Polar Biol (1998) 19: 250–256

ORIGINAL PAPER

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Profiles of soluble carbohydrates and their adaptive role in maritime Antarctic terrestrial arthropods

Received: 28 February 1997 / Accepted: 20 October 1997

Abstract The existence of seasonal changes in concentrations of water-soluble carbohydrates in arthropods (both freezing-tolerant and intolerant species) from Signy Island was demonstrated. Seasonal patterns of variation, imposed by seasonality of the maritime Antarctic environment, in the production of soluble carbohydrates in response to low temperatures and/or dehydration for a range of terrestrial arthropods were confirmed. The freshwater copepod *Pseudoboeckella poppei* exhibited much lower levels of soluble carbohydrates, with glycerol as the main component, and smaller seasonal fluctuations relative to the four terrestrial species. The two Antarctic mites (*Alaskozetes antarcticus* and *Gamasellus racovitzai*) accumulated glycerol (as a single-component cryoprotective system), in agreement with previous work reporting increased glycerol levels and lowering of the supercooling point in *A. antarcticus*. In the case of *G. racovitzai*, increased levels of glycerol may function in a different manner. The larval dipteran *Eretmoptera murphyi* and the collembolan *Cryptopygus antarcticus* have complex multi-component cryoprotective systems involving trehalose that may be related to low temperature acclimation and dehydration. These findings are discussed in relation to published work on single and multiple cryoprotective systems, supercooling points and the involvement of dehydration as a complementary stress in overwintering insects.

Introduction

The Antarctic ice-free ground represents 0.33% of conterminous Antarctica (Fox and Cooper 1994) and includes habitats at the limit for biological colonization and survival by animal and plant communities. For the purpose of biological investigations the Antarctic region is conventionally divided into three major biozones: sub, maritime and continental Antarctic (Holdgate 1977). The maritime zone is generally wetter than continental Antarctica, due to the oceanic influence, and supports a more diverse flora and fauna. Terrestrial communities contain a restricted number of arthropods: Acari, Collembola and two species of Diptera (see review by Convey and Block 1996). Survival of long periods of sub-zero temperatures, as well as freeze-thaw events, is necessary for all terrestrial biota in the maritime Antarctic (see Walton 1984; Block 1997). There exists widespread agreement in classifying strategies of insect cold tolerance as freezing tolerant or intolerant depending on the ability to survive extracellular ice formation (Bale 1987; Cannon and Block 1988; Block 1990, 1991). With the exception of the two species of Diptera, the majority of Antarctic arthropods are freezing intolerant, and capable of considerable depression of the supercooling point to temperatures below those experienced in field microhabitats (Cannon and Block 1988). Alternative strategies, such as cryptobiosis (often in the form of anhydrobiosis), may be used by micro-invertebrates and have been identified in three tardigrades from continental Antarctica (Sømme and Meier 1995) and maritime Antarctic nematodes (Pickup and Rothery 1991). Survival of intracellular freezing has been reported for the continental Antarctic nematode *Panagrolaimus davidi* (Wharton and Ferns 1995).

As in the continental Antarctic, the ability of species to overwinter in all or most life stages is widespread in the maritime Antarctic. That is the case for the widely distributed oribatid mite *Alaskozetes antarcticus* and the collembolan *Cryptopygus antarcticus*. Other species, such as the Diptera, show limited overwintering abilities (see review by Convey 1996; Convey and Block 1996). Seasonal variation in cold tolerance, partly related to variation in the concentration of potential cryoprotectants, has been shown in field samples of the freeze-intolerant *A. antarcticus*, *Halozetes belgicae* and *C. antarcticus* (Block 1980; Lee and Baust 1981; Cannon

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and Schenker 1985, Block 1997). Also, variation in summer cold-hardiness in *A. antarcticus* was linked to feeding activity and habitat food resources (Shimada et al. 1992). Not all species show these responses to carbohydrate levels: for example, the Antarctic mite *Gamasellus racovitzai* exhibited little variation in supercooling ability during starvation and temperature acclimation (Block and Sømme 1982), while *Belgica antarctica* showed seasonal variation in carbohydrates but no change in cold tolerance (Baust 1982; Baust and Lee 1983). Similarly, although carbohydrate levels varied seasonally in larvae of the chironomid *Erepmoptera murphyi*, these were assumed to be too low to contribute significantly to cold tolerance (Block et al. 1984).

Cryoprotection in Antarctic arthropods involves both single (glycerol or trehalose) and multi-component systems, in some cases acting in combination with other physiological mechanisms such as clearance of gut contents (to reduce potential ice nucleators) and partial dehydration (Burn 1984; Cannon and Block 1988; Block 1996; Block 1997). The metabolic costs of overwintering in Antarctic environments may be critical to survival. Polyol biosynthesis offers the metabolic advantage of preserving the total carbohydrate pool of the animal when triose (or hexose) cryoprotectants are synthesized by interconversion from stored glycogen (reviewed by Storey and Storey 1991). Variation in concentrations of specific compounds as a result of seasonal (or experimental) changes may not be solely cryoprotective and needs careful assessment. Although cryoprotection by polyols is widespread in arthropods, it is not necessarily a specific temperature response since it has also been associated with other stresses such as diapause and drought stress (see Pullin 1994) and perturbation by anoxia in larvae of the goldenrod gall fly *Eurosta solidagnis* (Storey and Storey 1990). Secondly, protection against freezing (and associated dehydration) involves several groups of compounds (carbohydrates, proteins, end-products of anaerobic metabolism), acting via colligative and non-colligative effects. This paper examines seasonal patterns of water-soluble carbohydrates in arthropods (both freezing tolerant and intolerant) from Signy Island (60°43′S, 45°38′W), maritime Antarctic.

Materials and methods

Samples of live arthropods were collected at Signy Island, mostly from Pageant Point, Gourlay Peninsula. *Eretmoptera murphyi* was obtained near the BAS research station (Factory Cove) and *Pseudoboeckella poppei* was collected from Sombre and Heywood Lakes. The latter species was identified as *Pseudoboeckella poppei* but for a taxonomic discussion on Antarctic copepods see Hessen et al. (1989). Sample size for all species fluctuated between 2 and 15 mg fresh weight with a variable number of individuals per sample. Table 1 summarizes the sampling schedule followed to obtain samples from five arthropod species (with separate life stages) in order to evaluate their carbohydrate status under the contrasting environmental conditions of winter, spring and summer.

Sample preparation

Samples were extracted in cold 70% aqueous ethanol and stored at −20°C. Betaine was added as an internal standard (0.5 μmol per sample) during sample preparation to evaluate recovery; betaine was chosen because it is not detected with the column used for carbohydrate analysis. Extracts (5.0 cm^3 total volume) were transported to the United Kingdom stored at −20°C.

Sample fractionation

Following centrifugation (4,000 rpm, 2 min) the aqueous ethanolic supernatant was separated and dried by centrifugal evaporation. For analysis, samples were resuspended in 1.5 cm^3 deionized distilled water, loaded onto micro-centrifuge filter tubes (0.45 μm) and centrifuged (4,000 rpm, 3 min). An aliquot of the extract (100–300 μl) was filtered and fractionated for determination of soluble sugars. Fractionation was achieved by solid phase extraction. Preconditioned Bond Elut SCX ion-exchange columns (100 mg) were placed in a VacElut vacuum chamber (24 samples capacity), applying reduced pressure to draw liquid through the columns (Varian, Palo Alto, California, USA). SCX columns were pretreated with 2 cm^3 methanol followed by 2 cm^3 methanol in 0.1 M HCl and 2 cm^3 0.1 M HCl (Moodie et al. 1989). An aliquot of the extract was loaded and eluted with distilled, deionized water. The eluant was collected, dried by centrifugal evaporation, resuspended in water and used for analysis.

Chromatographic analysis of soluble carbohydrates

An automated gradient HPLC system (Kontron Instruments), comprising the ternary pump system 325, autosampler 360 and the DECADE (Digital Electrochemical Amperometric Detector, ANTEC, Wutford, UK, Leyden B.V., The Netherlands) was used. The DECADE operates an integrated oven and was used in pulse mode with a three-electrode VT-03 cell (wall-jet design) incorporating 316 stainless steel auxiliary (AE), solid Ag/AgCl reference (RE) and Au working (WE) electrodes. Oxidative detection was used with working potentials of (E_1) +0.05V (detection potential), (E_2) +0.65V, (E_3) −0.60V and pulse times (t_1) 240 ms, (t_2) 110 ms, (t_3) 110 ms and a sample time (t_s) of 40 ms. A 10-μl injection loop was used. Instrument control and data analysis were performed

Table 1 Arthropod species sampled during (winter, spring) 1993 and summer 1994 at Signy Island

Species	Winter (Jul 1993)	Spring (Nov 1993)	Late summer (Feb 1994)
Alaskozetes antarticus (Acari)	Nymphs/adults	Adults	Adults
Gamasellus racovitzai (Acari)	Deutonymphs/adults	Adults	Adults
Eretmoptera murphyi (Diptera)	Larvae	Adults	Adults
Cryptopygus antarcticus (Collembola)	Mixed [a]	Mixed	Mixed
Pseudoboeckella spp. (Copepoda)	Adults	Adults	Adults

[a] Mixed instars

with the multitasking Kontron Data System 450-MT2. On-line degassing was provided by continuous vacuum filtration of up to three solvents with a metal-free degasser. Additionally, helium sparging and pressurizing of the mobile phases were available.

The analytical column used for carbohydrate analysis was a CarboPac MA1 analytical (4 × 240 mm) coupled with a guard column (4 × 50 mm, Dionex Corporation, Sunny dale, USA). Temperature was held constant at 30°C with flow rate of 0.4 cm^3 min^{-1}. For identification of unknown compounds, samples were run under four different programmes: isocratic at either 0.6 M, 0.48 M and 0.25 M NaOH for 35, 45 and 60 min respectively, and with a linear gradient from 0.25 to 0.70 M for 60 min. Retention times were compared with those from a library of pure standards. For routine analysis isocratic conditions were chosen (0.48 M NaOH) and mixtures of standards of known concentration (4 and 2 nmol injection) were prepared for each species and run together with the samples. Multipoint linear calibration was used for integration of the data. The routine running of standard mixtures between batches of samples allowed evaluation of column (drifting) and detector (sensitivity) performances. The use of electrochemical detection provided an extremely sensitive detection range (sub-nmol to nmol range) so that only a small aliquot from each 1.5-cm^3 sample (1:75 for all species with the exception of *Pseudoboeckella poppei*, 1:25) was required.

Evaluation of recovery of the internal standard betaine was performed with an AminoPac PA1 analytical (4 × 240 mm) coupled with a guard column (4 × 50 mm, Dionex Corporation) and electrochemical detection. Temperature was kept at 30°C with flow rate of 0.6 cm^3 min^{-1}. Elution was performed with degassed mobile phases: (i) 40 mM NaOH and 8 mM $Na_2B_4O_7$ (initial 15 min); (ii) 40 mM NaOH and 400 mM sodium acetate (15–50 min). The column was reconstituted after each run with 560 mM NaOH and 640 mM H_3BO_3 for 8 min. DECADE conditions were: E_1 +0.25V (detection potential), E_2 +0.85V, E_3 −0.65V; t_1 300ms, t_2 130 ms, t_3 120 ms, t_s 60 ms.

Gas chromatography (Hewlett Packard Gas Chromatograph Model 5890, Bracknell, UK) of water-soluble extracts was also performed to obtain confirmation of unknown compounds. The samples were initially mixed with methanol and dried under N_2 gas. The dry sample was resuspended in Sigmasil derivatizing agent (a mixture of trimethylchlorosilane, hexamethyldisilazane and pyridine in a 1:3:9 ratio for the preparation of trimethylsilyl derivatives; Sigma, Poole, UK). The derivatized sample (1 µl) was injected and separated with an Ultra 2 Hewlett Packard column (25 m, cross-linked with 5% PhMe silicone), with temperature ramping from 100 to 300°C over 1h. Data acquisition and processing were performed with the Kontron Data System 450-MT2.

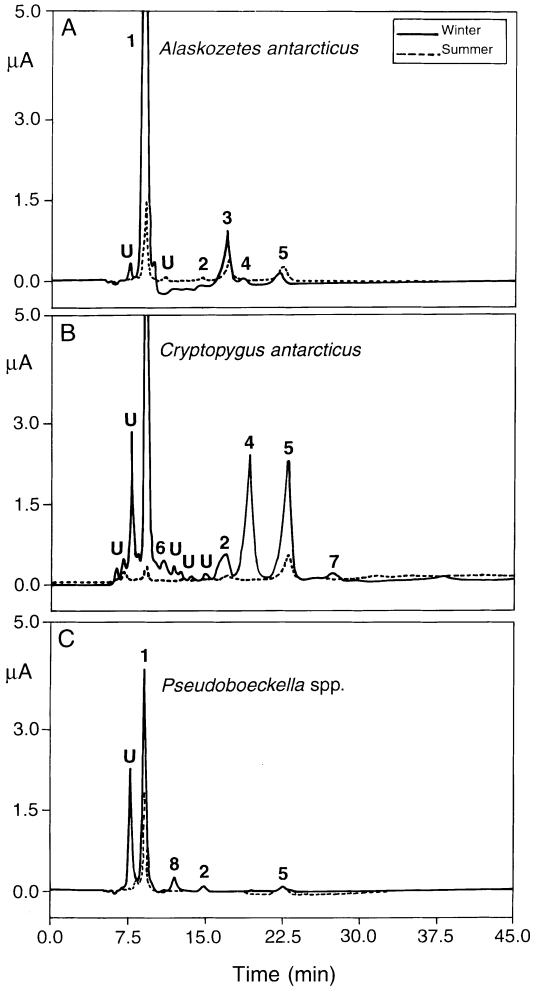

Fig. 1A–C HPLC chromatograms (overlay plot) from samples collected in winter (July 1993) and summer (February 1994) illustrating the seasonal change in the concentrations of soluble carbohydrates. **A** *Alaskozetes antarcticus*. **B** *Cryptopygus antarcticus*. **C** *Pseudoboeckella poppei*. (*1* glycerol, *2* arabitol, *3* ribitol, *4* mannitol, *5* glucose, *6* erythritol, *7* fructose, *8* fucitol, *U* unknown). DECADE detector range 5 µA

Results

Overlay chromatograms of a winter and a summer sample (similar fresh weights and dilution factor) are given in order to illustrate any observed differences in *A. antarcticus*, *C. antarcticus* and *Pseudoboeckella* spp. (Fig 1). Data are presented separately for each of the five species (Figs. 2–4). Seasonal changes are presented as histogram plots giving mean concentrations (and standard errors) for individual compounds for winter, spring and summer sets of samples. For each species, data were analysed statistically by single factor analysis of variance (ANOVA); statistical significance for individual compounds is included (Figs. 2–4), as well as for total soluble carbohydrates (Table 2).

The total concentrations of soluble carbohydrates were calculated from the data on individual compounds for all five species and are presented for winter, spring and summer (Table 2).

Terrestrial mites

Glycerol, fructose, glucose and ribitol were detected in *A. antarcticus* (Fig. 2A,B) in samples of adults and nymphs throughout the study, as well as trace amounts of arabitol and mannitol (possibly of plant origin). In the case of *Gamasellus racovitzai* (Fig. 3A,B) glycerol, glucose and trehalose were found in both adults and deutonymphs throughout the period, plus trace amounts of arabitol and erythritol (again these may be of plant origin). In both species, and for adults and nymphs, glycerol was the major soluble carbohydrate constituent, and also exhibited a marked seasonal fluctuation with significantly increased levels in winter (Figs. 2B,3B).

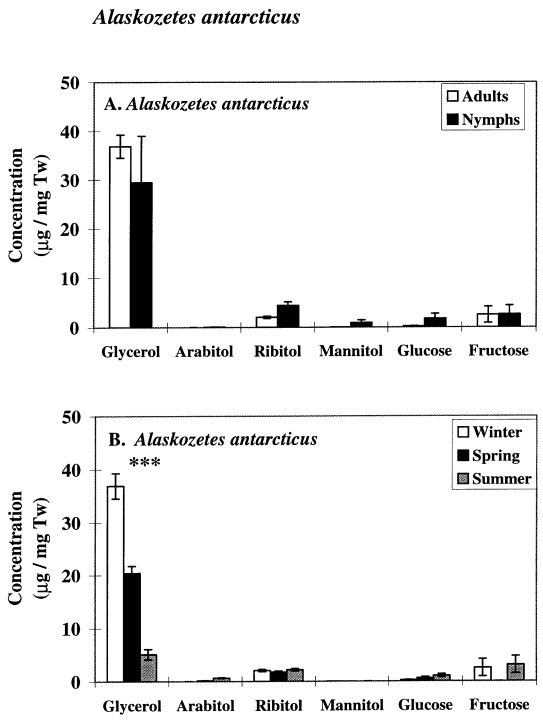

Fig. 2A,B *Alaskozetes antarcticus*. Mean concentrations (µg mg^{-1} total weight ± 1 SE) of soluble carbohydrates in: **A** winter samples of adults ($n = 24$) and nymphs ($n = 10$); **B** seasonal change of individual compounds in (adults) samples collected in winter ($n = 24$), spring ($n = 4$) and summer ($n = 11$). Statistical significance (single-factor ANOVA) for individual compounds is indicated (***$P < 0.001$)

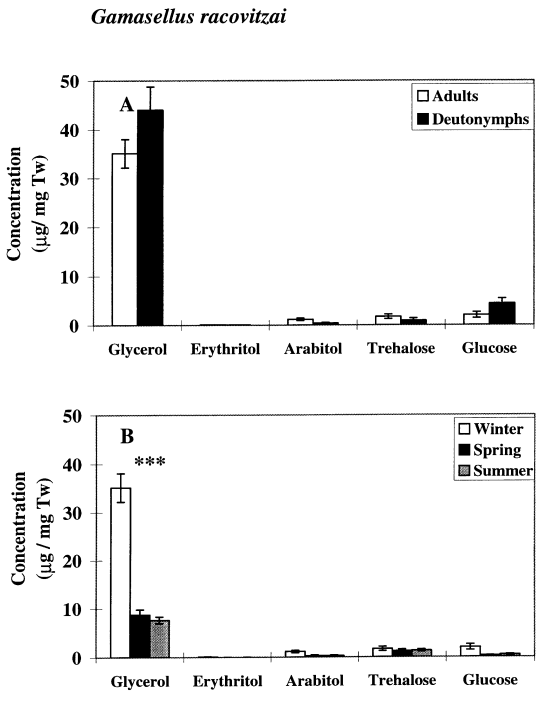

Fig. 3A,B *Gamasellus racovitzai*. Mean concentrations (µg mg^{-1} total weight ± 1 SE) of soluble carbohydrates in: **A** winter samples of adults ($n = 13$) and deutonymphs ($n = 13$); **B** seasonal change of individual compounds in (adults) samples collected in winter ($n = 13$), spring ($n = 8$) and summer ($n = 8$). Statistical significance (single-factor ANOVA) for individual compounds is indicated (***$P < 0.001$)

Dipteran species

Eretmoptera murphyi

A range of compounds were identified in samples of this dipteran, which were similar in larvae and adults (Fig. 4A). Glycerol, trehalose, mannitol and fructose were the main constituents. The presence of small amounts of sorbitol was also detected (possibly of plant origin). Seasonal fluctuations of the main constituents were marked, with significantly increased levels in larvae during winter. Trehalose, mannitol and fructose were present in high concentrations in winter and spring but virtually disappeared in summer.

Collembolan species

Cryptopygus antarcticus

Similar to *Eretmoptera murphyi*, this collembolan produced a range of soluble carbohydrates, with glycerol, trehalose, mannitol, glucose and fructose as the main constituents and present thoughout the year (Fig. 4B), while traces of erythritol and arabitol were also detectable (possibly of plant origin). Increased concentrations of the main constituents in winter samples were again apparent.

Freshwater copepod species

Pseudoboeckella poppei

Much lower levels of soluble carbohydrates (relative to the terrestrial species) were found in this copepod. Glycerol, glucose and fructose were the main constituents with traces of fucitol, arabitol, trehalose and mannitol (Fig. 4C). Interestingly, increased levels of glycerol were observed both in winter and summer.

An important consideration when interpreting the data is the presence of trace amounts of particular carbohydrates in field samples that may be of plant origin since most of the micro-arthropods analysed consume plant tissues as part of their diet. Also, peaks for unknown compounds were detected (particularly in *C. antarcticus*) that merit further investigation. These may correspond to amino or organic acids; although the CarboPac MA1 column is optimized for monosaccharides and sugar alcohols, some amino acid standards were also separated and electrochemically detected (unpublished data).

Discussion

In all species studied, both terrestrial and freshwater, the concentrations of soluble carbohydrates were higher in

winter than in spring/summer (Figs. 2–4). All the terrestrial arthropods showed winter levels of carbohydrates around 3.8–5.3% of fresh weight while much lower concentrations were detected in the freshwater copepod *Pseudoboeckella poppei* (Fig. 4C). This difference may reflect an amelioration of the freshwater relative to the terrestrial environment, but there is very little information regarding the physiology/biochemistry of adaptation of Antarctic freshwater organisms. The presence of glycerol in *Pseudoboeckella poppei* as the main constituent may indicate a passive osmoticum role. The lower levels of soluble carbohydrates may be a reflection of a more stable physical environment, relative to terrestrial habitats (Block 1984; Clarke et al. 1989).

The observed seasonal fluctuations in carbohydrate levels of *A. antarcticus* (Fig. 2A, increasing over winter, lowered in spring and depleted in summer) are in close agreement with published data (Block and Sømme 1982; Block and Convey 1995), particularly regarding glycerol. Previous work at Signy Island identified glycerol, ribitol and glucose in this species, as well as seasonal fluctuations in concentration with an inverse relationship between glycerol and glucose levels (Block and Sømme 1982). It has been experimentally established that in *A. antarcticus* glycerol production is triggered by low temperature acclimation (and dehydration), but not by photoperiod (Young and Block 1980). Survival of low winter temperatures depends on avoidance of freezing by supercooling and by the use of antifreezes. It has been proposed that there exists a linear relationship between glycerol concentration and supercooling ability, particularly above −25°C (Block and Convey 1995). Glycerol levels similar to those observed in winter samples of adults and nymphs, ca. 30–40 μg mg^{-1} fresh weight (Fig. 2A), will depress the supercooling point to ca. −30°C (Block and Convey 1995), and these are clearly of survival value since temperatures in this range may be experienced in soil habitats overwinter (Walton 1982).

In the case of *G. racovitzai* (Fig. 3A) concentrations of soluble carbohydrates, with glycerol as the main constituent, were in a similar range to *A. antarcticus*. Glycerol levels were marginally higher in deutonymphs relative to adults in winter samples (Fig. 3A), and were significantly depressed in spring and summer. These data contrast with previous work at Signy Island (Block and Sømme 1982), which identified glucose as the main constituent in deutonymphs and adults; these authors also established that starvation and low temperature acclimation during summer did not affect supercooling of this species significantly. Examination of the data from Block and Sømme (1982) indicates that glucose was the main constituent in non-acclimated and summer field samples, but following acclimation to −5°C there

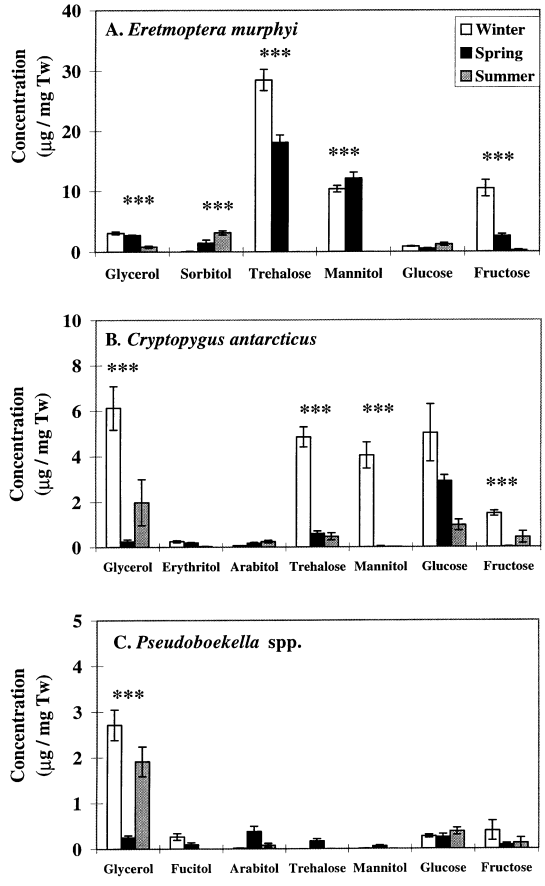

Fig. 4A–C Seasonal changes in concentrations (μg mg^{-1} total weight, mean ± 1 SE) of individual compounds of samples collected in winter, spring (1993) and summer (1994) of: **A** *Eretmoptera murphyii*; winter (larvae, $n = 24$), spring (adults, $n = 10$) and summer (adults, $n = 10$); **B** *Cryptopygus antarcticus*; winter ($n = 24$), spring ($n = 10$) and summer ($n = 10$); **C** *Pseudoboeckella* spp.; winter ($n = 24$), spring ($n = 9$) and summer ($n = 6$). Statistical significance (single-factor ANOVA) for individual compounds is indicated (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Table 2 Seasonal changes in concentrations (mean ± SE) of total soluble carbohydrates in Antarctic arthropods, at Signy Island (μg mg^{-1} total weight). Single factor analysis of variance was performed on the data for each species. Data set size and statistical significance are given

Species	Winter (Jul 1993)	Spring (Nov 1993)	Late summer (Feb 1994)	ANOVA
Alaskozetes antarcticus				
nymphs	39.23 (11.92)			
adults	41.67 (3.18)	22.86 (1.26)	12.08(1.82)	$F_{2,36}$ $P < 0.001$
Gamasellus racovitzai				
deutonymphs	49.72 (6.28)			
adults	40.03 (3.56)	10.50 (1.54)	9.69 (1.01)	$F_{2,26}$ $P < 0.001$
Eretmoptera murphyi[a]	53.29 (2.97)	37.52 (1.80)	5.35 (0.73)	$F_{2,41}$ $P < 0.001$
Cryptopygus antarcticus	37.76 (3.26)	4.29 (0.42)	7.14 (2.02)	$F_{2,34}$ $P < 0.001$
Pseudoboeckella poppei	3.66 (0.51)	1.28 (0.34)	2.48 (0.45)	$F_{2,36}$ $P < 0.05$

[a] Data for winter obtained from larvae

was an increase in glycerol levels in both deutonymph and adults, while glucose only increased in the former. Whether increased glycerol over winter plays a role in its survival in the natural habitat merits further investigation.

In larva and adults of the chironomid *Eretmoptera murphyi* the main constituents observed confirmed previous work at Signy Island on summer larvae, pupa and adult samples (Block et al. 1984), but the earlier work observed concentrations much lower than those reported here, particularly for winter and spring (although collection dates differ). However, the summer concentrations of carbohydrates in the present study are within the range of the earlier study. The larvae of the chironomids *Belgica antarctica* and *Eretmoptera murphyi* are known to be freeze-tolerant (see review by Convey and Block 1996). It has been found experimentally that following low temperature acclimation trehalose levels decreased while glycerol and glucose increased in *Eretmoptera murphyi* (Block et al. 1984). This contrasts with the present data (Fig. 4B), which show similar seasonal changes (a decline from winter to summer) for glycerol, trehalose, mannitol and fructose. These discrepancies may be attributable to differences between climatic and experimental cues. During seasonal acclimatization both low temperatures and desiccation are relevant factors, as has been shown for a sub-Antarctic population of *Eretmoptera murphyi* whose larvae have high body water content and low desiccation tolerance (Ring et al. 1990). In terms of desiccation tolerance *Eretmoptera murphyi* eggs are known to survive short periods of desiccation (Convey 1992). Trehalose (and proline) have been shown to accumulate in cold-acclimated larvae of the drosophilid *Chymomyza costata* (Moon et al. 1996).

The multi-component cryoprotective system demonstrated for the collembolan *Cryptopygus antarcticus* (Fig. 4B) has been described previously for populations at Signy Island (Sømme and Block 1982), Galindez Island and Rothera Point (Block 1982). The previous study at Signy Island also reported increased concentrations of these compounds in parallel with lowering of the supercooling point following experimental acclimation to −5°C. These previous studies report either glucose (Block 1982) or glycerol (Sømme and Block 1982) as the main constituents of the cryoprotective system. However, in the present study four main components (glycerol, trehalose, mannitol and glucose) were found in similar proportions, at concentrations higher than previously reported (particularly in winter samples) and exhibiting similar seasonal fluctuations (declining from winter to summer). Overwintering acclimatizaton of *C. antarcticus* may also comprise partial dehydration and clearing of gut contents (to reduce freezing potential) under certain circumstances (Cannon et al. 1985; Block 1996). A 4-year study (1984–1987) concluded that *C. antarcticus* may experience water stress in maritime Antarctic habitats with significant seasonal variations in body water content (Block and Harrison 1995). Clearly, the particular metabolic features essential (as opposed to marginal) for overwinter survival in this species require further research, as well as those allowing individuals to emerge from, and re-enter, a quiescent state in relatively short time scales.

Generally, the data from the current study are in agreement with those published for individual species, and illustrate the plasticity of organisms in terms of metabolic adaptation to environmental influences in polar habitats. Climatic, as well as dietary conditioning, need to be more rigorously investigated, particularly in polar research where energetic balance and nutrient limitations are fundamental constraints to colonization and survival in low temperature habitats. For example, a study on *A. antarcticus* at King George Island (South Shetland Islands) examined variation in summer cold hardiness and found that detrivores were inferior to algivores in their supercooling ability (Shimada et al. 1992). Similarly, dietary influences on cold hardiness were described for *C. antarcticus* at Signy Island by Sømme and Block (1982). Low atmospheric humidity proved advantageous for low temperature survival of the free-living oribatid mite *Antarcticola meyeri* from the continental Antarctic (Sugawara et al. 1995). Experimental studies with *Alaskozetes antarcticus* established that at saturating atmospheric humidity winter-collected mites exhibited a gradual loss of cold hardiness, with higher supercooling points and loss of glycerol, which was related to increased body water and the presence of potential nucleators throughout the year (Cannon 1986).

Regarding accumulation of low molecular weight solutes (sugars, polyhydric alcohols and other compounds) and freezing tolerance, three types of protective mechanisms may be involved: (a) osmotic adjustment to avoid freezing-induced dehydration, (b) a metabolic effect allowing metabolism to proceed at low temperatures, and (c) a cryoprotective function to protect cellular structures (biomembranes, proteins). In the case of C-compounds, the particular type accumulated is dependent on the normal carbohydrate metabolism of the species, while cellular localization is of considerable importance in explaining its role at low temperatures and its possible protective mechanism. It is hypothesized that in the case of maritime Antarctic arthropods these three mechanisms of protection are at work, although specific differences are beginning to emerge, as reported here. It is suggested that in species exhibiting partial dehydration, as an associated feature during acclimation to freezing stress (for a review on freezing/desiccation tolerance in arthropods see Block 1996), trehalose is present in the "cryoprotective system" as one of the main components. Trehalose is widely found in cyanobacteria, fungi, yeasts, nematodes, rotifers, tardigrades, plant seeds and crustacean cysts, which are all capable of surviving near-complete dehydration, i.e. loss of >99% of body water (see review by Crowe et al. 1992).

Most research on the survival of terrestrial organisms in the Antarctic has focused on identifying and quantifying cryoprotective strategies in terms of structural (stability of membranes and macromolecules) and

physiological (supercooling ability, water sequestration) qualities. Another essential consideration, much less investigated, is the metabolic viability of the strategy within existing microenvironments of the field habitat in terms of energetic budget and biosynthetic capability. In *Eurosta solidagnis* larvae, exposure to anoxia triggered the switching of polyol pools (e.g. from glycerol to sorbitol) and related metabolic pathways (glycogen breakdown, glycolysis, hexose monophosphate shunt), and was associated with lower consumption of ATP and additional NADPH generation (Storey and Storey 1990). Thus, polyol biosynthesis offers the metabolic advantage of preserving the total carbohydrate pool when triose (or hexose) cryoprotectants are synthesized by interconversion from stored glycogen (reviewed by Storey and Storey 1991). Whether Antarctic terrestrial arthropods are able to optimize cryoprotectant biosynthesis in this manner remains to be determined.

Acknowledgements I wish to thank the British Antarctic Survey for scientific and logistical support, M. Edworthy and M. Chalmers for assistance and sample collection in the field, Professor W. Block for advice throughout the study and Dr. P. Convey for comments on the manuscript.

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