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COMMENTARY

Eco-immunology in the cold: the role of immunity in shaping the overwintering survival of ectotherms

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ABSTRACT

The effect of temperature on physiology mediates many of the challenges that ectotherms face under climate change. Ectotherm immunity is thermally sensitive and, as such, environmental change is likely to have complex effects on survival, disease resistance and transmission. The effects of temperature on immunity will be particularly profound in winter because cold and overwintering are important triggers and regulators of ectotherm immune activity. Low temperatures can both suppress and activate immune responses independent of parasites, which suggests that temperature not only affects the rate of immune responses but also provides information that allows overwintering ectotherms to balance investment in immunity and other physiological processes that underlie winter survival. Changing winter temperatures are now shifting ectotherm immunity, as well as the demand for energy conservation and protection against parasites. Whether an ectotherm can survive the winter will thus depend on whether new immune phenotypes will shift to match the conditions of the new environment, or leave ectotherms vulnerable to infection or energy depletion. Here, we synthesise patterns of overwintering immunity in ectotherms and examine how new winter conditions might affect ectotherm immunity. We then explore whether it is possible to predict the effects of changing winter conditions on ectotherm vulnerability to the direct and indirect effects of parasites.

KEY WORDS: Winter, Climate change, Trade-offs, Immune system, Host–parasite interaction

Introduction

Ectothermic animals (see Glossary) face many challenges under climate change, including shifts in host–parasite interactions wrought by changes in environmental temperature (Rohr and Palmer, 2013). The relatively new field of eco-immunology (Sheldon and Verhulst, 1996) focuses on understanding immune function in an evolutionary, ecological and physiological context, and subsequently predicting population-level impacts of environmental change on disease resistance and transmission. In the context of changing temperatures, the goals of eco-immunologists and thermal biologists are necessarily intertwined, and similarly challenged by the complexity of predicting fitness from measures of thermal performance (Sinclair et al., 2016). Thus, the overlap between the sub-disciplines gets to the heart of our uncertainty about predicting the effects of climate change on ectotherms.

In temperate, polar and alpine regions, ectotherms spend the majority of their lives preparing for winter, or overwintering (Williams et al., 2015). Overwintering ectotherms might experience prolonged cold exposure, desiccation stress, nutrient stress and, potentially, hypoxia. Low temperatures slow rates of physiological activity, and many ectotherms enter states of dormancy, such as diapause, brumation or quiescence, to conserve energy (Williams et al., 2015). Furthermore, ectotherms may also be exposed to cold-active parasites that specialise in attacking ectotherms at low temperatures. For example, the fish parasite *Flavobacterium psychrophilum* kills a range of freshwater fishes below 10°C, with resulting economic losses for aquaculture (Starliper, 2011). Similarly, psychrophilic fungi (see Glossary; e.g. species of *Metarhizium* and *Beauveria*) are found in overwintering microhabitats and are associated with mortality of various species of insects in the spring (Bidochka et al., 1998). Thus, the effects of temperature on immunity and the ability to regulate immune activity to balance the response to parasites with multiple other physiological demands are likely to play an important role in the overwintering survival and subsequent reproductive fitness of ectotherms. However, we are only beginning to explore and understand this relationship between overwintering and immunity, and currently lack a framework within which to predict the impacts of immunity on ectotherm survival in the cold.

Here, we explore the effects of cold and overwintering on the thermal biology and activity of the immune system of terrestrial and aquatic invertebrate and vertebrate ectotherms. We focus on the integration of immunity with other physiological functions, and highlight that immune phenotypes during overwintering are likely to be representative of balancing trade-offs between energy conservation and the response to cold with the response to parasites. Furthermore, we highlight potential scenarios under climate change that will likely modify immune phenotypes and host–parasite interactions. In doing so, we identify three directions that we suggest will be essential for further understanding how the effects of temperature and season on immunity will impact the survival of ectotherms in a changing climate.

Thermal dependence of ectotherm immunity

In ectotherms, the rate of physiological activity is directly dependent on ambient temperature, in a non-linear fashion. Classically, the impact of temperature on physiological activity is described with a thermal performance curve (Fig. 1), where activity increases with increasing temperature in a curvilinear fashion towards an optimum, after which performance rapidly declines at higher temperatures (Sinclair et al., 2016). The ectotherm immune system relies on the temperature-dependent activity of enzymes and cells, and immune performance is therefore also constrained by temperature. Although the number of studies on the thermal performance of the ectotherm immune system is limited, immune activity generally conforms to a classic thermal performance curve and operates over a wide range of temperatures (Butler et al., 2013; Ferguson et al., 2016; Graham et al., 2017; Murdock et al., 2012; Zimmerman et al., 2017).

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Glossary

Acquired defence
Immune responses against a specific pathogen or parasite that the host has previously encountered. These defences are specialised and long term.

Cortisol
Steroid hormones released by vertebrates in response to stress. The specific molecule is cortisol in mammals and fish, and corticosterone in amphibians and reptiles. CORT is often quantified as a proxy for stress.

Ectotherm
Organism in which internal body temperature approximates the ambient temperature.

Immunocompetence
The ability of an organism to mount an appropriate response to a parasite.

Innate defence
Generalised immune response that provides immediate defence against parasites and pathogens and does not depend on the host's prior experience.

Melanisation response
Generalised immune defence by invertebrates that involves surrounding an invading parasite with the pigment melanin.

Phagocytosis
The engulfment and destruction of an invading parasite by an immune cell.

Phenology
Cyclic or seasonal variation in life-cycle events.

Pleiotropic
Multiple, seemingly unrelated phenotypic effects resulting from the expression of a single gene.

Psychrophilic
Microbes that are capable of growth and reproduction at low temperatures.

However, thermal performance differs among species, and may be adapted to particular climates. For example, *Drosophila melanogaster* from tropical Africa have weaker immune activity at low temperatures than the (presumably more cold-adapted) *D. melanogaster* from temperate North America (Lazzaro et al., 2008). This suggests that the thermal performance of the immune system is optimised to an animal's thermal environment, and there is the potential for immunity to be thermally co-evolved with local parasites.

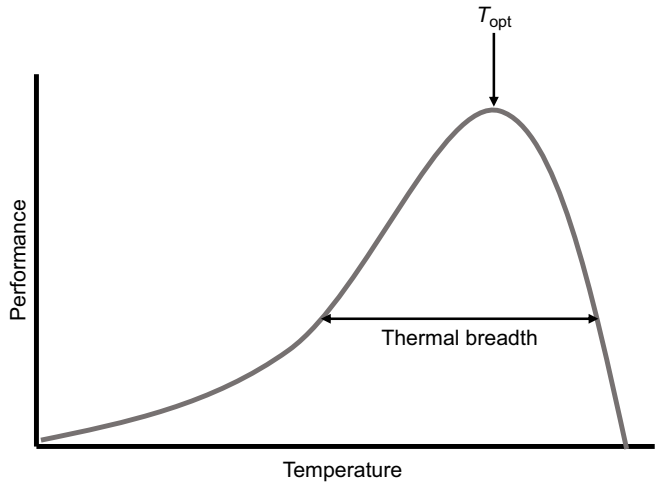

Fig. 1. Representative thermal performance curve. Performance increases non-linearly with increasing temperature, reaching an optimum (T_{opt}) and decreasing rapidly thereafter. Thermal breadth represents the range of temperatures at which performance reaches or exceeds a proportion of the optimum, and may be used to estimate the range of temperatures at which an organism can maintain a certain level of physiological performance.

The breadth of thermal performance and the thermal optimum of activity can also differ among different measures of immune activity, even when both are temperature dependent. In insects, the optimal temperatures of cell-mediated responses to parasites are often lower than the optimal temperatures of the biochemical reactions underlying these responses. For example, phagocytosis (see Glossary) and the broad-spectrum melanisation response (see Glossary) in mosquitoes (*Anopheles stephensi*) and crickets (*Gryllus veletis*) are optimal at 18°C (Ferguson et al., 2016; Murdock et al., 2012). Conversely, expression of the gene encoding nitric oxide synthase (responsible for producing the immune signalling molecule nitric oxide) and enzymatic activity underlying melanisation peak at 30°C in *A. stephensi* (Murdock et al., 2012). This suggests that different components of the immune system can be adapted to function at different temperatures to combat parasites with different thermal performance, or alternatively, to account for restructuring of the immune system under different thermal environments (which we explore further below). Importantly, these differences in thermal performance among immune measures re-emphasise the need to measure the thermal activity of multiple components of the immune system to understand how immunity behaves under different thermal environments (Adamo, 2004).

Exposure to low-temperature stress can also modify immune activity in a variety of ways (Chang et al., 2009; Chen et al., 2002; Fan et al., 2013; Sinclair et al., 2013). For example, repeated stressful cold exposure leads to increased survival of fungal infection in the fly *D. melanogaster* (Le Bourg et al., 2009) and the moth *Pyrrharctia isabella* (Marshall and Sinclair, 2011), possibly because cold-induced damage (including damage accrued during thawing and rewarming) triggers wounding responses that lead to secondary protection against parasites. By contrast, acute cold stress in tilapia (*Oreochromis aureus*) increases plasma cortisol levels (see Glossary) and correlates with decreased leukocyte phagocytic activity, suggesting that cold stress can be immunosuppressive (Chen et al., 2002). It remains unclear whether these effects of cold stress on immunity are by-products of damage and stress responses or adaptive cross-talk between stress responses and immune activity. Thus, it will be necessary to better understand the mechanisms underlying these connections to determine whether they have adaptive significance.

Immune activity is also plastic in the face of temperature changes, and acclimation/acclimatisation to low temperatures can modify winter immunity. Increases in immune activity are usually interpreted as prophylactic responses to parasite stress or tissue damage in the cold, or as compensatory responses to trade-offs between immune and other physiological activities (Sinclair et al., 2013). For example, in perch (*Perca fluviatilis*), acclimation to low temperatures shifts the phagocytic activity of immune cells towards increased activity at low temperatures (Marnila and Lilius, 2015). Similar acclimation responses also occur in immune cells of frogs (Marnila et al., 1995) and several other species of fish (summarised by Nikoskelainen et al., 2004). By contrast, decreases in activity might prevent or limit autoimmune damage during overwintering (Marnila and Lilius, 2015), or restructure immune investment in the face of constraints on resource and energy availability. For example, in *G. veletis*, cold acclimation increases cold tolerance but decreases low-temperature melanisation and humoral antibacterial activity, suggesting a trade-off between immunity and thermal tolerance (Ferguson et al., 2016), which may thus influence seasonal patterns of immune activity.

Seasonal patterns of immune activity associated with overwintering

Overwintering requires investment in protection against several stressors, including cold, and dormant ectotherms must conserve energy reserves (Williams et al., 2015); thus, animals may trade off immunity for energy savings that can be apportioned to cryoprotection. Winter is often considered immunosuppressive (Altizer et al., 2006), and immune activity is generally slower at low temperatures, as illustrated by classic thermal performance curves (Butler et al., 2013). However, although winter is associated with decreased diversity and density of parasites (e.g. infective stages of metazoan parasites in freshwater fish; Barber, 2012), low temperatures may also select for more-virulent parasites in the winter (DePaola et al., 2003) and the ability to invest more in immunity can increase overwintering survival (Krams et al., 2011). Thus, winter imposes a web of seasonal, integrated and often taxon-dependent pressures that shape investment in immunity and optimisation of survival against multiple stressors (Ferguson and Sinclair, 2017; Goessling et al., 2016; Kortet and Vainikka, 2008).

To balance trade-offs between immunity and multiple other physiological processes necessary for overwintering survival, ectotherms may reconfigure immune investment to yield distinct seasonal phenotypes of investment in different branches of immunity. For example, in many vertebrate ectotherms, innate defences (e.g. phagocytosis; see Glossary) are maintained or upregulated during winter, whereas acquired defences (e.g. antibody production; see Glossary) are suppressed, suggesting that costly activities are decreased in response to energetic or resource constraints (Abram et al., 2017; Goessling et al., 2016, 2017; Kortet and Vainikka, 2008; Le Morvan et al., 1998; Zimmerman et al., 2010). We do note, however, that few studies have compared the costs of innate and acquired defences during winter. Insects, which lack an adaptive immune response, have species-specific seasonal patterns of immune activity and disease susceptibility that likely also indicate immune reconfiguration during overwintering (Ferguson and Sinclair, 2017). These species-specific patterns suggest that seasonal reconfiguration is not only a product of expensive versus inexpensive responses but also a response to specific resource constraints and parasite pressures. Furthermore, the combinations of these stressors will vary depending on the physiology of the animal and both the abiotic and biotic environmental pressures of their overwintering microhabitats (Ferguson and Sinclair, 2017). To predict how winter will affect immunity in both vertebrate and invertebrate ectotherms, and the role of immune reconfiguration in overwintering survival, we clearly need to untangle the underlying reasons for immune reconfiguration in species where this has been observed, and the generalisability of these reasons among taxa.

Cold exposure may also prime an immune response to parasite stress in the spring. Increased temperatures in the spring often coincide with increased parasite pressure (Greenspan et al., 2017; Marcogliese, 2001), and the transition from cold to warm conditions may signal a prophylactic increase in immune activity. For example, frogs cooled from 26°C to 21°C are more susceptible to infection with the chytrid fungus *Batrachyium dendrobatidis*, than those that are warmed from 16°C to 21°C (Greenspan et al., 2017), indicating that increasing temperatures, specifically, can be immunostimulating. Fluctuating temperatures are also implicated in the stimulation of insect immune activity (Torson et al., 2015). In the context of climate change, it is unclear whether winters will remain immunostimulatory if mean temperatures increase above certain thresholds or whether seasonal patterns of immune activity are independent of absolute winter temperature.

Overwintering immunity is clearly more complex than just a wholesale suppression of activity, and seasonal immune phenotypes are likely to operate through a variety of mechanisms (Fig. 2). Because immunity changes during winter regardless of parasite exposure, we contend that cross-talk between responses to multiple overwintering stressors underlies programmed and prophylactic

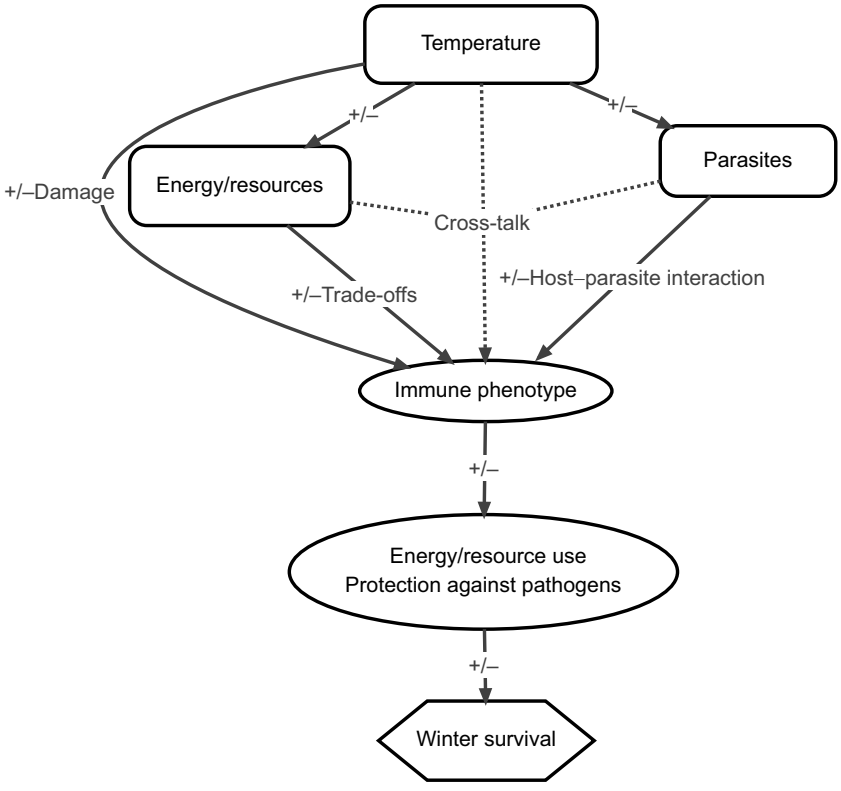

Fig. 2. The putative mechanisms underlying seasonal immune phenotypes and the interconnectedness of each with respect to changes in temperature. Dashed lines indicate prophylactic changes in immunity driven by trade-offs/energy constraints and parasites as selective pressures. Solid lines indicate direct effects of a variable on immunity. During overwintering, extreme temperatures could damage immune cells and tissues. Furthermore, energy/resource use and the presence of parasites during winter will also directly impact immune function. Overall, the resulting immune phenotype created through these mechanistic interactions will drive winter survival.

seasonal responses of the ectotherm immune system (Sinclair et al., 2013). Non-adaptive mechanisms will likely also contribute to overwintering immunocompetence (see Glossary), namely cold-induced damage to the immune system, by-products of stress responses or pleiotropic effects (see Glossary) of other seasonal preparations (Fedorka et al., 2013). Furthermore, infection itself can modify immune activity (e.g. parasite suppression of immune activity), and is subject to change under different thermal environments (Thomas and Blanford, 2003). However, although we hypothesise that these are the mechanisms underlying seasonal variation in immunity, their roles, and relative importance, in shaping immune phenotypes have yet to be fully explored.

Eco-immunology in changing winters

Winters are expected to become increasingly warm, variable and unpredictable as a consequence of climate change (Williams et al., 2015), which may modify the relative importance of the drivers of winter survival associated with immunity – namely, energy conservation and survival following infection (Fig. 2). Ectotherms are likely to experience increases in metabolic rate under higher or more variable thermal environments (Williams et al., 2015). Therefore, unless these conditions permit an increase in the ability to gather resources (unlikely for dormant ectotherms), winter climate change means that ectotherms will be under increased pressure to conserve their finite energy stores. Concurrently, infection and host–parasite interactions may either increase or decrease in intensity (Harvell et al., 2002). Higher temperatures are likely to permit increased growth of native and novel parasites that are suppressed by cold; conversely, cold-active parasites may no longer have the upper hand against their host under warmer or more variable conditions (Harvell et al., 2002; Williams et al., 2015). Therefore, overwintering success will depend on whether new winter conditions produce immune phenotypes that appropriately balance parasite defence with energy conservation.

Currently, we lack a framework to describe how changing winters will impact overwintering immunity. This is in part because experimental evidence suggests that responses will be species specific and highly dependent on the type of thermal environment that the animal experiences. Higher average temperatures could increase the rate of immune responses in

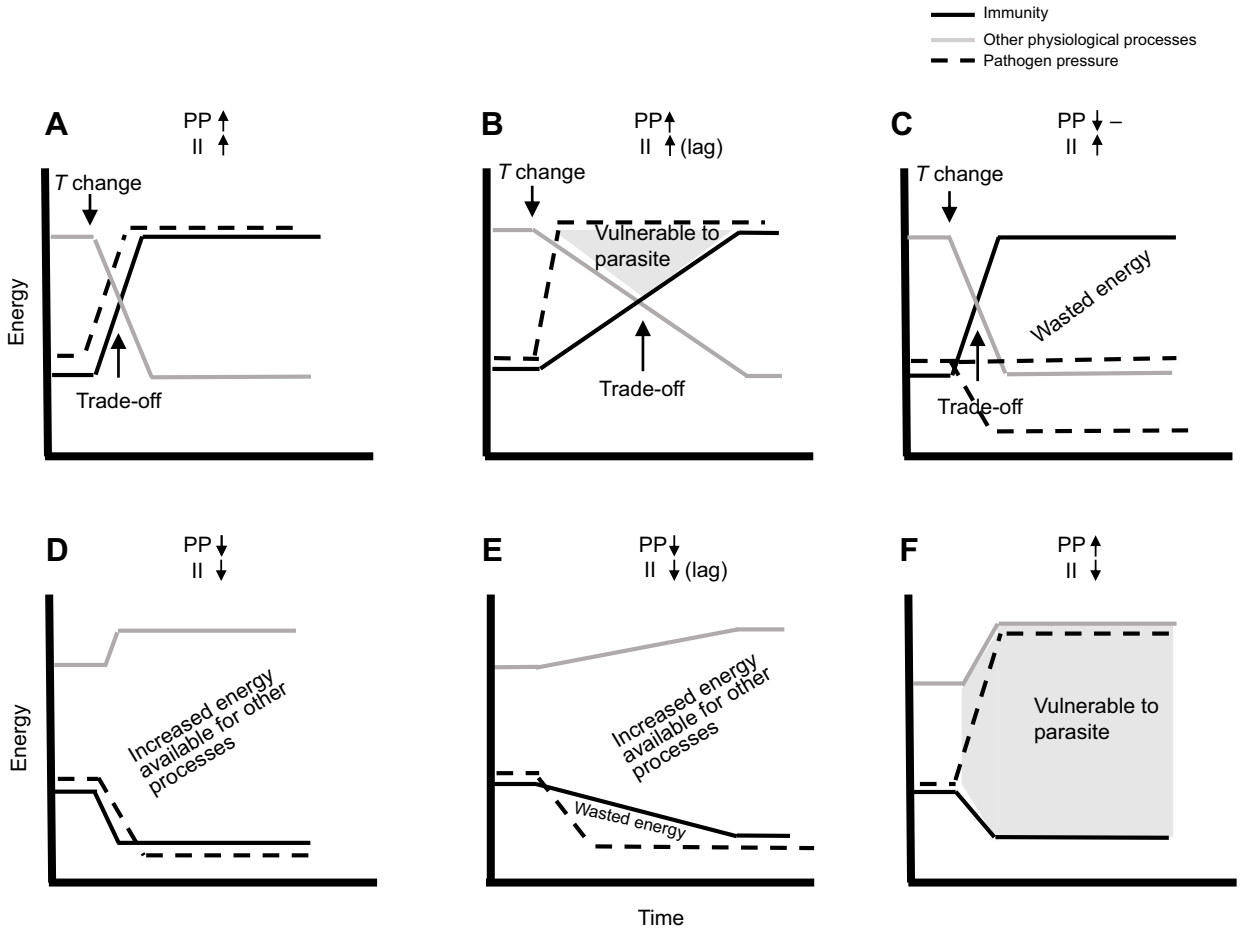

Fig. 3. Potential scenarios of changes in immune investment under temperature changes and the resultant trade-offs or vulnerability to parasites. Dashed lines represent potential increases or decreases in parasite pressure (either increase or decrease). *T*, temperature. (A) Immune investment (II) increases as parasite pressure (PP) increases, providing protection against infection but trading-off energy/resources with other physiological processes. (B) As in A, but with a lag between the increase in parasite pressure and immune investment, leaving a period of time in which ectotherms are still vulnerable to infection. (C) Parasite pressure decreases or remains unchanged but there is increased immune investment resulting from an unreliable cue in the environment, leading to wasted energy that cannot be used for other physiological processes. (D) Parasite pressure decreases and immunity is suppressed, providing energy savings with no trade-off for parasite protection. (E) As in D, but with a lag between the decrease in pathogen pressure and immune suppression (i.e. energy savings). (F) Parasite pressure increases but immunity is suppressed as a result of an unreliable cue in the environment, leaving the animal vulnerable to infection.

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some ectotherms (Martin et al., 2010; Sugahara and Eguchi, 2012), whereas variability in temperature may have more complex effects on immune activity (Colinet et al., 2015). For example, exposure to thermal variability during overwintering decreases immunocompetence in red-spotted newts (Raffel et al., 2006) and gopher tortoises (Goessling et al., 2017), but increases immunity and disease resistance in hellbender salamanders (Terrell et al., 2013) and insects such as *P. isabella* (Marshall and Sinclair, 2011). In yet other cases, seasonal immune phenotypes may be endogenously regulated and largely independent of temperature change (Gruber et al., 2014; Sandmeier et al., 2016). However, we have little knowledge of why variable overwintering temperatures provoke changes in immune activity, and consequently cannot yet predict the consequences of variable temperatures on ectotherm immunity and survival.

Each of the mechanisms underlying seasonal immunity is likely to contribute to changes in immune activity with climate change (Table 1). In particular, warmer and more variable temperatures may provide new signals about the environmental challenges that trigger physiological shifts to meet these demands (Colinet et al., 2015), thereby shaping new immune phenotypes. However, whether these signals are reliable and whether changes in immunity are adaptive to these new environments is unclear. Increases in immune investment may be beneficial if parasite pressures also increase (Table 1, Fig. 3). However, increased immune activity could also arise from 'miscues' in the environment of a nature similar to the deacclimation responses that inappropriately decrease cold tolerance in insects during warm spells in the winter, such that insects are left unprepared for re-entry into low temperatures (Sobek-Swant et al., 2011). If immunity increases without a mirrored increase in parasite pressure, it will serve only to increase energy and resource expenditure inappropriately, thereby compromising other physiological processes necessary for winter survival. Conversely, decreased or unchanged immune responses could leave ectotherms vulnerable to infection if parasite pressure rises (e.g. Rohr and Raffel, 2010), or instead protect energy savings and permit resources to be shunted to other physiological demands (Table 1, Fig. 3). Overall, our ability to predict overwintering survival will depend on our understanding of how each mechanism underlying seasonal immunity contributes to these changes.

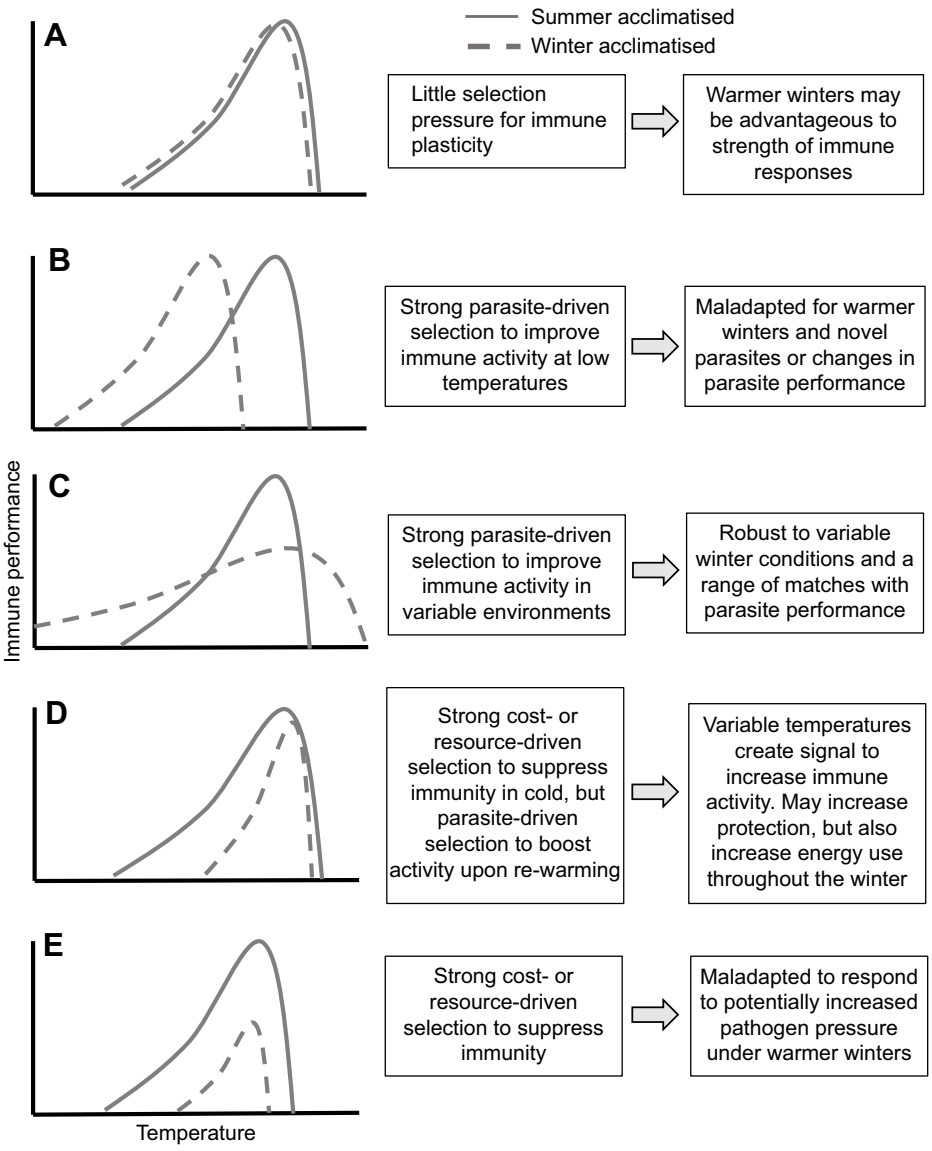

Fig. 4. Inferring adaptive significance and outcomes of climate change from thermal performance curves of immune activity. The thermal plasticity of immune activity compared among seasons can help us to generate hypotheses about the selective pressures underlying these changes and their adaptive significance. From here, we can create predictions about the impact of changing winters on protection against parasites or the energetic consequences of changes in immune activity. (A) A lack of thermal plasticity of immunity. (B) A shift in immune activity that favours performance at low temperatures. (C) Increased breadth of immune thermal performance. (D) Narrowing of the breadth of immune thermal performance. (E) Overall decrease or suppression of immune activity.

Table 1. Potential scenarios driving changes in immunity and success under warmer and more variable winters

Mechanism	Condition	Scenario	Outcome for immune system	Match to new winter demands
Damage	Warmer winters	Less exposure to damaging temperatures	No damage to immune system	+ for energy savings + for protection against parasites
	Variable winters	More exposure to damaging temperatures (e.g. repeated cold)	Increased damage to immune system	– for energy savings – for protection against parasites
		Increased opportunity to repair damage	Increased strength of immune system	– for energy savings + for protection against parasites
Trade-offs		Increases in metabolic rate and energy use	Potential for resources to be unavailable for immunity	– for protection against parasites
Cross-talk		New signals	No change if endogenous regulation	+/– for protection against parasites (see Fig. 2)
			Changes to configuration of and investment in immune activity	+/– for energy savings (see Fig. 2)
Host–parasite interactions		Increased if permitting more parasite growth	Potential for changes to immune phenotype if new parasites can suppress the immune system	+ for energy savings – for protection against parasites
		Decreased if cold-active parasites inhibited	Less immune activation if infection decreases	+ for energy savings

New directions in thermal eco-immunology

Thus far, the marriage of thermal biology and eco-immunology has characterised complex seasonal immune phenotypes and fluctuations in disease occurrence, and it will be appropriate to continue studies in this vein. However, we suggest a new focus on predicting how seasonal patterns of immunity will react to changing winters. We identify three directions that might propel the field towards these goals, detailed below.

Disentangling the mechanisms underlying immune phenotypes

The direction of change in immunity under climate change will be determined by the outcome of the processes underlying seasonal immune variation (Table 1, Fig. 3). Thus, before we can begin to predict how changing winters will modify immune phenotypes, we must understand the contributions of each putative mechanism (e.g. cross-talk versus damage) to seasonal immunity, and whether these mechanisms will maintain or change seasonal variation in immunity under changing winters. We expect that the relative contribution of these mechanisms to overwintering immunity will be species-specific; thus, mechanistic approaches will be most informative when used in a comparative format to determine the underlying traits (Williams et al., 2008). In this way, we may be able to characterise immune phenotypes through taxonomic, ecological or physiological diversity, thereby decreasing experimental workload and increasing predictive power.

Generating predictive power from thermal performance curves of immune activity

Using thermal performance curves (Sinclair et al., 2016) to explore seasonal changes in immune plasticity will help to generate hypotheses about the selective pressures that underlie this plasticity, and what the consequences of this adaptation will be under new environments (Fig. 4). For example, if immune plasticity is characterised by a shift towards increased immune activity at low temperatures, then this suggests that parasite activity at low temperatures is likely to have selected for this response (Fig. 4B). Consequently, the animal may be maladapted to warmer winters and parasites that favour warmer temperatures. The plasticity of thermal performance of various components of the immune system may be explored via acclimation conditions that mimic changing winter conditions (e.g. warmer, more variable) to determine the extent to which plasticity of immune phenotypes will make them robust to changing winters.

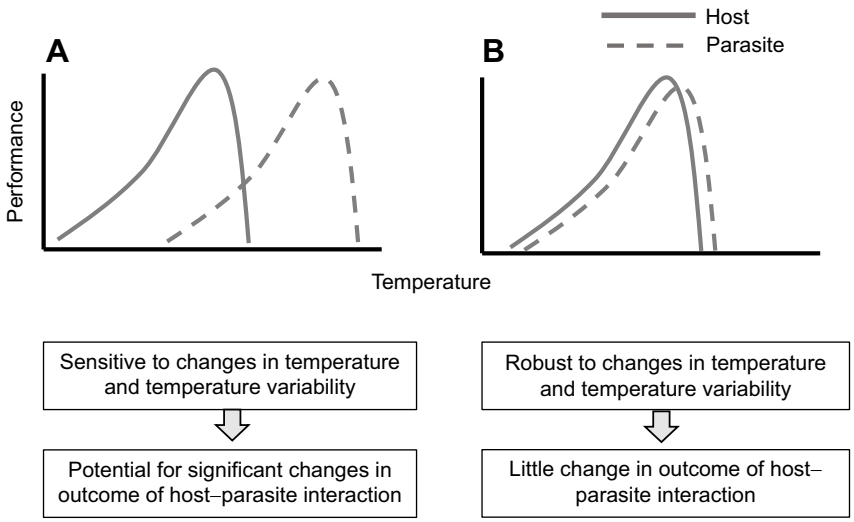

Fig. 5. The significance of mismatches and matches in thermal performance for the outcome of host–parasite interactions under conditions of climate change. The effects of temperature on parasite performance, and how this interacts with host performance, will be the most effective means of understanding the outcome of host–parasite interactions under different thermal environments. (A) Mismatches in thermal performance, where the outcome of the interaction (i.e. who performs best) is largely dependent on temperature (e.g. hosts win at low temperatures, parasites win at high temperatures). This suggests that, with increased variability in winter temperatures or overall increases in temperature, there is a strong potential for the outcome of this relationship to change. (B) Matched thermal performance, where changes in temperature during overwintering are unlikely to affect the outcome of the relationship, as the difference in performance never changes across temperature.

Incorporating parasite thermal biology into thermal eco-immunology

If we are to predict how changes in immunity will match the physiological demands of changing winters, we must also consider how immunity translates into survival (or a sub-lethal outcome) of a host–parasite interaction. Parasites are also ectotherms, and can therefore thermally acclimate to fine-tune infectivity and virulence (Altman et al., 2016; Raffel et al., 2013). Therefore, the outcome of a host–parasite interaction under different thermal environments will depend on the interactions in thermal performance of the host and parasite (Fig. 5; Altman et al., 2016; Gehman et al., 2018; Rohr and Raffel, 2010). Furthermore, parasites may override host investment in immunity through suppression of immune activity, or manipulate the thermal preferences of their hosts to gain a thermal advantage (Macnab and Barber, 2012). However, we know little of how overwintering conditions will change parasite physiology or phenology (see Glossary) (e.g. Paull and Johnson, 2014). Finally, we suggest that we must increase our use of thermally acclimated parasites in eco-immunological studies, and invite eco-parasitologists 'into the cold' to explore the consequences of changing winters on parasite biology.

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Competing interests

The authors declare no competing or financial interests.

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