergent ladybeetle (*Hippodamia convergens*) overwinters in massive aggregations that can reach tens of millions of individuals (Hagen, 1962). Overwintering convergent ladybeetles maintain their locomotor capacity, allowing them to re-form aggregations after perturbations and relocate if conditions become unfavorable (AS, personal observations). Convergent ladybeetle aggregations reduce water loss and beetles within aggregations maintain a constant water content (Hagen, 1962; Yoder and Smith, 1997), but whether these overwintering aggregations reduce metabolic rates and conserve winter energy stores has not been addressed.

Allometric scaling of metabolic rate is a powerful tool to detect and quantify the effects of group size on individual metabolic rate. Interspecific metabolic rates scale hypometrically with body mass with a slope between 0.67 and 0.75 when plotted on a logarithmic scale (Kleiber, 1932; Kolokotrones et al., 2010; Kooijman, 1986; Maino et al., 2014; West et al., 1997). Metabolic allometry extends to colonies of eusocial insects: in ant colonies of *P. californicus*, metabolic rate scales hypometrically with colony mass, but only in whole colonies (Waters et al., 2017), and not in groups of workers, wherein metabolic rates scaled isometrically with mass (Waters et al., 2010). Thus, hypometric scaling can be seen as a characteristic of functional units, both whole organisms and colonies, where larger mass results in lower metabolic rates per unit mass.

Reductions in metabolic rate in aggregating insects may be driven by decreases in locomotion. Locomotion is energetically costly (reviewed by Bonte et al., 2012), and may influence energetic costs of aggregations. For example, low metabolic rates in colonies of *P. californicus* are at least partially caused by decreases in activity levels of ants compared to smaller experimentally-reduced colonies (Waters et al., 2017). Low temperatures within physiological limits reduce locomotor activity in insects (Cloyed and Dell, 2020), and so by manipulating temperatures and measuring metabolic rates and activity we can assess the role of locomotion in driving differences in metabolic allometry across aggregation sizes.

We aimed to characterize the impact of aggregation size on overwintering energy use of *H. convergens*, to test the hypothesis that aggregations reduce energy use by lowering locomotor activity. Here, we ask three questions: 1) Does metabolic rate of individual beetles decrease with increasing aggregation size, such that whole-aggregation metabolic rates scale hypometrically with mass of beetles? 2) Does locomotor activity decrease with increasing aggregation size? 3) Do cool temperatures decrease the magnitude of group effects on locomotor activity and metabolic rates?

1.2 Material and Methods

Animal Husbandry

Adult overwintering *Hippodamia convergens* were collected from aggregations in Berkeley, CA (37°52'21.8"N, 122°14'18.5"W) in October 2018 and October 2019 for 15°C activity trials. Beetles were housed in plastic containers (15x15x5cm) with a small mesh top for aeration,

and a crumbled paper towel to hold moisture. Each box contained several hundred beetles, to allow aggregations to form. Boxes were stored at 15°C - 8°C ± 0.5°C in a gradual 10-step fluctuating cycle, on a short day 14:10 L:D cycle (I-36VL, Percival Scientific, IA, USA) for several weeks prior to the experiments. The temperatures of 15°C and 8°C represent the mean winter daily maxima and daily minima from microclimatic data for these beetle aggregations. Beetle aggregations were lightly sprayed with water every two weeks.

Respirometry Experiments

We used stop-flow respirometry to quantify oxygen consumption and carbon dioxide production rates ($\dot{V}O_2$ and $\dot{V}CO_2$) as a proxy for metabolic rates of beetle aggregations (aggregation sizes 1, 10, 25, and 50 beetles, N = 13-26 replicates per aggregation size, 69 groups in total) at 8°C and 15°C (protocol modified from Visser et al., 2018). Briefly, beetles were sorted into groups of 1, 10, 25 and 50 and put into 20mL glass chambers with a thin paper slip of Kimwipe for traction, and kept in dark conditions throughout the experiment. Beetle groups were left for one hour to form aggregations and acclimate to the chamber. Each beetle group was measured at both 8°C and 15°C (in random order) using a temperature-controlled chamber [Pelt-5, Sable Systems International (SSI), Las Vegas, NV, USA], and allowed at least one hour of acclimation after exposure to a new temperature. Respirometry chambers were connected to a flow multiplexer (RM8, SSI) and flushed for 3 min sequentially with dry, CO_2-free air, generated using a Drierite–Ascarite–Drierite column, at a flow rate of ~170mL min^{-1}, after which the chambers were sealed for 21min. After the incubation, dry and CO_2-free air carried the chamber bolus sequentially into the O_2 and CO_2 gas analyzers (FOXBOX, SSI) at a flow rate of 176mL min^{-1} for 3min/chamber, immediately followed by a two-minute baseline. The bolus was scrubbed of water vapor using a magnesium perchlorate column before entering the gas analyzers. We collected data using a UI2 interface (SSI) at a frequency of 1Hz. The multiplexer was programmed to cycle twice through five chambers with beetle groups, and a blank chamber with a paper slip of Kimwipe as a control. This resulted in two measurements for every beetle group per temperature, of which the lowest peak was used as a proxy for standard metabolic rate for analysis. Data was corrected using a Catmull-Rom correction to adjust for instrument drift and removed instrument noise with a Savitzky-Golay filter with a 15-step window. All corrections were carried out using the software ExpeData (SSI). Beetle groups were weighed immediately after the metabolic rate measurements (Mettler Toledo XP6 microbalance [±1 μg], Mettler-Toledo Inc., Columbus, OH, USA). Oxygen signal of individual beetles at 8°C (9.7% of total samples) was not detectable above the instrumental noise, so $\dot{V}CO_2$ was used for statistical analyses throughout ($\dot{V}CO_2 \sim \dot{V}O_2$, $R^2 = 0.989$). The sensitivity of metabolic rates to temperature was assessed using Q_{10} for each group and was calculated as:

$$Q_{10} = (^{R_2}/_{R_1})^{10/(T_2 - T_1)}$$

where R_1 and R_2 are $\dot{V}CO_2$ at T_1 (8°C) and T_2 (15°C) respectively.

Activity Experiments

We measured activity time of focal beetles across the full range of group sizes (1, 10, 25, 50) using a repeated measures design. Focal beetles (n=20) were haphazardly selected, weighed,

marked with blue (n=10) or white (n=10) acrylic paint, then allowed to recover from handling for 24hrs before the start of the experiment. Paint had no effects on mortality or aggregating behavior (0% mortality after one week at 8°C, aggregations continued to form; data not shown). Focal beetles were placed into their respective groups in 50mL vials and acclimated for 2h at 8°C or 15°C in darkness. Groups of 1 and 10 beetles had a total of one marked beetle each, and groups of 25 and 50 had two marked beetles of different colors at a time. After acclimation, activity of each focal beetle was observed and scored (0 = inactive, 1 = actively walking) every 5 minutes for an hour under red light through the incubator window. Activity scores were summed to give total activity time in minutes per hour for each focal beetle. This procedure was repeated until each focal beetle had been measured at each aggregation size in a random order, with each trial treated as a blocking factor. Activity experiments were conducted within 2 days for each temperature, and beetles were maintained at their fluctuating thermal regime when not used in the experiments.

Statistical Analysis

All statistical analyses were performed in R v.3.5.2 (R Team, 2018). Preliminary data exploration was performed according to (Zuur et al., 2010). Our general modeling approach was to fit saturated models with all interactions and sequentially simplify the models by dropping non-significant terms until the minimal adequate model was obtained, as suggested by Crawley, (2007).

Per capita metabolic rates were obtained by dividing $\dot{V}CO_2$ by the number of individuals in the group, normalized using natural log transformation and analyzed using a linear mixed model, where $\dot{V}CO_2$ was described as a function of mass, aggregation size, and temperature, with trial run as a random effect. Mass scaling exponents were obtained from the slopes of natural log-transformed group metabolic rates as a function of natural log-transformed mass at each temperature. Q_{10}s were analyzed using an ANOVA, with aggregation size as a categorical fixed factor.

Total time active of marked beetles across group size was analyzed using a Friedman's test for each temperature with a post-hoc analysis for significance (Galili, 2010). Analysis included group size as factor, and beetle ID (marked beetle identifier) as a random factor accounting for individual differences in activity levels. Chi-square tests and Pearson residuals were used to assess patterns of activity or inactivity of marked beetles across group sizes and their relative contribution to the pattern at each temperature.

1.3 Results

Respirometry Analysis

Metabolic rates of individual beetles were higher at 15°C than at 8°C and decreased with increasing group size ($F_{3,140}$ = 153.4, lnMass x Temp = $P < 0.001$, Fig. 1.1A). Metabolic rate scaled hypometrically with mass at both temperatures, but with a shallower slope at the cooler temperature (mass-scaling exponents 0.678 at 8°C and 0.798 at 15°C; $F_{3,140}$ = 689.7, lnMass x Temp $P < 0.001$, Fig. 1.1B). Mass scaling of metabolic rates was not seen within each aggregation size ($P > 0.05$, Fig. S1.1). Thermal sensitivity of metabolic rates increased with increasing group size from 2.86 in single individual beetles to 4.70 in groups of 50 beetles ($F_{3,68}$ = 4.44, $P = 0.006$, Fig. 1.2).
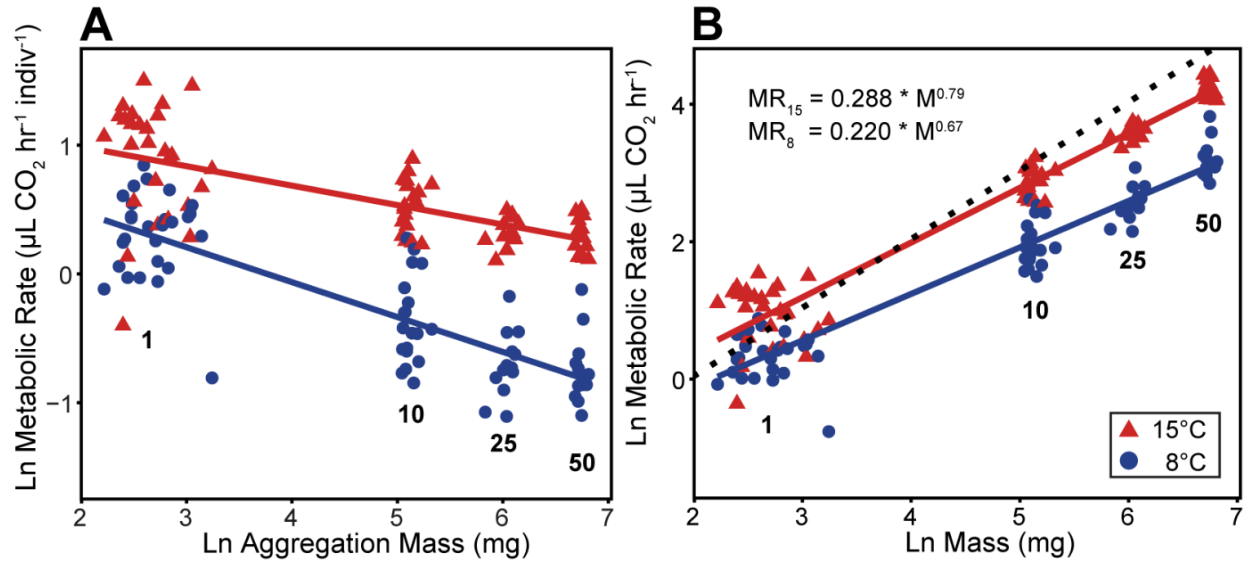
Fig. 1.1. Metabolic rate of diapausing convergent ladybeetles (*Hippodamia convergens*) at 15°C (red, triangles), and 8°C (blue, circles) in aggregations of varying sizes (1, 10, 25, and 50 individuals). A) *Per capita* metabolic rate as a function of group mass, data plotted on natural log scale. B) Mass scaling of metabolic rates. Data plotted on natural log scale, with allometric scaling equations from linear regressions. Dotted line represents an isometric mass-scaling of metabolic rate. Numbers under each cluster of points represent number of individuals in each aggregation.
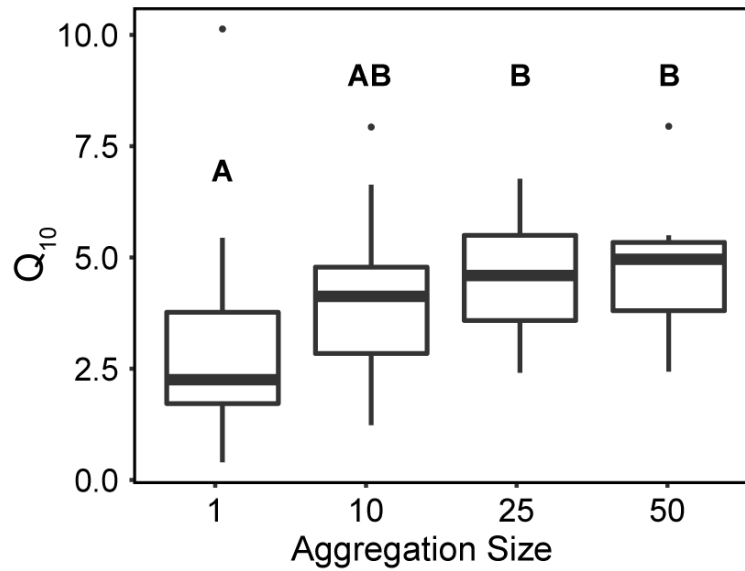
Fig. 1.2. Thermal sensitivity of metabolic rates of diapausing convergent ladybeetles (*Hippodamia convergens*) across aggregations sizes (1, 10, 25, and 50 individuals). Q_{10} calculated from metabolic rates at 8°C and 15°C. Letters indicate significant differences between group sizes, $P<0.05$. Boxplots show quartiles, and median values.

Activity Analysis

      Activity at 15°C was not significantly different across aggregation sizes (Friedman statistic = 4.3286, df = 3, P = 0.228, Fig. 1.3A). However, at 8°C, marked beetles were active between 0 – 15 minutes per hour, and activity time decreased with increasing aggregation size (Friedman statistic = 16.677, df = 3, P = 0.0008, Fig. 1.3B). Beetle mass and beetle ID did not affect activity time (Mass; Kruskal-Wallis $\chi2$ = 36.257, P = 0.3638, Beetle ID; Kruskal-Wallis $\chi2$ = 20.297, P = 0.4395). We then compared observed vs expected frequencies of active and inactive beetles at each temperature. At 8°C, single individual beetles were more active and beetles within aggregations of 50 were less active than expected ($\chi2$ = 14.762, df = 3, P = 0.002, Fig. 1.4A), while for beetles at 15°C activity frequencies did not differ from expected values across aggregation sizes ($\chi2$ = 5.416, df = 3, P = 0.143, Fig. 1.4B).
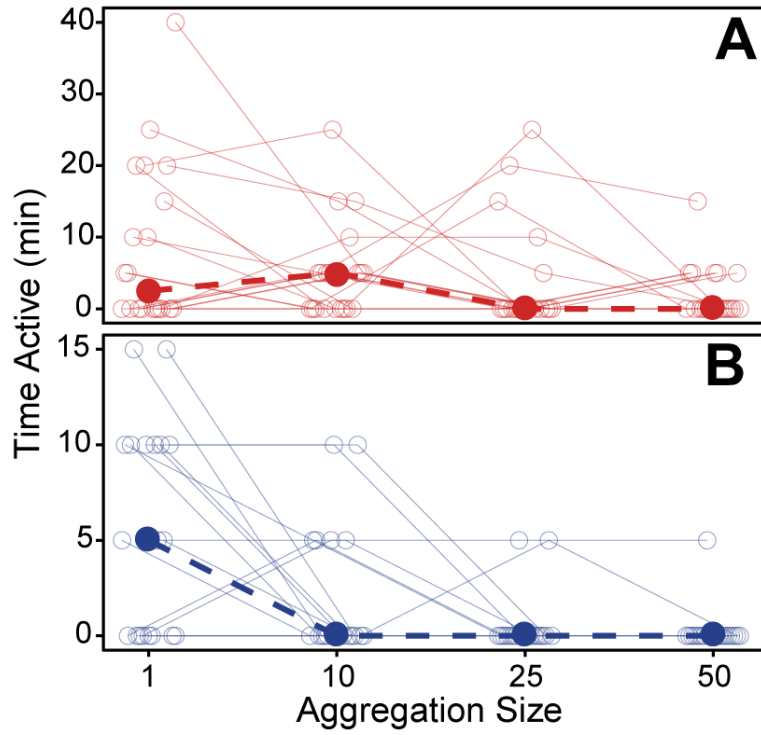
Fig. 1.3. Activity time of diapausing convergent ladybeetles (*Hippodamia convergens*) at 15°C (A), and 8°C (B) in aggregations of varying sizes (1, 10, 25, and 50 individuals). Median active time (closed circles) of marked beetles (n=20, open circles), solid lines track each marked beetle. Note the time active axis differs between temperatures.
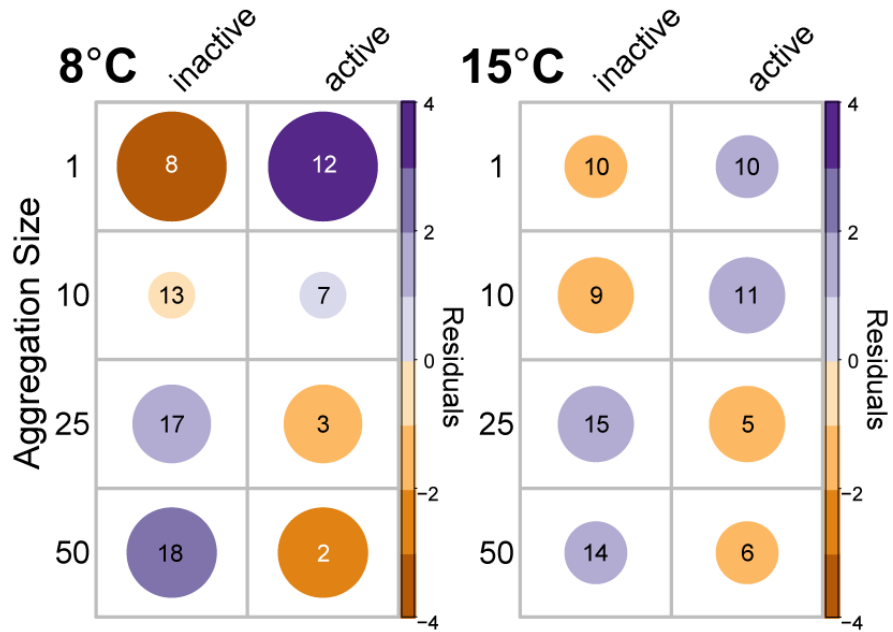
Fig. 1.4. Observed versus expected frequencies of active and inactive beetles (*Hippodamia convergens*) in aggregations of different sizes (1, 10, 25, and 50 individuals) at 8°C (left) and 15°C (right). Bubble size and color represent weighted Pearson residuals from Chi-square tests on frequencies of active or inactive marked beetles. Numbers within bubbles are observed frequencies of marked beetles at each aggregation size (n=20).

Table 1.1. Overview of current literature of group effects on metabolic rates in dormant insect aggregations

Species	Aggregation size (no. individuals)	Energy use (MR/mg/hr)[a]	Energy saved (%)[b]	Scaling Factor[c]	References
Hippodamia convergens (15°C)	1	0.189	57.14	0.79	Current study
	10	0.103			
	25	0.082			
	50	0.081			
Hippodamia convergens (8°C)	1	0.101	71.33	0.67	Current study
	10	0.044			
	25	0.03			
	50	0.029			
Pyrrhocoris apterus	1	0.052	36.54	0.88	(Su et al., 2007)
	5	0.044			
	10	0.041			
	20	0.037			
	50	0.033			
Parastrachia japonensis	1	0.256	41.40	0.85	(Tojo et al., 2005)
	10	0.152			
	20	0.163			
	40	0.150			
Stenotarsus subtilis	15	0.185	78.37	0.42	(Tanaka et al., 1988)
	30	0.15			
	90	0.085			
	210	0.04			

[a] Energy use units varied across studies. We standardized units of mass to mg. Original units for mass-specific metabolic rates: Current study (CO_2/mg/hr), Su et al., 2007(W/g), Tojo et al., 2005 (uL O_2/g/hr), and Tanaka et al., 1988 (uL O_2/g/hr).
[b] Energy saved is the percent change between the largest and smallest aggregation size tested.
[c] Scaling factors were calculated from linear regressions of natural log transformed metabolic rates and mass across all aggregation sizes.

1.4 Discussion

Aggregating behavior is often associated with mating systems, aposematic/defense, and modifications to microclimatic conditions (Danks, 2002; Sillén-Tullberg, 1990, 1988; Stamp and Bowers, 1988; Susset et al., 2018). However, many aggregations occur during winter, when energy conservation is linked to survival, and few studies have explored the role of group effects on energy use and overwintering success (Su et al., 2007; Tojo et al., 2005). Here, we have quantified the effects of aggregation size on energy use and locomotor activity in overwintering ladybeetle aggregations, and explored the effects of temperature on these group effects. Metabolic savings that aggregations provide to overwintering organisms may represent an underexplored energy use strategy, with direct implications for overwintering success.

Energetic Benefits of Aggregations

Ladybeetles within overwintering aggregations expended less energy than single individual beetles. *Per capita* beetle metabolic rates decreased by 57% and 71% with increasing aggregation size at warm and cool temperatures respectively (Fig. 1.1A, Table 1.1). These fall within the range of metabolic savings seen in overwintering aggregations of *Pyrrhocoris apterus* and *Parastrachia japonensis* (Su et al., 2007; Tojo et al., 2005; Table 1.1) and the tropical fungus beetle *Stenotarsus subtilis* (Tanaka et al., 1988; Table 1.1). Diapausing organisms already suppress their metabolic rates in order to conserve energy, and these group effects provide additional energetic savings that may result in greater energy stores at the end of winter (Hahn and Denlinger, 2011, 2007; Irwin and Lee, 2003; Sinclair, 2015). Thus, aggregating during winter may provide significant energy savings, with related fitness benefits. *H. convergens* aggregations can reach tens of millions of individuals, but our results showed that *per capita* metabolic rates plateaued at aggregation sizes of 25 and above (Fig. 1.1A). This suggests that the magnitude of metabolic savings we detected may generalize to larger aggregations in nature, but this remains to be tested in wild beetles.

Aggregation size modulated the effects of temperature on energy use, suggesting that aggregation size will determine the impact of thermal variation. Contrary to our predictions, the impact of aggregation on metabolic rate was more pronounced at the cooler temperature (8°C, Fig. 1.1A) than at warmer temperatures, giving rise to an exaggerated thermal sensitivity of metabolism in large aggregations (Fig. 1.2). Expected Q_{10}s (thermal sensitivities) for biological processes are in the range of 2-3 (Somero et al., 2017), and large aggregations had an average Q_{10} of 4.70, meaning that metabolism would almost increase 5-fold for a 10°C increase in temperature. This is much higher than the thermal sensitivities of 2.1 found in aggregations of tropical fungus beetles (Tanaka et al., 1988). Winter mean temperatures are increasing in temperate regions as a result of anthropogenic climate change (Williams et al., 2015b). The lower degree of metabolic suppression that we documented at the warmer temperature suggests that the energetic benefits of overwintering aggregations may decrease as climate warms, potentially having a disproportionate impact on aggregating species by increasing energy stress and reducing overwintering success.

Metabolic Scaling of Overwintering Aggregations

Beetle aggregations show a hypometric mass scaling of metabolic rates at both temperatures (Fig. 1.1B). A similar hypometric scaling is also seen in whole ant colonies but not

in groups of workers (Waters et al., 2017), suggesting that collections of individuals do not necessarily generate hypometric scaling. Our results suggest that the energetic profile of ladybeetle aggregations resembles that of a colony rather than a congregation of individuals. We also calculated mass-scaling exponents for published studies of insect aggregations and found that hypometric scaling was the norm in overwintering aggregations (Table 1.1). However, metabolic rates of groups of non-aggregating insects are seldom reported (Burggren et al., 2017). Thus, it is uncertain whether group effects on metabolic rates are an evolved life-history trait in insect overwintering aggregations and insect colonies, or alternatively, a non-adaptive consequence of grouping, for example due to supply limitation. We encourage future studies to address this gap in knowledge and determine if hypometric scaling is an adaptive strategy tied to life-history, or a non-adaptive consequence of grouping behavior.

Mechanisms Underlying Energy Savings

Locomotion is energetically expensive and contributes to metabolic outputs (Bonte et al., 2012). We found some evidence that reduced locomotor activity may have accounted for some portion of the metabolic suppression in large aggregations, but only at low temperatures. Overwintering *H. convergens* aggregations may reduce locomotion after finding other conspecifics using chemical cues (Wheeler and Cardé, 2014). At warmer temperatures, even though aggregations still formed and activity was generally low, active time and proportion active did not differ significantly across aggregation sizes (Fig. 1.3, Fig. 1.4), ruling out locomotor activity as a driver of reduced metabolic costs of aggregating beetles at warm temperatures. We thus found partial support for our hypothesis that aggregations reduce metabolic costs by reducing locomotor activity, and suggest that other mechanisms must contribute to the reduction in metabolic rates of aggregations compared to single individual beetles at warm temperatures.

Relative humidity within aggregations is a likely mechanism driving metabolic suppression, acting either as a cue or token stimuli that stimulates metabolic suppression, or as a direct regulator of energetic costs via the stress response. Low relative humidity ameliorates metabolic suppression in large aggregations of tropical fungus beetles (Tanaka et al., 1988), suggesting that high relative humidity in aggregations is a cue to induce metabolic suppression. However, *P. japonensis* aggregations still showed metabolic suppression in aggregations under desiccating conditions (Tojo et al., 2005). As an alternative to humidity acting as a cue for metabolic suppression, desiccation stress in single individual beetles may elevate metabolic demands relative to beetles in aggregations, as seen with thermal stress (Williams et al., 2016a). Aggregations of *H. convergens* have reduced water loss compared to individual beetles (Yoder and Smith, 1997), suggesting that desiccation stress may contribute to the metabolic pattern across groups.

Evolutionary Context

Energy saving strategies are most likely to evolve in life-history stages where energy is limited, and fitness is enhanced by low metabolic rates. Correspondingly, aggregations reduce metabolic rate in dormant insects with a fixed energy budget (Su et al., 2007; Tojo et al., 2005), but not in active and feeding insect aggregations when fitness is enhanced by rapid growth and development (Schoombie et al., 2013). This shows the need to take into account the context of the aggregations to predict the impacts of aggregations on energetics. The energy-saving properties of

overwintering aggregations provide an additional benefit that may explain the multiple times overwintering aggregations have evolved in insects.

Although metabolic suppression has fitness enhancing effects by conserving energy, large aggregations with reduced locomotor activity may be more vulnerable to predation. This could explain why most large overwintering aggregations involve insects with aposematic coloration and chemical defenses against predation (Riipi et al., 2001). Predation risk induces energetic costs associated with increased escape responses (Khater et al., 2016). By lowering the risk of predation, aposematic aggregations reduce the need to be prepared for an escape and the costs linked to this behavior. Given the fitness advantages of overwintering energy saving, release of predation risk may have facilitated the selection for deeper metabolic suppression in aposematic aggregations. However, further testing is needed between cryptic and aposematic aggregations to determine if there is a link between aposematism and group effects on metabolic rates.

Conclusions

Metabolic suppression in aggregating insects is an overwintering energy use strategy often overlooked in the field of winter physiology, but there is growing evidence that it may be widespread (Su et al., 2007; Tojo et al., 2005). Here we demonstrated marked metabolic suppression in aggregating ladybeetles, and identified locomotion as one of the potential mechanisms driving these metabolic savings at cool temperatures. This energy saving strategy is reduced at warm temperatures, which suggests that the benefits of overwintering aggregations may be reduced as winters warm. We encourage more studies to address the mechanism driving this energy saving strategy and better understand metabolic plasticity dependent on group size.

Chapter 1-2 Transition

Energy conservation is linked to survival and fitness of overwintering ectotherms, and is particularly critical in winter. In Chapter 1, I showed that increasing overwintering aggregation size drives energy conservation partly because of reduced locomotor activity, and group effects are larger in cooler temperatures. The allometric mass scaling of metabolic rates of aggregations showed similar trends to those found in ant colonies, where hypometric scaling show lower per capita energy demands with increasing mass. I also explore the literature on insect aggregations, and found that documented dormant aggregations show decreases in energy use compared to single individuals. These results highlight the need to include behavioral adaptations into overwintering energy use strategies. This group strategy for energy conservation may provide an additional selective advantage for the evolution of large overwintering aggregations. The rest of this dissertation will continue to explore energy use strategies under a potential winter tradeoff between survival and future reproduction. In summary, the findings from Chapter 1 shed light into the gap of knowledge regarding the nuanced role of energy use strategies and fitness. In the following chapters I continue to address my central hypothesis that energy allocation is regulated to meet energetic demands under both predictable and unpredictable environments.

Chapter 2 now takes a theoretical approach to understanding energy allocation strategies under a winter trade-off between survival and future reproduction. The relative importance of energy reserves at the end of winter varies across all life, with some organisms allocating post-winter reserves into reproduction (capital breeding), and others do not (income breeding). However, winter conditions may pose stress that requires energetically expensive responses to improve survival. In the next chapter I develop a general theoretical model to create new predictions of energy allocation, given the life history context across the income-capital continuum, and the role of predictable and stochastic components of seasonal environments.

Chapter 2

Energy allocation trade-off between somatic maintenance and future reproduction under predictable seasonal and unpredictable stochastic conditions during winter

2.1 Introduction

Seasonality poses one of the strongest and most widespread selective pressures organisms face (Williams et al., 2017). Seasonality is defined by an annual cycle of alternating periods of permissive conditions for growth and reproduction, i.e. growing season, followed by a period of adverse conditions. Seasonal abiotic and biotic selection pressures shape major life-history strategies including migration and dormancy, all of which are prevalent across the tree of life (Varpe, 2017). Adverse seasons, such as winters, usually have limited or no food available and can have lethal climatic conditions, like low temperatures, that apply strong selective pressures that shape fitness landscapes of many traits. The predictability of alternating growing and adverse seasons has allowed evolution to consistently select for certain life-history traits, such as increased energy reserves and stress hardiness, that prepare the organism for the adverse seasons (Kivelä et al., 2013; Sinclair, 2015; Wilsterman et al., 2021). However, even though recent work has shown that stochasticity in seasonal environments, like season endpoint or mortality risk, can also play an important role in life-history evolution (Ejsmond et al., 2018; Kivelä et al., 2016), theoretical work often oversimplifies the adverse season, leaving a large gap in our understanding of how organisms cope with a combination of predictable (seasonal) and unpredictable (stochastic) variation.

Stochastic extreme events, like heat spells or cold snaps, are becoming more frequent (Diffenbaugh et al., 2017; IPCC, 2021; van der Wiel and Bintanja, 2021) and can have a substantial impact on fitness (Bentz et al., 2010; Bozinovic et al., 2016; Vázquez et al., 2015). However, there is evidence that some organisms are able to respond to extreme events via adaptive plasticity (Campbell-Staton et al., 2017), moderating selective pressures at least partially. Seasonal extreme events can also have a temporal component of predictability, where the probability of extreme events vary within a season (Fu et al., 2013; Vergni and Todisco, 2011). Furthermore, timing of seasonal transitions represents another stochastic component to seasonality. Organisms transitioning from adverse seasons into growing seasons must undergo large physiological shifts including upregulation of reproductive and digestive systems (Roberts et al. in prep). Thus, phenological mismatches when falling out of synchrony with the growing season can lead to decreasing fitness (Reed et al., 2013).

Energy reserves can determine survival and fitness over winter, particularly for dormant animals (Sinclair, 2015). Prior to winter, many animals accumulate somatic energy reserves, which are used to fuel metabolic demands during winter (Hahn and Denlinger, 2011; Wilsterman et al., 2021). Energy depletion can cause winter mortality, if somatic energy reserves cannot be replenished due to dormancy (Biro et al., 2021; Klockmann and Fischer, 2019; Roberts et al., 2021; Vesterlund et al., 2014). Winter energy reserves can also be used to increase cold tolerance, thus increasing survival after stress exposure. Cold tolerance mechanisms can include upregulation of cold-responsive genes closely tied to energy production, and proteins like heat shock proteins,

modification of lipid membrane composition and fluidity to restore membrane function, and protective modifications to the cytoskeleton (reviewed by Clark and Worland 2008; and Toxopeus and Sinclair 2018). Another prevalent cold tolerance strategy is the production and accumulation of cryoprotectants, usually low molecular weight sugars and alcohols that reduce the freezing point (Storey and Storey, 1983). Additionally, evidence suggest a direct energetic cost associated with the recovery after stressful conditions associated with cold temperatures, including repair from oxidative damage, clear metabolic by-products, repair or clear damaged proteins (Štětina et al., 2018). Additionally, recent studies have also shown indirect costs in longevity and fecundity after recovery from cold stress (Scaccini et al., 2020). Thus, somatic maintenance costs during winter are determined by both the basal costs of metabolism and any energy invested in stress tolerance, which can be modulated in response to cold exposure.

Energy reserves remaining at the end of winter can also influence future reproductive fitness, depending on the relative importance of stored versus recently acquired reserves for reproduction across the capital to income breeding continuum (Jönsson, 1997). Examples of extreme capital breeders often have non-functional mouthparts, coupling their energy reserves to their reproductive fitness, while extreme income breeders only develop their reproductive systems until they start feeding, both extremes are relatively common in some insect orders (Coleoptera and Lepidoptera for capital breeders, Diptera and Hemiptera for income breeders). However, most organisms fall within the continuum, and energy allocation between maintenance and reproduction is not likely fixed. Evidence suggests an energy allocation trade-off between survival and reproduction, where high frequency of extreme events results in less fecundity than individuals exposed to a single extreme event without differences in survival (Koštál et al., 2019; Marshall and Sinclair, 2018; Rukke et al., 2018). Theory predicts that seasonality can modulate selection for these breeding strategies, with long growing seasons favoring income breeding, and short growing seasons selecting for capital breeding (Sainmont et al., 2014). However, most models lack the multifarious selective pressures during adverse seasons that may reveal energy allocation trade-offs and their subsequent consequences on reproductive success. More precisely, we currently lack a theoretical framework to test and generate new predictions on how capital and income breeders respond to changing seasonal stochastic conditions.

Here I explore how organisms can maximize fitness given energy allocation trade-offs between somatic maintenance and future reproduction under stochastic environmental variation. I assess optimal energy allocation under (1) increasing probability of stochastic lethal events, (2) temporal variation in probability of stochastic events through winter, and (3) stochastic end of winter. I assess effects of these three environmental changes on fitness for both income and capital breeders. The results of this work will help identify breeding strategies that are particularly sensitive to changes in stochastic conditions and identify which type of stochastic seasonal conditions have a greater impact on energy allocation trade-offs and fitness. Identifying the selective pressures of environmental stochasticity on different life-history strategies will assist our understanding of current seasonal population dynamics and species distributions.

The general model will be framed around overwintering organisms for ease of interpretation, but the results and predictions should be generalizable to any system where energy reserves are not replenishable, individuals may experience environmental stress that can be survived through energy investment, and energy reserves are linked to future fitness either directly through reproduction or growth. This model is particularly relevant to organisms with metabolically active tissues, sufficient plasticity in stress tolerance, and are able to respond to stress.

2.2 Methods

Overview of the models

I considered a simple winter with ten time periods T (called 'weeks' from now on for simplicity, but they can be interpreted as any unit of time). Individuals start winter with a set amount of energy reserves, x_{start}, with maximum possible reserves, x_{max}, and must spend one energy unit per week that represents weekly baseline metabolic costs, c. Each week, individuals in the model determine their cold tolerance state, tol, based on the probability of lethal cold events, p_c, at the expense of using up more of their energy reserves, x. Individuals that invest into cold tolerance (considered somatic maintenance) gain immunity to cold events for that week, while individuals that do not to invest become cold susceptible and die if a cold event occurs. Finally, individuals die from starvation if energy reserves fall below a critical level, x_{crit}.

All individuals start winter as cold susceptible, and can switch allocation decisions weekly. There is a low constant background weekly mortality risk, μ, due to additional factors such as disease or predation and is independent of cold tolerance state. Parameters c and μ remain constant in the current models, but are included as a foundation for future investigations. Full descriptions of the parameters are in Table 2.1.

I used stochastic dynamic programming (SDP) models paired with forward simulation models for income and capital breeders through winter to compare optimal allocation strategies under stochastic seasonal conditions. The SDP model optimizes an individual's allocation decisions on their cold tolerance state through winter. The model calculates fitness under varying stochastic conditions, and produces an optimal decision matrix that maximizes fitness for each energy pool available for allocation, EP, for each timepoint. Then I ran forward simulation models where individuals allocate their reserves optimally through each week given their energy reserves through winter. The final model outputs for each energy pool EP are: weekly optimal decisions given the energy reserve at each timepoint, total energy invested into cold tolerance (allocation into somatic maintenance), and total energy left (allocation into future reproduction) at the end of winter, and survival. The order of events in the models are as follows: each week, an individual spends the baseline metabolic cost of that week, and the cost of becoming cold tolerant or not. At the end of the week, fitness is calculated given the new energy state, taking into account the mortality risk μ, and the probability of a cold event p_c happening. The individuals die when x falls to x_{crit} or a lethal cold event occurs while they are cold susceptible.

I compiled my models into three modules, each matching a research objective on energy allocation strategies: Module 2.1 tests the effects of changes in probability of stochastic lethal events, Module 2.2 tests the effects of temporal variation in probability of stochastic events, and Module 2.3 tests the effects of stochastic end of the season. All models and data processing were conducted in R 4.0.2 (R Team, 2020). Further details on fitness functions, SDP and forward simulation models, and modules are described below.

Fitness Functions for breeding strategies

Fitness in the models is calculated based on the expected fitness at the end of winter (terminal fitness) for each breeding strategy. I modeled three breeding strategies, one for income breeders and two for capital breeders where the terminal fitness is a function of energy reserves after winter, $T+1$, and independent of cold tolerance state (Fig. 2.1).
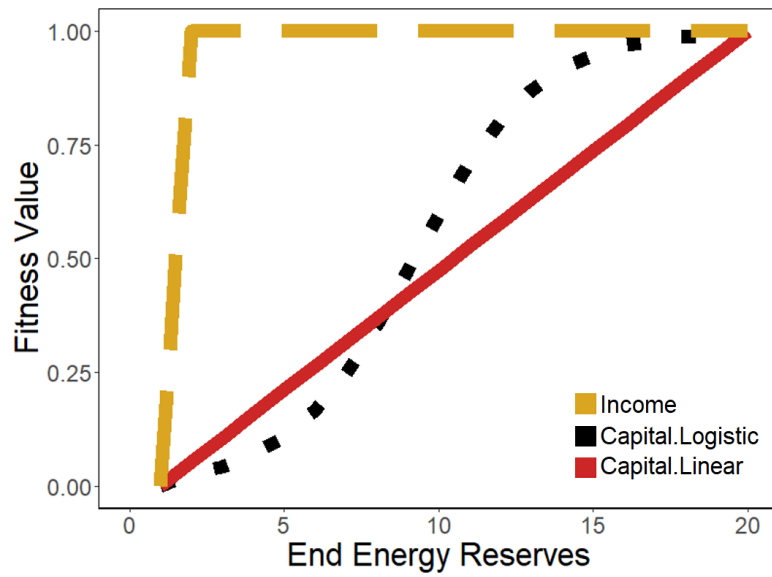
Fig. 2.1. Terminal fitness functions for Income (yellow), Capital.Logistic (black), Capital.Linear (red) given the end energy reserves at the end of winter.

For income breeders, I defined their terminal fitness as:

$$F_{income}(x, tol, T + 1) = \begin{cases} 1 & if\ x > x_{crit} \\ 0 & if\ x \leq x_{crit} \end{cases} \quad (1)$$

where individuals receive zero fitness when energy reserves fall below x_{crit} and receive maximum fitness if energy reserves end above x_{crit}. The terminal conditions are either survival or starvation, as income breeders do not rely on end energy reserves for future reproduction.

For capital breeders, I tested two terminal fitness functions where the end energy reserves increase fitness linearly or nonlinearly. I defined the linear terminal fitness as:

$$F_{capital.linear}(x, tol, T + 1) = \begin{cases} 1 & if\ x \geq x_{sat} \\ \alpha x\ +\ \beta & if\ x_{crit} < x < x_{sat} \\ 0 & if\ x \leq x_{crit} \end{cases} \quad (2)$$

where fitness increases linearly above x_{crit} with increasing energy reserves up to the limit x_{sat} when fitness is maximized. One example of capital breeders with a linear terminal fitness function is when number of eggs increase with energy reserves up to a maximum egg capacity.

For capital breeders with a logistic function, I defined their terminal fitness as:

$$F_{capital.logistic}(x, tol, T + 1) = \begin{cases} 1 & if\ x \geq x_{sat} \\ 1/(1 + 100e^{-0.5x}) & if\ x_{crit} < x < x_{sat} \\ 0 & if\ x \leq x_{crit} \end{cases} \quad (3)$$

where fitness increases exponentially above x_{crit} with increasing energy reserves, then plateaus at x_{sat}, when fitness is maximized. This terminal function represents capital breeders that have an initial slow increase in fitness with increasing reserves (for example, when a certain amount of reserves are required for the first clutch or offspring), after which fitness increases rapidly with ending energy reserves until plateauing (for example, due to constraints on egg production or costs of very high energy storage). Finally, more offspring may lead to diminishing fitness gains due to high competition at high clutch/offspring densities.

SDP and forward simulation models

In order to find the investment strategy given an energy state that maximizes fitness, I calculated potential fitness $F(x,tol,t)$, describing the optimal decision at each energy reserve and tolerance state for each time t and each breeding strategy. Optimal decisions are those resulting in the maximum fitness for that week when comparing tolerance investment decisions.

The individuals' fitness function through winter is:

$$F(x, tol, t) = \max_i \left(\underbrace{(1 - \mu)}_{survive} \left\{ \underbrace{[(1 - p_c)F(x - e_i - c, tol, t + 1)]}_{no\ cold\ event} \right. \right.$$

$$\left. \left. + \underbrace{[(p_c)F(x - e_i - c, tol, t + 1)]}_{cold\ event} \right\} \right) \quad (4)$$

where $F \in \{F_{income}, F_{capital.linear}, F_{capital.logistic}\}$ and $i \in \{invest, save\}$.

I used the standard method of backward iteration (Clark and Mangel, 2000) to solve the SDP models. After solving for F, I extracted the optimal decisions for each week that maximize the fitness, and used forward simulation models where individuals allocate their reserves optimally

through each week, given energy reserves through winter, testing individuals with all possible starting reserves, x_{start}. To ease interpretation of the results, I calculated the total energy pool available for allocation, *EP* as:

$$EP = x_{start} - x_{crit} - w_{cost} \qquad (5)$$

where x_{start} is the energy reserves at *t=1, x_{crit}*. Is the critical energy reserves before starvation, and w_{cost} is the minimum amount of energy required to survive winter, in other words the sum of weekly baseline metabolic costs for a whole winter.

Module 2.1: Impact of increased probability of cold on energy allocation

Winters can vary in probability of extreme cold events. For example, populations in low altitudes and latitudes are less likely to experience extreme cold events than populations at higher altitudes. However, the frequency of extreme cold also varies between years.

In the model, lethal cold events occur with a constant weekly probability p_c, and I tested values ranging from 0.01 to 0.52 in 0.01 increments. This wide range of probabilities span from mild winters with only a 9.5% chance of at least one lethal cold event happening by the end of winter to very harsh winters where there is 99.9% of at least one cold event and high chances of multiple lethal cold events occurring through winter.

I simulated 500 individuals through winter for each possible starting reserve x_{start} and each probability of cold p_c for each breeding strategy. From each forward simulation model, I extracted total energy invested into cold tolerance and total energy left for future reproduction at the end of winter. For each p_c, I also extracted the minimum energy pool required to invest into cold tolerance at least once (maintenance thresholds). Maintenance thresholds show the affinity for energy reserves for each breeding strategy given the risk of cold, with high maintenance thresholds showing that investment into tolerance occurs only when individuals have a large energy pool for allocation, and vice versa. These maintenance thresholds were then used to assess sensitivity to winters with different cold risks.

Table 2.1. Parameters used in the models

Parameter	Values	Description
α	0.0526	Income Breeder terminal fitness slope
β	-0.0526	Income Breeder terminal fitness y-intercept
x	1-30	Energy reserves through winter
x_{start}	1-30	Starting winter energy reserves
x_{end}	1-20	End energy reserves after winter
x_{max}	30	Maximum possible energy reserves
x_{crit}	1	Critical energy reserves
x_{sat}	20	Energy reserves when capital breeder reach maximum terminal fitness
EP	0-18	Energy pool available for allocation through winter, taking into account the minimum baseline cost of winter and the critical energy reserves
tol	1, 2	Cold susceptible or cold tolerant state
μ	0.001	Weekly background mortality
$p_c(t)$	0.01-0.51 0.01-0.30	Range of constant weekly probabilities of cold for Module 2.1 Range of weekly probabilities of winter scenarios for Module 2.2 and 2.3
T	10	Winter length (in weeks)
t	1-10	Time point within winter
$T+1$	11	Time point after winter
p_{cont}	$P_{cont} = \begin{cases} 1 & t \leq 7 \\ -0.25t & t > 8 \end{cases}$	Probability of the season continuing
c	1	Base weekly energy cost
w_{cost}	10	Minimum energy cost required to survive winter, as the sum of basal winter costs for a whole winter

Module 2.2: Temporal variation in probabilities of cold

Winters can vary temporally in the distribution of extreme cold events. For example, populations in alpine environments that experience large snow cover may be largely buffered from extreme cold events during the middle of the winter, exposing them to cold at either shoulder of the season, while populations without a snow cover may experience higher probabilities of cold events during the middle of winter. I also tested winters with a constant probability of cold for comparison and simplicity. I tested these three winter scenarios that have distinct temporal patterns of probabilities of cold events (Fig. 2.2). In these scenarios, probabilities of cold events p_c varied through time from 0.01 to 0.30 with peaks at the beginning and at the end of winter (Shoulder scenario), during mid-winter (Mid-winter scenario), or a constant probability of 0.15 (Constant scenario). All three winter scenarios have comparable probabilities of at least one cold event happening by time T (whole winter has a 42% chance of at least one lethal cold event, and Mid and Shoulder scenarios have only 1.42% difference to the Constant scenario). The individuals' fitness function is the same as in Eq.4 but p_c now varies through time as $p_c(t)$.

I simulated 500 individuals through winter for each possible starting reserve x_{start} and each winter scenario for both income breeders and capital breeders with a logistic fitness function. From the forward simulation models, I extracted the timing of investment into cold tolerance given their energy pool, total energy invested into cold tolerance. I then simulated ten winters to obtain the proportion alive at the end of winter given for each energy pool.
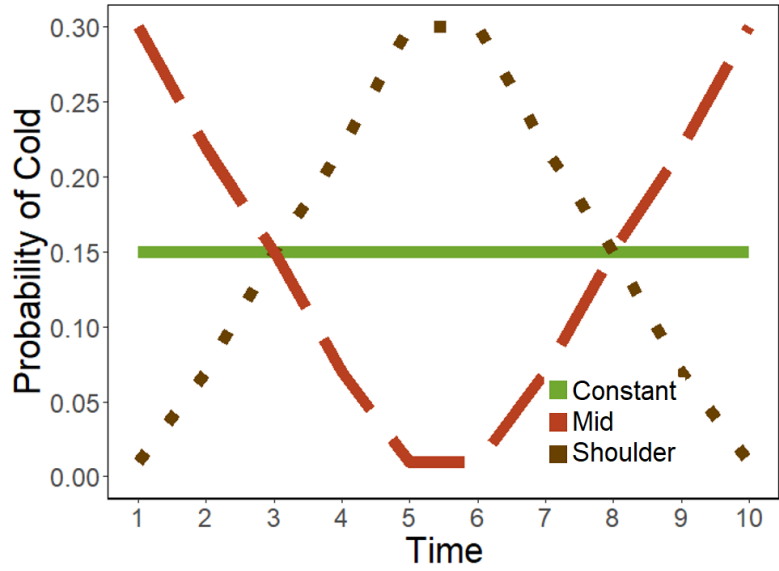
Fig. 2.2. Temporal variation in probabilities of cold through three theoretical winter scenarios.

Module 2.3: Stochastic end of the season

Winter-Spring transitions are often variable across years, leaving organisms unable to fully prepare for the onset of spring. To include the effects of a stochastic end of the season to organismal fitness, I modified Eq.4 to include the probability of the season ending before *T*:

$$F(x, tol, t) = \max_i \left(\underbrace{(1-\mu)}_{\text{survive}} \left\{ \underbrace{\begin{bmatrix} p_{cont} \underbrace{[(1-p_c(t))F(x-e_i-c, tol, t+1)]}_{\text{no cold event}} \\ + \underbrace{[(p_c(t))F(x-e_i-c, tol, t+1)]}_{\text{cold event}} \end{bmatrix}}_{\text{season continues}} + \underbrace{[(1-p_{cont})F(x-e_i-c, tol, T+1)]}_{\text{season ends}} \right\} \right) \quad (6)$$

where $F \in \{F_{income}, F_{capital.logistic}\}$ and $i \in \{invest, save\}$. I introduce the probability of the season continuing p_{cont}, and the probability of the season ending $1-p_{cont}$. Stochastic end of the season begins after *t=7*, and increases linearly until *T+1* (Fig. 2.3).

I first simulated 500 individuals through winter for each possible starting reserve x_{start} and each winter scenario for both income breeders and capital breeders with a logistic fitness function. I then extracted the timing of investment into cold tolerance for each winter scenario (constant, mid, and shoulder) and to compare the effects of stochastic end and fixed end winters, I simulated an additional five fat and five lean populations of 500 individuals through winter, each with normally distributed starting energy reserves (Mean fat reserves= 25, Mean lean reserves = 16, both with a standard deviation of 3 energy units) for income and capital breeders with a logistic fitness function. Each winter scenario consisted of 10 replicates. I then obtained survival at *t=7* as a short winter, and at *T+1* as a long winter, and compared survival between individuals behaving optimally for a long winter or a winter with a stochastic end.
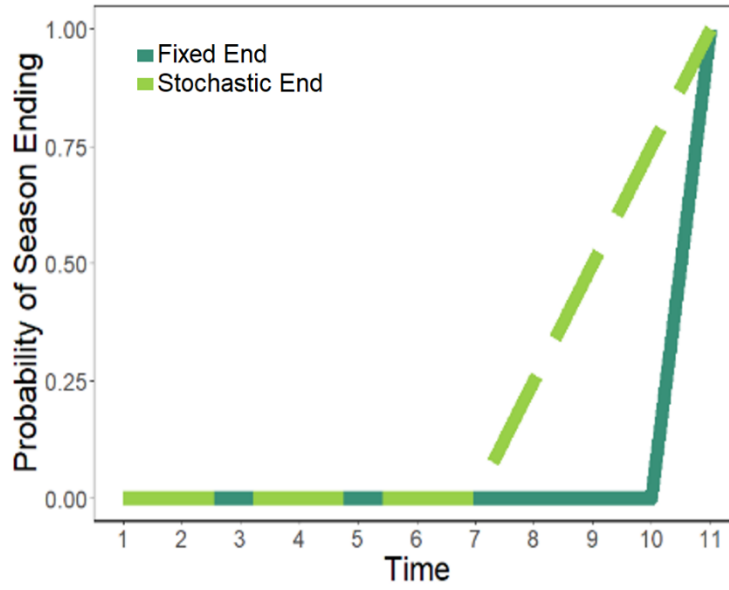
Fig. 2.3. Probability of the season ending through winter. Winters with a fixed end (solid line) always end after week 10, and winters with a stochastic end (dashed line) can end after week 7 with increasing probability.

2.3 Results

Module 2.1: Impact of increased probability of cold on energy allocation

Winter harshness determines the optimal maintenance allocation strategy. The optimal maintenance allocation (as the total energy invested into cold tolerance) of income breeders does not change with increased probability of cold (Fig. 2.4A). However, increasing probability of cold drives capital breeders to shift their investment into cold tolerance at a leaner state (Fig. 2.4A). Income breeders invest into cold tolerance as soon as they have any extra energy over the baseline cost of winter, prioritizing survival since end energy reserves do not enhance fitness (Fig. 2.4A). So, the energy pool required to start investing into maintenance in the form of cold tolerance (maintenance threshold), is low for income breeders under all winters (Fig. 2.4B). Capital breeders have a high maintenance threshold in winters with low probability of cold, because investment into cold tolerance comes at a cost of decreasing their end energy reserves and fitness, and on any given day there is a very low risk of a cold event (Fig. 2.4B). However, as the probability of cold increases, the maintenance threshold decreases for both capital breeders, with Capital.Linear being highly sensitive to winters with low probability of cold (Fig. 2.4B). To visualize the trade-off between somatic maintenance and future reproduction, I plotted the total amount of energy allocated to cold tolerance (maintenance allocation) against the energy saved for future reproduction (end energy reserves) for each energy pool available. Optimal energy allocation shifts from future reproduction into somatic maintenance only for capital breeders, revealing that the energy allocation trade-off is modulated by stochastic cold events (Fig. 2.5).
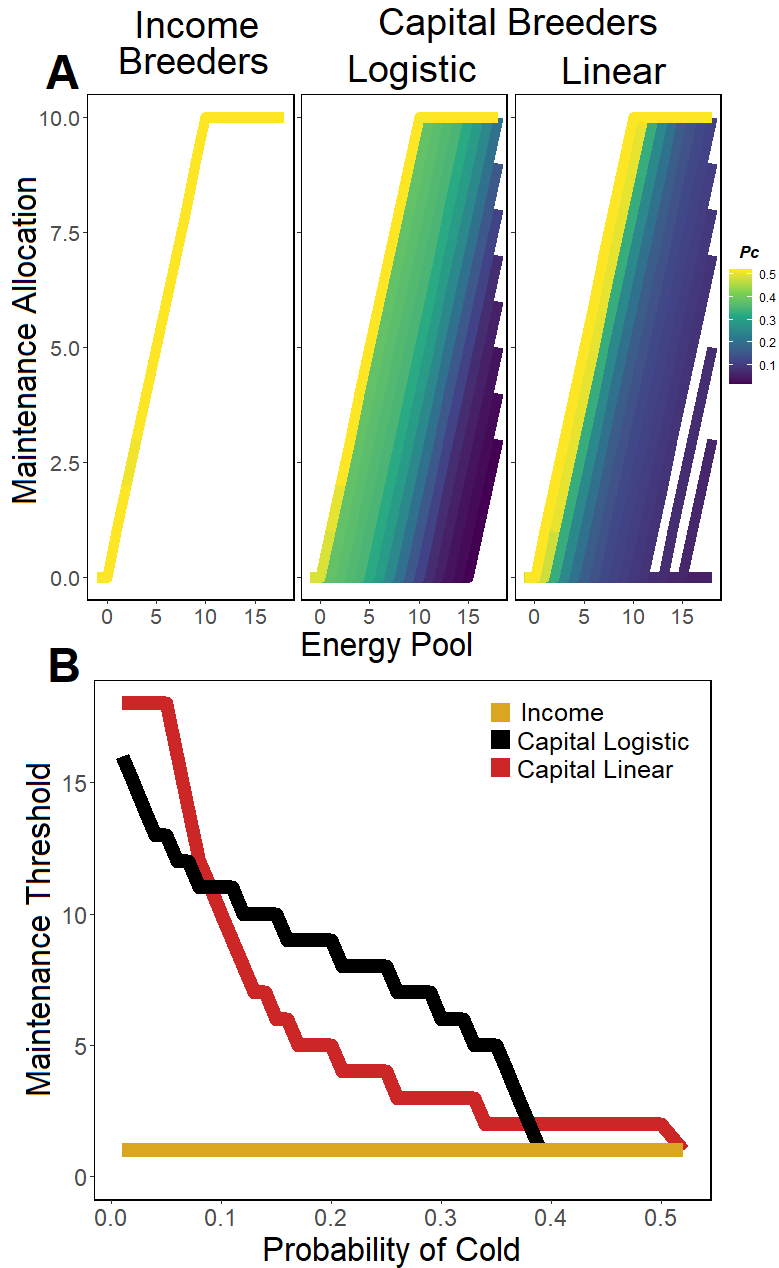
Fig. 2.4. Maintenance allocation and sensitivity across probabilities of cold for income and capital breeders. A) Energy invested into cold tolerance (maintenance allocation) across energy pool for Income (left), Capital.Logistic (center), and Capital.Linear (right), across a range of probabilities of lethal cold 0.01 – 0.51 (blue to yellow). B) Energy pool required to start investing into maintenance in the form of cold tolerance (maintenance threshold) across probability of cold for Income (yellow), Capital.Logistic (black), and Capital.Linear (red).
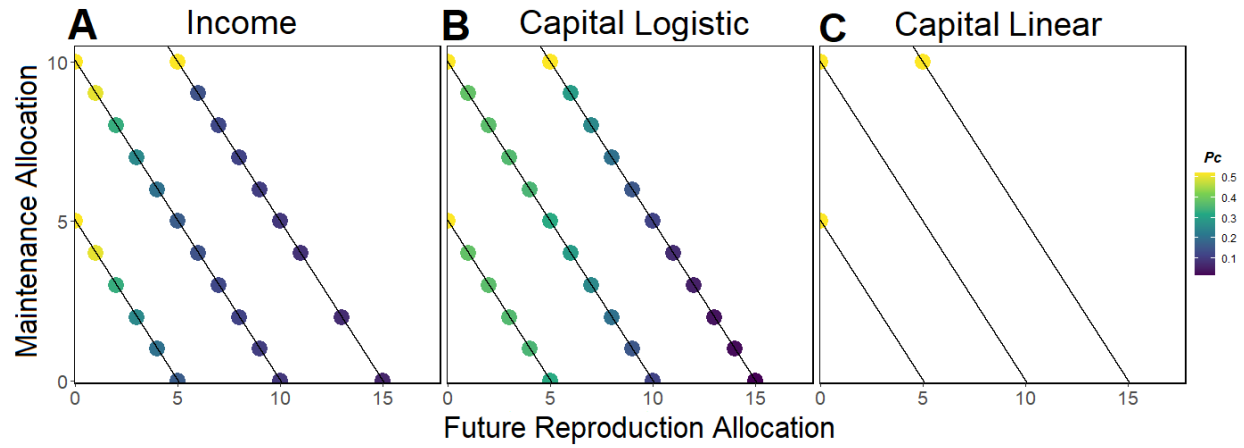
Fig. 2.5. Energy allocation trade-off between maintenance and future reproduction across probabilities of cold (*Pc*) for A) income, B) Capital.Logistic, and C) Capital.Linear. Solid lines represent the energy pool at the start of winter, and each dot represents the total number of energy units allocated to either future reproduction or maintenance at the end of winter.

Module 2.2: Impact of temporal patterns in probability of cold events on energy allocation strategies

Capital breeder decrease their maintenance threshold by 40% when winters have a temporal pattern in probability of cold (Fig. S2.1). When individuals behave optimally through winter, both income and capital investment in maintenance closely match the time periods with high probability of cold in mid and shoulder winters (Fig. 2.6). During mid and shoulder winters, capital breeders allocate the same amount of energy into maintenance, even though the timing of investments vary widely.

However, during constant winters, both income and capital breeders invest more than under mid and shoulder winters, and investment timing skews towards the end of winter. Replicating forward simulations using the full range of starting energy reserves revealed that both capital and income breeders survive better when the timing of cold is predictable (mid and shoulder winters) compared to when probability of cold is evenly distributed across the season (constant winters) (Fig. 2.7). Income breeders have higher survival under mid and shoulder winters because the timing of investments closely match the pattern of probability of cold while also keeping a low maintenance threshold (Fig. 2.7A, Fig. S2.1).

On the other hand, capital breeders under mid and shoulder winters have higher survival because of both targeting their investments to times that have high probability of cold and a decrease in their maintenance threshold, allowing leaner individuals to survive cold events relative to individuals in constant winters (Fig. 2.7B, Fig. S2.1). Additionally, for income breeders, the temporal pattern of cold does not affect survival above a threshold of ~10, because they have enough energy to invest (and are protected from cold) all winter. Conversely, for capital breeders the temporal pattern of cold does not affect survival below a threshold of ~5, because they do not invest (and are susceptible to cold) all winter. Thus, the level of energy available differentially affects the responses of income versus capital breeders to temporal pattern of cold.
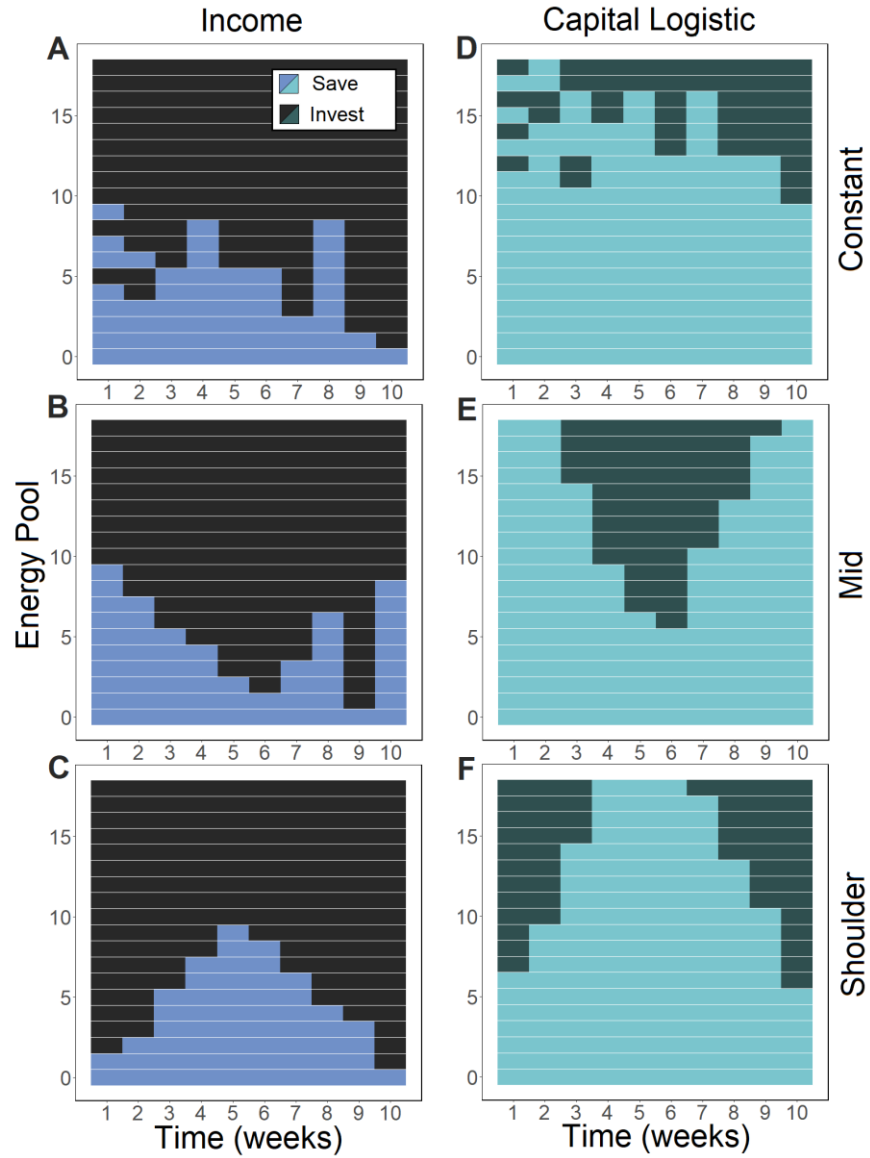
Fig. 2.6. Timing of investment decisions through winter for each starting energy pool in Income (A-C) and Capital.Logistic (D-F) across winter scenarios of constant probability of cold (top), mid-winter cold (mid), and shoulder (bottom). Decision of investing into cold tolerance (dark shade) and not investing into cold tolerance (light shade).
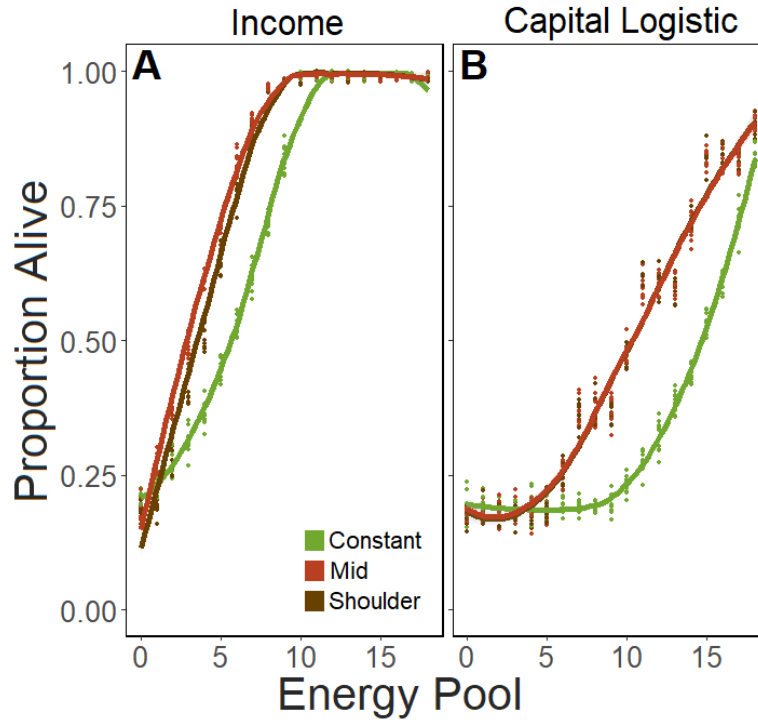
Fig. 2.7. Survival across starting energy pools for allocation for Income (A) and Capital.Logistic (B) under different winter scenarios: constant probability of cold (green), mid-winter cold (red), and cold shoulders (brown).

Module 2.3: Impact of stochastic end of season on energy allocation

When simulating individuals with a given energy pool through winters with a stochastic end, both income and capital breeders shift their investment timing, with cold tolerance investments generally shifted earlier in the winter (Fig. 2.8), while still generally matching the time periods with high probability of cold in mid and shoulder winter scenarios as seen in Fig. 2.6. This pattern is most pronounced in capital breeders that did not invest during the last time step, even when the probability of cold was very high (as in the shoulder winter).

Lean income breeders rely on winters ending early, as they over-invest energy reserves, leading to starvation if winter lasts for the entire season. Shoulder winters with a stochastic end decrease survival at week 10 when compared to individuals behaving optimally for a fixed winter (Fig. S2.2). Interestingly, this occurs only for lean income breeders and fat capital breeders, suggesting that energetic constraints are present in both breeding strategies, just at different energy pool sizes. However, when comparing survival at week 7 (corresponding to a short winter), survival is higher when individuals use the investment strategy for a stochastic end scenario relative to full length winters (Fig. S2.3), especially capital breeders with high energy pool under constant winters, as most of their investment into cold tolerance occurs before the end of winter (Fig. 2.8D).

I then simulated populations with varying starting energy reserves, high mean (fat) or low mean (lean) through short or long winters, to compare how effective are investment strategies for a fixed or stochastic end winter for each winter scenario (Fig. 2.9). For fat income breeders, a strategy optimized for a stochastic end of winter (compared to a fixed-end strategy) had no impact on survival under all winter scenarios and whether the winter was long or short (Fig. 2.9A,B), because all individuals had enough energy reserves to invest into cold tolerance every week, regardless of winter length. This matches Fig. 2.7A, in which individuals above the threshold of energy were not impacted by variation in timing of cold.

For fat capital breeders, populations optimized for a stochastic end of winter (compared to a fixed end strategy) increased survival if winter ended early (Fig. 2.9A), but had little impact on survival when winter was long and cold occurred either uniformly (Constant) or in the middle of winter (Mid) (Fig. 2.9B). However, when the probability of cold was highest on the shoulders, fat capital breeders using a strategy optimized for a stochastic end of winter greatly decreased survival relative to a fixed winter end strategy. In this scenario, investment is low at the tail end of winter, hoping winter would end early (Fig. 2.8F), but if the last day did occur then there was a very high probability of a cold event occurring and they would die.

Lean populations had generally low survival for both income and capital breeders (Fig. 2.9C,D). Similar to fat capital breeders, income breeders optimized for a fixed winter length enjoy higher survival in shoulder and mid scenarios in both long and short winters, with shoulder exposure scenarios having the highest survival. Survival for lean capital breeders is low across most winter scenarios due to little or no investment into cold tolerance. However, this is not the case in shoulder scenarios and short winters, mainly due to a large portion of the high-risk weeks not occurring at the tail end of winter. In summary, in most cases, investment strategies for stochastic end were beneficial in short winters (Fig. 2.9A,C), and had little effect or were detrimental in long shoulder winters (Fig. 2.9B,D).
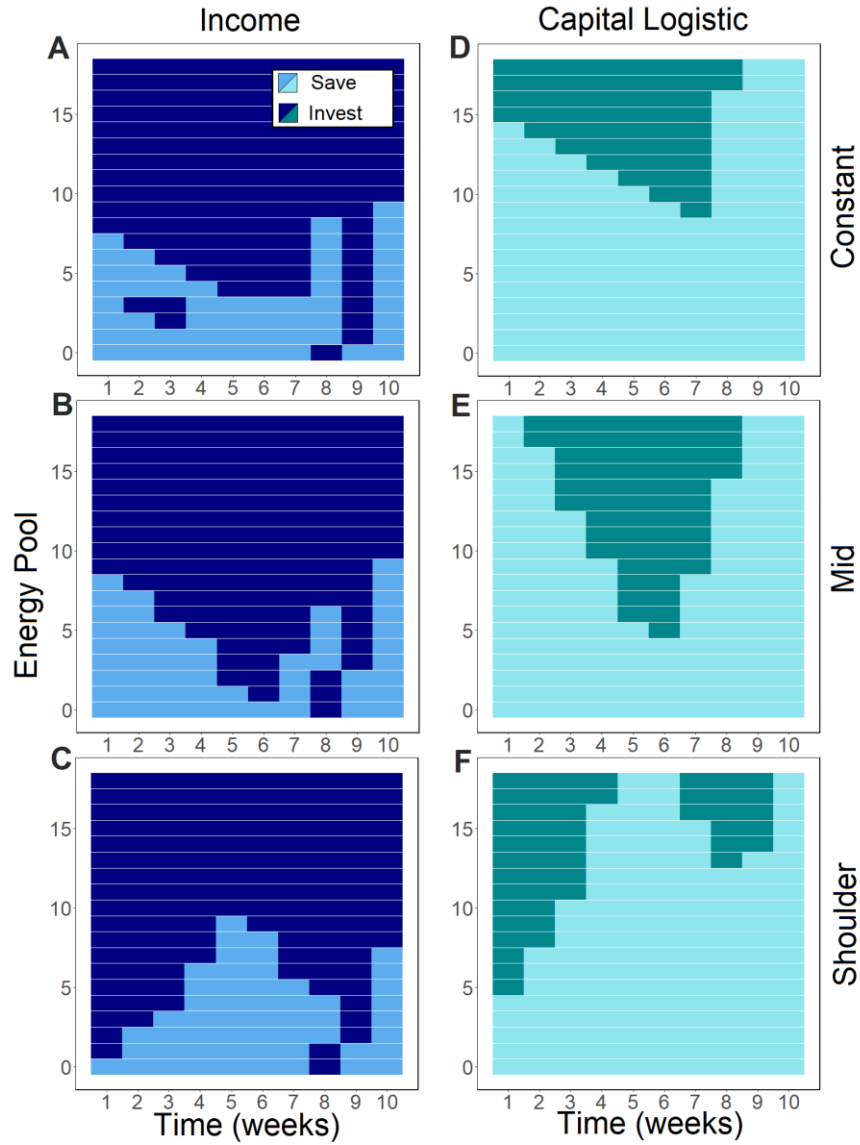
Fig. 2.8. Timing of investment decisions optimized for each starting energy pool under stochastic winter end in Income (A-C) and Capital.Logistic (D-F) across winter scenarios of constant probability of cold (top), mid-winter cold (mid), and shoulder (bottom). Decision of investing into cold tolerance (dark shade) and not investing into cold tolerance (light shade).
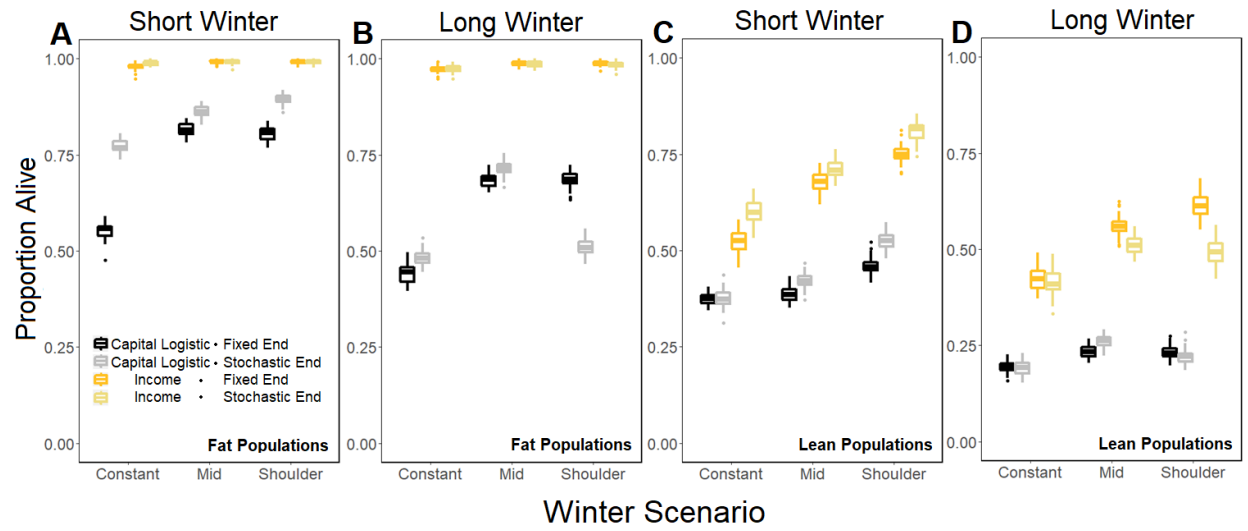
Fig. 2.9. Survival of populations varying in starting energy pools (Fat: A,B, Lean: C,D) optimized for a fixed winter length (Income: black, Capital.Logistic: yellow) or stochastic end on the season (Income: grey, Capitall.Logistic: light yellow) under short winters (A,C) or long winters (B,D) and across winter scenarios.

2.4 Discussion

In this study I assessed how organisms with different breeding strategies can maximize their fitness given a combination of predictable seasonal and unpredictable stochastic conditions during winter. My analyses explore how organisms invest their energy reserves when facing a trade-off between somatic maintenance, in this case, investment in traits that confer cold tolerance, and future reproduction.

Stochastic events modulate life-history trade-offs

Energy reserves held at the start of winter are essential for both income and capital breeders (Jönsson, 1997; Varpe et al., 2009). When reserves are high, both income and capital are able to maximally invest into both somatic maintenance and reproduction, swamping the energy trade-off as predicted by van Noordwijk and de Jong (1986). However, when reserves are limited, a trade-off between maintenance and future reproduction is modulated by stochastic events. For income breeders, all energy available for allocation is invested into cold tolerance regardless of cold risk, as they do not rely on winter reserves for their reproduction. Thus, they become susceptible to increasing stochastic events only when energy reserves are insufficient to invest every week. Capital breeders, on the other hand, will risk death in order to reserve energy for reproduction, until the cold risk reaches a threshold. A recent field-manipulation study on *Chrysomela aeneicollis* (Coleoptera: Chrysomelidae)*, a capital breeder, shows that beetles overwintering under no snow conditions (exposed to extreme cold events) die with relatively high energy reserves compared to beetles that die under snowy conditions (Roberts et al., 2021). However, it is difficult to separate whether this is due to high allocation of energy reserves for future reproduction or just mortality due to extremely cold winter conditions, regardless of energy reserve levels.

My results predict shifts in energy allocation as winters vary in the probability of stochastic events. The advantage of capital breeders over income breeders occurs early in the growing season, where capital breeders do not rely on spring resources to start reproduction (Varpe, 2017). However, in environments with high cold risk, my model suggests that capital breeders decrease their investment in reproduction while joining income breeders in relying more on early spring conditions, potentially increasing competition, and making growing seasons more restrictive. In my model there was little incentive   e for income breeders to be plastic, but for capital breeders it was beneficial to shift investment away from reproduction and into maintenance depending on the probability of cold in the environment. This suggests that populations with limited plasticity in their allocation strategies due to physiological or genetic constraints, or those who rely heavily on reproductive allocation such as obligate capital breeders (like several moths in the family Saturniidae) may be more susceptible to increasing frequency of extreme events.

Optimal investment patterns match temporal patterns of cold risk

Dormancy is often linked with stress tolerance, especially in overwintering organisms where there is usually a gradual increase in stress tolerance, for example via accumulation of cryoprotectants (Teets and Denlinger, 2013; Wilsterman et al., 2021). Although seasonal acclimation and cold tolerance mechanisms are active and rich fields of inquiry (Sinclair and Marshall, 2018; Toxopeus and Sinclair, 2018; Williams et al., 2015b), studies have mainly documented temporal patterns of cold tolerance, without examining what drives the observed

pattern (Marshall et al., 2014; Watanabe, 2002; Williams et al., 2004). My model predicts that temporal patterns of lethal events within seasons can drive large shifts in the timing and magnitude of investment in somatic maintenance. Populations that endure winters with a consistent pattern of cold events may evolve investment strategies that match these seasonal patterns. Environments with distinct temporal patterns may include mountain passes with high variation in the winter shoulders, but the persistent snow layer buffers and reduces extreme events during mid-winter for soil-dwelling organisms. On the other hand, high latitude areas with low precipitation during winter may expose organisms to more extreme cold events during the mid-winter. My results show that capital breeders benefit more than income breeders when investment is optimized to varying cold risk through winter, as they are able to target their investments more effectively in mid and shoulder winter scenarios. In these scenarios, capital breeders both increased survival due to better timed investments and increased future reproduction due to higher end energy reserves than in winters with no temporal pattern of cold. These results suggest that capital breeders would be more prevalent in seasonal environments with a temporal pattern of stochastic events than environments with a uniform distribution of cold. However, it is important to note that income breeder populations starting winter relatively lean invest into cold tolerance more effectively when there is a temporal variation of cold risk, mirroring fat capital populations. These similarities highlight how energy constraints within a life-history context still has comparable fitness consequences, albeit capital breeders having the benefit of early spring reproduction.

Environments such as oceanic areas, on the other hand, where extreme cold events may be more unpredictable throughout the season (Chown et al., 2004; Sinclair et al., 2003), are energetically costly for organisms to tolerate. In these environments, organisms may be unable to accurately time investments in stress tolerance because of energic constraints, especially capital breeders. Therefore, based on these results, I predict income breeders will be favored in environments with uniform distributions of extreme events, as they may have more potential energy available for tolerance than capital breeders. Theoretical studies propose that capital breeding strategies are favored in highly seasonal environments (Sainmont et al., 2014) with some support from empirical evidence in fish (Mcbride et al., 2015). Although, models also predict capital breeding to be favored over income strategies in stochastic environments (Ejsmond et al., 2015; Stephens et al., 2014), I encourage future studies to assess the distribution of income-to-capital breeding strategies across seasonal landscapes while taking into account temporal distribution of extreme events.

Costs and benefits of optimal investment strategies for stochastic end of the season

Throughout their life cycles, organisms face season-specific selective pressures, thus seasonal transitions play a large role in determining organismal fitness (Varpe et al., 2007; Visser and Gienapp, 2019). Here I explored how stochastic end to the season affect energy allocation strategies, and my results confirm that stochastic season end can have major effects on energy allocation and survival. Firstly, my model predicts that populations that prepare for a stochastic end of the season will shift their investments in tress tolerance away from the end of the season. Increases in temperature, as seen in the winter-spring transitions, can decrease cold tolerance (Baust and Miller, 1970; Bennett and Lee, 1989; Bourget, 1983). However, how seasonal transitions affect interannual variation in cold hardiness remains an open question.

Stochastic end of the season can pose added risks or benefits, depending on the temporal pattern of cold risk and how lean organisms enter winter. My model suggests that investing

optimally for a stochastic end may be maladaptive in winters where the end of the season has a high risk of cold events while also having an uncertain end of the season. These conditions are likely to co-occur in some environments, for example snow melt exposes ground to spring freezes after development may have already started (Kearney, 2020; Taulavuori et al., 2011; Walter et al., 2013). My results show that both lean income and fat capital breeders in these conditions show high mortality when the season is long, as no investment occurs while the probability of cold is high. However, optimality for stochastic end may be adaptive for capital breeders when winters have a uniform distribution of probabilities of cold events. Investment strategies for fat capital breeder populations show a large increase in survival while still allocating energy into future reproduction when the season ends early. Growing season fitness for capital breeders is also predicted to be highest during early spring, when the offspring fitness value is highest relative to offspring later in the season (Varpe et al., 2007).

Conclusions and future directions

My model raises new questions about the costs of mismatches that may occur when temporal patterns change, in other words when winter scenarios change from one to another. Climate change is increasing the frequency of extreme events (IPCC, 2021), but the temporal variation of extreme events within seasons remains an urgent area to be explored as it will likely be an important factor affecting population dynamics. I can predict that locations that are expected to experience a decline in snow cover will likely shift from a period where extreme events occur more often during the seasonal shoulders to one in which there is a peak in extreme events during mid-winter. And the opposite trend is predicted in environments where snow is rarely present and expected to increase.

Finally, the relative importance of energy reserves to fitness is a key question in seasonal biology, and yet very few studies have quantified their effects in seasonal transitions. My model clearly shows that sensitivity to stochastic events depends on the relative importance of energy reserves at the end of winter (terminal fitness) of the organism. Based on my results, I may expect capital breeders with a linear terminal fitness to be uncommon in environments with relatively high cold risk because most of their energy allocation has shifted towards tolerance at the cost of reproductive fitness. Thus, capital breeders may be especially susceptible to climate change as the frequency of extreme events are increasing. I call for more studies to characterize the nuanced role of end energy reserves on spring fitness given the diversity and plasticity of breeding strategies in the income-to-capital continuum, to better inform population dynamic models under climate change.

Chapter 2-3 Transition

In Chapter 2 I showed that allocation strategies and fitness are driven by both predictable and stochastic seasonal conditions. My results show that energy allocation shifts with increasing probability of stochastic events, including a stochastic end of the season, especially for capital breeders. This suggests that capital breeders may be especially susceptible to climate change. My results support my central hypothesis that energy allocation is regulated and modulated by life history and environmental conditions. These results are the outcome of a generalized model with simple theoretical parameters, and they lay the groundwork for new predictions and hypothesis for allocation strategies in seasonal environments.

Chapter 3 now takes the theoretical model developed in Chapter 2, and applies the overwintering physiology of the willow leaf beetle *Chrysomela aeneicollis*, and their seasonal microclimatic conditions. In this chapter I test the role of snow cover on fitness and allocation strategies using real ecophysiological parameters for a capital breeder. Snow cover modulates both predictable and stochastic conditions during winter. Wet winters have a deep layer of snow that buffers temperature fluctuations for organisms overwintering underground. This stable environment has low probability of lethal cold events occurring, but snow cover can also lead to longer winter phenology and cause more energy drain. On the other hand, dry winters that lack the snow buffering are characterized by a higher probability of lethal cold events, while the lower temperatures decrease the total baseline energy costs of winter. However, organisms must prepare for stressful conditions, and these responses are energetically costly. In Chapter 3 I investigate a realistic case scenario of environmental stochasticity and its impact on energy allocation strategies and a trade-off between survival and future reproduction.

Chapter 3

Winter allocation strategies and cross-seasonal fitness shift in response to stochastic winter conditions in a montane beetle

3.1 Introduction

Integrating fitness across seasons is key to understanding how seasonal transitions affect entire life cycles (Varpe, 2017). Changes in winter climate can impact organisms directly by determining survival in winter, and indirectly by determining reproductive fitness the following growing season (Marshall et al., 2020). Winter survival and future reproduction are strongly determined by energetics for animals that overwinter in dormancy (Wilsterman et al., 2021). Winter energy reserves are used for somatic maintenance and they can improve overwintering survival in two main ways. First, enough energy reserves are required to maintain baseline metabolic needs when resources are often not replenishable (Hahn and Denlinger, 2011; Wilsterman et al., 2021). Winter baseline costs vary widely across space and time, with temperature often playing the largest role in baseline metabolic costs for ectotherms (Marshall et al. 2020; Roberts et al. 2021). Energy reserve depletion can drive overwintering mortality (Biro et al., 2021; Klockmann and Fischer, 2019; Roberts et al., 2021; Vesterlund et al., 2014). Second, winter energy reserves must also be used to fuel energetically expensive processes allowing organisms to prepare for or recover from stress (e.g. cold exposure). Mechanisms of cold tolerance span from molecular, cellular, and tissue level. Molecular and cellular mechanisms include upregulation of cold-responsive genes and proteins, and lipid membrane modifications to maintain function (Clark and Worland, 2008). At the tissue level, mechanisms include the production and accumulation of cryoprotectants that prevent cold-induced damage (Toxopeus and Sinclair, 2018). All these mechanisms require energy, although how much remains an open question in the overwintering literature. Plasticity can also play a role in cold tolerance, where repeated cold exposures, including those occurring during seasonal changes, can improve cold tolerance via cold hardening (Teets and Denlinger 2013). Additionally, evidence points to direct energetic costs associated with recovery after stressful conditions like cold temperatures, including repair from oxidative damage, clearing metabolic by-products, and repair or clearance of damaged proteins (MacMillan et al., 2012; Štětina et al., 2018; Williams et al., 2016b). Recent studies have also shown indirect costs in longevity and fecundity after recovery from cold stress (Scaccini et al., 2020). During the transition out of winter, organisms undergo major physiological shifts including up-regulation of genes involved in reproductive and digestive systems (Roberts et al. in prep), preparing them to resume reproduction and activity in the growing season. Greater energy reserves are also associated with higher fecundity (Boggs and Freeman, 2005; Ellers and Van Alphen, 1997). Thus, overwintering success is linked to energetics directly through baseline costs of metabolism, energetic costs associated with cold exposure, and future reproductive success.

Winter conditions can modulate a trade-off between somatic maintenance and future reproduction (Irwin and Lee, 2003; Sinclair, 2015). Energy acquisition, or the amount of energy reserves accumulated prior to winter, determines the energy available for allocation, while winter conditions can determine allocation strategies into cold tolerance and maintenance, at the cost of future reproduction (Jönsson, 1997; Varpe, 2017). In other words, an increase of energy allocation into one trait results in a decrease in energy allocated into another, as seen in the traditional "Y-

Model" of resource allocation (van Noordwijk and de Jong, 1986; Zera and Harshman, 2001). Both empirical and theoretical evidence suggests that extreme events and energy reserves can modulate this trade-off, where high frequency of extreme events results in reduced fecundity compared to that of individuals exposed to fewer or lower magnitude of extreme events, without differences in survival (Marshall and Sinclair 2018, Chapter 2).

Snow cover can alter both magnitude of cold exposure and mean temperatures, especially for organisms overwintering underground (Kearney, 2020; Zhu et al., 2019). Stochastic extreme events like cold snaps and heat waves are becoming more frequent (IPCC, 2021) and can lead to substantial fitness costs (Bentz et al., 2010; Bozinovic et al., 2016; Vázquez et al., 2015). Seasonal extreme events often vary temporally (Fu et al., 2013; Vergni and Todisco, 2011) and interannually (Zhang et al., 2001). Winters with deep snow cover (Wet) have high baseline winter costs and low risk of lethal cold events. High baseline costs are due to the relatively long season and high mean temperatures, compared to air temperatures, while snow buffers temperature fluctuations reducing the risk of cold events (Roberts et al., 2021). On the other hand, winters with little or no snow cover (Dry) have low baseline winter costs due to low temperatures and the relatively short season, but large temperature fluctuations leave organisms exposed to high risk of lethal cold events (Roberts et al., 2021). With climate change altering precipitation patterns resulting in wetter and drier winters, we need a better understanding of how stochastic cold events and changing snow cover, and thus maintenance costs, will impact optimal energy allocation strategies and selective pressures on populations. Recently, I have shown that stochastic events can modulate optimal energy allocation strategies in a theoretical model, especially when these stochastic events have a temporal pattern during the season (Chapter 2). However, we still have limited understanding about how environmental conditions that naturally modulate fluctuations in risk of cold and baseline costs, like snow cover, affect the trade-off between winter survival and future reproduction. I hypothesize that snow cover impacts energy allocation trade-offs and optimal allocation strategies between winter survival and future reproduction and thus fitness in overwintering ectotherms. This study builds on the previous work by incorporating organism-specific parameters for energy reserves, rates of energy use, and microclimate conditions.

In this study, I focus on the willow beetle *Chrysomela aeneicollis* (Coleoptera: Chrysomelidae) to study energy allocation trade-offs imposed by snow cover. This study system offers detailed natural history knowledge gathered in the last four decades, specifically their overwintering physiology, seasonal transition into the growing season, and microclimate data. Adult beetles accumulate large energy reserves before entering dormancy under the soil (Smiley and Rank, 1986). Beetle cold tolerance is seasonally up-regulated, responds to the environment, and is attained using mechanisms that may be energetically costly (Boychuk et al., 2015; Roberts et al., 2021). During the Spring transition, beetles develop their reproductive systems quickly, using energy reserves stored prior to winter (Roberts et al. in prep), enabling us to link post-winter energy reserves to future reproductive investment. Previous work on *C. aeneicollis* also shows large genetic variation in metabolic enzymes across both latitudinal and altitudinal gradients (Rank, 1992a), making this a promising system to explore potential genetic links to energy allocation trade-offs. Additionally, long-term population surveys and high-resolution microclimate data for a large array of field sites across multiple drainages and elevations from 1981 to 2018 (Dahlhoff et al., 2019) allow me to parameterize my model with ecologically relevant microclimate conditions, and interpret results in light of interannual population dynamics.

Here I develop a model to systematically explore how 1) intensity of stochastic events, 2) amount of baseline winter costs set by average microclimate temperatures, and 3) their interaction

in realistic snow cover scenarios affect energy use, survival, and allocation strategies between maintenance and future reproduction using the overwintering biology and environment of *C. aeneicollis*. Throughout this study, I make the distinction between resource-based trade-offs and fitness trade-offs: a resource-based trade-off solely encompasses the energy allocation strategies between maintenance and future reproduction given starting energy reserves, while fitness trade-off takes into account both fitness components of winter survival and reproduction during the growing season. The results of this work yield new predictions for population and distribution models in seasonal environments, and identify populations that may be particularly susceptible to changes in winter conditions due to energy allocation trade-offs and fitness.

3.2 Methods

Study system

     C. aeneicollis occurs across the Eastern Sierra Nevada Mountains, California, US, along an elevational gradient (2700– 3400 m) in riparian and bog habitat. Beetles are univoltine (one generation per year), and their life cycle closely matches the phenology of their host plant, willow (*Salix sp.*) (Rank, 1992b), on which they are specialist feeders. Reproduction occurs during early summer, eggs hatch and larvae grow and pupate from June to August, and emerge as new adults in late August to mid- September. New adults feed and build their lipid reserves until they enter dormancy, which can last up to 9 months burrowed 5-10cm under the soil (Smiley and Rank, 1986). During winter, beetles are freeze tolerant (50% of beetles surviving 1 hour frozen at −15°C) (Boychuk et al., 2015). Environmental and beetle parameters for the model are described in Table 3.1.

Model overview

     The model assumes winters of 30 weeks in length, T. Beetles start winter with a set amount of energy reserves, x_{start}, with maximum possible reserves, x_{max}, and must spend weekly baseline metabolic costs, c. Each week, individuals choose their cold tolerance state, tol, based on the probability of lethal cold events, p_c, and the expected energetic costs of maintenance which include the weekly metabolic cost and a weekly tolerance cost, e. The weekly tolerance cost describes the energetic cost of becoming cold tolerant, and was estimated to be 1% of x_{max}, which is lower than the lowest weekly baseline cost tested. Thus, becoming cold tolerant comes at the expense of using up more of their energy reserves, x, that can be allocated into future reproduction at the end of winter. Beetles that invest into cold tolerance gain immunity to cold events for that week, while beetles that do not invest become cold susceptible and die if a cold event occurs. Finally, individuals die from starvation if energy reserves fall below a critical level, x_{crit}, and energy reserves remaining at the end of winter, x_{end}, determine their future reproductive fitness. All individuals start winter as cold susceptible, and can make a new decision about investment in cold tolerance every week. Full descriptions of the parameters are in Table 3.1.

Table 3.1. Parameters used in the models.

| Parameter	Values	Description
x	0.055-2mg	Lipid reserves
x_{start}	0.45-2mg	Lipid reserves at the start of winter
x_{max}	2mg	Maximum possible lipid reserves (estimated from Roberts in prep.)
x_{crit}	0.055mg	Critical lipid reserves (Roberts et al. 2021)
x_{end}	0.0-1.10mg	Lipid reserves at the end of winter
e	0.02mg Sensitivity analysis includes: 0.005mg 0.01mg 0.02mg	Weekly cold tolerance cost in lipids, estimated as 1% of maximum reserves
$c(t)$	**Module 3.1:** High winter baseline cost = 0.0166mg Low winter baseline cost = 0.0122mg **Module 3.2:** $c(t) = (24 * 0.3e^{(Temp*0.12)}) / 2000$	Weekly baseline metabolic cost in grams of lipid (Roberts et al. 2021) Module 3.2 calculates baseline costs from winter microclimate temperatures using energy use model from Roberts et al. 2021
tol	1, 2	Cold susceptible or cold tolerant state
T	30 weeks	Total Winter length
t	1-30 weeks	Timepoint within winter
$T+1$	31	Timepoint after winter
$p_c(t)$	High risk: 0 - 0.03 Low risk: 0 - 0.01	Weekly risk of lethal cold derived from whole winter probability of at least one cold event occurring (Roberts et al. 2021) Module 3.1: risks peak during mid winter. Risks in seasonal shoulders are found in the Appendix 3. Module 3.2: dry winter uses the high risk function with peak during mid winter and wet winter uses the low risk function with peaks during seasonal shoulders.

I used stochastic dynamic programming (SDP) paired with stochastic individual based simulation models for beetles through winter to compare optimal allocation strategies under stochastic seasonal conditions. The SDP model optimizes an individual's decisions on when to invest in cold tolerance throughout winter. I used the model to calculate expected fitness under varying cold risk and winter baseline costs to produce the optimal allocation decisions that maximizes fitness for each energy reserve available for each week during winter. Then the individual based simulation models run a population of beetles that allocate their reserves optimally through each week given their energy reserves through winter. The final model outputs for each energy reserve are: total energy left at the end of winter, survival, and total allocation into future reproduction and somatic maintenance.

The order of events in the model are as follows: each week, beetles spend the baseline metabolic cost of that week and (if they choose to invest in cold tolerance) the cost of becoming cold tolerant. At the end of the week, expected fitness is calculated given the new energy state and the cold tolerance state, taking into account the probability of a cold event p_c happening. The beetles die when energy reserves, x, fall to x_{crit}, or a lethal cold event occurs while they are cold susceptible.

Module 3.1: Fitness under different risks of lethal cold and costs of winter

Winter parameters

I estimated high and low baseline winter costs for winter using Roberts et al. 2021 energy use model and estimates of baseline winter costs using field experimental plots (37°28′09.1″N, 118°43′22.5″W, 2826m elevation). In short, I used a thermal performance curve for metabolic rates for diapausing *C. aeneicollis* (Fig. 3.1A) to estimate a total baseline winter cost (Fig. 3.1B) based on daily mean temperatures for a wet snowy (high cost) or a dry (low cost) field plot. I then divided the total winter baseline costs into high or low constant weekly costs for a 30 week-long winter. Risk of lethal cold events were estimated from the whole-winter probability of lethal events across an elevational gradient (Roberts et al. 2021). I used two overall cold probabilities based on the highest and lowest cold risks of at least one cold event occurring across the elevational gradient (42% at the cold mid-elevation sites, and 12% at the mild low-elevation sites). I then distributed the cold risk to a distinct temporal pattern, where cold risk peaks during mid-winter (Fig. 3.1C), while maintaining the same whole-winter risk. I also tested a temporal pattern where cold risk peaks during the seasonal shoulders (Appendix 3).
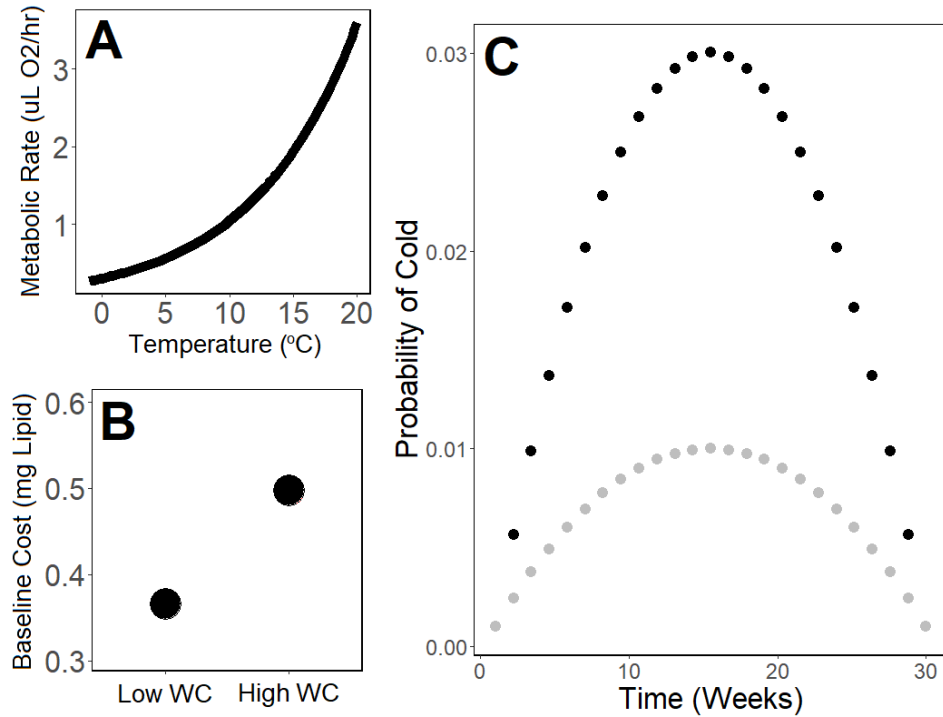
Fig. 3.1. Schematic of parameters used in Module 3.1. A) Thermal performance curve of metabolic rates for diapausing *C. aeneicollis*. (Roberts et al. 2021). B) Total baseline winter costs calculated from experimental field plots for a wet snowy plot (high baseline cost) and a dry plot (low baseline cost) (Roberts et al. 2021). C) Cold risk for harsh (black) or mild (grey) winters, where whole winter probability of at least one lethal cold event is 42% for harsh winters and 12% for mild winters (Roberts et al. 2021). Equations for A, C, and for cold risk peak during seasonal shoulders are found in Appendix 3.

Post-winter fitness

      C. aeneicollis uses lipids at the end of winter for future reproduction. I defined end of winter fitness as a function of lipid reserves, *x*, at the end of winter, *T+1*, and it is independent of the cold tolerance state, *tol*. I derived the winter fitness function from a binomial general linear model with gravidity as the dependent variable and end lipid reserves after one week of emergence as fixed effects (Fig. 3.2).

      The fitness function at the end of winter (terminal fitness function for SDP models) is then defined as:

$$F(x, tol, T + 1) = \begin{cases} 1/(1 + 19e^{-8x}) & if \ x > x_{crit} \\ 0 & if \ x \le x_{crit} \end{cases} \tag{7}$$

where beetles receive zero fitness when energy reserves fall below x_{crit} and increase fitness above this critical level with increasing lipids at the end of winter.

Model Scenarios

      I modeled winter scenarios with varying cold risk and winter baseline costs to compare optimal allocation strategies and fitness. I modeled three winter scenarios: 1) change the cold risk (PC) while maintaining a high baseline winter cost, 2) change in total baseline winter cost (WC) while maintaining a high cold risk, and 3) coupling cold risk and winter costs, a winter with low cold risk with a high baseline winter cost (Low PC/High WC; simulating snowy conditions), or high cold risk with a low baseline winter cost (High PC/Low WC; simulating dry conditions) (Table 3.2).

      I used stochastic dynamic programming (SDP) paired with individual-based simulation models to obtain the weekly optimal energy allocation decisions for each starting lipid reserves and each winter scenario. Optimal decisions were calculated by solving the SDP model using the backwards iteration method (Clark and Mangel, 2000) where an optimal decision results in the maximum fitness for that week. At the end of each week, fitness is calculated given the new energy state, taking into account the probability of a cold event p_c happening. Beetle fitness through winter is:

$$F(x, tol, t) = \max_i \left\langle \left\{ \underbrace{\left[\left(1 - p_c(t)\right) F(x - e_i - c, tol, t + 1) \right]}_{\text{no cold event}} + \underbrace{\left[\left(p_c(t)\right) F(x - e_i - c, tol, t + 1) \right]}_{\text{cold event}} \right\} \right\rangle \tag{2}$$

where i $\in$ {invest, conserve}, and p_c varies through time. Fitness is reduced to 0 any time beetles reach the critical lipid reserves x_{crit}, or a cold event occurs when cold susceptible.

      After solving for the SDP model, I used simulation models where beetles behave optimally through each week given their lipid reserves through winter. I simulated 100 individuals through winter for each possible starting reserve *x,* under high or low cold risk and baseline winter costs, under each winter type. From the simulations I extracted for each possible level of starting reserves: lipids left at the end of winter, proportion alive, energy allocation into maintenance (Maintenance Allocation = whole winter cost + total energy invested into cold tolerance), and energy allocation into future reproduction (Reproduction Allocation = total lipids left at the end of winter). All models and data processing were conducted in R 4.0.2 (R Team, 2020).
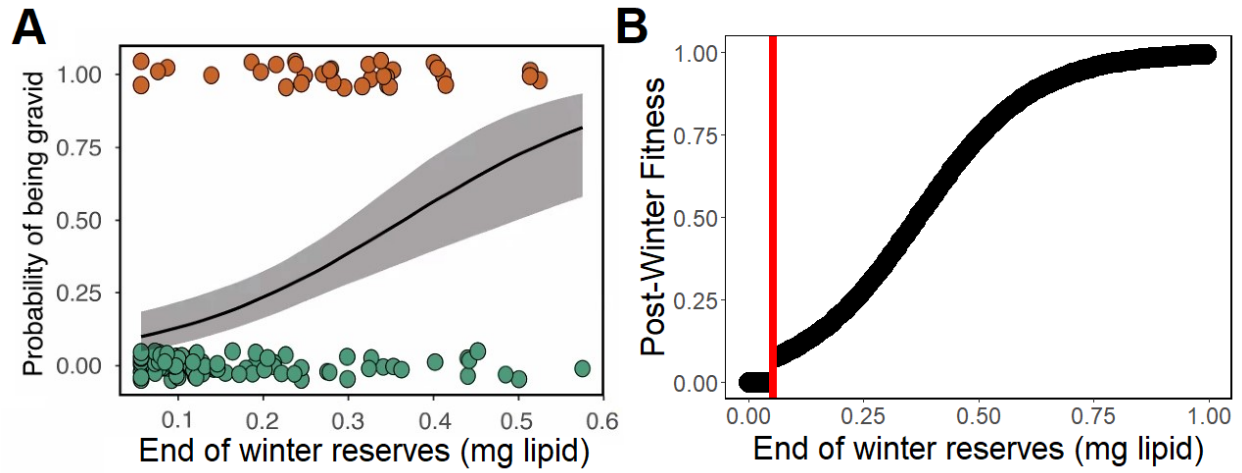
Fig. 3.2. Post-winter fitness function for *C. aeneicollis* relative to lipid reserves at the end of winter. A) Probability of gravidity after emergence and lipid reserves (from Roberts et al. in prep). B) Fitness function given lipid state at the end of winter based on end lipid reserves and probability of being gravid. Red line points to the critical reserves before starvation occurs.

Table 3.2. Winter scenarios testing a combination of winter conditions for Module 3.1

| Winter Scenario	Variables Tested	Treatment Levels
Scenario 1	Change in PC	High PC/High WC Low PC/High WC
Scenario 2	Change in WC	High WC/High PC Low WC/High PC
Scenario 3	Coupling PC and WC	High PC/Low WC Low PC/High WC

Module 3.2: Fitness under realistic winter scenarios

Winter parameters

Snow alters both energy use and cold stress, so I used real microclimate conditions from a mid-elevation site in Rock Creek, California (3067m) to parameterize the models with varying levels of baseline energy use and cold risk, to mimic ecologically relevant variation in microclimate conditions associated with snow variation. I used a long-term mid-elevation microclimate dataset to identify a wet and a dry winter. To standardize and simplify the microclimatic data for the model, winters were determined to start and end after the first and last day reached a daily mean temperature of 5°C. I established 2014 as a dry winter due to the short winter length and large temperature fluctuations during mid-winter, and 2019 as a wet winter due to the long season length and consistent snow cover through winter (established by the period of time that soil temperatures remained stable and close to 0°C; Fig. 3.3A). To account for the difference in winter length and subsequent difference in baseline winter costs between dry and wet years, I first calculated total baseline winter energy costs, as in Module 3.1, and then calculated the baseline winter costs for the first 210 days (30 weeks). The residual baseline costs after the first 210 days were then redistributed evenly across winter. Finally, I summed daily costs into weekly costs that resulted in clear and realistic energetic cost differences between wet and dry winters (Fig. 3.3B). I used the same whole-winter risk of lethal cold events as in Module 3.1, but applying the more realistic scenarios where dry winters have a high risk (42%) and peaks during mid-winter, and wet winters have low risk (12%) and peaks during the seasonal shoulders, when snow pack is minimal and temperature fluctuations are the highest (Fig. 3.3C).

SDP Models and Simulations

I used the same fitness equations and simulation models as in Module 3.1 for a dry and a wet winter, with the added factor of baseline metabolic costs, c, changing weekly calculated from microclimate data through time (Fig. 3.3B). I also conducted a sensitivity analysis for cold tolerance cost, e, with weekly costs of 0.005mg, 0.01mg, and 0.02mg of lipid. All models and data processing were conducted in R 4.0.2 (R Team, 2020).
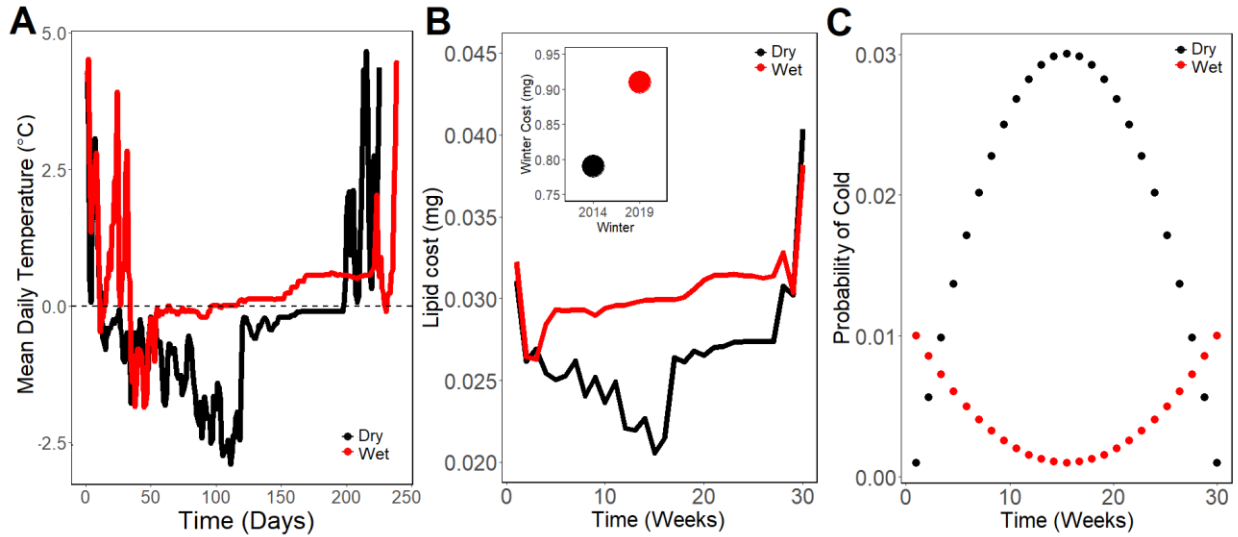
Fig. 3.3. Schematic of winter parameters for realistic winters varying in snow cover. A) Mean daily temperatures for a dry winter (black), and a wet winter (red). Dashed line at 0°C. B) Weekly lipid costs calculated from the daily temperature costs and metabolic rate curve as in Module 3.1 for a dry (black), and a wet (red) winter. Inset shows the total baseline winter costs. C) Weekly cold risk for dry (black) and wet (red) winters, where whole winter probability of at least one lethal cold event for a dry winter is 42%, and wet winter is 12%.

3.3 Results

Module 3.1: Effects of risks of lethal cold and baseline winter costs on fitness and allocation strategies

Under scenario 1, different risks of cold (PC) had no effect on lipids left for future reproduction when beetles start with few lipid reserves, but once beetles start winter with enough lipid reserves to invest into cold tolerance, colder winters result in beetles having less lipids for reproduction than low PC winters (Fig. 3.4A). As mortality is jointly determined by cold risk and starvation (falling below x_{crit}), colder winters result in lower survival when beetles are very lean as they do not allocate energy into cold tolerance and are exposed to lethal events (Fig. 3.4B).

Under Scenario 2, with different baseline winter costs (WC), beetles undergoing winters with a low cost have more lipids for reproduction at the end of the season than beetles under costly winters (Fig. 3.4C). This difference decreases with increasing starting lipids. Differences in baseline winter costs alone have little effect on survival across most starting reserves (Fig. 3.4D). Mortality due to cold affects lean beetles, similar to the High PC response in Scenario 1. However, beetles under low WC can survive with less starting reserves than High WC and also invest in cold tolerance at a lower threshold leading to an increase in survival at moderate starting lipid levels. The shift in threshold results in lipids left converging between High WC and Low WC over a range of moderate starting lipid levels (Fig. 3.4C,D).

In Scenario 3, when cold risk and baseline winter costs were coupled to simulate snow cover variation, (High PC/Low WC, and Low PC/High WC), lean beetles have more lipids for reproduction under colder winters but with a cost in survival, while fat beetles end with more lipids for reproduction under mild winters relative to cold winters (Fig. 3.4E). The pattern seen in lean beetles is largely determined by WC as seen in Fig. 3.4C, with no tolerance investment and higher lipids left under low WC compared to high WC for a given starting lipid. The pattern in fat beetles is driven by PC as in Fig. 3.4A, with tolerance investment occurring at a lower threshold under high PC than at low PC for a given starting lipid. Interestingly, when beetles start winter with enough reserves to maximize allocation to both cold tolerance and baseline maintenance the interaction between PC and WC on lipids left for reproduction is mostly lost in Scenario 1, as can be seen by all lines converging at the highest range of starting lipids. Similar to Fig. 3.4B, mortality in Scenario 3 is largely driven by high PC (Fig. 3.4F), and mortality due to starvation remains higher in leaner beetles under high WC as seen in Fig. 3.4D. Together, these results suggest that changes in PC primarily affect fat beetles, while changes in WC primarily affect lean beetles, and these trends are lost when comparing survival when beetles have enough lipid reserves to maximally invest in both reproduction and survival. All results in Scenario 1-3 are comparable when risk of cold peaks during the seasonal shoulders (Appendix 3).
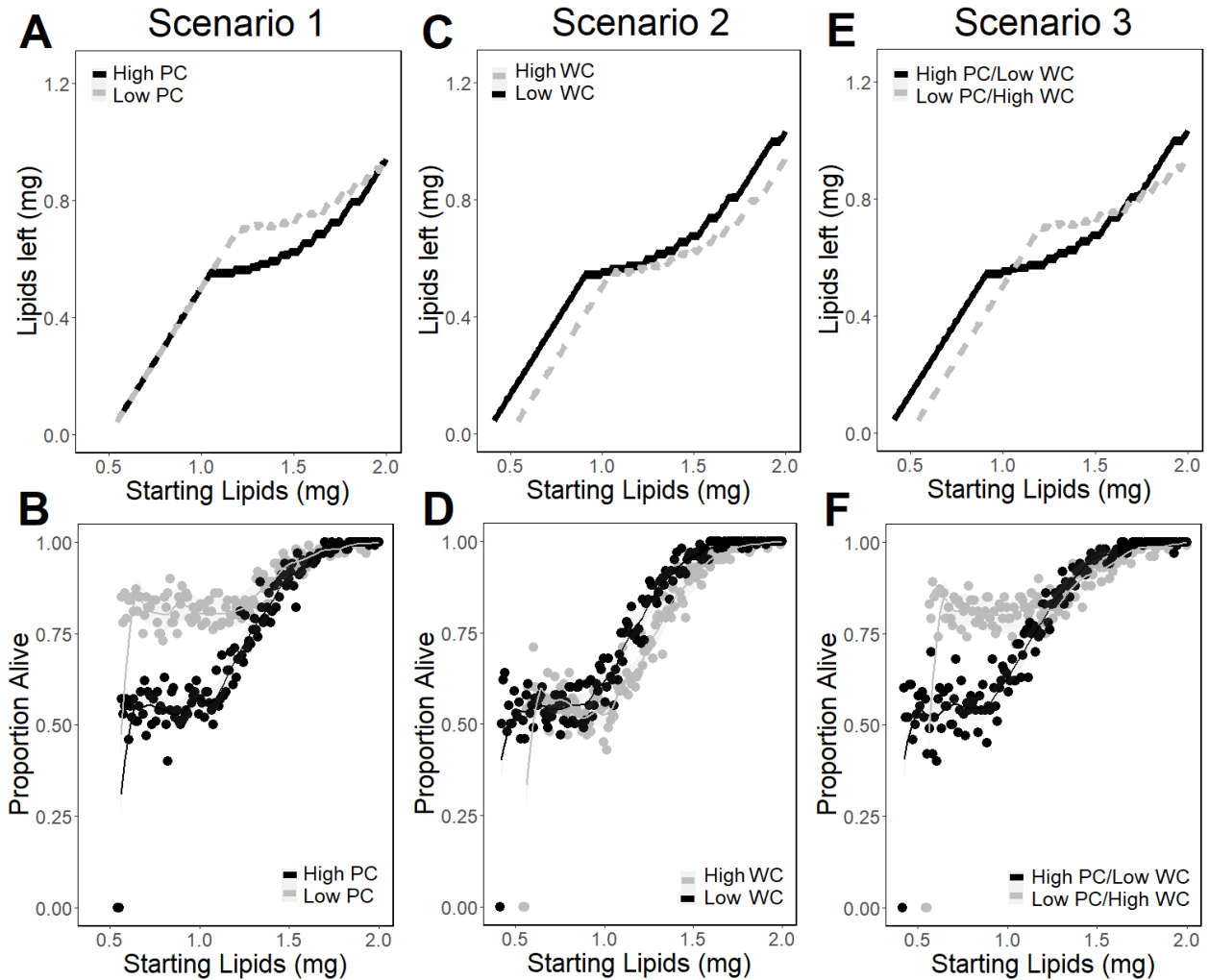
Fig. 3.4. Lipids left at the end of winter (top row), and survival (bottom row) of beetles as a function of starting lipids when changing probability of lethal cold PC (Scenario 1, A,B), baseline winter costs WC (Scenario 2, C,D), or both (Scenario 3, E,F) for winter with a mid-winter peak in cold risk. Data from simulated populations of 100 individuals for each possible starting lipid, and allocating optimally into cold tolerance for their specific winter conditions. Scenarios for peak cold risk during seasonal shoulders are found in Appendix 3.

Somatic maintenance allocation in winter consists of the sum of energy invested into tolerance and energy used for baseline maintenance. Reproduction allocation consist of the lipids left at the end of winter. I thus compared the optimal allocation strategies into overall somatic maintenance and reproduction at the end of winter given variation in cold risk and baseline energy costs.

The amount of energy allocated into maintenance is largely determined by the starting lipids acquired before winter (Fig. 3.5). When winters differed only in cold risk, maintenance allocation does not differ and remains constant when beetles start winter with few starting reserves, because beetles do not invest any energy into cold tolerance and have the same baseline maintenance cost in both conditions (Fig. 3.5A). Reproduction allocation is favored until beetles had started winter with enough lipid reserves to start investing into the cold tolerance component of the maintenance allocation. The minimum starting lipid reserves before investing into cold tolerance, termed tolerance investment threshold, is lower when PC is high (Fig. 3.5A). This is because during high PC winters, beetles optimally invest into cold tolerance at a leaner state due to the high probability of lethal cold events, at the cost of future reproduction. On the other hand, during low PC winters, beetles shift the tolerance investment threshold to a fatter state, and prioritize future reproduction over maintenance due to the lower risk of lethal cold. These results suggest that increasing cold risk can alter the allocation strategies away from reproduction and towards somatic maintenance, but only in beetles that start winter with moderately high lipid reserves. Lastly, when beetles start winter with large amounts of lipid reserves, the resource-based trade-off is reduced as seen by both winter allocation strategies allocating similar amounts given the same starting reserves. These results show that beetles had enough lipid reserves to maximize somatic maintenance under both winter scenarios.

When comparing the role of WC on allocation strategies, beetles starting winter with few lipids have higher maintenance costs under high WC, relative to low WC scenarios (Fig. 3.5B). As beetles start winter with more reserves, maintenance allocation increases slightly sooner under low WC as they invest in cold tolerance at a lower threshold because they have more discretionary energy for a given level of starting lipid compared to high WC (Fig. 3.5A). This suggests that increases in WC can alter the allocation strategies away from reproduction and towards somatic maintenance, but mostly in beetles that start winter with low lipid reserves.

Finally, when comparing scenarios where changes in PC and WC are coupled as they would be in the face of snow cover variation (Low PC/High WC; High PC/Low WC), allocation strategies between maintenance and future reproduction are dependent on the interaction between starting lipids and winter conditions (Fig. 3.5C). Lean beetles allocate more towards reproduction in High PC/Low WC conditions, when compared to beetles with the same starting reserves under Low PC/High WC. However, this trend is reversed when beetles have more starting lipids, where for the same starting reserves, beetles in High PC/Low WC invest more energy into maintenance (in the form of cold tolerance) than beetles under Low PC/High WC. Note that because the tolerance investment threshold is lower under High PC/Low WC, lipid reserves allocated into maintenance intersect the high baseline winter costs, before beetles under Low PC/High WC even start investing into tolerance (Fig. 3.5C). These results show that under winters with coupled baseline costs and cold risk, allocation into maintenance or reproduction is favored depending on the starting lipids and the winter type.
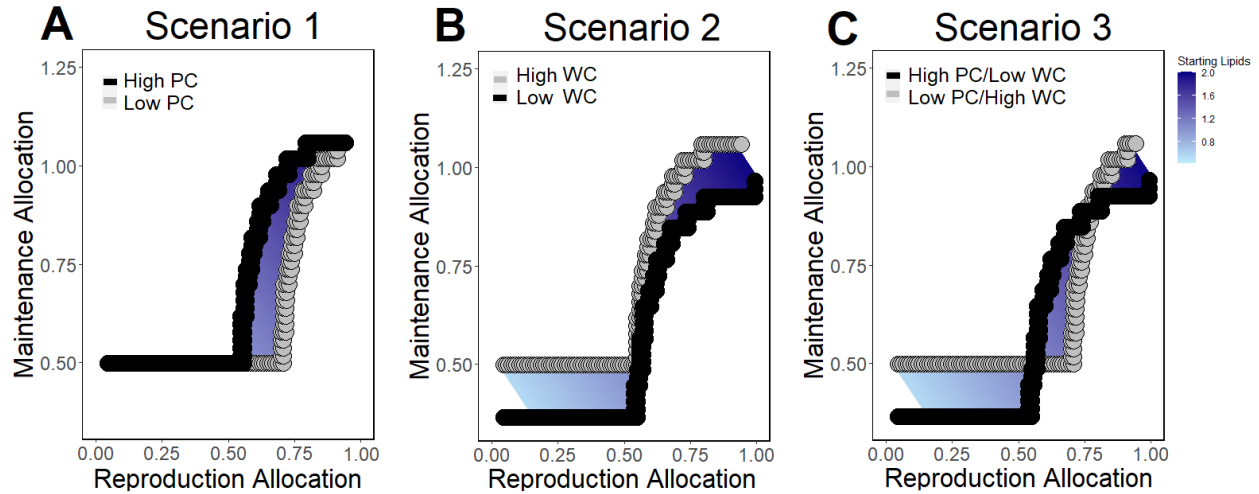
Fig. 3.5. Optimal energy allocation between maintenance and future reproduction across all possible starting winter lipids when changing probability of lethal cold (A), baseline winter costs (B) or both (C). Data from simulated populations of 100 individuals for each starting lipid. Maintenance allocation is the sum of energy invested into cold tolerance and winter energy cost, and reproduction allocation is the energy left at the end of winter. Individuals allocated optimally for their specific winter scenarios under a mid-winter peak in cold risk. Blue gradient shows each possible starting reserves. Scenarios for shoulder winters are found in Appendix 3.

Here I tested how optimal allocation strategies respond to changes in PC, WC, and their interaction, and my results suggest that energy allocation strategies are driven by resource acquisition and winter conditions as seen in Scenarios 1 and 2. However, the interaction between starting reserves and winter conditions can also determine which winter will favor reproduction or maintenance. In other words, when PC varies more than WC, the resource-based trade-off is revealed only when beetles have enough starting reserves to allocate into cold tolerance (seen as a difference in the tolerance investment threshold), and no resource-based trade-off is seen in lean beetles (seen as both allocation strategies fully overlapping).When WC varies more than PC, the resource-based trade-off is strongest in lean beetles, as allocation into tolerance is limited under already high baseline costs. Finally, when both PC and WC are coupled, the strength and allocation strategy depends on both starting reserves and winter conditions, with High PC/Low WC favoring reproduction allocation in lean beetles, while Low PC/High WC favoring reproduction at moderate lipid reserves.

Module 3.2: Effects of snow cover on fitness and allocation strategies under realistic winter conditions

Lastly, I tested the role of snow cover on fitness and optimal allocation strategies under realistic winter scenarios, linking baseline energy costs and risk of lethal cold to environmental conditions. Dry winters were characterized by low baseline costs calculated from microclimate data for a year with little snow cover, high cold risk based on mid-high elevations, and a temporal pattern of the cold risk reaching a peak during mid-winter. Wet winters, on the other hand, were characterized by high baseline costs calculated from microclimate data for a year with snow cover, low cold risk based on low elevations, and the cold risk reaching a peak during the seasonal shoulders when snow cover is absent (Fig. 3.3). I predicted that maintenance allocation in wet winters was primarily driven by high baseline winter costs, while in dry winters the maintenance allocation would be dominated by tolerance costs.
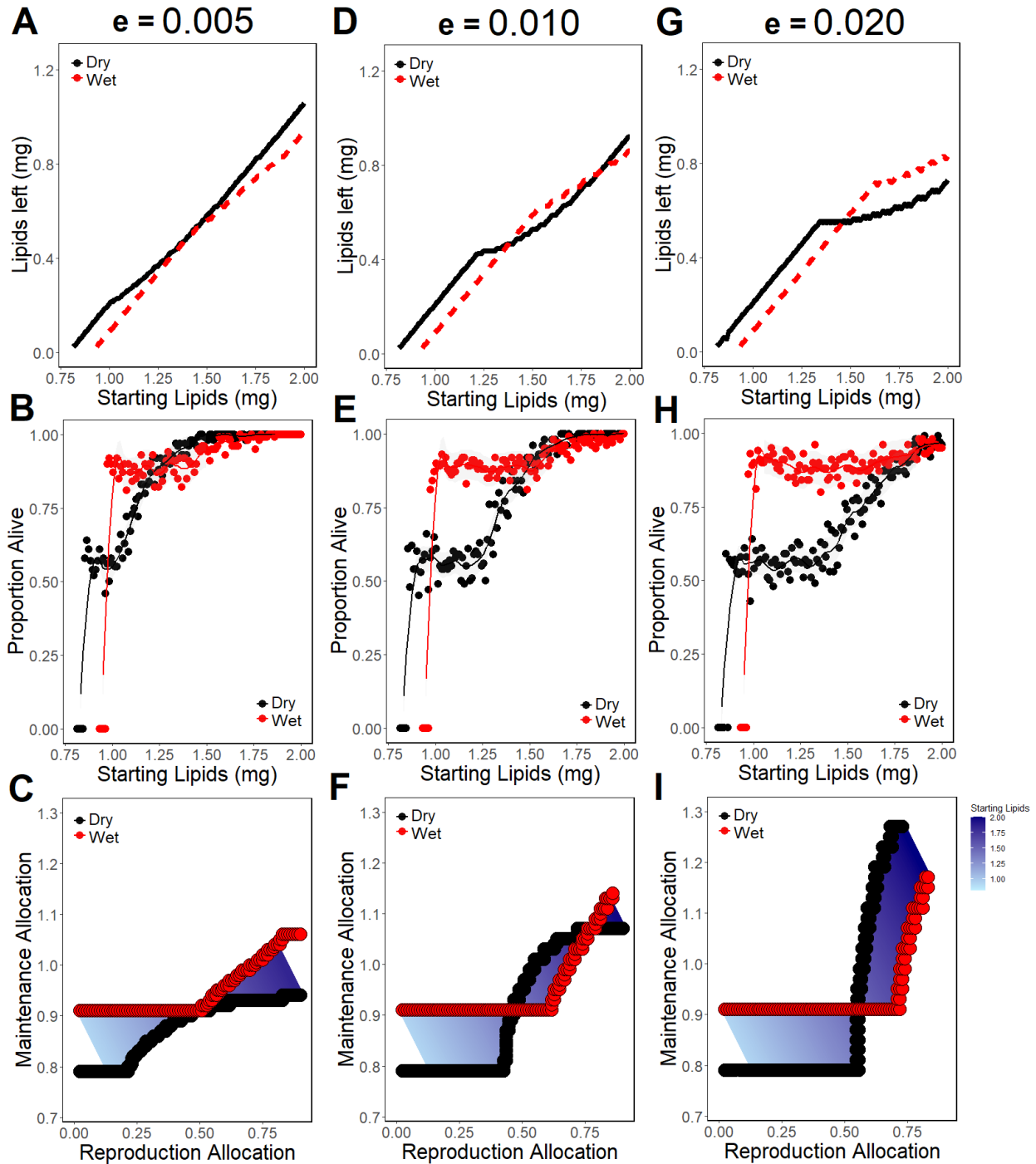
When comparing lipids left for reproduction, survival, and allocation strategies between wet and dry winters, starting winter reserves again largely determined beetle fitness. Beetles starting winter lean have more end lipid reserves but lower survival in dry winters compared to wet winters (Fig. 3.6A-B). These results reveal a potential fitness trade-off between winter survival and reproduction in the growing season. Conversely, beetles starting winter with more lipid reserves have more lipids for reproduction (Fig. 3.6A) with few differences in survival between wet and dry winters (Fig. 3.6B). Snow cover shifts optimal allocation strategies where lean beetles in dry winters invest more into reproduction than beetles in a wet winter given the same starting lipids, primarily due to differences in baseline winter costs (Fig. 3.6C). Fat beetles in dry winters, on the other hand, invest more into maintenance than beetles in a wet winter given the same starting lipids. This switch is driven by a higher PC in dry winters than in wet winters, resulting in lower tolerance investment thresholds in dry winters at the cost of reproduction. My results suggest that for a given population of beetles that vary in starting lipids, dry winters will favor future reproduction of lean beetles albeit having high mortality, while wet winters will favor future reproduction of fat beetles without the survival cost associated with cold exposure.
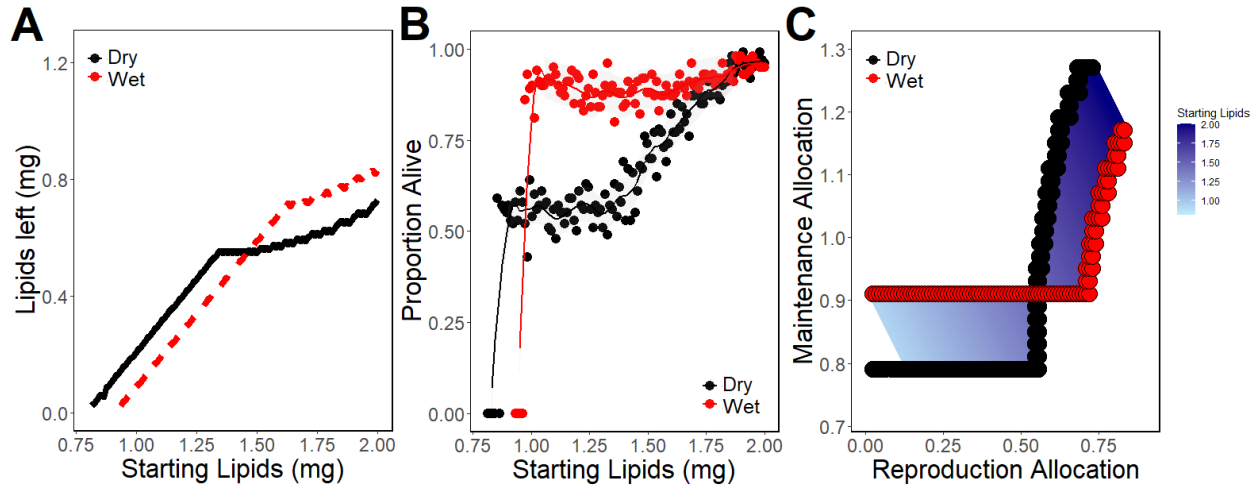
Fig. 3.6. Response to winter conditions on lipids left (A), survival (B), and allocation strategies (C) between two realistic winter scenarios, a dry winter (black), and a wet winter (red). Data from simulated populations of 100 individuals for each starting lipid Individuals allocated optimally for their specific winter scenarios. Maintenance allocation is the sum of energy invested into cold tolerance and winter energy cost, and future reproduction is the energy left at the end of winter. Blue gradient shows each possible starting reserves.

Finally, I conducted a sensitivity analysis of cold tolerance cost, e, to assess how the energetic cost of stress tolerance impacted allocations to survival and reproduction in dry and wet winters. Very low tolerance costs ($e = 0.005$) result in beetles in dry winters consistently having higher reserves for reproduction than in wet winters, except for beetles with intermediate starting reserves between 1.25mg-1.5mg (Fig. 3.7A). At these lipid levels, beetles in both dry and wet winters have equivalent lipids left for reproduction. Additionally, low tolerance costs result in beetles requiring lower starting lipids to invest into cold tolerance, improving the survival of very lean beetles during dry years (Fig. 3.7B). Allocation strategies under low tolerance costs show that beetles in wet winters consistently invest more into maintenance than beetles in dry winters, with the exception of beetles with moderate starting reserves (Fig. 3.7C). At this lipid level, beetles in both dry and wet winters invested equivalent amounts into maintenance and reproduction.

As tolerance cost increases, the lower range of starting lipids in which beetles favor reproduction in dry winters increases: only a small window between 1.00mg-1.10mg starting lipids under low tolerance cost, to a wide window between 1.00mg-1.50mg when tolerance costs are high (Fig. 3.7D). However, this reproductive advantage in dry winters comes at a survival cost (Fig. 3.7E), revealing a specific window of starting lipids where a trade-off between survival and lipids left for reproduction occurs. Interestingly, maintenance allocation of fat beetles in dry winters crosses that of wet winters (at which point beetles in dry winters have invested the equivalent amount to a baseline cost for a wet winter) (Fig. 3.7F). After this intersection, beetles in dry winters allocate more into maintenance than beetles in wet winters, until beetles had enough starting lipids that wet winters become more costly again due to the added costs of high baseline costs and tolerance investment. Finally, at high tolerance costs, lean beetles and now moderately fat beetles have more lipids left for reproduction in dry winters compared to wet winters (Fig. 3.7G), at a survival cost (Fig. 3.7H), while fat beetles in wet winters have more reserves left without a survival cost. When cold tolerance cost is high, fat beetles in dry winters consistently invest more in maintenance than reproduction relative to beetles in wet winters (Fig. 3.7I), and only beetles with high starting reserves are able to fully invest into maintenance and have high survival (Fig. 3.7H). It is important to note that the energetic cost of tolerance has little effect on survival under wet winters for most starting reserves, with the only exception of the leanest beetles that suffer starvation at a higher starting lipid compared to dry winters. These results show that tolerance costs and starting reserves can modulate both resource-based and fitness trade-offs; whereby higher tolerance costs result in a wider range of starting reserves over which the trade-off between winter survival and future reproduction is revealed.

Fig. 3.7. Sensitivity analysis to determine the effect of low (A-C), mid (D-F), and high (G-I) tolerance cost, *e*, on lipids left for reproduction, survival, and allocation across all possible starting winter lipids under dry (black) and wet (red) winter scenarios. Data from simulated populations of 100 individuals for each starting lipid reserve. Maintenance allocation is the sum of energy invested into cold tolerance and winter energy cost, and future reproduction is the energy left at the end of winter. Individuals allocated optimally for each winter scenario. Blue gradient shows each possible level of starting lipids.

3.4 Discussion

In this study, I illustrate how resource allocation strategies and life-history trade-offs could be modulated by snow cover, in ecologically relevant conditions. I parameterized a general theoretical model of allocation strategies under stochastic conditions with the overwintering physiology and microclimatic data of the willow-leaf beetle *C. aeneicollis*. My results provide insights into how changes in cold risk and baseline winter costs affect fitness in an overwintering ectotherm. Snow alters life-history fitness trade-offs between winter survival and spring reproduction, enhancing winter survival but in some cases reducing growing season reproduction. These results highlight the importance of the interplay between starting winter reserves and microclimates, and their role in seasonal transitions between winter and the growing season that link entire life cycles.

Fitness under changing winters

Winters are dynamic seasons and vary in risk of extreme events and baseline costs (Marshall et al. 2020). My model supports recent conclusions that increased risk of cold events can have indirect effects on fitness by reducing energy available for future reproduction (Chapter 2), but also adds ecologically relevant context and reveals the range of starting reserves over which increasing cold risk may impact allocation decisions. My model results agree with my original hypothesis that increased cold risk and baseline winter costs impact energy allocation strategies. Interestingly, the type and magnitude of the selective pressures that winter conditions pose to beetles are strongly dependent on starting energy reserves. Increased cold risk primarily affects beetles with high starting reserves by decreasing future reproduction. My results support conclusions from empirical studies that show extreme events reduce reproductive fitness in insects (Koštál et al., 2019; Marshall and Sinclair, 2018, 2010; Rukke et al., 2018).

On the other hand, increased baseline winter costs affect lean beetles due to both low reproductive fitness and high mortality due to starvation. This conclusion is supported by both field and laboratory studies, where a wide range of organisms from bees, beetles, butterflies, and several fish species entering winter with few energy reserves have high mortality due to energy depletion or large decreases in post-winter reproductive success (Biro et al., 2021; Klockmann and Fischer, 2019; Kooka et al., 2007; Roberts et al., 2021; Takegaki and Takeshita, 2020; Treanore and Amsalem, 2020). Finally, there is also support for decreased reproductive success due to high winter maintenance costs in hibernating brown bears (López-Alfaro et al., 2013).

My models also suggest that the outcome of overwintering populations is dependent on the winter conditions, but also on a population's range of starting reserves. The variation in energy acquisition, and resulting energy storage prior to winter, within a population can determine whether or not organisms will express a fitness trade-off between winter survival and reproduction during the growing season. Population variation in energy reserves may be constrained by ontogenetic stages, like eggs, that have a limited amount of variation in energy reserves relative to other life stages (Donoughe, 2022; Honěk, 1993), highlighting the importance of the life-history context. However, we need more information on natural variation in overwintering starting energy reserves within and between populations, to better understand how changing winters will impact population dynamics and life-history trade-offs.

Impacts of snow cover on allocation strategies and fitness

Snow cover mediates both cold exposure and temperature fluctuations (Kearney, 2020; Roberts et al., 2021), which in turn determine energy use by overwintering ectotherms. Also, energy use during winter is a well-known component of fitness and biogeographical limits (Fitzpatrick et al., 2019; Marshall et al., 2020). However, how overwintering organisms allocate energy in response to the environment remains an open area of research, and my model points to some predictions on population dynamics by linking winter energy reserves and the following growing season.

Given a sufficiently high energetic cost of cold tolerance, my results suggest that dry winters will cause high mortality for lean beetles, and reproductive deficits for fat beetles, suggesting two distinct ways that droughts may drive population declines. These results provide an explanation for observations from long-term population surveys of *C. aeneicollis*, which illustrate up-slope range contractions and local extirpation of populations during long droughts, with populations recovering in wet years (Dahlhoff et al., 2019). Conversely, snow cover reduced fecundity in goldenrod gallflies (*Eurosta solidaginis*, Diptera: Tephritidae) that overwintered as pre-pupae in galls beneath snow, relative to free-standing galls, by increasing baseline energy costs (Irwin and Lee, 2003). My model suggests that either these pre-pupae were lean at the start of winter, relative to their total winter energy costs, or that the costs of cold tolerance were sufficiently low that the increased cold stress associated with exposed galls did not reduce the energy available for reproduction.

Snow cover varies along elevational gradients (Grünewald et al., 2014; Rice et al., 2011), thus optimal allocation strategies will likely change across elevations. However, cold exposure peaks at mid-elevations, while high elevations are buffered due to persistent snow cover (Roberts et al., 2021). This suggests that for sufficiently fat beetles, increased cold exposure at mid-elevations, particularly in dry years, could reduce reproductive investments in spring. Baseline energy costs decrease with elevation (Roberts et al. 2021), but growing season length declines, meaning that populations at lower elevations may have greater ability to accumulate starting reserves. However, beetles will use more of those reserves on baseline maintenance costs, at least partially canceling out the impacts of elevation on baseline energy costs. In natural populations, fecundity declines with increasing elevation (Dahlhoff et al., 2019), suggesting that the short growing seasons or low oxygen availability at high elevations, or a combination of both, may overwhelm the impacts of decreased baseline energy costs on overwintering energetics. Finally, the prospects of prolonged droughts that may cause a sequence of dry years is particularly grim if primary productivity also declines during the summer (i.e. willow habitat decreases due to decreased precipitation). Less resources during the growing season can lead to populations starting winter with less reserves, exposing them to high cold risk and high mortality in the subsequent dry winter. This illustrates the importance of considering winter in the context of the full life cycle, in order to predict fitness and thus population dynamics.

My results confirmed a fitness trade-off between winter survival and growing season reproduction, which was modulated by snow cover. Dry winters favored future reproduction at the cost of survival, while wet winters showed high survival at the cost of reproduction, albeit driven by high baseline costs. Snow cover, thus, may alter selective gradients on cold tolerance and energy conservation (Roberts et al., 2021). Interannual variation in snow cover may then maintain genetic variation in traits related to cold tolerance and metabolism, potentially leading to balanced polymorphisms that fluctuate seasonally (Bergland et al., 2014; Machado et al., 2021). However,

increasing droughts that are driving high risks of cold exposure may lead to loss of this genetic variation. I encourage future studies to look into signatures of selection between populations under different snow cover regimes.

Finally, my sensitivity analysis of tolerance costs showed significant effects on both fitness and resource-based trade-offs under realistic winter scenarios. My results highlight a large gap in our knowledge regarding the tangible energetic costs of stress tolerance when energy allocation and conservation are under strong selection (as energy reserves relate to mortality due to starvation, and future reproductive success). In order to better understand the evolution of life-history trade-offs in natural systems, we need to quantify the energetic costs of stress tolerance and their fitness consequences.

Impacts of climate change on energy allocation strategies

Anthropogenic climate change is modifying seasonal stochasticity in both frequency of extreme events and mean temperatures (IPCC, 2021; Osland et al., 2021), thus affecting the risk of lethal cold and baseline winter costs (Marshall et al., 2020). Predicted declines in snow cover will expose overwintering organisms to high cold risk (Zhu et al., 2019) putting strong selective pressures on cold hardiness. My results suggest that energy allocation in dry winters will also shift away from future reproduction. Also, predicted increases in mean temperatures will alter overall baseline winter costs driving increased maintenance costs. My models suggest that temperate species will have to allocate more energy towards somatic maintenance which in turn will impact growing season reproductive fitness. These results are also supported by theoretical work on hibernating brown bears under climate change scenarios (Robbins et al., 2012). Thus, temperate populations may face increased selective pressures associated with winter allocation constraints during climate change (Marshall et al., 2020).

Finally, it is possible that some habitats may experience increased snow cover during climate change. Under this scenario, snow cover can lengthen winters while also reducing the growing season. This scenario would be particularly detrimental to lean organisms starting winter because of the high baseline winter costs, the subsequent reduction in reproductive allocation, and a shorter time to increase energy reserves for the following winter. However, mortality would be predicted to be mostly due to starvation and not due to stochastic extreme events, allowing most individuals with enough reserves to survive. Although this scenario may be unlikely in the native range of *C. aeneicollis* (but see Polade et al., 2017), it may be of importance in areas where snow was a rare event, and is increasing in frequency.

Assumptions of the model

My models relied on parameters from mid-elevation beetle populations, especially how reserves at the end of winter translate into post-winter fitness. With the large body of work showing examples of local adaptation (Pelini et al., 2009; Shik et al., 2019; Williams et al., 2015a), including *C. aeneicollis* (Dahlhoff et al., 2019; Rank, 1992b; Rank et al., 2020), we must explore in greater detail how winter reserves affect future fitness between populations given the large variation in life-history strategies and environmental conditions.

Furthermore, the models assume a single tolerance cost, when in fact overwintering strategies of stress tolerance vary widely, and very little empirical evidence exists of how much of the somatic energy reserves are allocated into tolerance. My sensitivity analysis showed that the

tolerance cost can determine allocation strategies that affect specific ranges of starting reserves, particularly in dry winter scenarios when cold risk is high. However, more research is sorely needed to understand the long-term energetic costs of cold tolerance, and stress in general during winter (Lavy et al. 1997; Colinet et al. 2006; MacMillan et al. 2012; but see Lehmann et al. 2016). This is essential to revealing potential trade-offs and will point towards which organisms may face strong selection in a changing seasonal landscape.

Final Remarks

      Energetics are well-known to link seasonal transitions and fitness, but very few studies address the role of stochastic conditions on energy allocation, especially under realistic winter conditions. My model clearly shows that both winter conditions, in the form of cold risk and baseline costs, and starting reserves drive distinct optimal energy allocation strategies under an allocation trade-off model between winter survival and reproduction during the growing season. I also show how ecologically relevant snow cover scenarios modulate this trade-off and identified tolerance costs to be a significant component driving allocation strategies in stochastic environments. Finally, I encourage future studies to look at natural variation in both energy reserves and environmental stochasticity, as they both may play a major role in shaping distinct selective pressures in particular life stages and seasons. Understanding these interacting factors will be key to better inform population models and their response to ongoing climate change.

Conclusions

Winter can pose serious challenges to organisms with selective pressures that affect winter survival and future reproduction in the growing season. The selective pressures vary widely across landscapes, but a unifying set of adaptations to cope with harsh winter conditions involve energy use strategies. Energy use is tightly linked to fitness while also directly affected by the environment. Energy conservation during winter has a large impact on winter survival, and energy left at the end of winter can be used for future reproduction. However, energy must be allocated to become stress hardy, and sustain metabolic needs for a prolonged period of time without food. Even though a robust body of work covers seasonal adaptations and energy use strategies, the role of stochastic events on energy use and their cross-seasonal consequences remains a challenging issue in field. This dissertation addressed part of this gap by exploring how aggregations can also act as an energy conservation strategy (Chapter 1), the role of stochastic environments on energy allocation (Chapter 2), and how natural variation in snow cover can reveal tradeoffs between survival and reproduction as a result from different allocation strategies (Chapter 3).

Chapter 1 demonstrated that overwintering aggregations reduce metabolic rates, serving as an additional energy conservation strategy during winter. This strategy is partially driven by reduced locomotor activity, and other mechanisms driving these group effects remain an open question. This energy saving strategy is thermally sensitive, with steeper effects at cooler temperatures. We encourage future studies to take into account how warmer temperatures caused by climate change will impact energy use at the individual level, but also in overwintering aggregations.

Chapter 2 and 3 reveal that the resource-based allocation trade-off between maintenance and future reproduction in winter can be modulated by stochastic events. My models lay the theoretical framework to start disentangling the nuanced role of energy allocation during winter when overwintering organisms face unpredictable environments. At the same time, my results raise new questions about the potential fitness costs of mismatch between timing of investment and stochastic events that may occur when temporal patterns change. Recently, we have seen increased frequency of extreme events around the globe due to climate change. This underlines the urgency in understanding the temporal distribution of extreme events within seasons that will likely be an important factor affecting populations. In addition to environmental variation, we must characterize variation in starting energy reserves, their use through winter, and their relative role for future reproduction, as these are traits likely under strong selection in highly seasonal environments. My models clearly show, especially in Chapter 3, that cold risk, winter baseline costs, and starting reserves can drive a trade-off between winter survival and reproduction given that energy is a limiting resource during winter and tolerance has a direct energetic cost from energetic reserves.

In summary, this dissertation supports that energy use strategies and allocation is tightly regulated and likely to be under natural selection in predictable and unpredictable environments, allowing organisms to meet changing energetic demands during winter. However, the fitness benefits of alternative strategies are highly context specific. Thus, I encourage future studies to further explore the nuanced role of energy use on cross-seasonal fitness given the diversity and

plasticity of life history strategies across the tree of life. Adding this to the field will improve our understanding on population dynamics under variable environments, and future predictions with ongoing climatic changes. This dissertation advanced the field of overwintering biology by addressing key questions on energy use strategies, allocation decisions, and their links to fitness across seasons. Together, this body of work provides new insights into how allocation decisions may be shaped by evolution to overcome environmental unpredictability and physiological constraints while also meeting their life-history energetic demands.

References

Baust, J., Miller, L.K., 1970. Variation in glycerol content and its influence on cold hardiness in the Alaskan carabid beetle *Pterostichus brevicornis*. J. Insect Physiol. 16, 979–990.

Beatty, C.D., Bain, R.S., Sherratt, T.N., 2005. The evolution of aggregation in profitable and unprofitable prey. Anim. Behav. 70, 199–208. https://doi.org/10.1016/j.anbehav.2004.09.023

Bennett, L.E., Lee, R.E., 1989. Simulated winter to summer transition in diapausing adults of the lady beetle (*Hippodamia convergens*): supercooling point is not indicative of cold-hardiness. Physiol. Entomol. 14, 361–367. https://doi.org/10.1111/j.1365-3032.1989.tb01103.x

Benoit, J.B., Del Grosso, N.A., Yoder, J.A., Denlinger, D.L., 2007. Resistance to dehydration between bouts of blood feeding in the bed bug, *Cimex lectularius*, is enhanced by water conservation, aggregation, and quiescence. Am. J. Trop. Med. Hyg. 76, 987–993. https://doi.org/10.4269/ajtmh.2007.76.987

Bentz, B.J., Rgnire, J., Fettig, C.J., Hansen, E.M., Hayes, J.L., Hicke, J.A., Kelsey, R.G., Negron, J.F., Seybold, S.J., 2010. Climate change and bark beetles of the western United States and Canada: Direct and indirect effects. Bioscience 60, 602–613. https://doi.org/10.1525/bio.2010.60.8.6

Bergland, A.O., Behrman, E.L., O'Brien, K.R., Schmidt, P.S., Petrov, D.A., 2014. Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. PLoS Genet. 10, e1004775. https://doi.org/10.1371/journal.pgen.1004775

Biro, P.A., Post, J.R., Beckmann, C., 2021. Autumn lipid reserves, overwinter lipid depletion, and high winter mortality of rainbow trout in experimental lakes. Can. J. Fish. Aquat. Sci. 78, 738–743. https://doi.org/10.1139/cjfas-2020-0276

Boggs, C.L., Freeman, K.D., 2005. Larval food limitation in butterflies: Effects on adult resource allocation and fitness. Oecologia 144, 353–361. https://doi.org/10.1007/s00442-005-0076-6

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., Schtickzelle, N., Stevens, V.M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T.G., Chaput-Bardy, A., Clobert, J., Dytham, C., Hovestadt, T., Meier, C.M., Palmer, S.C.F., Turlure, C., Travis, J.M.J., 2012. Costs of dispersal. Biol. Rev. 87, 290–312. https://doi.org/10.1111/j.1469-185X.2011.00201.x

Bourget, E., 1983. Seasonal variations of cold tolerance in intertidal mollusks and relation to environmental conditions in the St. Lawrence Estuary. Can. J. Zool. 61, 1193–1201. https://doi.org/10.1139/z83-162

Boychuk, E.C., Smiley, J.T., Dahlhoff, E.P., Bernards, M.A., Rank, N.E., Sinclair, B.J., 2015. Cold tolerance of the montane Sierra leaf beetle, *Chrysomela aeneicollis*. J. Insect Physiol. 81, 157–166. https://doi.org/10.1016/j.jinsphys.2015.07.015

Bozinovic, F., Medina, N.R., Alruiz, J.M., Cavieres, G., Sabat, P., 2016. Thermal tolerance and survival responses to scenarios of experimental climatic change: changing thermal variability reduces the heat and cold tolerance in a fly. J. Comp. Physiol. B Biochem. Syst.

Environ. Physiol. 186, 581–587. https://doi.org/10.1007/s00360-016-0980-6

Brower, L.P., Williams, E.H., Fink, L.S., Zubieta, R.R., Ramírez, M.I., 2008. Monarch butterfly clusters provide microclimatic advantages during the overwintering season in Mexico. J. Lepid. Soc. 62, 177–188.

Burggren, W., Souder, B.M., Ho, D.H., 2017. Metabolic rate and hypoxia tolerance are affected by group interactions and sex in the fruit fly (*Drosophila melanogaster*): New data and a literature survey. Biol. Open 6, 471–480. https://doi.org/10.1242/bio.023994

Campbell-Staton, S.C., Cheviron, Z.A., Rochette, N., Catchen, J., Losos, J.B., Edwards, S. V., 2017. Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. Science (80-.). 357, 495–498. https://doi.org/10.1126/science.aam5512

Chapman, J.A., Romer, J.I., Stark, J., 1955. Ladybird beetles and army cutworm adults as food for grizzly bears in Montana. Ecology 36, 156–158. https://doi.org/10.2307/1931444

Chown, S.L., Sinclair, B.J., Leinaas, H.P., Gaston, K.J., 2004. Hemispheric asymmetries in biodiversity - A serious matter for ecology. PLoS Biol. 2. https://doi.org/10.1371/journal.pbio.0020406

Clark, C., Mangel, M., 2000. Dynamic State Variable Models in Ecology. Oxford University Press.

Clark, M.S., Worland, M.R., 2008. How insects survive the cold: Molecular mechanisms - A review. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 178, 917–933. https://doi.org/10.1007/s00360-008-0286-4

Cloyed, C.S., Dell, A.I., 2020. The body size and temperature dependence of organismal locomotion. Ecology 101, 3114. https://doi.org/10.1002/ecy.3114

Colinet, H., Hance, T., Vernon, P., 2006. Water relations, fat reserves, survival, and longevity of a cold-exposed parasitic wasp *Aphidius colemani* (Hymenoptera: Aphidiinae). Environ. Entomol. 35, 228–236. https://doi.org/10.1603/0046-225X-35.2.228

Crawley, M.J., 2007. The R Book. John Wiley & Sons Ltd.

Dahlhoff, E.P., Dahlhoff, V.C., Grainger, C.A., Zavala, N.A., Otepola-Bello, D., Sargent, B.A., Roberts, K.T., Heidl, S.J., Smiley, J.T., Rank, N.E., 2019. Getting chased up the mountain: High elevation may limit performance and fitness characters in a montane insect. Funct. Ecol. 33, 809–818. https://doi.org/10.1111/1365-2435.13286

Danks, H. V., 2002. Modification of adverse conditions by insects. Oikos 99, 10–24. https://doi.org/10.1034/j.1600-0706.2002.990102.x

Diffenbaugh, N.S., Singh, D., Mankin, J.S., Horton, D.E., Swain, D.L., Touma, D., Charland, A., Liu, Y., Haugen, M., Tsiang, M., Rajaratnam, B., 2017. Quantifying the influence of global warming on unprecedented extreme climate events. Proc. Natl. Acad. Sci. U. S. A. 114, 4881–4886. https://doi.org/10.1073/pnas.1618082114

Donoughe, S., 2022. Insect egg morphology: evolution, development, and ecology. Curr. Opin. Insect Sci. 50, 100868. https://doi.org/10.1016/j.cois.2021.12.008

Ejsmond, M.J., McNamara, J.M., Søreide, J., Varpe, Ø., 2018. Gradients of season length and mortality risk cause shifts in body size, reserves and reproductive strategies of determinate

growers. Funct. Ecol. 32, 2395–2406. https://doi.org/10.1111/1365-2435.13191

Ejsmond, M.J., Varpe, Ø., Czarnoleski, M., Kozłowski, J., 2015. Seasonality in offspring value and trade-offs with growth explain capital breeding. Am. Nat. 186, E111–E125. https://doi.org/10.1086/683119

Ellers, J., Van Alphen, J.J.M., 1997. Life history evolution in *Asobara tabida*: Plasticity in allocation of fat reserves to survival and reproduction. J. Evol. Biol. 10, 771–785. https://doi.org/10.1007/s000360050053

Fitzpatrick, M., Zuckerberg, B., Pauli, J., Kearney, M., Thomson, K., Werner, L., Porter, W., 2019. Modeling the distribution of niche space and risk for a freeze-tolerant ectotherm, *Lithobates sylvaticus*. Ecosphere 10, e02788.

Fu, G., Yu, J., Yu, X., Ouyang, R., Zhang, Y., Wang, P., Liu, W., Min, L., 2013. Temporal variation of extreme rainfall events in China, 1961-2009. J. Hydrol. 487, 48–59. https://doi.org/10.1016/j.jhydrol.2013.02.021

Galili, T., 2010. Post hoc analysis for Friedman's Test (R code) [WWW Document]. URL https://www.r-statistics.com/2010/02/post-hoc-analysis-for-friedmans-test-r-code/

Grünewald, T., Bühler, Y., Lehning, M., 2014. Elevation dependency of mountain snow depth. Cryosphere 8, 2381–2394. https://doi.org/10.5194/tc-8-2381-2014

Güven, Ö., Gollüoglu, H., Ceryngier, P., 2015. Aestivo-hibernation of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in a mountainous area in southern Turkey: Is dormancy at high altitudes adaptive? Eur. J. Entomol. 112, 41–48. https://doi.org/10.14411/eje.2015.002

Hagen, K.S., 1962. Biology and ecology of predaceous Coccinellidae. Annu. Rev. Entomol. 7, 289–326. https://doi.org/10.1146/annurev.en.07.010162.001445

Hahn, D.A., Denlinger, D.L., 2011. Energetics of insect diapause. Annu. Rev. Entomol. 56, 103–121. https://doi.org/10.1146/annurev-ento-112408-085436

Hahn, D.A., Denlinger, D.L., 2007. Meeting the energetic demands of insect diapause: Nutrient storage and utilization. J. Insect Physiol. 53, 760–773. https://doi.org/10.1016/j.jinsphys.2007.03.018

Hodek, I., 2012. Diapause / Dormancy, in: Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). pp. 275–341.

Honěk, A., 1993. Intraspecific variation in body size and fecundity in insects : A general relationship. Oikos 66, 483–492.

IPCC, 2021. Climate Change 2021: The Physical Science Basis, Intergovernmental Panel on Climate Change, Geneva.

Irwin, J.T., Lee, R.E., 2003. Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis*. Oikos 100, 71–78. https://doi.org/10.1034/j.1600-0706.2003.11738.x

Jönsson, K.I., 1997. Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78, 57–66.

Kearney, M.R., 2020. How will snow alter exposure of organisms to cold stress under climate

warming? Glob. Ecol. Biogeogr. 29, 1246–1256. https://doi.org/10.1111/geb.13100

Khater, M., Murariu, D., Gras, R., 2016. Predation risk tradeoffs in prey: effects on energy and behaviour. Theor. Ecol. 9, 251–268. https://doi.org/10.1007/s12080-015-0277-5

Kivelä, S.M., Välimäki, P., Gotthard, K., 2016. Evolution of alternative insect life histories in stochastic seasonal environments. Ecol. Evol. 6, 5596–5613. https://doi.org/10.1002/ece3.2310

Kivelä, S.M., Välimäki, P., Gotthard, K., 2013. Seasonality maintains alternative life-history phenotypes. Evolution (N. Y). 67, 3145–3160. https://doi.org/10.1111/evo.12181

Kleiber, M., 1932. Body size and metabolism. Hilgardia J. Agric. Sci. 6, 315–353.

Klockmann, M., Fischer, K., 2019. Strong reduction in diapause survival under warm and humid overwintering conditions in a temperate-zone butterfly. Popul. Ecol. 61, 150–159. https://doi.org/10.1002/1438-390X.1016

Kolokotrones, T., Van Savage, Deeds, E.J., Fontana, W., 2010. Curvature in metabolic scaling. Nature 464, 753–756. https://doi.org/10.1038/nature08920

Kooijman, S.A.L.M., 1986. Energy budgets can explain body size relations. J. Theor. Biol. 121, 269–282. https://doi.org/10.1016/S0022-5193(86)80107-2

Kooka, K., Yamamura, O., Andoh, T., 2007. Rate of energy depletion and overwintering mortality of juvenile walleye pollock in cold water. J. Fish Biol. 71, 1714–1734. https://doi.org/10.1111/j.1095-8649.2007.01638.x

Koštál, V., Grgac, R., Korbelová, J., 2019. Delayed mortality and sublethal effects of cold stress in *Drosophila melanogaster*. J. Insect Physiol. 113, 24–32. https://doi.org/10.1016/j.jinsphys.2019.01.003

Lavy, D., Nedved, O., Verhoef, H.A., 1997. Effects of starvation on body composition and cold tolerance in the collembolan *Orchesella cincta* and the isopod *Porcellio scaber*. J. Insect Physiol. 43, 973–978. https://doi.org/10.1016/S0022-1910(97)00011-5

Lehmann, P., Pruisscher, P., Posledovich, D., Carlsson, M., Käkelä, R., Tang, P., Nylin, S., Wheat, C.W., Wiklund, C., Gotthard, K., 2016. Energy and lipid metabolism during direct and diapause development in a pierid butterfly. J. Exp. Biol. 219, 3049–3060. https://doi.org/10.1242/jeb.142687

López-Alfaro, C., Robbins, C.T., Zedrosser, A., Nielsen, S.E., 2013. Energetics of hibernation and reproductive trade-offs in brown bears. Ecol. Modell. 270, 1–10. https://doi.org/10.1016/j.ecolmodel.2013.09.002

Machado, H.E., Bergland, A.O., Taylor, R., Tilk, S., Behrman, E., Dyer, K., Fabian, D.K., Flatt, T., González, J., Karasov, T.L., Kim, B., Kozeretska, I., Lazzaro, B.P., Merritt, T.J.S., Pool, J.E., O'brien, K., Rajpurohit, S., Roy, P.R., Schaeffer, S.W., Serga, S., Schmidt, P., Petrov, D.A., 2021. Broad geographic sampling reveals the shared basis and environmental correlates of seasonal adaptation in drosophila. Elife 10, 1–21. https://doi.org/10.7554/eLife.67577

MacMillan, H.A., Williams, C.M., Staples, J.F., Sinclair, B.J., 2012. Reestablishment of ion homeostasis during chill-coma recovery in the cricket *Gryllus pennsylvanicus*. Proc. Natl.

Acad. Sci. U. S. A. 109, 20750–20755. https://doi.org/10.1073/pnas.1212788109

Maino, J.L., Kearney, M.R., Nisbet, R.M., Kooijman, S.A.L.M., 2014. Reconciling theories for metabolic scaling. J. Anim. Ecol. 83, 20–29. https://doi.org/10.1111/1365-2656.12085

Marshall, K.E., Gotthard, K., Williams, C.M., 2020. Evolutionary impacts of winter climate change on insects. Curr. Opin. Insect Sci. 41, 54–62. https://doi.org/10.1016/j.cois.2020.06.003

Marshall, K.E., Sinclair, B.J., 2018. Repeated freezing induces a trade-off between cryoprotection and egg production in the goldenrod gall fly, *Eurosta solidaginis*. J. Exp. Biol. 221. https://doi.org/10.1242/jeb.177956

Marshall, K.E., Sinclair, B.J., 2010. Repeated stress exposure results in a survival-reproduction trade-off in *Drosophila melanogaster*. Proc. R. Soc. B Biol. Sci. 277, 963–969. https://doi.org/10.1098/rspb.2009.1807

Marshall, K.E., Thomas, R.H., Roxin, A., Chen, E.K.Y., Brown, J.C.L., Gillies, E.R., Sinclair, B.J., 2014. Seasonal accumulation of acetylated triacylglycerols by a freeze-tolerant insect. J. Exp. Biol. 217, 1580–1587. https://doi.org/10.1242/jeb.099838

Mcbride, R.S., Somarakis, S., Fitzhugh, G.R., Albert, A., Yaragina, N.A., Wuenschel, M.J., Alonso-Fernández, A., Basilone, G., 2015. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish Fish. 16, 23–57. https://doi.org/10.1111/faf.12043

Murakami, Y., Hasegawa, E., Watanabe, S., 2019. Effects of color morph on aggregation formation for hibernation in an extremely color polymorphic ladybug, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Entomol. Ornithol. Herpetol. Curr. Res. 8, 10–13. https://doi.org/10.35248/2161-0983.19.8.219

Osland, M.J., Stevens, P.W., Lamont, M.M., Brusca, R.C., Hart, K.M., Waddle, J.H., Langtimm, C.A., Williams, C.M., Keim, B.D., Terando, A.J., Reyier, E.A., Marshall, K.E., Loik, M.E., Boucek, R.E., Lewis, A.B., Seminoff, J.A., 2021. Tropicalization of temperate ecosystems in North America: The northward range expansion of tropical organisms in response to warming winter temperatures. Glob. Chang. Biol. 27, 3009–3034. https://doi.org/10.1111/gcb.15563

Pelini, S.L., Dzurisin, J.D.K., Prior, K.M., Williams, C.M., Marsico, T.D., Sinclair, B.J., Hellmann, J.J., 2009. Translocation experiments with butterflies reveal limits to enhancement of poleward populations under climate change. Proc. Natl. Acad. Sci. U. S. A. 106, 11160–11165. https://doi.org/10.1073/pnas.0900284106

Polade, S.D., Gershunov, A., Cayan, D.R., Dettinger, M.D., Pierce, D.W., 2017. Precipitation in a warming world: Assessing projected hydro-climate changes in California and other Mediterranean climate regions. Sci. Rep. 7, 1–10. https://doi.org/10.1038/s41598-017-11285-y

R Team, 2020. R.

R Team, 2018. R.

Rank, N.E., 1992a. Host plant preference based on salicylate chemistry in a willow leaf beetle (*Chrysomela aeneicollis*). Oecologia 90, 95–101. https://doi.org/10.1007/BF00317814

Rank, N.E., 1992b. A hierarchical analysis of genetic differentiation in a montane leaf beetle *Chrysomela aeneicollis* (Coleoptera: Chrysomelidae). Evolution (N. Y). 46, 1097–1111.

Rank, N.E., Mardulyn, P., Heidl, S.J., Roberts, K.T., Zavala, N.A., Smiley, J.T., Dahlhoff, E.P., 2020. Mitonuclear mismatch alters performance and reproductive success in naturally introgressed populations of a montane leaf beetle. Evolution (N. Y). 74, 1724–1740. https://doi.org/10.1111/evo.13962

Reed, T.E., Jenouvrier, S., Visser, M.E., 2013. Phenological mismatch strongly affects individual fitness but not population demography in a woodland passerine 131–144. https://doi.org/10.1111/j.1365-2656.2012.02020.x

Rice, R., Bales, R.C., Painter, T.H., Dozier, J., 2011. Snow water equivalent along elevation gradients in the Merced and Tuolumne River basins of the Sierra Nevada. Water Resour. Res. 47, 1–11. https://doi.org/10.1029/2010WR009278

Riipi, M., Alatalo, R. V, Lindstro, L., 2001. Multiple benefts of gregariousness cover detectability costs in aposematic aggregations. Nature 413, 512–514. https://doi.org/https://doi.org/10.1038/35097061

Robbins, C.T., Lopez-Alfaro, C., Rode, K.D., Toien, Ø., Lynne Nelson, O., 2012. Hibernation and seasonal fasting in bears: The energetic costs and consequences for polar bears. J. Mammal. 93, 1493–1503. https://doi.org/10.1644/11-MAMM-A-406.1

Roberts, K.T., Rank, N.E., Dahlhoff, E.P., Stillman, J.H., Williams, C.M., 2021. Snow modulates winter energy use and cold exposure across an elevation gradient in a montane ectotherm 6103–6116. https://doi.org/10.1111/gcb.15912

Rukke, B.A., Sivasubramaniam, R., Birkemoe, T., Aak, A., 2018. Temperature stress deteriorates bed bug (*Cimex lectularius*) populations through decreased survival, fecundity and offspring success. PLoS One 13, 1–16. https://doi.org/10.1371/journal.pone.0193788

Sainmont, J., Andersen, K.H., Varpe, Ø., Visser, A.W., 2014. Capital versus income breeding in a seasonal environment. Am. Nat. 184, 466–476. https://doi.org/10.1086/677926

Scaccini, D., Vanishvili, L., Tirello, P., Walton, V.M., Duso, C., Pozzebon, A., 2020. Lethal and sub-lethal effects of low-temperature exposures on *Halyomorpha halys* (Hemiptera: Pentatomidae) adults before and after overwintering. Sci. Rep. 10, 1–9. https://doi.org/10.1038/s41598-020-72120-5

Schoombie, R.E., Boardman, L., Groenewald, B., Terblanche, J.S., Glazier, D.S., Van Daalen, C.E., Clusella-Trullas, S., 2013. High metabolic and water-loss rates in caterpillar aggregations: evidence against the resource-conservation hypothesis. J. Exp. Biol. 216, 4321–4325. https://doi.org/10.1242/jeb.095554

Shik, J.Z., Arnan, X., Oms, C.S., Cerdá, X., Boulay, R., 2019. Evidence for locally adaptive metabolic rates among ant populations along an elevational gradient. J. Anim. Ecol. 88, 1240–1249. https://doi.org/10.1111/1365-2656.13007

Sillén-Tullberg, B., 1990. Do predators avoid groups of aposematic prey? An experimental test. Anim. Behav. 40, 856–860. https://doi.org/10.1016/S0003-3472(05)80986-8

Sillén-Tullberg, B., 1988. Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. Evolution (N. Y). 42, 293–305. https://doi.org/10.1111/j.1558-

5646.1988.tb04133.x

Sinclair, B.J., 2015. Linking energetics and overwintering in temperate insects. J. Therm. Biol. 54, 5–11. https://doi.org/10.1016/j.jtherbio.2014.07.007

Sinclair, B.J., Addo-Bediako, A., Chown, S.L., 2003. Climatic variability and the evolution of insect freeze tolerance. Biol. Rev. Camb. Philos. Soc. 78, 181–195. https://doi.org/10.1017/S1464793102006024

Sinclair, B.J., Marshall, K.E., 2018. The many roles of fats in overwintering insects. J. Exp. Biol. 121. https://doi.org/10.1242/jeb.161836

Smiley, J.T., Rank, N.E., 1986. Predator protection versus rapid growth in a montane leaf beetle. Oecologia 106–112.

Somero, G.N., Lockwood, B.L., Tomanek, L., 2017. Biochemical Adaptation : Temperature. pp. Chapter 3.

Stamp, N.E., Bowers, M.D., 1988. Direct and indirect effects of predatory wasps (*Polistes sp.*: Vespidae) on gregarious caterpillars (*Hemileuca lucina*: Saturniidae). Oecologia 75, 619–624. https://doi.org/10.1007/BF00776428

Stephens, P.A., Houston, A.I., Harding, K.C., Boyd, I.L., McNamara, J.M., 2014. Capital and income breeding: the role of food supply. Ecology 95, 882–896. https://doi.org/10.1890/13-1434.1

Štětina, T., Hůla, P., Moos, M., Šimek, P., Šmilauer, P., Koštál, V., 2018. Recovery from supercooling, freezing, and cryopreservation stress in larvae of the drosophilid fly, *Chymomyza costata*. Sci. Rep. 8, 1–13. https://doi.org/10.1038/s41598-018-22757-0

Storey, J.M., Storey, K.B., 1983. Regulation of cryoprotectant metabolism in the overwintering gall fly larva, *Eurosta solidaginis*: Temperature control of glycerol and sorbitol levels. J. Comp. Physiol. B 149, 495–502. https://doi.org/10.1007/BF00690008

Su, Y.-L., Lu, Z.-Z., Song, J., Miao, W., 2007. Effect of overwintering aggregation on energy metabolism in the firebug, *Pyrrhocoris apterus* (Heteroptera : Pyrrhocoridae). Acta Entomol. Sin. 50, 1300–1303.

Susset, E.C., Hemptinne, J.L., Danchin, E., Magro, A., 2018. Overwintering aggregations are part of *Hippodamia undecimnotata*'s (Coleoptera: Coccinellidae) mating system. PLoS One 13. https://doi.org/10.1371/journal.pone.0197108

Takegaki, T., Takeshita, F., 2020. Winter mortality of young mudskipper fish: Effects of size, temperature and energy depletion. J. Exp. Mar. Bio. Ecol. 530–531. https://doi.org/10.1016/j.jembe.2020.151436

Tanaka, S., Wolda, H., Denlinger, D.L., 1988. Group size affects the metabolic rate of a tropical beetle. Physiol. Entomol. 13, 239–241. https://doi.org/10.1111/j.1365-3032.1988.tb00928.x

Taulavuori, K., Bauer, E., Taulavuori, E., 2011. Overwintering stress of *Vaccinium vitisidaea* in the absence of snow cover. Environ. Exp. Bot. 72, 397–403. https://doi.org/10.1016/j.envexpbot.2011.02.009

Teets, N.M., Denlinger, D.L., 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiol. Entomol. 38, 105–116. https://doi.org/10.1111/phen.12019

Tojo, S., Nagase, Y., Filippi, L., 2005. Reduction of respiration rates by forming aggregations in diapausing adults of the shield bug, *Parastrachia japonensis*. J. Insect Physiol. 51, 1075–1082. https://doi.org/10.1016/j.jinsphys.2005.05.006

Toxopeus, J., Sinclair, B.J., 2018. Mechanisms underlying insect freeze tolerance. Biol. Rev. 93, 1891–1914. https://doi.org/10.1111/brv.12425

Treanore, E., Amsalem, E., 2020. The effect of intrinsic physiological traits on diapause survival and their underlying mechanisms in an annual bee species *Bombus impatiens*. Conserv. Physiol. 8, 1–12. https://doi.org/10.1093/conphys/coaa103

Turnock, W.J., Wise, I.L., 2004. Density and survival of lady beetles (Coccinellidae) in overwintering sites in Manitoba. Can. Field-Naturalist 118, 309–317. https://doi.org/10.22621/cfn.v118i3.10

van der Wiel, K., Bintanja, R., 2021. Contribution of climatic changes in mean and variability to monthly temperature and precipitation extremes. Commun. Earth Environ. 2, 1–11. https://doi.org/10.1038/s43247-020-00077-4

van Noordwijk, A.J., de Jong, G., 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Am. Nat. 128, 137–142. https://doi.org/10.1086/284547

Varpe, Ø., 2017. Life history adaptations to seasonality. Integr. Comp. Biol. 57, 943–960. https://doi.org/10.1093/icb/icx123

Varpe, Ø., Jørgensen, C., Tarling, G.A., Fiksen, Ø., 2009. The adaptive value of energy storage and capital breeding in seasonal environments. Oikos 118, 363–370. https://doi.org/10.1111/j.1600-0706.2008.17036.x

Varpe, Ø., Jørgensen, C., Tarling, G.A., Fiksen, Ø., 2007. Early is better: seasonal egg fitness and timing of reproduction in a zooplankton life-history model. Oikos 116, 1331–1342. https://doi.org/10.1111/j.2007.0030-1299.15893.x

Vázquez, D.P., Gianoli, E., Morris, W.F., Bozinovic, F., 2015. Ecological and evolutionary impacts of changing climatic variability. Biol. Rev. 92, 22–42. https://doi.org/10.1111/brv.12216

Vergni, L., Todisco, F., 2011. Spatio-temporal variability of precipitation, temperature and agricultural drought indices in Central Italy. Agric. For. Meteorol. 151, 301–313. https://doi.org/10.1016/j.agrformet.2010.11.005

Vesterlund, S.R., Lilley, T.M., van Ooik, T., Sorvari, J., 2014. The effect of overwintering temperature on the body energy reserves and phenoloxidase activity of bumblebee *Bombus lucorum* queens. Insectes Soc. 61, 265–272. https://doi.org/10.1007/s00040-014-0351-9

Visser, B., Williams, C.M., Hahn, D.A., Short, C.A., López-Martínez, G., 2018. Hormetic benefits of prior anoxia exposure in buffering anoxia stress in a soil-pupating insect. J. Exp. Biol. 221. https://doi.org/10.1242/jeb.167825

Visser, M.E., Gienapp, P., 2019. Evolutionary and demographic consequences of phenological mismatches. Nat. Ecol. Evol. 3, 879–885. https://doi.org/10.1038/s41559-019-0880-8

Walter, J., Jentsch, A., Beierkuhnlein, C., Kreyling, J., 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. Environ. Exp. Bot. 94, 3–8.

https://doi.org/10.1016/j.envexpbot.2012.02.009

Watanabe, M., 2002. Cold tolerance and myo-inositol accumulation in overwintering adults of a lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). Eur. J. Entomol. 99, 5–9. https://doi.org/10.14411/eje.2002.002

Waters, J.S., Holbrook, C.T., Fewell, J.H., Harrison, J.F., 2010. Allometric scaling of metabolism, growth, and activity in whole colonies of the seed-harvester ant *Pogonomyrmex californicus*. Am. Nat. 176, 501–510. https://doi.org/10.1086/656266

Waters, J.S., Ochs, A., Fewell, J.H., Harrison, J.F., 2017. Differentiating causality and correlation in allometric scaling: Ant colony size drives metabolic hypometry. Proc. R. Soc. B Biol. Sci. 284. https://doi.org/10.1098/rspb.2016.2582

West, G.B., Brown, J.H., Enquist, B.J., 1997. A general model for the origin of allometric scaling laws in biology. Science (80-.). 276, 122–126. https://doi.org/10.1126/science.276.5309.122

Wheeler, C.A., Cardé, R.T., 2014. Following in their footprints: cuticular hydrocarbons as overwintering aggregation site markers in *Hippodamia convergens*. J. Chem. Ecol. 40, 418–428. https://doi.org/10.1007/s10886-014-0409-1

Williams, C.M., Buckley, L.B., Sheldon, K.S., Vickers, M., Pörtner, H.O., Dowd, W.W., Gunderson, A.R., Marshall, K.E., Stillman, J.H., 2016a. Biological impacts of thermal extremes: mechanisms and costs of functional responses matter. Integr. Comp. Biol. 56, 73–84. https://doi.org/10.1093/icb/icw013

Williams, C.M., Chick, W.D., Sinclair, B.J., 2015a. A cross-seasonal perspective on local adaptation: Metabolic plasticity mediates responses to winter in a thermal-generalist moth. Funct. Ecol. 29, 549–561. https://doi.org/10.1111/1365-2435.12360

Williams, C.M., Henry, H.A.L., Sinclair, B.J., 2015b. Cold truths: How winter drives responses of terrestrial organisms to climate change. Biol. Rev. 90, 214–235. https://doi.org/10.1111/brv.12105

Williams, C.M., Marshall, K.E., MacMillan, H.A., Dzurisin, J.D.K., Hellmann, J.J., Sinclair, B.J., 2012. Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. PLoS One 7. https://doi.org/10.1371/journal.pone.0034470

Williams, C.M., McCue, M.D., Sunny, N.E., Szejner-Sigal, A., Morgan, T.J., Allison, D.B., Hahn, D.A., 2016b. Cold adaptation increases rates of nutrient flow and metabolic plasticity during cold exposure in *Drosophila melanogaster*. Proc. R. Soc. B Biol. Sci. 283. https://doi.org/10.1098/rspb.2016.1317

Williams, C.M., Ragland, G.J., Betini, G., Buckley, L.B., Cheviron, Z.A., Donohue, K., Hereford, J., Humphries, M.M., Lisovski, S., Marshall, K.E., Schmidt, P.S., Sheldon, K.S., Varpe, Ø., Visser, M.E., 2017. Understanding evolutionary impacts of seasonality: An introduction to the symposium. Integr. Comp. Biol. 57, 921–933. https://doi.org/10.1093/icb/icx122

Williams, J.B., Ruehl, N.C., Lee, R.E., 2004. Partial link between the seasonal acquisition of cold-tolerance and desiccation resistance in the goldenrod gall fly *Eurosta solidaginis*

(Diptera: Tephritidae). J. Exp. Biol. 207, 4407–4414. https://doi.org/10.1242/jeb.01320

Wilsterman, K., Ballinger, M.A., Williams, C.M., 2021. old A unifying, eco-physiological framework for animal dormancy. Funct. Ecol. 35, 11–31. https://doi.org/10.1111/1365-2435.13718

Yoder, J.A., Denlinger, D.L., Wolda, H., 1992. Aggregation promotes water conservation during diapause in the tropical fungus beetle, *Stenotarsus rotundus*. Entomol. Exp. Appl. 63, 203–205. https://doi.org/10.1111/j.1570-7458.1992.tb01574.x

Yoder, J.A., Smith, B.E., 1997. Enhanced water conservation in clusters of convergent lady beetles, *Hippodamia convergens.* Entomol. Exp. Appl. 85, 87–89. https://doi.org/10.1046/j.1570-7458.1997.00237.x

Zera, A.J., Harshman, L.G., 2001. The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. 95–126.

Zhang, X., Hogg, W.D., Mekis, É., 2001. Spatial and temporal characteristics of heavy precipitation events over Canada. J. Clim. 14, 1923–1936. https://doi.org/10.1175/1520-0442(2001)014<1923:SATCOH>2.0.CO;2

Zhu, L., Ives, A.R., Zhang, C., Guo, Y., Radeloff, V.C., 2019. Climate change causes functionally colder winters for snow cover-dependent organisms. Nat. Clim. Chang. 9, 886–893. https://doi.org/10.1038/s41558-019-0588-4

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

# Appendix 1: Supplementary information for Chapter 1
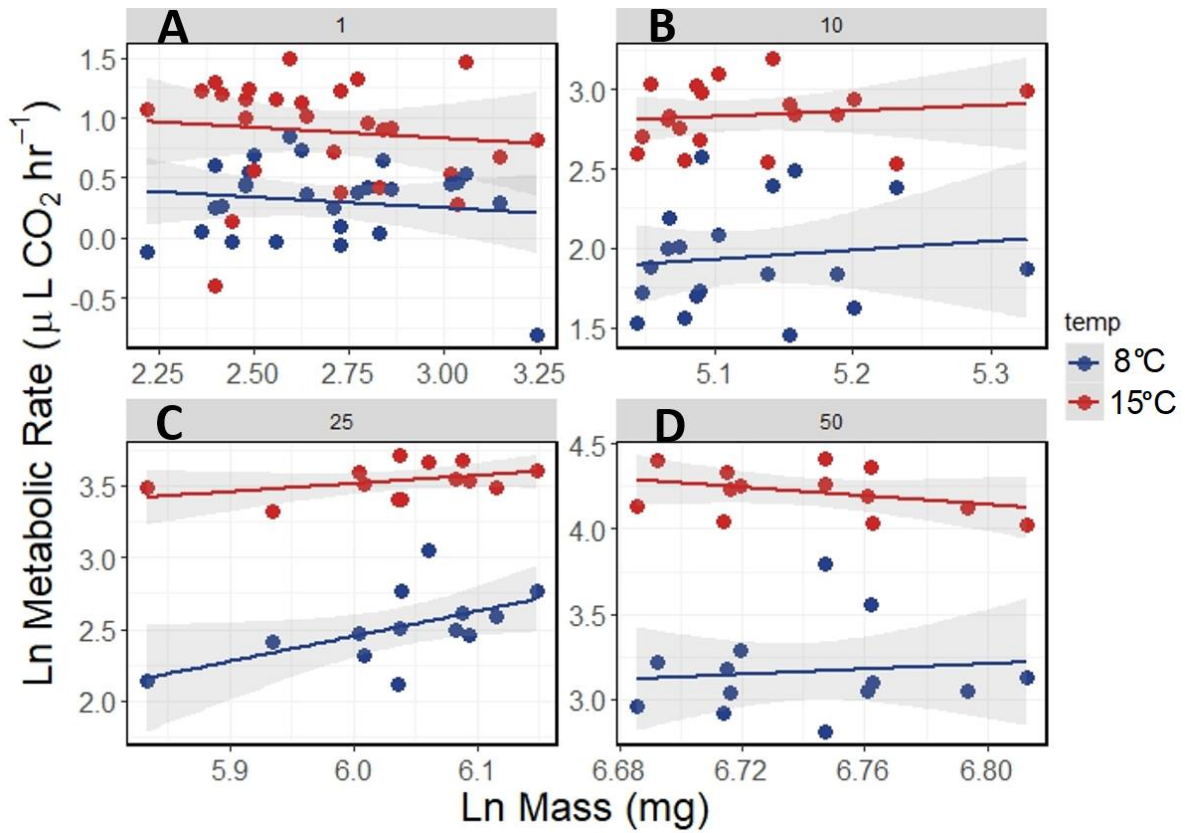
Fig. S1.1. Allometric scaling of metabolic rates and mass within each aggregation size (A-D). Data plotted on natural log axis, with group metabolic rates at 15°C (red), and 8°C (blue). Metabolic rates do not scale with mass within each group size ($F_{15,128} = 164.6$, $P > 0.05$).
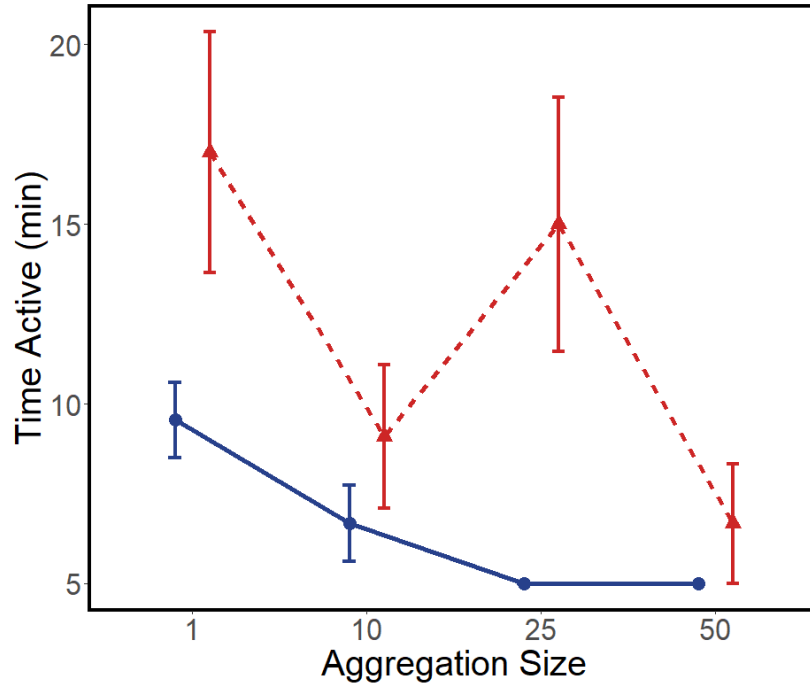
Fig. S1.2 Mean active time per hour from the subset of marked beetles (*Hippodamia convergens*) that were active at any point at each temperature (8°C, blue solid line and 15°C red dotted line). Activity time was not significantly different across aggregation sizes, but it was higher at 15°C compared to 8°C (P = 0.005).

Appendix 2: Supplementary information for Chapter 2
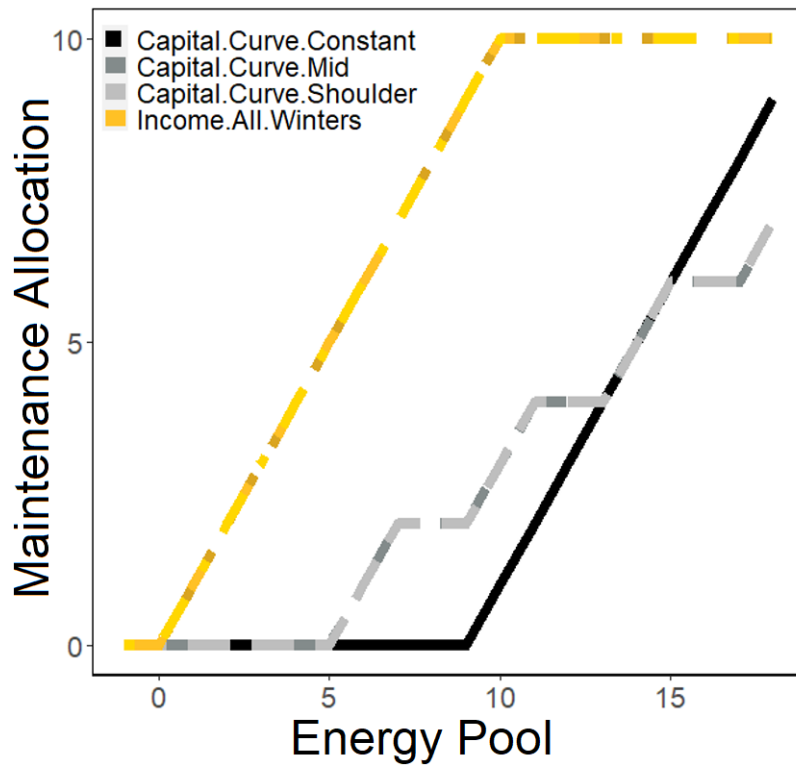
Fig. S2.1. Total energy invested into cold tolerance with increasing starting energy pools for Income (yellow) and Capital.logistic (black) under varying winter cold scenarios: constant p_c (dark shade), mid winter cold (light shade), and cold winter shoulders (light shade).
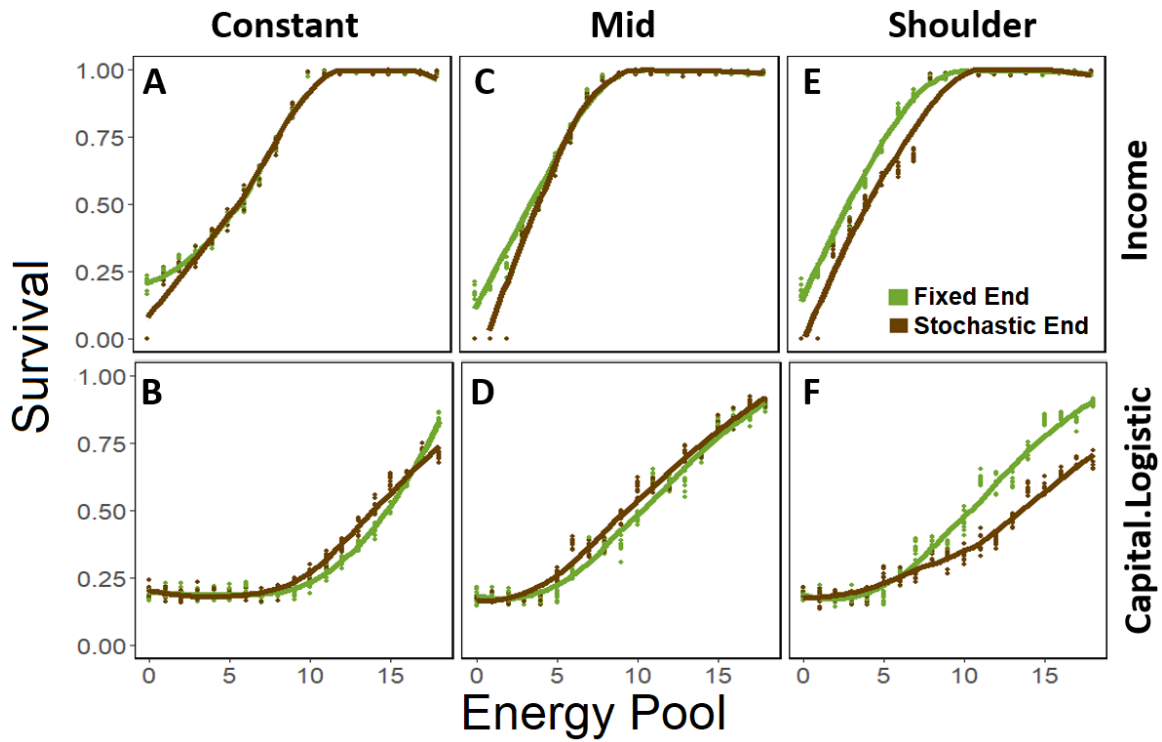
Fig. S2.2. Survival at week 10 (full winter) across starting energy pools for allocation under different winter scenarios, constant probability of cold (left), mid-winter cold (middle), and cold shoulders (right) for income breeders (top) and capital breeders (bottom), under winters with stochastic end (green) or fixed length (brown).
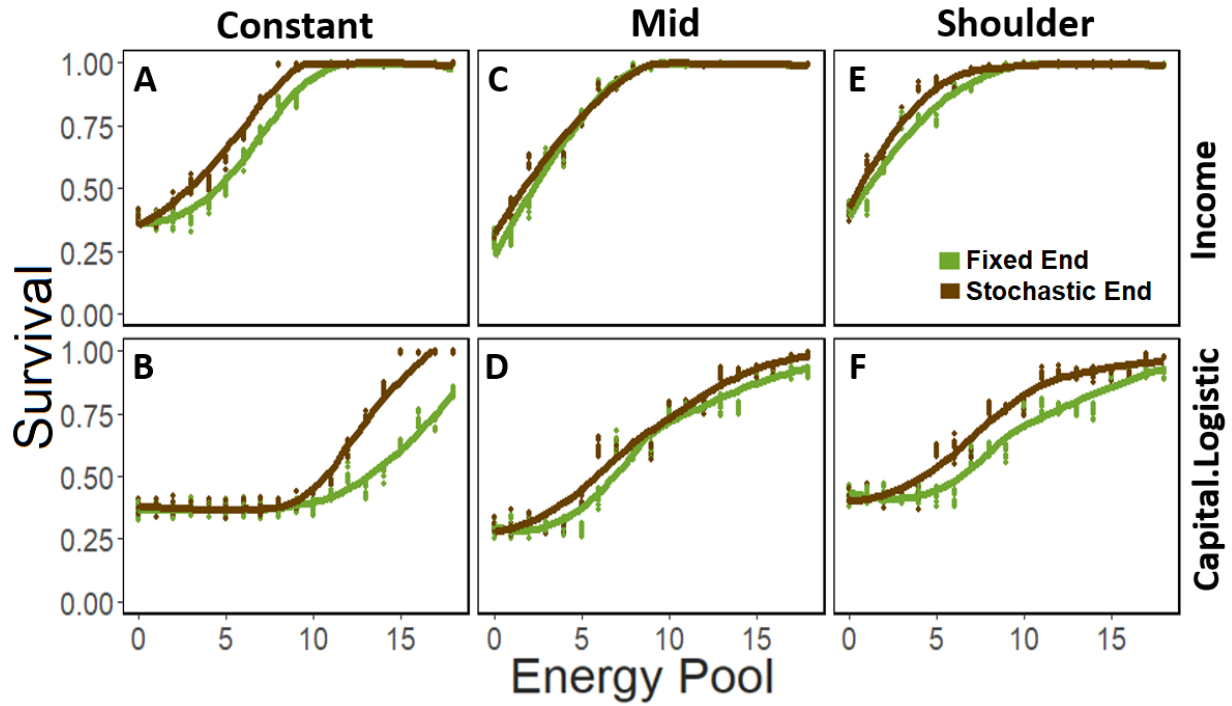
Fig. S2.3. Survival at week 7 (short winter) across starting energy pools for allocation under different winter scenarios, constant probability of cold (left), mid-winter cold (middle), and cold shoulders (right) for income breeders (top) and capital breeders (bottom), under winters with stochastic end (green) or fixed length (brown).

Appendix 3: Supplementary information for Chapter 3

Cold risk functions obtained using dcode.fr based on known parameters of whole-winter risk of at least one lethal event (42% for harsh winters and 12% for mild winters) (Roberts et al., 2021), and fitted into either mid-winter peak risk, or seasonal shoulder peak risk.
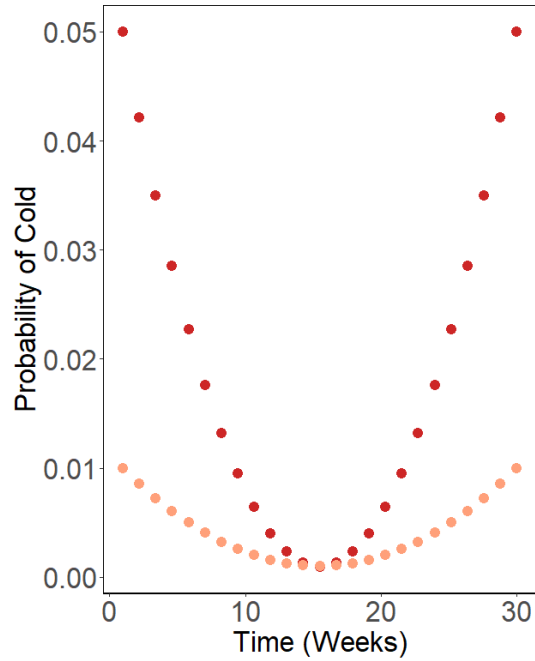
Mid-winter high and low risk functions:

$$p_{c.high}(t) = -1.38 \cdot 10^{-4} t^2 + 4.28 \cdot 10^{-3} t - 3.14 \cdot 10^{-3}$$
$$p_{c.low}(t) = -4.28 \cdot 10^{-5} t^2 + 1.32 \cdot 10^{-3} t - 2.85 \cdot 10^{-4}$$

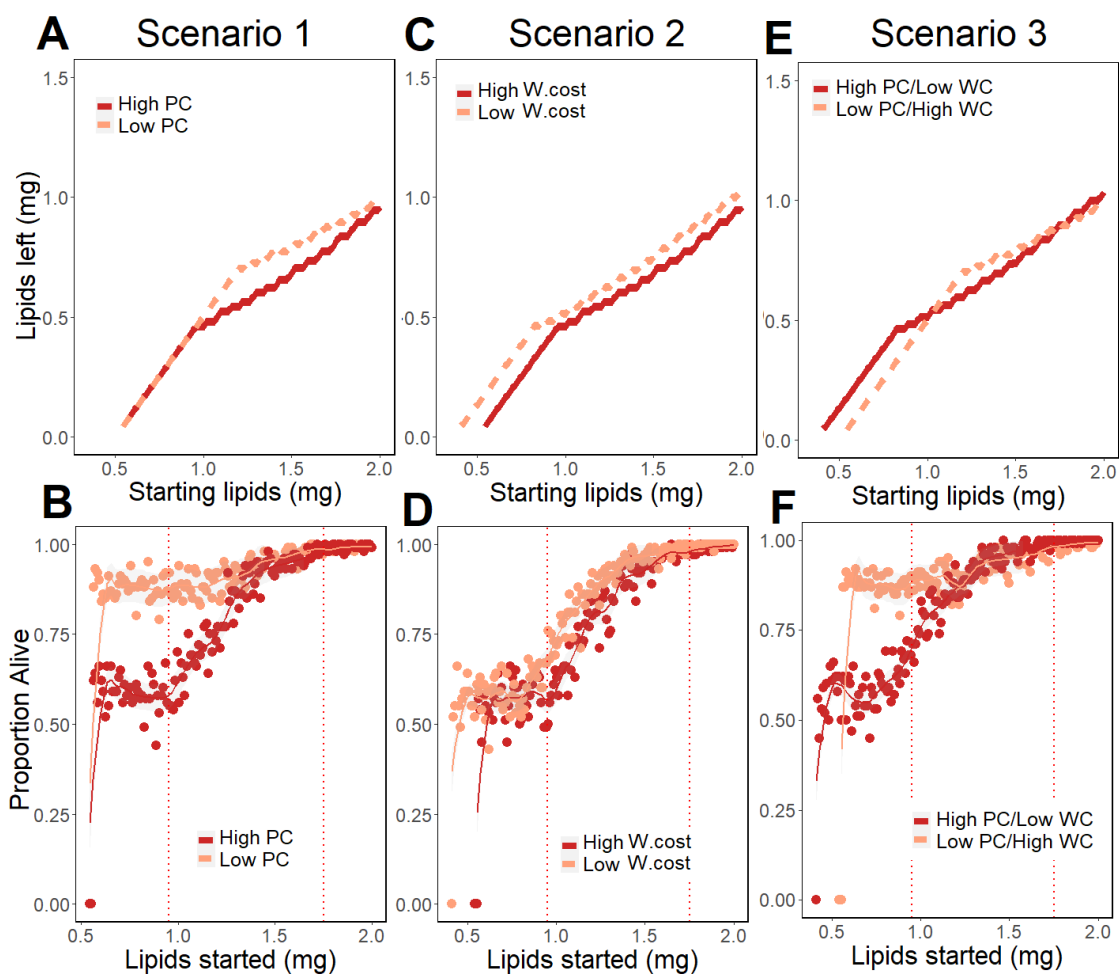Shoulder high and low risk functions:

$$p_{c.high}(t) = 2.33 \cdot 10^{-4} t^2 - 7.23 \cdot 10^{-3} t + 0.057$$
$$p_{c.low}(t) = 4.28 \cdot 10^{-5} t^2 - 1.32 \cdot 10^{-3} t + 0.0112$$

where t is time.

Fig. S3.1. Cold risk for harsh (dark red) or mild (pink) winters with peak cold risk during seasonal shoulders. Whole winter probability of at least one lethal cold event is 42% for harsh winters and 12% for mild winters (Roberts et al. 2021).

Fig. S3.2. Lipids left and survival across starting winter lipids when changing probability of lethal cold PC (Scenario 1, A,B), baseline winter costs WC (Scenario 2, C,D), or both (Scenario 3, E,F) for winters with a shoulder-winter peak in cold risk. Data from simulated populations of 100 individuals for each possible starting lipid, and allocating optimally into cold tolerance for their specific winter conditions.
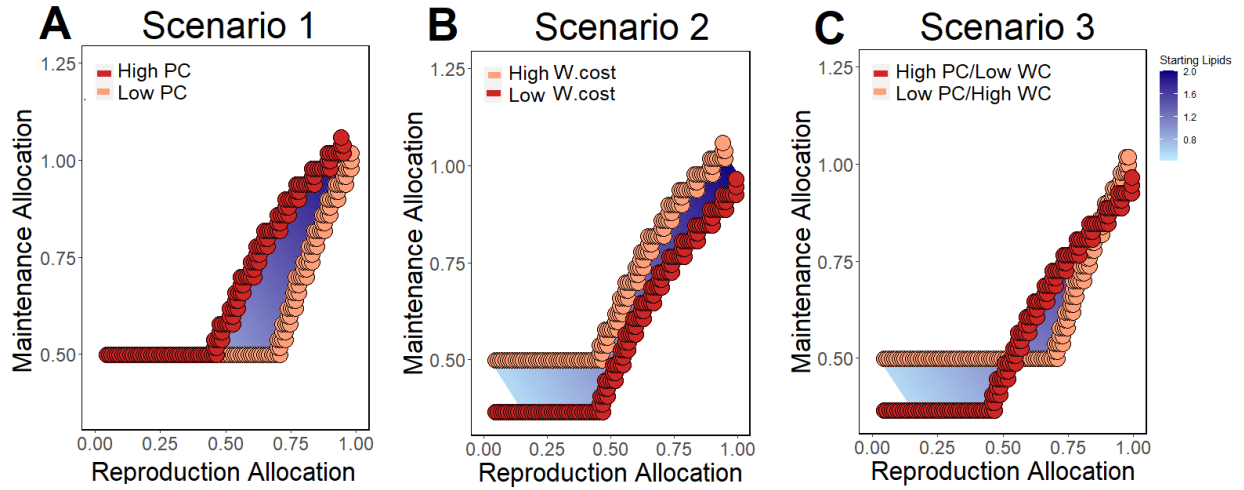
Fig. S3.3. Optimal energy allocation between maintenance and future reproduction across all possible starting winter lipids when changing probability of lethal cold (A), baseline winter costs (B) or both (C) for winters with a shoulder-winter peak in cold risk. Data from simulated populations of 100 individuals for each starting lipid. Maintenance allocation is the sum of energy invested into cold tolerance and winter energy cost, and reproduction allocation is the energy left at the end of winter. Individuals allocated optimally for their specific winter scenarios. Blue gradient shows each possible starting reserves.