DIET AND ACCLIMATION EFFECTS ON THE COLD TOLERANCE AND SURVIVAL OF AN ANTARCTIC SPRINGTAIL

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ABSTRACT. The effect of cold acclimation (5 to $-20°C$) and diet (moist alga; ± distilled water) on the cold tolerance and survival of an Antarctic isotomid collembolan, *Cryptopygus antarcticus*, were investigated using samples collected on Signy Island in 1983. Increased supercooling ability and potential cryoprotectant concentrations, and decreased survival rates were associated with low ($\leqslant -10°C$) temperatures. Both algal and distilled water diets resulted in a substantial loss of supercooling ability at 5°C. Hypotheses concerning the role of water and ice nucleators in this species are discussed in relation to these findings.

INTRODUCTION

Cryptopygus antarcticus Willem (Collembola: Isotomidae) is the largest and most abundant arthropod of bryophyte-dominated communities on Signy Island (60° 43′ S, 45° 38′ W), in the maritime Antarctic (Tilbrook, 1970; Block, 1982). *Cryptopygus* are commonly encountered in almost all terrestrial habitats (free of permanent snow and ice cover) on Signy Island; from exposed Antarctic fellfields (dominated by lichen–moss (*Usnea–Andreaea*) association) to nutrient-rich ornithogenic sites, i.e. close to penguin rookeries.

Collembola are generally considered non-selective feeders, but field-collected *Cryptopygus* showed a degree of selectivity in that living moss was virtually absent from their guts (Burn, 1984). Unicellular green algae, dead moss, fungal hyphae and mineral particles constituted the majority of identifiable food items; however, unidentifiable material was the most frequent component (Burn, 1984). A clear correlation between microscopically visible gut contents and supercooling ability was demonstrated for the collembolan *Tetracanthella wahlgreni* Linnaniemi (Sømme and Conradi-Larsen, 1977), and from this and other studies on micro-arthropods (e.g. Block and Zettel, 1980; Block and Sømme, 1982; Sømme and Block, 1982), it has emerged that cold hardiness is built up in a two-step process. First, food residues (containing potential ice nucleators) are eliminated from the gut, and then cryoprotective compounds (e.g. polyhydric alcohols such as glycerol) are accumulated. Both of these processes occur in response to low-temperature cues (Young and Block, 1980). This hypothesis is supported by field observations, which showed that feeding activity in *Cryptopygus* declines with the onset of subzero temperatures, and that the absence of gut contents in winter-collected specimens is correlated with enhanced low-temperature survival in the laboratory (Burn, 1981).

It was suggested that the increased supercooling ability of *Cryptopygus* individuals fed a diet of purified green algae resulted from the absence of nucleator material in this food, and that conversely, individuals fed a diet of moss turf homogenate showed poor supercooling ability as a result of the presence of efficient nucleators (Sømme and Block, 1982).

Water uptake through the ventral tube vesicles is a well-established phenomenon in Collembola (Noble-Nesbitt, 1963; Eisenbeis, 1982), and it has been demonstrated that compensatory water uptake, following artificially induced dehydration, can occur

very rapidly, i.e. within minutes (Verhoef and Witteveen, 1980). The finding, that winter-acclimatized *Cryptopygus* show an extensive loss of supercooling ability when given access to (distilled) water (Cannon and others, 1985), suggests an alternative hypothesis to that of 'gut-clearing'. Namely, that seasonal patterns in cold hardiness of *Cryptopygus* result from changes in the activity of (unknown) ice nucleators, which though they are always present within the organism, are activated and de-activated by alterations in the distribution and quantity of freezable water pools, i.e. bulk versus bound water (Storey and others, 1981; Baust and Zachariassen, 1983; Storey, 1983).

The aims of these experiments were to test certain assumptions underlying these hypotheses, especially concerning cold hardiness and survival, and the role of diet and water availability in cold hardiness.

Materials and Methods

Field-fresh (ff) *Cryptopygus*, of mixed instars, were collected from beneath flat rocks, downslope from a penguin rookery on the Gourlay Peninsula of Signy Island (see Cannon and others, 1985). The insects were transported in dry containers, and placed at 5 ± 2°C for sorting. Supercooling point (SCP) and liveweight determinations were made within 2–3 h of collection. Individual supercooling points were measured by attaching the insects (using a thin film of grease) to fine Cu-con thermocouples, and recording the electrical outputs on a 6-channel, mains-operated Linseis L 2001 chart recorder. A continuous measurement of body temperatures enabled the determination of the initiation of the SCP exotherm, which is caused by the release of latent heat of fusion when the animal freezes. A cooling rate of *c.* 1 deg min^{-1} was obtained using the freezing-mixture method of Block and Sømme (1982).

To investigate *Cryptopygus* survival under controlled temperature (CT) conditions, the insects were taken from 5°C and immediately placed in the following temperatures: 5, 0, −5, −10, −15 and −20°C. Each survival sample consisted of five batches of 50 individuals, and the exposure durations used were 2, 7 and 14 days. To assess survival, the insects were kept for 72 h at 5°C in saturated conditions, after removal from the acclimation experiment. Only those individuals which showed normal locomotory movements after this recovery period were classed as survivors.

To determine the concentrations of potential cryoprotectant (i.e. antifreeze) compounds, *c.* 2–3 mg samples comprising 50–100 live insects of mixed sizes were weighed and then macerated in 70% ethanol. The extracts were stored and transported at −20°C to the B.A.S. (Cambridge), where they were analysed by gas–liquid chromatography (Block and Sømme, 1982).

In the survival and acclimation experiments, the insects were placed inside dry, 2-dram glass vials which had mesh-covered ventilation holes in their tops to allow gaseous exchange. The vials were placed inside 500 ml screw-top glass jars which contained wetted filter paper to provide a saturated (RH 100%) atmosphere throughout. In the feeding and water experiments, the insects were placed inside 25-ml plastic containers containing shreds of nylon mesh, which reduced the surface tension of the water in those treatments where it was available to the insects. These containers also had mesh-covered ventilation holes, and were likewise placed inside screw-top glass jars to provide a saturated atmosphere. In the feeding experiment, a sample of *Cryptopygus* collected on 15 August 1983 was used for four separate treatments; (A) field-fresh SCP were determined; (B) the insects were given access to double-distilled water (only) for 15 days before SCP were determined; (C) the insects were given access to unwashed (but moist) algal thallus, *Prasiola crispa* (Lightf.) Menegh, for 15 days before SCP were determined; (D) the insects had no access to liquid water, and all of them died within the 15-day period.

In those treatments where the insects were in contact with liquid water, care was taken to exclude the possibility of inoculative freezing in subsequent SCP determinations. The insects were individually removed from the treatment containers, and given a short (*c.* 10 min) period on dry filter paper before being attached to the thermocouples.

Smoothed frequency distributions of the SCP data were plotted using three-point running means (after Salt, 1970) with individual SCP summed over one deg intervals. One effect of such running means is to extend the upper and lower extremes of a distribution. Mean ($\pm$SD) and median (M) SCP were calculated for each entire distribution, i.e. there was no division into high and low groups (see Cannon and others, 1985). One-way analysis of variance was used to test SCP data and cryoprotectant data for significance. Where more than two treatments were involved, the sum of squares simultaneous test procedure (SS–STP) (Sokal and Rohlf, 1969) was used to test for differences between sets of means. The survival data were subjected to a two-way analysis of variance.

Results and Discussion

Survival experiments

The results (Fig. 1), given in terms of mean ($\pm$SD) percentage survival, showed different patterns for the two collection dates, i.e. experiments A and B. Although only eighteen days separated these two winter field samples, the supercooling potential of the two batches of insects was very different. Whereas on 23 August 1983 (A), 48% had SCP less than -20°C (median SCP $= -19.6$°C), on 5 August 1983 (B), only 8% of the insects had SCP below -20°C (median SCP $= -11.7$°C). These differences in supercooling potential may have resulted from contrasting ambient field temperatures during the periods immediately prior to the two collection dates, i.e. there was a brief thaw on 4 August 1983 (Table I).

In experiment A the survival values were highly significantly different between the two treatments (2 and 14 days) ($F = 34.0$; $P < 0.001$), and also between acclimation temperatures ($F = 9.6$; $P < 0.001$). However, there was no significant interaction between the acclimation temperature and the duration of exposure ($F = 1.1$;

Table I. 24-hourly maximum and minimum screen air temperatures (°C), for August 1983. Signy Island synoptic meteorological station.

Date	*Maximum*	*Minimum*	*Date*	*Maximum*	*Minimum*
1	2.6	−0.3	16	−5.3	−16.1
2	2.4	−0.3	17	−9.5	−16.3
3	2.4	−3.4	18	−0.8	−10.1
4	3.9	0.8	19	−0.2	−2.5
5	3.0	−0.8	20	−0.3	−12.0
6	0.5	−8.5	21	−11.0	−14.9
7	−3.6	−11.2	22	−4.7	−13.2
8	1.3	−1.6	23	−2.3	−7.6
9	0.8	−2.9	24	−3.4	−8.0
10	2.7	−0.3	25	−3.0	−8.4
11	3.0	−2.9	26	4.0	−8.9
12	3.1	−0.7	27	3.2	−1.1
13	2.1	−4.5	28	−0.8	−7.2
14	0.2	−5.1	29	−5.8	−12.3
15	−0.6	−9.0	30	−4.2	−15.4
			31	−0.7	−6.2

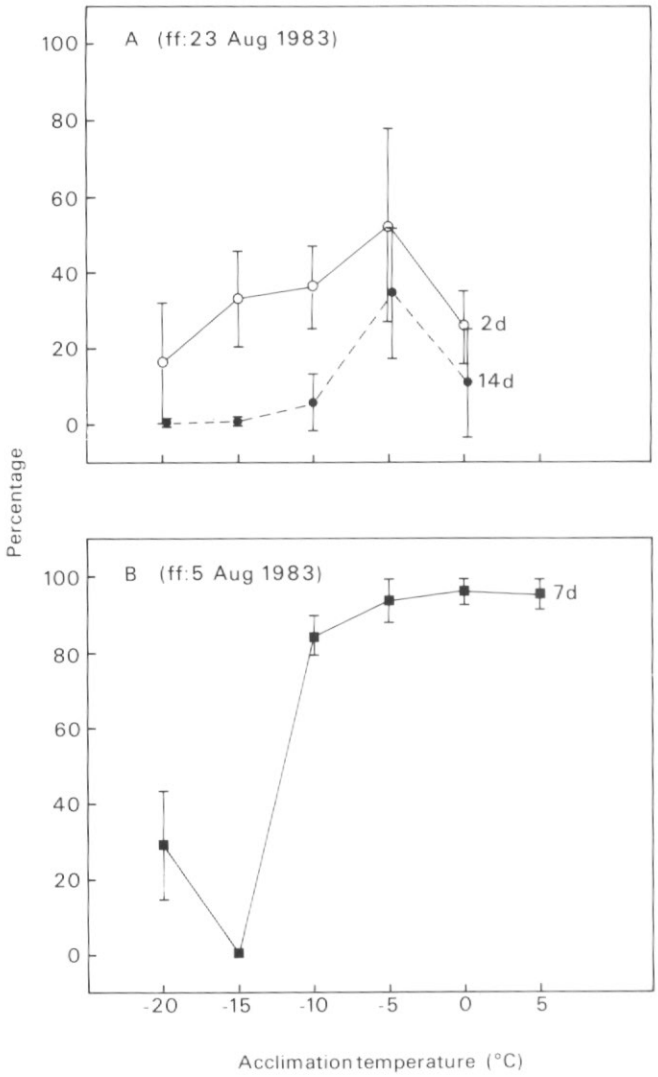

Fig. 1. Percentage survival rates ($\bar{x} \pm SD$) for *Cryptopygus antarcticus* under controlled acclimation temperatures in the range 5 to −20°C (A) Field-fresh (ff) on 23 August 1983 and given 2-day (○) and 14-day (●) treatments. (B) Field-fresh (ff) on 5 August 1983 and given 7-day treatment (■).

$P > 0.05$). In both the 2- and 14-day treatments the highest survival rates were at −5°C, which reflects the small proportion of insects with SCP below *c.* −10°C in the 23 August 1983 field sample. The low survival rates at 0°C are unexpected.

In experiment B, the survival rates remained high over the acclimation range 5 to −10°C, but had declined to zero at −15°C. It is not apparent why all of the insects died at this temperature, given a mean survival of 29% at −20°C, but this may have been caused by chance nucleation effects.

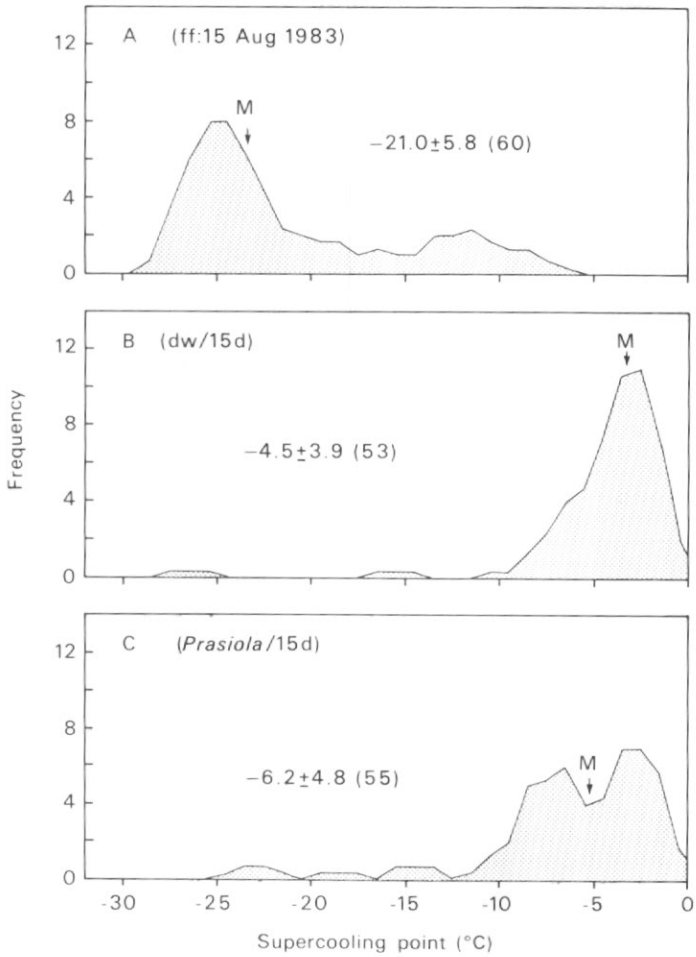

Fig. 2. Smoothed frequency curves, using 3-point running means, of supercooling point (SCP) data for *Cryptopygus antarcticus*. (A) Field fresh (ff) on 15 August 1983. (B) With access to double-distilled water (dw) (only) for 15 days. (C) With access to moist *Prasiola crispa* alga for 15 days. Figures refer to mean (±SD) SCP (*n*); M = median SCP.

Feeding experiment

Of the field-fresh insects collected on 15 August 1983, 68% exhibited SCP below −20 °C, with a mean SCP of −21.0°C (median SCP = −23.3°C) (Fig. 2A). However, after 15 days of treatment B the SCP distribution had altered, with the majority of insects now having SCP greater than −5°C, and with a mean SCP of −4.5°C (median = −3.3°C) (Fig. 2B). Although none of the insects in treatment D survived for comparison, these results are similar to those discovered in separate experiments using cold-hardy *Cryptopygus* (Cannon and others, 1975), i.e. a rapid loss of cold hardiness associated with water uptake. Where *Prasiola* alga was available for grazing (Fig. 2C), the response after 15 days was generally similar to that for the distilled-water treatments, i.e. the mean SCP was −6.2°C, although the mean SCP for the two treatments (i.e. B and C) is significantly different ($P < 0.05$). The suggestion of

bimodality (Fig. 2C) is due to a greater proportion of insects with SCP in the range −5 to −10°C, possibly as a consequence of having fed on alga. If this was the case, then the algal diet (deliberately unpurified in this experiment) is either not nucleator-free or, as in the case of the distilled-water treatment, it activated nucleators which have previously remained sequestered in the body under the cold, dry, field conditions experienced over winter.

A large proportion of SCP in treatments B and C (Fig. 2) were higher than −5°C, but there was only one individual with an SCP above −1°C, and the modal range was between −2 and −3°C. Supercooling points as high as −1°C have been reported previously for *Cryptopygus* (Schenker, 1984; Cannon and others, 1985) and the loss of supercooling potential associated with hydration (water uptake) has been shown in locusts; hydrated *Schistocerca gregaria* Forskal showed a mean (±SD) SCP of −1.7±0.3°C (Cloudsley Thompson, 1973); and ladybird beetles (Baust and Morrissey, 1975). The mean supercooling points of field-fresh insects can also be greater than −5°C. For example, adult beetles, *Perimylops antarcticus* Muller 1884, from the sub-Antarctic island of South Georgia had mean SCP of −3.6°C (Block and Sømme 1983).

Although the body-fluid melting points for *Cryptopygus* were not ascertained in these experiments, they were probably similar to those for alpine Collembola (seven species), which showed mean melting points of *c.* −0.5°C when not cold-hardened (Zettel, 1984).

An alternative explanation for these extreme changes in cold hardiness in the laboratory might be that bacteria, one species of which has been shown to possess ice nucleator activity (Maki and others, 1974; Vali and others, 1976), are developing in those treatments where moisture was present. Further investigations into the possible effects of bacteria and other micro-organisms on the cold-hardiness of micro-arthropods such as *Cryptopygus* are required to resolve this issue.

Acclimation experiment

The SCP distribution for field-fresh insects on 5 August 1983 was essentially unimodal, with 91% of the SCP occurring between −6 and −16°C (Fig. 3A). The effects of the seven-day acclimations at 5, 0 and −5°C were slight, with no significant change ($F = 0.8$; $P > 0.05$) in supercooling potential occurring. However, the SCP distributions (B, C and D in Fig. 3) exhibit the same characteristic form, i.e. skewed to the right with a long tail extending towards −25°C. Although the mean (and median) SCP for the 5, 0 and −5°C treatments show slight downward trends (Fig. 3), the duration of the exposures was either too short or the temperatures were too high to induce significant effects ($P > 0.05$). When SCP data from the −10°C treatment are included with that from the other treatments in an overall ANOVA test, there is a highly significant ($F = 7.5$; $P < 0.001$) added variance component among acclimation treatments, for supercooling ability. In addition, the −10°C treatment improved the supercooling ability of the insects more than any other treatment.

Although it is possible that the significant effect of the −10°C treatment (Fig. 3E) occurred in part because of selective mortalities, i.e. of insects with SCP in the upper range of the field-fresh distribution (Fig. 3A), it is evident that an overall lowering of SCP also occurred.

The effects of these acclimation treatments, plus that of another at −20°C, on the concentration of potential cryoprotectant compounds are presented in Table II. A measure of the total potential cryoprotection provided by the range of antifreeze compounds present in an organism, termed the hydroxyl equivalent (E^{OH}), was

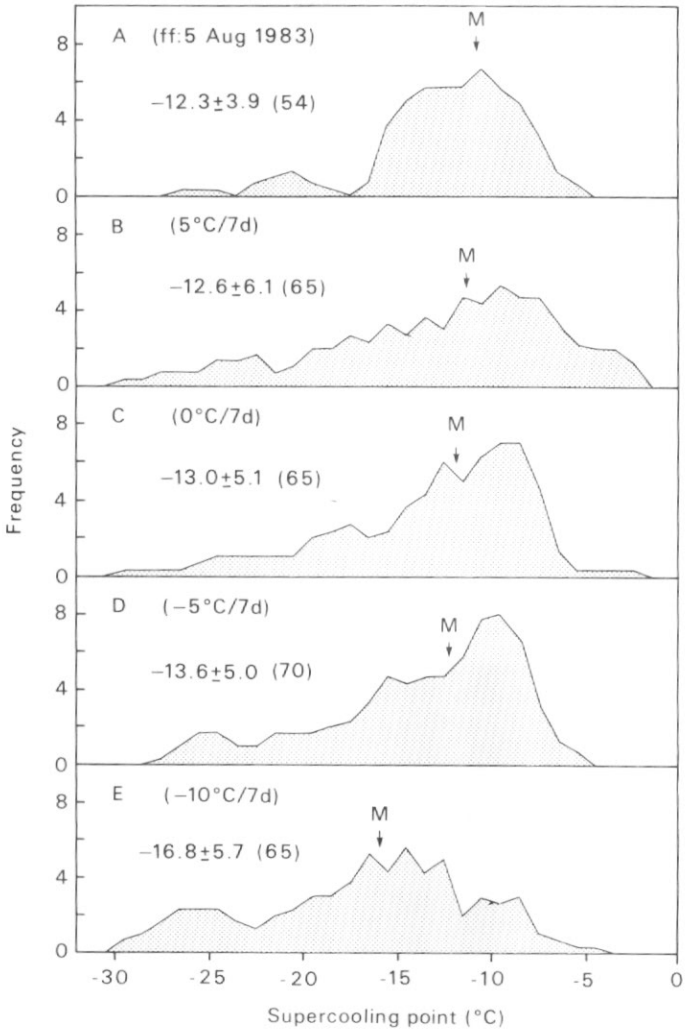

Fig. 3. Smoothed frequency curves, using 3-point running means, of supercooling point (SCP) data for *Cryptopygus antarcticus* after 7-day acclimation experiments under controlled temperatures: (A) Field-fresh (ff) on 5 August 1983; (B) 5°C; (C) 0°C; (D) −5°C; (E) −10°C. Figures refer to mean (±SD) SCP (*n*); M = median SCP.

formulated by Baust and Lee (1983). The E^{OH} values (Table II), are summations per sample of the concentration of each chemical compound (e.g. glycerol) in $\mu g\ mg^{-1}$ fresh weight, multiplied by the number of hydroxyl (OH^-) groups contributed by that particular molecule. In this case the E^{OH} values have not been corrected for changes in water content, but given the saturated conditions of the treatments these would not vary greatly. Over the five acclimation treatments, i.e. from field-fresh to −10°C, there is a highly significant ($F = 14.3$; $P < 0.001$) effect on total cryoprotection (E^{OH}), with increasingly higher values appearing at lower acclimation temperatures. Significant differences (at the 5% level) between individual treatment means are indicated by different lines underscoring the treatments (Table II). These results show that under these particular experimental conditions, temperature differences of 10 deg

Table II. Mean concentrations (in $\mu g\ mg^{-1}$ fresh weight) of potential cryoprotectant compounds for *Cryptopygus antarcticus* in a 7-day acclimation experiment (see Fig. 3). $n = 3$ samples per acclimation temperature, except for $-20°C$ where $n = 1$. *figure in parentheses is the number of OH^- groups per compound; TR = trace amounts ($< 0.1\ \mu g\ mg^{-1}$ fresh weight); E^{OH} = hydroxyl equivalent (see text for explanation); ff = field fresh on 5 August 1983. Treatment lines underscored by the same line are not significantly different at the 5% level.

	Treatment (acclimation temperature, °C)					
*Compound**	*A (ff)*	*B* (5)	*C* (0)	*D* (−5)	*E* (−10)	*F* (−20)
Glycerol (3)	0.1	0.4	0.3	0.2	0.2	0.2
Mannitol (6)	TR	0.2	0.9	1.1	1.4	22.8
Sorbitol (6)	0.1	0.2	0.4	0.3	0.5	2.2
Myo-inositol (6)	TR	0.7	0.4	0.1	0.7	1.0
Fructose (5)	1.7	0.8	4.0	2.8	9.2	62.3
Glucose (5)	5.3	6.6	7.1	10.9	7.9	5.2
Trehalose (8)	1.1	1.8	1.9	2.3	3.1	3.4
E^{OH} ($\bar{x} \pm SD$)	46.8 ± 22.9	59.2 ± 4.5	81.4 ± 7.0	96.9 ± 18.7	126.0 ± 9.2	521.3

are required for differences in cryoprotective compounds to be significant ($P < 0.05$). The $-20°C$ treatment is excluded from this analysis because, due to insufficient survivors, only a single sample was taken. Nonetheless, this $-20°C$ result indicates that the trend towards increasing cryoprotectant build-up is expressed using a fixed time period, i.e. seven days, in this experiment. However, if samples from the same treatment temperature were taken regularly over time, a similar cryoprotectant build-up might be expected, especially at subzero temperatures. In other words, cryoprotection would show a (species-specific) time–temperature interaction pattern, in much the same way that supercooling does (Salt, 1966*a*, *b*). The high concentrations of mannitol and fructose (Table II) which occur in the $-20°C$ sample suggest that synthesis of these compounds might follow a non-linear course in *Cryptopygus*, with decreasing temperatures. Evidence (R. J. C. Cannon, unpublished) from field samples collected regularly throughout the year on Signy Island suggests that high concentrations of certain polyols and sugars (e.g. sorbitol, mannitol and fructose) may be synthesized (and broken down) rapidly in *Cryptopygus*.

The results from these experiments (Table II) differ from those of similar (though mostly longer) experiments on *Cryptopygus* reported by Sømme and Block (1982), particularly with regard to glycerol concentration. The paucity of glycerol in the present experiments is nevertheless consistent with the results from field samples collected during the period March 1983 to March 1984 (above).

Water availability experiment

In this experiment 94% of field-fresh insects collected on 27 October 1983 showed SCP greater than $-20°C$, with a mean SCP of $-9.8°C$ (Fig. 4A). After five days without access to food or liquid water, the mean SCP had shifted downwards by 3.3 deg ($P < 0.001$) to $-13.1°C$ (Fig. 4B). The proportion of insects with SCP below $-20°C$ also increased slightly, to 15%. After five days with access only to distilled water there was a small but significant ($P < 0.05$) decrease in SCP, with the mean SCP

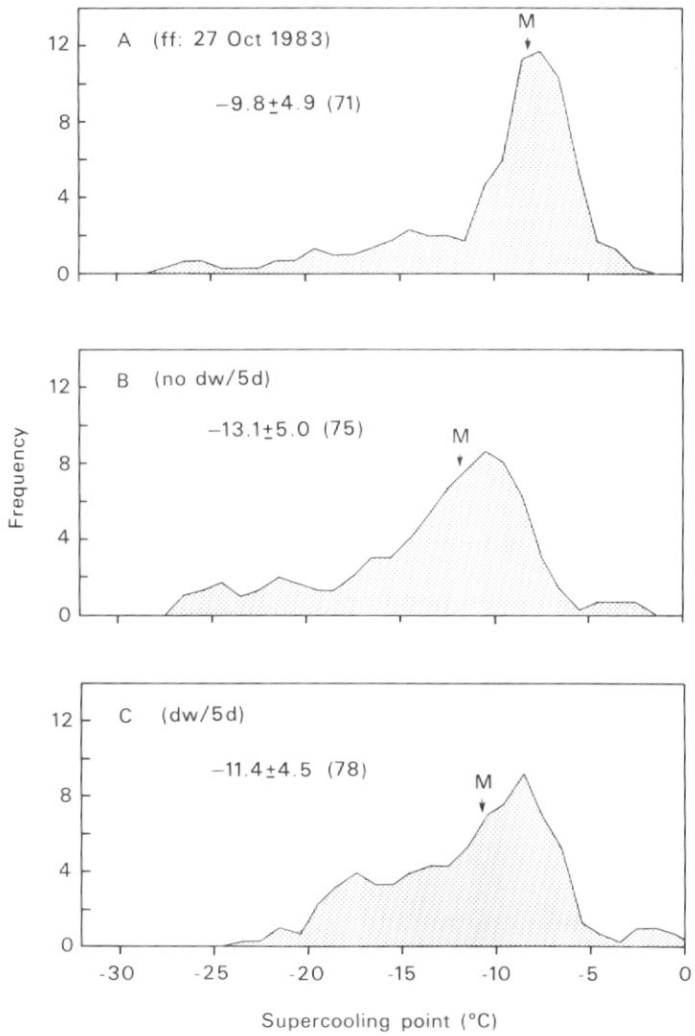

Fig. 4. Smoothed frequency curves, using 3-point running means, of supercooling point (SCP) data for *Cryptopygus antarcticus* at 5°C. (A) Field-fresh (ff) on 27 October 1983. (B) With no access to liquid water (no dw) for 5 days. (C) With access to double-distilled water (dw) (only) for 5 days. Figures refer to mean (±SD) SCP (n); M = median SCP.

at −11.4°C (Fig. 4C). The proportion of insects with SCP below −20°C was very small (4%) after this treatment. The mean SCPs for the two treatments (B and C) were significantly different ($P < 0.05$).

The effects of the three treatments on the concentrations of potential cryoprotectants were varied (Table III). Once again the hydroxyl equivalent index (E^{OH}) illustrates that both of the 5-day treatments (B and C) produced a significant ($P < 0.05$) increase in potential antifreeze levels. The increase was greatest in treatment B, where no liquid water was available to the insects, and this is largely due to an increase in the concentration of trehalose (Table III). Differences in body water concentrations may

Table III. Mean concentrations (in $\mu g\ mg^{-1}$ fresh weight) of potential cryoprotectant compounds for *Cryptopygus antarcticus* in a 5-day water availability experiment (see Fig. 4). $n = 3$ samples per treatment, except for B where $n = 2$. Treatment means underscored by different lines are significantly different at the 5% level. ff = field-fresh; dw = distilled water; d = day.

*Compound**	*ff* (27 *Oct* 1983)	*no dw*/5 *d*	*dw only*/5 *d*
Glycerol (3)	0.4	0.7	0.6
Mannitol (6)	0	1.0	1.0
Sorbitol (6)	0	0.7	0.7
Myo-inositol (6)	0.4	0	0.4
Fructose (5)	0	0	TR
Glucose (5)	1.8	3.9	1.7
Trehalose (8)	0.4	8.1	2.3
E^{OH^-} ($\bar{x} \pm SD$)	15.4 ± 1.7	89.9 ± 12.5	40.9 ± 10.0

have influenced cryoprotectant levels between treatments B and C, although these a[illegible] unlikely to have differed by a factor of two.

The relatively small improvements in supercooling ability in treatments B and C may have been due to experimental conditions. Sømme and Block (1982) showed that individually starved *Cryptopygus* exhibited a greater proportion of SCP $\leqslant -15°C$ than those stored in bulk, as in these experiments, and this was thought to have occurred because the insects stored in bulk could consume shed exuviae and frass. However, when cold-hardened *Cryptopygus* were stored in bulk in the absence of available distilled water, the SCP remained low (mostly $< -20°C$) (Cannon and others, 1985).

Conclusions

In the 5-day distilled-water treatment (Fig. 4C) there were relatively few insects with SCP above $-5°C$, whereas in the 15-day distilled-water treatment (Fig. 2B) the majority of SCP were above $-5°C$. Although recent experiments (G. D. Collett, pers. comm.) have confirmed the time-dependence of this effect (i.e. the loss of supercooling potential associated with water uptake), five days was sufficient time for the process to occur at 5°C. A possible alternative explanation involves the field-fresh condition of the insects. Those collected on 15 August 1983 (Fig. 2A) were cold-hardened and (mostly) non-feeding, whereas those collected on 27 October 1983 (Fig. 4A) were n[illegible] cold-hardened and (presumably) feeding. Why this effect should depend on the insects being in a cold-hardy winter condition is not clear, but it may be related to the internal distribution of body water.

It should be emphasized that in the present experiments (and those of Cannon and others, 1985), the insects were not maintained floating on the surface of distilled water (a relatively impenetrable barrier), but were given water distributed as a thin film on shreds of nylon mesh. This factor, together with the requirement for cold-hardened field-fresh insects, may explain the novelty of the phenomenon shown in Fig. 2.

The problem of whether decreased supercooling ability is associated with the uptake of water or the intake of (gut) nucleators (including bacteria), or to a combination of both, is almost impossible to unravel under field conditions, where both these processes may occur simultaneously. In effect, these phenomenon may have been simulated in the feeding experiment, where the differences between the effects of distilled water and alga diets on supercooling ability were minor. However, it has been

demonstrated experimentally that the availability of distilled water alone does result in such effects (Cannon and others, 1985), but until more is known about the location and activity of biological ice-nucleating agents, this issue is unresolved.

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