# Parasites and low temperatures

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SUMMARY

Low temperatures affect the rate of growth, development and metabolism of parasites and when temperatures fall below 0 °C may expose the parasite to the potentially lethal risk of freezing. Some parasites have mechanisms, such as diapause, which synchronise their life cycle with favourable seasons and the availability of hosts. Parasites of endothermic hosts are protected from low temperatures by the thermoregulatory abilities of their host. Free-living and off-host stages, however, may be exposed to subzero temperatures and both freezing-tolerant and freeze-avoiding strategies of cold hardiness are found. Parasites of ectothermic hosts may be exposed to subzero temperatures within their hosts. They can rely on the cold tolerance adaptations of their host or they may develop their own mechanisms. Exposure to low temperatures may occur within the carcass of the host and this may be of epidemiological significance if the parasite can be transmitted via the consumption of the carcass.

Key words: Temperature, freezing, cold tolerance, diapause.

## EFFECTS OF LOW TEMPERATURES ON ANIMALS

Temperature is an important environmental influence on the biology of all animals. The responses to temperature are complex but at its simplest temperature affects the rate at which life processes (growth, metabolism, reproduction etc.) proceed. Every animal has an optimum temperature at which these are at their maximum. As the temperature declines the rate of metabolism slows, due to the decrease in kinetic energy imparted to reactions. This is reflected in changes in the level of activity displayed by the animal. At low temperatures the animal first displays cold stupor, in which its motion becomes disorientated and normal processes are disrupted, and then cold coma, in which activity (and development) ceases altogether (Fig. 1). Proteins are not denatured by low temperatures (as they are by high temperatures) and thus the effects are potentially reversible. If the temperature falls below the melting point of its body fluids (usually just below 0 °C), however, the animal may freeze. This review will focus upon the response of parasites to subzero temperatures where they are exposed to freezing or the risk of freezing.

When an animal freezes water within its body undergoes a change in state from a liquid to a solid. This is obviously a dramatic event to occur within a living organism. Although it is not fully understood why freezing is usually lethal to cells, there are a number of proposed mechanisms (Storey & Storey, 1988; Lee, 1991; Karlsson, Cravalho & Toner, 1993). Death may result from the mechanical disruption, during ice formation, of the plasma membrane of cells or the membranes of organelles.

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Ice forming in a solution results in the concentration of salts in the unfrozen portion as water molecules join the growing ice crystals. If this occurs extracellularly this will raise the osmotic gradient between the intracellular and extracellular compartments and result in cellular dehydration, which may prove lethal. Despite these potentially lethal effects there are several groups of animals which can survive ice formation in their bodies.

Freezing-tolerant animals include: many species of terrestrial insects; intertidal invertebrates such as barnacles, bivalves and gastropods; some terrestrial hibernating amphibians and reptiles; centipedes, slugs and nematodes (Storey & Storey, 1996). The cold-tolerance adaptations of insects are the best understood. Freezing-tolerant insects produce ice-nucleating proteins which ensure freezing at a high subzero temperature (Duman *et al.* 1991); although the gut, and other sites, may also act as sites of ice nucleation (Block, 1995). Freezing at a relatively high temperature ensures that it is a gentle process, allowing the cells of the animal to adjust to the stresses involved and reducing the chances of intracellular freezing. Insects also produce sugars and polyols, such as trehalose and glycerol, which act as cryoprotectants. These depress the melting point and decrease the amount of ice formed at a given temperature, reducing cellular dehydration (Lee, 1991). Thermal hysteresis proteins are produced by freezing-tolerant insects which act to inhibit the recrystallization which may damage cells by the growth of ice crystals (Knight & Duman, 1986).

Freezing avoidance is the alternative strategy adopted by cold-hardy animals. Freeze-avoiding insects maintain their bodies in a supercooled (still liquid) state at temperatures below the melting point of their fluids. If freezing occurs (at the supercooling point) it is lethal. This involves removing or

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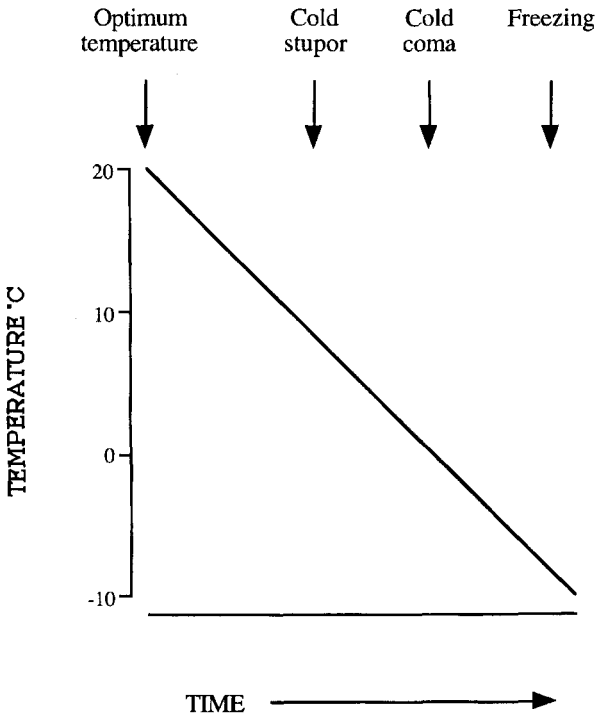



Fig. 1. The effect of decreasing temperature on a hypothetical animal (adapted from Vannier, 1994).

inactivating sources of ice nucleation, such as emptying the gut during winter and avoiding contact with external ice. Sugar and polyols are synthesized but in this case act as antifreezes, depressing the melting and supercooling points and masking or inactivating ice nucleators (Lee, 1991). Thermal hysteresis proteins are thought to stabilize the supercooled state by attaching to and inhibiting the growth of ice crystals (Duman *et al.* 1991). Antarctic fish are at constant risk of freezing since the temperature at which their blood melts is above that of the surrounding sea water and ice crystals in the water threaten to seed ice formation. They produce antifreeze proteins which attach to the surface of ice crystals thus preventing their growth and the freezing of the blood (Davenport, 1992).

Freeze-avoiding insects die when they freeze whereas freezing-tolerant insects do not. The third possibility is that the insect dies before it freezes. This is known as chilling injury or prefreeze mortality (Bale, 1993). Causes of chilling injury may include cold-induced phase changes in lipid membranes (Quinn, 1985), thermoelastic stress (McGrath, 1987), effects on protein structure and disruptions in metabolism which result in lethal imbalances (Storey & Storey, 1988).

## EFFECTS OF LOW TEMPERATURES ON PARASITES

Endoparasites of birds and mammals gain protection from low temperatures by living within an endothermic host. The host generates its own heat and maintains a higher temperature within its body than that in the surrounding environment. This is achieved firstly by burning fuel (food) to generate heat through metabolism and secondly by mechanisms which reduce the loss of heat to the environment, such as insulation (fur, feathers and fat beneath the skin). These parasites will only be exposed to low temperatures if they leave the host during the free-living phase of their life cycle or if the host dies. Ectoparasites of endothermic hosts may still gain some thermal protection from their host if they are in close proximity to the skin and protected from the exterior by fur or feathers. This thermal protection will be less the further away the parasite is from the skin of its host and will be lost altogether if the parasite detaches from the host. Ectothermic animals (the majority of animals) are at the same temperature as their environment. A parasite of an ectothermic host thus experiences, and has to survive, low temperatures in the environment. If the host is exposed to subzero temperatures and is in danger of freezing, so are its parasites.

Low temperature may affect the host/parasite relationship in ways other than its potentially lethal effects on the parasite. The reproduction and activity of hosts will be low during cold seasons and they are thus likely to be unavailable for infection by parasites. The transmission of parasites will also be directly affected by low temperatures. The development of eggs, other free-living stages and of parasitic stages within an ectothermic intermediate host is related to temperature and development will not occur, or proceed slowly, at low temperature. The population dynamics and seasonal infection cycles of parasites are thus likely to be driven directly or indirectly by temperature both by its effects on parasite development and on the seasonal availability of hosts (Kennedy, 1975; Poulin, 1998).

The use of low temperatures is also important to parasitologists for the preservation of parasite cultures. The techniques used are often unrelated to the parasite's natural response to low temperatures (for example by freezing in liquid nitrogen) and are hence beyond the scope of this review.

## LOW TEMPERATURES AND PARASITE LIFE CYCLES

In temperate and polar regions low temperatures are associated with seasonality and are more likely to be encountered during the winter. Parasites can improve their chances of locating a host and/or surviving the winter by a life cycle which is adjusted to this seasonal pattern. This allows the parasite to survive the cold season and to synchronize the production of infective stages both with conditions favourable to their survival and development and to the availability of hosts to infect. This is achieved by a variety of mechanisms.

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### *Diapause in nematodes*

The existence of a diapause in some species of parasitic nematode is indicated by the pattern of seasonal development and/or the response of a developmental event to temperature and other stimuli. Insects synchronize their life cycles with favourable seasons via a diapause which is hormonally regulated. Although hormone regulation in nematode diapause has yet to be demonstrated, there is experimental evidence for the existence of diapause mechanisms (Evans, 1987; Womersley, Wharton & Higa, 1998).

The eggs of some species of plant-parasitic nematodes show increased hatching after chilling, which may produce a pattern of invasion of host roots in the spring and maturation and egg production by adults in the summer (Evans & Perry, 1976). Different isolates vary in their responses, suggesting adaptation to local conditions, which may allow survival of a dry season rather than overwintering. There may be variation in the stimuli necessary to produce egg hatching even within a single population, allowing a proportion of eggs to hatch immediately and a proportion to overwinter and hatch in a second season. This improves the persistence of the population and reduces competition for feeding sites on the host (Jones, Tylka & Perry, 1998). Photoperiod is a more reliable seasonal indicator than temperature and there is evidence for a photoperiod-induced diapause in the eggs of *Globodera rostochiensis*, mediated by its effect on the host plant (Hominick, 1986).

A period of chilling is required for the hatching of the eggs of the animal-parasitic nematode *Nematodirus battus*, with infective larvae overwintering within the eggshell and hatching *en masse* in the spring (Evans & Perry, 1976). In Britain, eggs which have developed on pasture fail to hatch if kept at 20 °C but a mass hatch is produced if they are chilled at 5 °C before exposure to 20 °C. Conditions in autumn induce a diapause which is terminated by chilling over winter and a rise in temperature in spring. Infective larvae overwinter within the egg and hatch in spring ready to infect lambs. This response is so predictable that formulae have been derived which forecast the timing and severity of pasture contamination with hatched larvae (Thomas, 1974).

### *Arrested development in trichostrongyle nematodes*

In some species and strains of trichostrongyle nematodes 3rd-stage infective larvae ($L_3$s) acquired by hosts during autumn do not immediately develop into adults but remain as 4th-stage larvae ($L_4$s) throughout the winter. This phenomenon is called hypobiosis or arrested development (Gibbs, 1986; Eysker, 1997). The $L_4$s resume development in the spring, in response, it is thought, to hormonal changes in the host at the start of the breeding season (Gibbs, 1986). Infective larvae are thus available on pasture at a time when new susceptible hosts (e.g. lambs and calves) start feeding. Low temperatures experienced by $L_3$s on pasture during autumn trigger the winter inhibition of $L_4$s. The process thus appears to be a diapause. The controlling mechanisms are unknown but the identification of a protein which is diagnostic for inhibited $L_4$s of *Ostertagia ostertagi* (Cross, Klesius & Williams, 1988) suggests an underlying molecular mechanism.

### *Infective larvae*

To continue their life cycle parasites need to infect a host. The infective larva is thus in a state of developmental arrest and only resumes development once physiological stimuli are received which indicate entry into or the proximity of a suitable host. This ensures that development is prevented when conditions are likely to result in the death of the parasite (outside the host) and resumes when conditions are favourable (inside the host). This also has the effect of synchronizing the development of the parasite with the availability of hosts. A common physiological trigger which indicates entry into the intestine of a vertebrate is an increase in carbon dioxide concentration at an appropriate temperature and pH (Rogers, 1960; Lackie, 1975).

### *Ectoparasitic arthropods*

Ticks (Ixodides) remain attached to their host for varying periods of time, depending on the species, but may detach after engorgement and be exposed to low temperatures off their host. *Ixodes uriae* is the predominant tick infecting seabirds on the Antarctic peninsula. It has a circumpolar distribution in both the Arctic and Antarctic and is also found in more temperate regions. It has a three-host life cycle which usually takes 4–5 years to complete in Antarctica due to the limited seasonal availability of hosts (Eveleigh & Threlfall, 1974). It has only occasionally been reported from the continental Antarctic, despite the presence of suitable hosts, and its distribution may be limited by its inability to tolerate very low temperatures off the host. *Ixodes uriae* can overwinter in all stages of its life cycle. It also shows behavioural diapause, which is a suspension of host-seeking behaviours, and morphological diapause: a delay in embryogenesis of the developing egg, metamorphosis of larvae and nymphs after engorgement or a delay in female oviposition (Lee & Baust, 1987). The ticks attach to hosts during nesting and engorgement is completed within 1 week, after which they detach (Eveleigh & Threlfall, 1974). They have access to their hosts for only a brief period of the year and it is likely that *I. uriae* must overwinter three times to

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complete its life cycle: as an egg or unengorged larva, an engorged larva and as an engorged nymph or pharate adult (Lee & Baust, 1987). Apart from the brief time spent feeding, the ticks are off the host and exposed to ambient temperatures.

Three-host ixodid ticks spend most of their time off the host and most of those of medical and veterinary importance in the USA overwinter off-host. The lone star tick, *Amblyomma americanum*, overwinters as a nymph in diapause, whilst the American dog tick, *Dermacentor variabilis* overwinters in any stage. The Eastern blacklegged tick, *Ixodes scapularis*, in the northern part of its range has a 2-year life cycle, overwintering as nymphs and adults. The ranges of these three species overlap and may partly be explained by their response to climatic factors such as low temperature (Burks *et al.* 1996).

Arthropod ectoparasites which remain attached to their host during winter will be protected against low temperatures. The Antarctic flea *Glaciopsyllus antarcticus* infects a number of migratory seabirds. No live fleas were found in nest material during the winter, suggesting that they overwinter on their hosts (Whitehead *et al.* 1991).

## FREE-LIVING STAGES AND OFF-HOST SURVIVAL

The free-living stages of parasites and ectoparasites off their host are faced with low temperatures and the risk of freezing, whether their host is an ectotherm or an endotherm. In this section I will consider the cold-tolerance mechanisms of the free-living or off-host stages of a number of parasites.

### *Globodera rostochiensis*

The potato cyst nematode *Globodera rostochiensis* evolved along with its potato host in the high Andes of South America. It is exposed to subzero temperatures in its natural alpine habitat and in many parts of its present range. After mating, the female becomes gravid, with 200–300 eggs being retained within her uterus. She then dies and her body is chemically hardened by a quinone-tanning process to form a cyst. *Globodera rostochiensis* overwinters as a 2nd-stage larva ($L_2$) within the eggshell within the cyst. This represents a complex situation for its cold tolerance since both the eggshell and the cyst wall could act as barriers to ice nucleation.

The eggshell prevents inoculative freezing in water (that is ice forming outside the egg from seeding the freezing of the contents) and allows the enclosed $L_2$ to supercool and survive temperatures as low as −38 °C (Wharton, Perry & Beane, 1993). Hatched $L_2$s, however, freeze by inoculative freezing and are killed. The larvae within the egg can thus use a freeze-avoiding strategy and supercool in the presence of external ice because the eggshell acts as a barrier to inoculative freezing. This interpretation is supported by studies using differential scanning calorimetry (DSC). During cooling DSC thermograms of cysts show three exotherms (production of heat), which indicate freezing events, and during warming three endotherms (absorption of heat), which indicate the melting of compartments of different composition (Wharton & Ramløv, 1995). These indicate the freezing and melting of three water compartments: the water surrounding the cyst (exotherm I, endotherm VI), the water between the cyst wall and the eggs (exotherm II, endotherm V) and the water contained within the eggs (exotherm III, endotherm IV) (Fig. 2). This explanation is supported by the disappearance of exotherm II after removal of eggs from the cyst and the merging of endotherms V and IV after heating cysts to 70 °C. Heating destroys the permeability barrier of the eggshell and allows mixing between these two compartments. The peak of the egg exotherm (−38 °C) corresponds with the mean supercooling point of eggs (−38.2 °C), determined by cryomicroscopy (Wharton *et al.* 1993; Wharton & Ramløv, 1995). The perivitelline space contains trehalose (Clarke & Hennessy, 1976), which is known to act as an antifreeze in some animals (Lee, 1991).

The eggshell is likely to act as a barrier to inoculative freezing of the eggs of many parasites. This may be of particular importance for parasites which spend all of the free-living phase of their life cycle within the egg and which are exposed to subzero temperatures. The walls of protozoan cysts, and of other encysted parasite stages, are also likely to act in this way. Retained cuticles or sheaths can also act as barriers to inoculative freezing (Wharton & Allan, 1989; Wharton & Surrey, 1994).

### *Trichostrongylus colubriformis*

Trichostrongyle nematodes infect sheep and other ruminants. The infective $L_3$s can overwinter on pasture and are exposed to subzero temperatures in parts of their range. Wertejuk (1959) reported that a variety of species survived temperatures as low as −28 °C on Polish pastures. The $L_2/L_3$ moult is incomplete and the $L_3$ retains the cuticle of the previous stage as a sheath, which is shed upon ingestion by the host. This process is known as exsheathment but is actually an ecdysis (Wharton, 1986).

The apparent cold-hardiness strategy of the $L_3$s depends on the conditions under which they are exposed to low temperatures. If the surface water is removed and water loss prevented by covering with liquid paraffin the $L_3$s supercool, with a mean supercooling point of −24.0 °C (Wharton & Allan, 1989). They thus adopt a freeze-avoiding strategy under these conditions. The situation in which the nematodes are free of surface water and yet not

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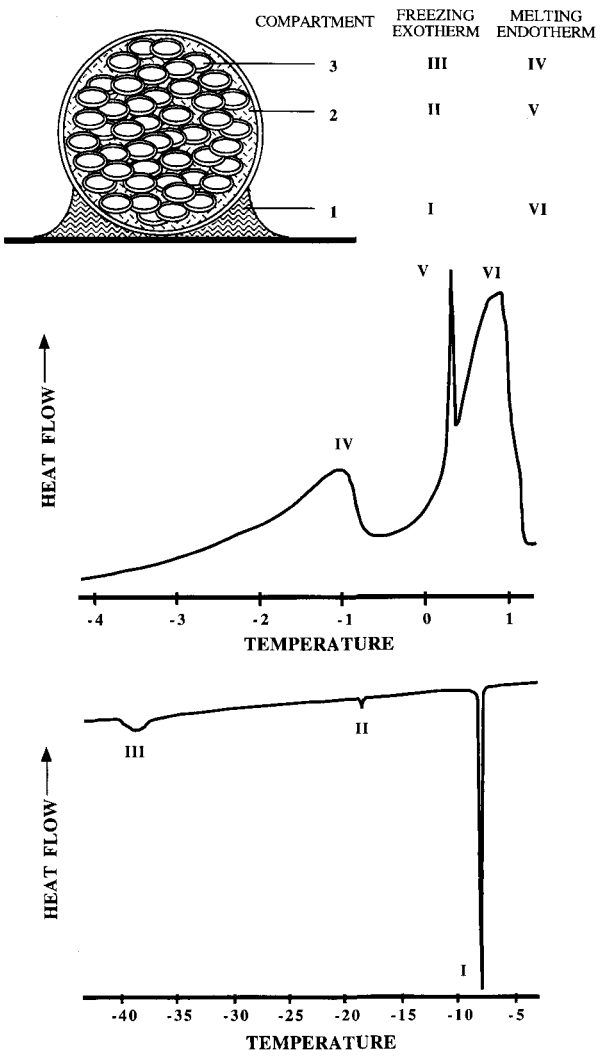



Fig. 2. The water compartments (top) and associated exotherms during freezing (middle) and endotherms during melting (bottom) in DSC thermograms of the cysts of *Globodera rostochiensis*. The compartments are: (1) the water surrounding the cyst, (2) the water between the cyst wall and the eggs and (3) the water within the eggs (redrawn from Wharton & Ramløv, 1995).

desiccated is, however, likely to be transient under natural conditions (Wharton, 1995). It is more likely that the nematode will continue to lose water and, if it is able to survive, enter a state of anhydrobiosis (see Perry, this volume). *Trichostrongylus colubriformis* $L_3$s can survive anhydrobiotically if they lose water slowly (Allan & Wharton, 1990). In a state of anhydrobiosis there is no water to freeze and hence the nematodes are also likely to be resistant to low temperatures.

Nematodes are essentially aquatic organisms, requiring at least a film of water for activity. Exposure to subzero temperatures is likely to occur whilst the nematodes are in contact with water and they are at greater risk of inoculative freezing than are terrestrial arthropods (Wharton, 1995). In water, the sheath can prevent inoculative freezing and allow the $L_3$ to supercool in the presence of external ice. A proportion of $L_3$s do freeze, however, but survive and thus show a degree of freezing tolerance (Wharton & Allan, 1989). In water the $L_3$s thus display both freeze avoidance and freezing tolerance. In arthropods these two strategies are often considered to be distinct (Zachariassen, 1985); although there have been reports of insects shifting their cold-tolerance strategy in successive seasons (Horwarth & Duman, 1984). If the sheaths of *T. colubriformis* $L_3$s are removed there is a shift from freeze avoidance to freezing tolerance but an overall decrease in cold tolerance (Wharton & Allan, 1989). This supports the suggested role of the sheath in reducing inoculative freezing.

### *Entomopathogenic nematodes*

Studies on the cold tolerance of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* have been initiated because of the need to develop a long-term storage method for these biological control agents of insect pests. Poor storage ability is a major impediment to their widespread commercial application (Friedman, 1990). The limited success of desiccation as a storage method (Womersley, 1990; Perry, this volume) has led to an interest in freezing as an alternative. Small quantities have been stored by freezing in liquid nitrogen (Popiel & Vasquez, 1991) but a technique for the storage of commercial quantities is more likely to be based on their natural cold-tolerance abilities.

The sheath of the infective larva of *Heterorhabditis zealandica* prevents inoculative freezing and allows the larva to supercool (Wharton & Surrey, 1994). Survival is, however, lower than would be predicted if each larva which did not freeze survived. They are thus chill tolerant (Bale, 1993) and suffer prefreeze mortality. The larvae are more cold tolerant if reared on an insect host (*Galleria mellonella*) than if grown in artificial culture, suggesting a nutritional component. Acclimation at low temperature, however, does not enhance their cold tolerance (Surrey, 1996).

*Heterorhabditis bacteriophora*, *Steinernema feltiae*, *S. anomoli*, *S. riobravis* and *S. glaseri* infective larvae are freezing tolerant and survive inoculative freezing from the surrounding water (Brown & Gaugler, 1996, 1998) but there is some evidence for prefreeze mortality in *H. bacteriophora* (Brown & Gaugler, 1996). Freezing tolerance in *H. bacteriophora* and *S. feltiae* was enhanced by acclimation at low temperatures and by the addition of glycerol (Brown & Gaugler, 1996).

### *Ticks*

The Antarctic tick *Ixodes uriae* does not tolerate freezing but survives subzero temperatures by a freeze-avoiding strategy (Lee & Baust, 1987). Gly-

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cerol is present in *I. uriae* but at low levels and there is no seasonal variation in glycerol levels or changes in response to acclimation. This suggests that the synthesis of sugars or polyols as antifreezes does not play a role in the cold tolerance of this species (Lee & Baust, 1987). This tick appears to maintain its cold hardiness throughout the year, with the exception of a brief period during which feeding and the processing of a blood meal occurs. Engorged ticks have higher supercooling points than unengorged ticks, presumably because of ice nucleating agents in the blood meal. The processing and digestion of the meal must remove or mask these ice nucleators and thus restore the supercooling capacity (Lee & Baust, 1987).

The lower lethal temperatures of three North American hard ticks, *Amblyomma americanum*, *Dermacentor variabilis* and *Ixodes scapularis*, are much higher than their supercooling points. This indicates prefreeze mortality with chilling injury resulting in death before the supercooling point was reached (Burks *et al.* 1996). The lower lethal temperature was, however, much lower than temperatures recorded during winter in tick hibernaculae and they can thus survive temperatures encountered during winter. Inoculative freezing occurs at much higher temperatures, however, and results in lethal freezing, which may be the major cause of cold-related mortality in the field. This may indicate a preference for dry overwintering sites but ticks are susceptible to desiccation and require fairly moist sites for off-host survival (Needham & Teal, 1991). Microhabitat selection may thus have to balance the risk of desiccation with that of inoculative freezing (Burks *et al.* 1996).

The soft tick *Argus reflexus* is native to parts of Europe and the Near East but is an introduced species in Central Europe. It overwinters off its host, the rock pigeon. The hard tick *Ixodes ricinus* is native to Central Europe and parasitizes a range of mammals, birds and reptiles. Dautel & Knulle (1997) compared the cold hardiness of these two ticks, which were recorded as having been exposed to temperatures as low as −13 °C in an experimental attic. All postembryonic stages of *A. reflexus* had a marked supercooling capacity with supercooling points below −20 °C throughout the year. They did not survive freezing and thus employ a freeze-avoiding strategy. Engorgement did not appear to affect supercooling. The supercooling capacity of *I. ricinus* was very similar, although there was some evidence that engorgement elevated supercooling points in this species. Lower lethal temperatures were generally similar to the corresponding supercooling point for both species, indicating that there was little prefreeze mortality. Inoculative freezing in contact with ice resulted in increased mortality. The introduced species, *A. reflexus*, had a greater degree of cold tolerance than the native *I. ricinus*, despite the former being a native of areas where it is unlikely that it is exposed to very low overwinter temperatures. The supercooling capacity of *A. reflexus* may be a consequence of its ability to survive prolonged periods of starvation and low water availability (Dautel & Knulle, 1997).

### *Insect parasitoids*

Insect parasitoids which overwinter outside their host may be exposed to subzero temperatures. The Braconid parasitoid, *Cotesa glomeratus* parasitizes a wide range of lepidopterous larvae but one of its most common hosts is the large white butterfly, *Pieris brassicae*. *Cotesa glomartus* overwinter as final instar larvae in diapause, with pupation delayed until spring. The larvae accumulate glycerol and have mean supercooling points between −25 °C and −26 °C. This may indicate a freeze-avoiding strategy, but a decline in survival at subzero temperatures above the supercooling point indicates that they also suffer prefreeze mortality (Pullin, 1994). *Athrycia cinerea*, a parasitoid of the bertha armyworm, overwinters as pupae. The mean supercooling points of large puparia were −21·1 °C and of small puparia −27·3 °C (Turnock & Bilodeau, 1992). They also use a freeze-avoiding strategy, with some indication of prefreeze mortality. The hymenopteran egg parasite *Anastatus disparis* infects eggs of the gypsy moth. Larvae consume the entire egg contents and overwinter as mature larvae within the chorion of the host egg. The larvae are killed by freezing but have a mean supercooling point of −28·8 °C and thus have a freeze-avoiding strategy (Sullivan, Griffiths & Wallace, 1997).

### *Other examples of cold tolerance in parasite free-living stages*

The freezing survival of larvae of the plant-parasitic nematode *Meloidogyne hapla* is enhanced after acclimation at low temperatures (Forge & MacGuidwin, 1990). The response is reversed if it is returned to higher temperatures (Forge & MacGuidwin, 1992*a*) and survival is enhanced at low water potentials (Forge & MacGuidwin, 1992*b*). It is not known whether this nematode survives by freezing tolerance or freeze avoidance. Forge & MacGuidwin (1992) suggest that in soil, the nematodes may desiccate rather than freeze due to freeze concentration effects. *Meloidogyne hapla* will freeze by inoculative freezing (Sayre, 1964); although this may not be the case at low water potentials in soil. The free-living Antarctic nematode *Panagrolaimus davidi* freezes by inoculative freezing at osmolalities which are much higher than those likely to occur by freeze concentration effects (Wharton & To, 1996).

The freezing survival of *Ditylenchus dipsaci* infective larvae is similar in contact or not in contact

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with water (Wharton, Young & Barrett, 1984), which may indicate freezing tolerance. Cryomicroscopy confirms that they freeze by inoculative freezing and are freezing tolerant (D. A. Wharton, unpublished observations). *Aphelenchoides ritzemabosi* also has some freezing tolerance ability (Asahina, 1959). There are other records of plant-parasitic nematodes surviving subzero temperatures (Miller, 1968; Antoniou, 1989) but the mechanisms by which they do so are unknown.

The eggs and hatched $L_3$s of the animal-parasitic nematode *Nematodirus battus* supercool in the absence of surface water. The degree of supercooling is increased after low-temperature acclimation (Ash & Atkinson, 1986), this is accompanied by an increase in trehalose concentration (Ash & Atkinson, 1983). Whether this nematode survives inoculative freezing in water is unknown but it might be expected that the eggshell would allow unhatched larvae to supercool in the presence of external ice, whereas hatched larvae would freeze.

*Elaphostrongylus rangiferi* $L_1$s, a nematode parasite of reindeer, survive freezing, at −80 °C or −20 °C in faeces and at −20 °C in water for 360 days (Lorentzen & Halvorsen, 1986). $L_3$s from a number of species of trichostrongyle nematodes have been reported to survive subzero temperatures under a variety of conditions (Wharton & Allan, 1989). The mechanisms by which these nematodes, and of others reported in the literature, survive are unknown but it is likely that if in contact with water they will freeze by inoculative freezing, unless this is prevented by the presence of a sheath or an eggshell. If free of surface water they may be able to supercool or to survive in a state of anhydrobiosis (Wharton, 1995). We clearly need data from a much wider range of species before we can determine the distribution of cold tolerance strategies in nematodes.

The cold tolerance mechanisms of the free-living stages of most other groups of parasites remain to be determined.

## LOW TEMPERATURES AND PARASITIC STAGES

Parasites may be exposed to subzero temperatures within the host if the host is an ectotherm and is itself periodically exposed to these temperatures or within the carcass of an endothermic host after the host has died. The cold-tolerance strategy of the parasite may be dictated by that of its host. A parasite of a freeze-avoiding host can rely on the host's ability to supercool. The only requirement would be that the parasite did not initiate ice nucleation above the temperature at which nucleation is initiated within the body of the host itself. Since the parasite will always be smaller than its host the supercooling point of the parasite is likely to be below that of the host, as long as the parasite does not contain any ice-nucleating agents. A parasite of a freezing tolerant host, however, may also be subject to freezing unless it is able to inhabit parts of the host which do not freeze.

*Wetanema* sp. is a thelastomatid nematode which inhabits the hind gut of *Hemideina maori*, a large orthopteran insect which lives under rock slabs in the alpine zone of the South Island of New Zealand. The host is regularly exposed to temperatures below −5 °C, and occasionally to as low as −11 °C, and is freezing tolerant (Ramløv, Bedford & Leader, 1992; Sinclair, 1997). All stages of *Wetanema* are found within their host throughout the winter, suggesting that the nematode can survive freezing of its host in the field (Tyrrell *et al.* 1994). The gut of the host contains potent ice nucleators (Sinclair, Worland & Wharton, 1999) and hence *Wetanema* will experience freezing. In laboratory studies live nematodes were recovered from hosts which had been frozen to −61 °C. The lower lethal temperature of the host is about −11 °C (Ramløv *et al.* 1992). *Wetanema* is thus able to survive much lower temperatures than its host. The freezing of *Wetanema* has also been observed directly on a microscope cold stage (Tyrrell *et al.* 1994). The nematode froze by inoculative freezing from the external medium, with freezing starting at the posterior end of the body and travelling forwards. Nematodes observed to freeze on the cold stage survived for six hours or more. The nematode is thus freezing tolerant and mirrors the cold tolerance strategy of its host; suggesting the coevolution of cold tolerance strategies of the parasite and its host.

There are few other reports of parasites surviving freezing within live hosts. The hymenopteran parasitoid *Syndipnus* sp. survives ice nucleation within its host the Arctic sawfly *Amauronematus* (Humble & Ring, 1985). The filarid nematode *Pelecitus fulicaeatrae* lives near the tendons of the ankles of its bird host and may be exposed to subzero temperatures during the winter within their host since they are located close to the surface of the skin and away from major blood vessels (Bartlett, 1992).

Many other freezing-tolerant hosts carry parasites. The parasite could avoid the risk of freezing within its host by being outside the host in the free-living phase of its life cycle during periods when the host is likely to freeze. However, the free-living stages of the parasite will be in a similar environment to that of the host and exposed to the same, or greater, risk of freezing. For parasites remaining in the host they may be exposed to freezing within the host's tissues. The wood frog, *Rana sylvatica* is widely distributed in N. America from the southern USA to above the Arctic Circle in Alaska. It overwinters under leaf litter where its surroundings often undergo extensive freezing. *Rana sylvatica* is freezing tolerant with about 65 % of its body water being converted into ice and it survives temperatures as low as −6 °C (Layne & Lee, 1995). The freezing process is slow, often

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taking more than 24 h for completion. Survival is aided by the initiation of freezing at a high subzero temperature by inoculative freezing through the skin, or by nucleators such as bacteria in the gut, and by the production of glucose as a cryoprotectant (Storey & Storey, 1996; Lee & Costanzo, 1998).

*Rana sylvatica* has an extensive parasite fauna with a variety of protozoa, trematodes, cestodes and nematodes parasitizing sites including the intestine, lungs, liver, mesenteries, body cavity and musculature (McAllister *et al.* 1995; Yoder & Coggins, 1996). Survival of these parasites within the host during freezing may involve tolerating freezing themselves or survival in sites within the host which do not freeze. Ice formation in *R. sylvatica* appears to be restricted to the body cavity and extracellular spaces. Ice formation has been monitored in live animals using proton magnetic resonance imaging (Rubinsky *et al.* 1994). Freezing starts at the periphery and moves inwards. Water from the body organs appears to be sequestered into ice in the body cavity. This results in an osmotic dehydration of the organs (Storey & Storey, 1996; Lee & Costanzo, 1998). Parasites infecting the body organs could thus have less contact with ice than those in the body cavity but some ice does still form in the muscles, heart and other organs (R. Lee, personal communication). They may also be subjected to osmotically-induced dehydration in these sites.

The lungworm *Rhabdias ranae*, which parasitizes *R. sylvatica* and several other amphibian hosts, appears to be freezing tolerant (Woodhams *et al.* 2000). Live *R. ranae* were found in the lungs of frogs that survived freezing at −4 °C in the laboratory, and isolated lungworms tolerated *in vitro* freezing to the same temperature. Lungworms cooled on a cryomicroscope stage froze internally at −3 °C, shortly after contacting ice crystals in the suspension medium, whereas worms submerged in paraffin oil supercooled extensively (to −21 °C). Given its high susceptibility to ice inoculation, it is likely that *R. ranae*, like its host, survives exposure to subzero temperatures by tolerating internal freezing.

There are few reports on the cold tolerance of parasites within freeze-avoiding hosts. The hyperparasitoid *Tetrastichus galactopus* survives low temperatures within overwintering larvae of its host, the parasitoid *Cotesia glomeratus*, which can supercool but also shows some prefreeze mortality (Pullin, 1994). The egg parasitoid *Anaphes* sp. overwinters within eggs of its host, the carrot weevil *Listronotus oregonensis*. *Anaphes* sp. relies upon the supercooling of its host egg and freezing is fatal. Parasitized eggs synthesize glycerol and fructose which may enchance their supercooling capacity by acting as antifreezes (Hance & Boivin, 1993). Pupae of the cabbage root fly *Delia radicum* parasitized by the hymenopteran parasitoid *Trybliographa rapae* showed more variability in their supercooling points than unparasitized hosts, with more with high supercooling points and fewer with low supercooling points. This may indicate an adverse effect of the parasitoid on the cold tolerance of its host but parasitized hosts still have sufficient cold tolerance to survive temperatures they are likely to encounter in the field (Block, Turnock & Jones, 1987).

Parasites may also survive freezing within the frozen carcasses of their host. This is of biological relevance if new hosts are infected by consuming infective stages of the parasite when they eat the carcass. A number of parasites are transmitted in this way and hence survival of infective stages within the carcass of a frozen host may be an important epidemiological factor in some situations.

*Trichinella* spp. infect a variety of mammals and are transmitted by the ingestion of larvae encysted in the musculature. Arctic isolates of *Trichinella* (*T. nativa*) are more tolerant of freezing than are isolates from more temperate latitudes (Smith, 1984; Kapel *et al.* 1999). The ability to survive freezing at very low temperatures is enhanced by preconditioning at a higher (but still subzero) temperature (Smith, 1984, 1987). This may indicate an acclimation response, for example involving the production of cryoprotectants. Viable infective larvae have been recovered from arctic fox tissues after freezing at −18 °C for 4 years (Kapel *et al.* 1999). The mechanisms involved in the cold tolerance of this nematode are unknown.

Other reports of parasites surviving freezing within the host carcass are for *Pelecitus fulicaeatrae* a filarid nematode of the American coot (Bartlett, 1992), *Dirofilaria raemeri* a parasite of marsupials (Spratt, 1972) and *Anisakis simplex* within its fish intermediate host (Gustafson, 1953). *Anisakis* larvae infect a variety of fish and are unlikely to be exposed naturally to freezing. Although its ability to survive a degree of freezing stress (Gustafson, 1953; Wharton and Aalders, unpublished observations) is of no adaptive significance, it is important since fish and fish products which are to be consumed raw (e.g. as sushi) or lightly cooked (e.g. by cold smoking) are made safe for human consumption by freezing. It is therefore necessary to determine the temperature and time of freezing which will ensure the killing of larvae. This is also true for the use of freezing in this way for other parasites such as *T. spiralis* and *Echinococcus granulosus*.

## CONCLUSIONS

In this review I have described how subzero temperatures affect parasites in a number of situations. Free-living and off-host stages are exposed to low temperatures in the environment outside the host. Parasites of ectothermic hosts may be exposed to freezing, or the risk of freezing, within the host. Finally, parasites may be exposed to freezing within

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the carcass of the host after it dies. These events could be important in determining the epidemiology of the parasite. Some parasites respond to low temperatures with strategies which synchronize their life cycle with favourable seasons and the availability of hosts. Parasites may take advantage of the cold tolerance adaptations of their host and they may have cold tolerance adaptations of their own. However, for most groups of parasites we have limited information on their cold tolerance mechanisms. Nematodes and ticks have been the most studied, but even for these groups a very limited range of species have been investigated. We have little or no information on the cold tolerance mechanisms of most other groups of parasites.

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