A COMPARATIVE STUDY OF INVERTEBRATE SUPERCOOLING AT SIGNY ISLAND, MARITIME ANTARCTIC

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ABSTRACT. The capacity for supercooling and the cryoprotective contents of 19 species of invertebrates collected from inshore marine (eleven species), freshwater (two species) and terrestrial (six species) habitats in summer 1981–82 were examined at Signy Island. All the species tested were freezing susceptible and individuals died at their supercooling points. Aquatic species generally exhibited poor supercooling ability (−5 to −9°C) compared with the terrestrial arthropods (−8 to −12°C), of which four species were high capacity supercoolers (<−20°C). Potential cryoprotectants were in lower concentrations (mostly <0.1% of fresh weight) in aquatic species than in terrestrial forms, where a four-component (glycerol, myo-inositol, glucose and trehalose) profile was identified. Summer levels of cryoprotectants were not thought to influence individual supercooling in any species. Considering the annual temperature range for each of the habitat categories (*c.* 3 deg for inshore marine, *c.* 5 deg for freshwater lakes and *c.* 62 deg (−26.5 to +35.8°C) for terrestrial sites), it is unlikely that any of the aquatic invertebrates tested would be at risk from freezing in maritime Antarctic conditions. Most of the terrestrial species avoid lethal freezing by extensive supercooling, but nucleation by food retained in the gut will increase the probability of freezing even in summer acclimatized animals. The high incidence of freezing susceptibility in the Antarctic fauna, especially that of the land, may indicate that supercooling confers a selective advantage on potential colonists over freezing tolerance.

INTRODUCTION

Two strategies for overcoming the problems caused by freezing temperatures have been adopted by invertebrate poikilotherms (see review by Block (1982)). Some species are freezing tolerant, being able to survive extracellular ice formation, while others are freezing susceptible and avoid ice crystallization by supercooling (the capacity to maintain their body fluids in the liquid phase below the freezing point). Freezing tolerant forms frequently have poor supercooling ability and ice nucleators present in the haemolymph in winter aid protective extracellular freezing in the temperature range from *c.* −2 to −12°C (Zachariassen and Hammel, 1976). Compounds such as glycerol may confer a degree of protection from ice crystals during freezing and thawing in such animals. In freezing susceptible species, supercooling may be enhanced by low molecular weight solutes (polyhydric alcohols, sugars, etc.), depending on their concentration in the body fluids, thereby avoiding freezing, which is always lethal. Nucleating agents (possibly proteins or peptides) may also be present in freezing suceptible animals but these are either removed or masked within the intercellular matrix during supercooling (Zachariassen, 1982). The temperature at which whole-body freezing occurs in the supercooled state is termed the supercooling point.

However, the situation is not always clear-cut and freezing tolerant insects (particularly Coleoptera) with relatively low supercooling points have been found in Arctic and alpine habitats (Miller, 1982; Ring, 1982).

In the Antarctic, previous investigations concentrated on land arthropods in which freezing susceptibility is widespread (Block and Sømme, 1982; Sømme and Block,

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1982). A single species of insect, the Antarctic midge *Belgica antarctica* Jacobs, is freezing tolerant but only in the larval stage (Baust and Edwards, 1979). Freezing resistance has been little studied in marine invertebrates (Rakusa-Suszczewski and McWhinnie, 1976) and not at all in the freshwater fauna. The purpose of the present study was to compare the potential for avoiding freezing by supercooling in a range of invertebrates representative of terrestrial, freshwater and inshore marine habitats. The work was undertaken during the austral summer 1981–82 at Signy Island, which is typical of much of the maritime Antarctic zone (Holdgate, 1977), where no freeze-tolerant species had been discovered. The aims of the study were to relate the supercooling capacity of selected species to their normal habitat temperatures and to determine the levels of any possible cryoprotectants that occur.

METHODS

Fauna

A total of 19 invertebrate species was examined, all except two being arthropods. They comprised six terrestrial species, two freshwater and eleven marine forms, the latter including an intertidal annelid (Enchytraeidae) and a gastropod mollusc. The taxa are listed in Table I, each being referred to by the generic name throughout this paper except the enchytraeid, which was not determined, but appears to be referable to one species (B. Christensen, pers. comm.)

Samples of animals were collected in the field by hand sorting and micro-aspiration (terrestrial habitats), by vertical-haul netting and water bottle sampling (freshwater habitats), and by hand collection of sea-weeds, etc. by SCUBA diving techniques (inshore marine habitats). The terrestrial species were obtained from sites around Factory Bluffs, Cemetery Flats and Gourlay Peninsula, the freshwater species from Sombre Lake and a pool at Hillier Moss (near Signy Island Reference Site 2), while the marine forms were from sites in Borge Bay, in particular near Bare Rock, in water between 10 and 20m deep. All specimens for experimental work were maintained in their appropriate medium either at *c.* 0°C (aquatic species) or at 3–5°C (terrestrial species) for up to 8h before being used. Specimens were tested shortly after field collection in order that the results would be representative of field animals as far as possible.

Supercooling points

Supercooling points of individuals of all species were measured following the method of Block and Sømme (1982) using a freezing mixture (1.5:1 v/v) of $CaCl_2 . 6H_2O$ and snow to produce a cooling rate of *c.* 1 deg min^{-1}. A six-channel Linseis recorder and various sizes of copper–constantan thermocouples (30–40s.w.g.) monitored the body temperature of the experimental animals. The supercooling point was read as the point of origin of the small, but significant, rise in body temperature that occurred through latent heat emission during spontaneous freezing. All the aquatic specimens were damp-dried on filter paper before attachment to the thermocouples. For the larger species, e.g. amphipods, isopods pycnogonids, etc., each individual was located in the base of a small polythene tube with the thermocouple tip being lowered to make a firm contact on the ventral surface.

Cryoprotectants

Extracts of polyhydric alcohols and sugars were prepared for chromatography by macerating, in 70% ethanol, single individuals of most of the larger species (all the marine taxa except the enchytraeid) and samples composed of several individuals (all

Table I. Invertebrate taxa tested for supercooling potential from terrestrial, freshwater and inshore marine habitats at Signy Island. Live weights of individuals of some taxa are indicated. nd: not determined.

Habitat category	*Group*	*Species*	*Live weight (mg) per individual*	*Habitat*
Terrestrial	Insecta, Collembola	*Cryptopygus antarcticus*	$2–120 \times 10^{-3}$	Ubiquitous
		Parisotoma octooculata	$3–61 \times 10^{-3}$	Guano-enriched areas
		Archisotoma brucei	nd	Shore-line
	Arachnida, Acari	*Stereotydeus villosus*	nd	Moist areas with some plant cover
		Gamasellus racovitzai	$10–145 \times 10^{-3}$	Mosses, fellfields; predator
		Alaskozetes antarcticus	$13–197 \times 10^{-3}$	Rocky areas (enriched) preferred
Freshwater	Crustacea, Anostraca	*Branchinecta gaini*	4–11	Lakes, nekton
	Crustacea, Copepoda	*Pseudoboeckella poppei*	nd	Lakes, pools
Marine	Oligochaeta, Enchytraeidae	nd	1–5	Intertidal
	Crustacea, Amphipoda	*Oradarea bidentata*	81–187	Epifauna on rocks and weeds
		Oradarea ocellata	nd	,,
		Bovallia gigantea	nd	,,
		Paraphimedia integricauda	nd	,,
		Paradexamine fissicauda	nd	,,
		Cheirimedon femoratus	nd	Infauna of sediments
	Crustacea, Isopoda	*Serolis polita*	51–92	Sandy substrates
		Glyptonotus antarcticus	nd	,,
	Arachnida, Pycnogonida	*Nymphon orcadense*	101–292	Bottom dwelling
	Gastropoda, Opisthobranchiata	*Philine* (?) *gibba*	nd	Sediments

terrestrial and freshwater species together with the enchytraeid worm). The range of fresh weights obtained using a Cahn electro-balance for the former animals is given in Table I, while the latter micro-faunal samples were all slightly greater than 1 mg fresh weight. A minimum of three samples per taxon was assayed but five to six samples were possible for larger specimens. GLC techniques were applied after derivatizing the samples in trimethylsilyl reagent with pyridine (Sigma Sil-A) (Sweeley and others, 1963), using a Pye-Unicam GCD instrument with a Chrompack CP^{tm} Sil 5 non-polar capillary column and internal standards. A Hewlett-Packard Integrator 3380A was also used (see Block and Sømme (1982) for details).

RESULTS

Supercooling capacity

Frequency histograms of individual supercooling points for the six terrestrial species are shown in Fig. 1. Those for the two freshwater crustaceans are given in Fig. 2 together with the data for three of the marine arthropods and the intertidal enchytraeid worm. Separation of the data into a high group (HG) (>−20°C) and a low group (LG) (<−20°C) was undertaken following earlier work on Antarctic micro-arthropods (Bløck and Sømme, 1982). The division between the two groups was at −20°C.

In the terrestrial invertebrates (Fig. 1), the shape of the supercooling point distribution varies considerably. Of the three collembolans, only *Cryptopygus* was capable of supercooling below −20°C and the mean supercooling points of the HG did not vary significantly between species. Of the three mite species, only the oribatid *Alaskozetes* was able to resist temperatures below −20°C by supercooling in all its life stages. This species possessed both a HG and a LG in the samples examined, whereas the mesostigmatid *Gamasellus* and the prostigmatid *Stereotydeus* (with one slight exception) exhibited only a HG in their supercooling point distributions. The mean supercooling points of all the HGs in the mites were not significantly different and the mean LG supercooling points of the various life stages of *Alaskozetes* were also similar. Similarly, the two life stages of *Gamasellus* had almost identical supercooling ability with the majority of the measured points falling within a narrow 2–3 deg band. This contrasts with most of the other terrestrial species, which showed a broader range of supercooling powers (with the exception of *Archisotoma*).

The aquatic species, both marine and freshwater (Fig. 2), show a consistent presence of a HG in their unimodal distributions. Individuals in the arthropod samples had a remarkable similarity of supercooling ability with mean supercooling points ranging from −5.7 (the pycnogonid *Nymphon*) to −8.9°C (the copepod *Pseudoboeckella*). Data for amphipods, other than *Oradarea bidentata*, were very few and generally similar to those of *O. bidentata* (mean supercooling points ranging from −2.8 for *Cheirimedon femoratus* to −8.5°C for *Bovallia gigantea*), that they are not considered further. Only the enchytraeid showed any extension of the supercooling range below −20°C (but only in three individuals in a total of 33), the overall mean being −11.9°C.

A comparison of mean (± SD) supercooling points for the invertebrates studied at Signy Island is made in Fig. 3. For *Alaskozetes* and *Gamasellus*, mean values were calculated from the data for all life stages tested. The species are grouped according to major habitat categories: terrestrial, freshwater and inshore marine. It can be seen that LG supercooling points (<−20°C) occurred only in four of the terrestrial species and that the supercooling points of their HGs (overall mean of *c.* −10°C) were generally lower than the aquatic species (overall means of *c.* −8 and

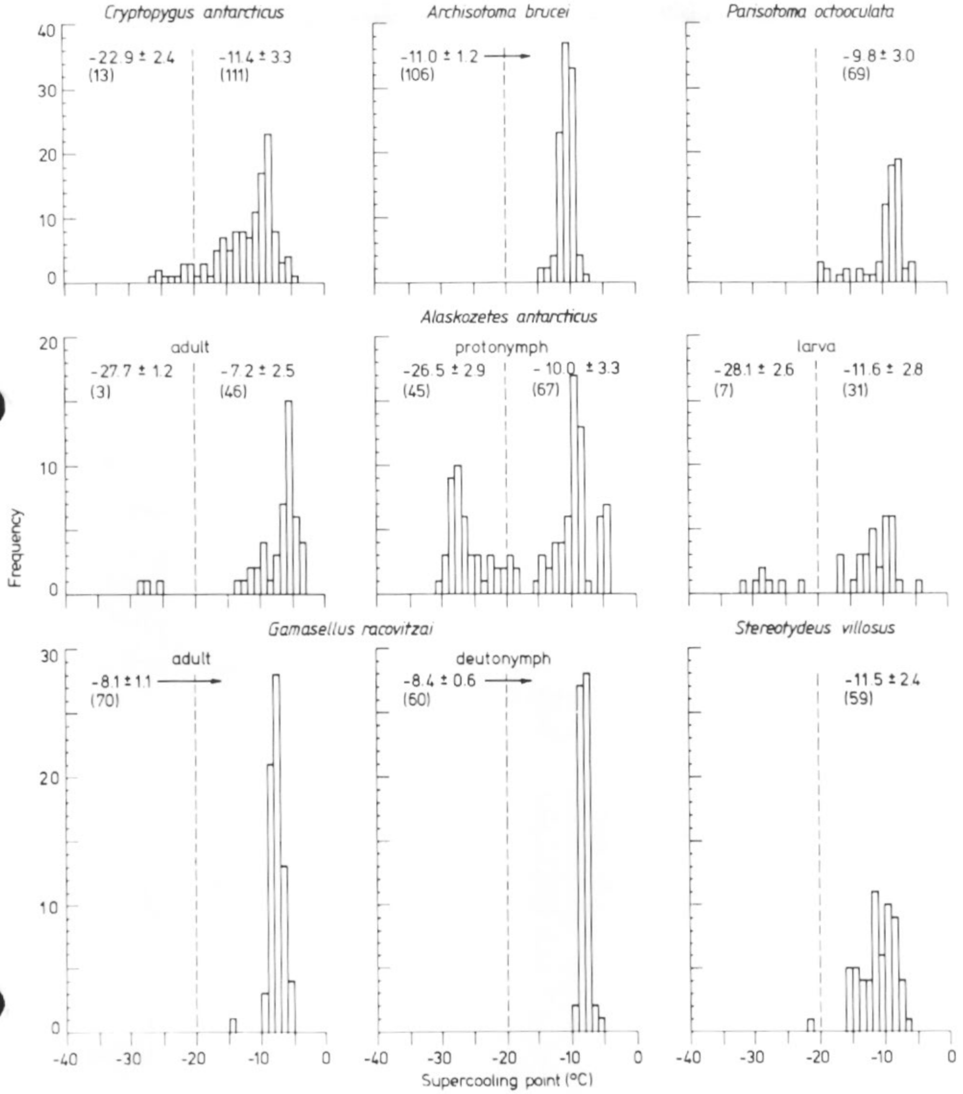

Fig. 1. Supercooling point distribution histograms for six species of terrestrial arthropods (three Collembola and three Acari) at Signy Island during December–January of the 1981–82 austral summer. The mean (±SD) supercooling points and the number of observations (n) are shown for the high group (HG) and low group (LG) with the division at −20°C.

−6°C for freshwater and marine respectively). These forms have the greatest capacity for avoiding freezing by supercooling. Most (four species out of six) of the terrestrial invertebrates showed evidence of an ability to shift their supercooling range to below −20°C (i.e. LG), and these clearly have a greater capacity to avoid lethal freezing when it occurs. The exception in the aquatic species was the enchytraeid with an overall mean supercooling point of *c.* −12°C. The freezing susceptibility of the Antarctic enchytraeid is in contrast to the freezing tolerance displayed by intertidal invertebrates of arctic and temperate regions (Aarset, 1982).

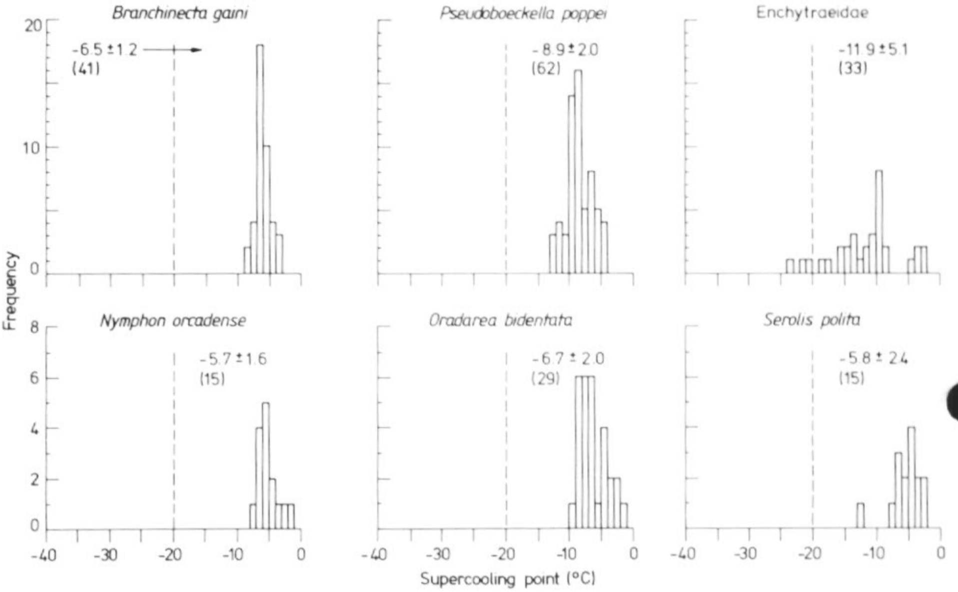

Fig. 2. Supercooling point distribution histograms for six taxa of aquatic invertebrates (two freshwater crustaceans, two marine crustaceans, one pycnogonid and one intertidal enchytraeid) at Signy Island during December–January of the 1981–82 austral summer. The mean (± SD) supercooling points and the number of observations (*n*) are shown for the HG.

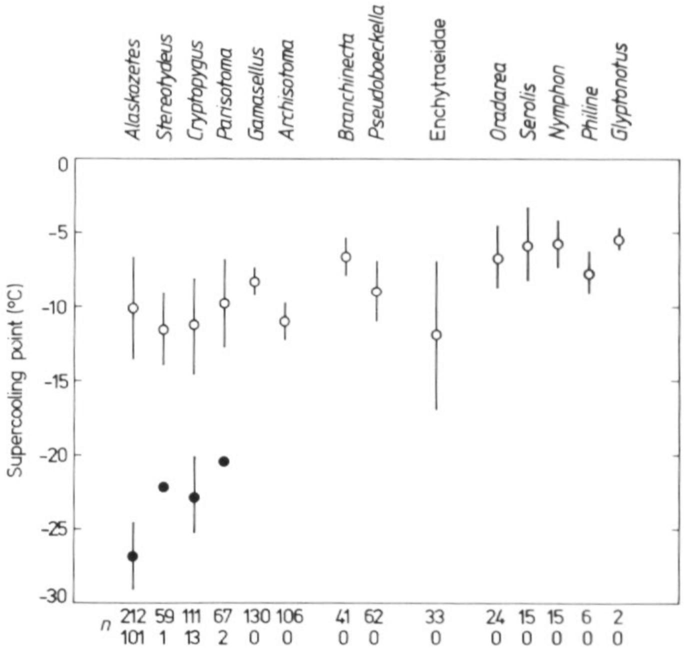

Fig. 3. Comparison of the mean HG and LG supercooling points of 14 invertebrate taxa from terrestrial, freshwater and inshore marine habitats at Signy Island in the austral summer 1981–82. *n*: number of data points in the HG and LG respectively.

Potential cryoprotectants

Three polyhydric alcohols (polyols) and three sugars were detected in extracts of twelve species examined (Table II). No compound was found in a concentration in excess of 1.1% of fresh weight, which was the average level of glucose in the enchytraeid samples. Mean concentrations of most compounds were <0.1% of fresh weight, although some increased to 0.4% by weight. Glycerol, glucose and trehalose were found in all samples from all the taxa, whilst ribitol and fructose occurred in eleven species. Myo-inositol was found in eight taxa. On a species basis, there is no distinct pattern of occurrence of cryoprotectants but the terrestrial species appear to possess a four-component cryoprotectant profile, consisting of glycerol, myo-inositol, glucose and trehalose. Each of these were in excess of 0.1% by weight. This range of compounds contrasted with the aquatic taxa in which only glycerol was detected at concentrations >0.1% of fresh weight in only two of the five species. The enchytraeid was, again, unusual in having 11 μg mg^{-1} fresh weight of glucose in addition to ribitol, myo-inositol and fructose, all >0.1% by weight.

The compounds found in the present study do not reflect the profiles reported earlier from field mites and Collembola (Block and Sømme, 1982; Sømme and Block, 1982), except in the case of *Alaskozetes*. Here, the 1979–80 summer samples are broadly similar to the 1981–82 results (Table II). The conclusion that there is a more diverse spectrum of potential cryoprotectants available to terrestrial invertebrates than is found in either freshwater or marine forms, supports the experimental evidence that the former have a much greater capacity for supercooling.

DISCUSSION

Freezing was lethal to 19 species of invertebrates from three major habitats at Signy Island and individuals rely entirely on supercooling as an avoidance mechanism. In samples from field populations, collected in summer, the average supercooling was between −6 and −12°C (Fig. 3), although in four terrestrial species individuals were able to extend their supercooling to well below −20°C. None of the aquatic forms from both freshwater and inshore marine systems exhibited a LG (low group) in terms of supercooling. Although the present data are derived from summer

Table II. Concentrations of sugars and polyols in extracts of twelve invertebrates from terrestrial, freshwater and marine habitats at Signy Island in 1981–82. −, no trace; +, trace but <0.1% fresh weight; *, >0.1%; **, >0.2%; ****, >0.4%; *****, >1.0%.

Taxon	*Glycerol*	*Ribitol*	*Myo-inositol*	*Glucose*	*Fructose*	*Trehalose*
Cryptopygus	+	−	+	**	+	**
Parisotoma	+	+	−	+	+	+
Archisotoma	+	+	*	+	+	+
Stereotydeus	+	+	+	*	+	*
Gamasellus	+	+	****	*	+	+
Alaskozetes	**	+	*	*	−	+
Branchinecta	+	+	−	+	+	+
Pseudoboeckella	*	+	+	+	+	+
Enchytraeidae	+	*	*	*****	*	+
Oradarea	*	+	+	+	+	+
Serolis	+	+	−	+	+	+
Nymphon	+	+	−	+	+	+

acclimatized animals, it is important to relate the results to the environmental temperatures experienced by the fauna in the field and also to winter levels of supercooling where data exist.

The annual temperature range (absolute maximum and minimum) recorded in Borge Bay (D. G. Bone, pers. comm.) where the marine animals were collected, the average mid-depth water temperature in three freshwater lakes (Heywood, 1968) and that at the surface of a moss turf community (Walton, 1982) at Signy Island are depicted in Fig. 4. Mean supercooling points for species from each of the habitat categories are shown for comparison. The terrestrial arthropods are subdivided into the two supercooling groups and data from winter samples are included. The aquatic habitats at Signy Island have relatively narrow annual temperature ranges, with the inshore marine environment experiencing only *c.* 3.3 deg change compared to the lakes where *c.* 5 deg is usual. Terrestrial communities undoubtedly experience the largest variation in temperature of all the habitats throughout the year (*c.* 62 deg). Although the duration of a particular low (or high) temperature level may be short, species here probably have to survive such a range and extremes at least once within their life cycle. In terms of supercooling, both the inshore marine fauna and the lake and pool invertebrates have a sufficient capacity, even in summer, to avoid freezing over a temperature range representative of the annual one. The position for terrestrial invertebrates is more complex in that, due to feeding activity and the retained food in the arthropod gut promoting, in effect, self-nucleation under certain conditions, a bimodal separation of individual supercooling points results (Fig. 4). Temperatures below −5°C are unlikely to occur even on the ground surface during November–March at Signy Island (Walton, 1982) and, therefore, the supercooling ability demonstrated for summer specimens in this study would ensure survival for the majority of their populations. In winter, both supercooling point groups show a downward shift relative to summer levels and the LGs of most species would avoid lethal freezing, some to <−30°C. The HGs would be more at risk from freezing in winter, and some individuals would succumb.

A single sample of *Nymphon orcadense* (Pycnogonida) collected in winter 1982 had a mean (±SD) supercooling point of -5.4 ± 1.2°C ($n = 23$) (A. D. Hemmings, pers. comm.), both this and its cryoprotectant profile being very similar to the summer sample. The enchytraeid, occupying an intertidal habitat, will experience lower temperatures than benthic and other marine invertebrates and its supercooling capacity reflects this in being intermediate between the truly aquatic species and the terrestrial forms.

It is concluded that, although there is a graded response across the habitats to subzero temperatures, the aquatic species are well protected by their powers of supercooling to avoid freezing. The terrestrial invertebrates, on the other hand, may be subjected to much greater thermal variation during the year and supercooling may be inhibited by the presence of gut contents even at times in winter when a proportion of their populations may be at risk from freezing.

Supercooling is a widespread phenomenon in invertebrates (see review by Sømme (1982)) and is apparently a successful strategy for a wide range of species inhabiting low temperature environments where winter survival is linked to tolerance or avoidance of freezing temperatures. Few freezing tolerant species have been recorded (Block, 1982). In the Antarctic, freezing susceptibility is common in invertebrates but they also exhibit considerable powers of supercooling, which are manifest in the mostly terrestrial fauna studied to date. This may be due to the fact that the land fauna of the Antarctic region is grossly impoverished by comparison with other continents and the immigration routes of potential colonists from warmer

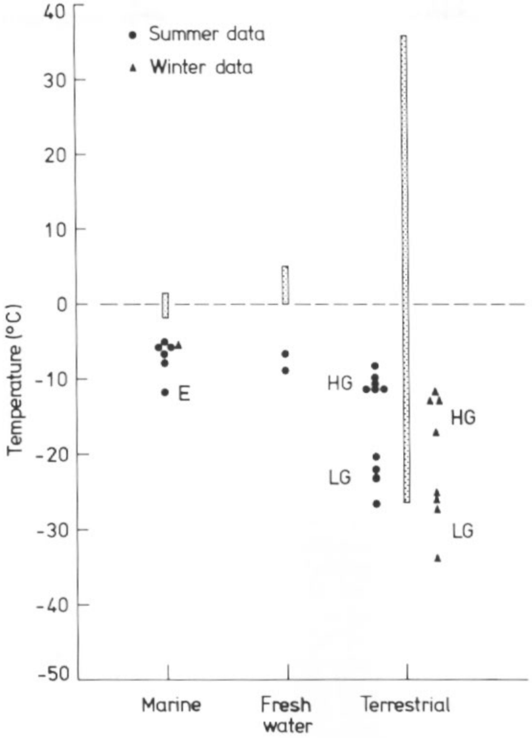

Fig. 4. Comparison of the mean supercooling points of 18 species of invertebrates with the annual temperature range for inshore marine, freshwater and terrestrial habitats at Signy island. ●, summer data; ▲, winter data; E, enchytraeid; HG, high group; LG, low group.

northern areas are long and rigorous. Supercooling may thus be more advantageous for a species colonizing an environment colder than whence it originated. No novel physiological or biochemical adaptations appear to be required, merely the extension and development of existing mechanisms. An example of this is provided by the chironomid midge (*Eretmoptera murphyi* Schaeffer), which was accidentally introduced to Signy Island from either the Falkland Islands or South Georgia (Block and others, 1984). Its life stages, especially the larva, have sufficient supercooling capacity to have survived for 17 years in the new habitat and reproduction by parthenogenesis occurs. It may be more difficult for freeze tolerant forms to become colonizers. In this strategy, particular proteins are required to perform the nucleating function as temperature declines (ice nucleating proteins, cf. Duman and others, 1982; Zachariassen, 1982). It is essential to the survival of such animals that nucleation takes place at relatively high subzero temperatures.

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