

# ECOPHYSIOLOGICAL STUDIES OF TERRESTRIAL FREE-LIVING NEMATODES ON SIGNY ISLAND

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## INTRODUCTION

Nematodes are amongst the most water-dependent of terrestrial invertebrates, relying upon the films of water surrounding soil particles and plant structures for both nutrition and mobility. They have a cuticle which is generally highly permeable to water and therefore show a poor ability to resist dehydration when subjected to atmospheric humidities significantly below saturation. As nematodes are amongst the most abundant metazoans in terrestrial ecosystems, and especially within Antarctic fellfield communities, they present an ideal model for the examination of one of the basic hypotheses of the BAS Fellfield Ecology Research Programme (Block and others, 1980): that survival in such communities is as much limited by water availability as by the direct effects of temperature.

Previous nematological work in the maritime Antarctic has consisted largely of taxonomic studies (e.g. Spaul, 1972; Maslen, 1979), population ecology (e.g. Spaul, 1973; Maslen, 1981) and the energetics (Caldwell, 1981) of the free-living nematodes of moss-turf communities. In contrast the present work, conducted on Signy Island from December 1985 to March 1988, has primarily examined nematodes of fellfield habitats using an ecophysiological approach. More specifically this has involved the investigation of cold-hardiness on a seasonal basis in a range of nematode species, and an examination of the ways in which species differ in their ability to resist and withstand water loss under various environmental conditions.

## MATERIALS AND METHODS

### *Choice of habitats and species*

Preliminary surveys revealed *Andreaea* moss carpets to be good sources of *Plectus antarcticus* de Man, *Teratocephalus tilbrookii* Maslen and three species of *Eudorylenus*, namely *E. spauli* Loof, *E. coniceps* Loof and *E. pseudocarteri* Loof. Exposed *Andreaea aurantiaco-atra* (= *U. fasciata*) thalli contained large numbers of a previously unknown and as yet undescribed species, *Ditylenchus* sp. B. This latter species provides an ideal contrast with the nematode species inhabiting *Andreaea*, which clearly can gain a greater degree of shelter from their environment. A further species, *Coomansus gerlachei* de Man, added a further comparison as it was obtained from *Drepanocladus*, a wet moss-turf found in much less exposed localities.

Sampling was carried out from specific areas on or around Factory Bluffs, the most mature of the main fellfield study sites. Care was taken to ensure that substrate collected in the field was not exposed to any unnecessary environmental extremes following sampling. Prior to extraction of the nematodes, the material was allowed to equilibrate to 5°C, a process taking 1–2 hr in summer, but often 8–10 hr for frozen winter samples.

Extraction, using a modified Baermann technique (Whitehead and Hemming, 1965), may require 8 hours or more to obtain nematodes from *Andreaea*, but with

*Usnea* and *Drepanocladus* good numbers of nematodes could usually be obtained within 3 hours. This extraction period, during which the temperature and hydration of the environment are necessarily altered, presents greater difficulties in interpreting the results when similar work on nematodes is compared with that on the more readily accessible terrestrial microarthropods.

#### *Cold stress*

Examining nematode cold-hardiness by means of super-cooling points (SCPs) is difficult due to the small sizes of the worms to be studied (down to 0.2  $\mu\text{g}$  fresh weight). The approach of Wharton and Rowland (1984) using direct observation of freezing was rejected in favour of recording freezing exotherms using thermocouples. However, as the heat liberated from such a small mass is minimal, the thermocouples needed to be constructed from very fine diameter wire (75  $\mu\text{m}$ ). To prevent ice-formation in one worm seeding freezing in another, nematodes were used singly on a thermocouple. This technique had the advantage of precise recording of SCP temperatures and the facility to relate individual SCPs to specific worms.

#### *Water stress*

The ability of nematodes to resist water loss was examined in two species, *Ditylenchus* sp. B. and *T. tilbrooki*. The nematodes were dehydrated on clean glass microscope slides, after removing any surface water by means of fine strips of filter paper. Atmospheric humidity was controlled by using glycerol solutions (Grover and Nicol, 1940), or silica gel crystals, in small desiccating chambers. Nematode water contents were then estimated by first arresting any further water exchange by immersing the worms in liquid paraffin, and secondly by measuring the refractive index of the body contents using interference microscopy (Ellenby, 1968). An indirect measurement of water content was then made, given that a 1% decrease in body water content produces a constant increase in refractive index (Barer, 1953).

To monitor survival of water loss, nematodes were dehydrated within petroleum jelly ring cells on glass slides (Ellenby, 1943). After varying periods of dehydration, survival was assessed by adding water to the cells and maintaining the slides in a saturated atmosphere by placing them on moist filter paper within a petri dish. The criterion used to assess survival was movement, a distinction being made between rapid active swimming and sluggish reactions to gentle physical agitation. Recovery was not usually immediate on rehydration, so survival was assessed after 2, 5 and 10 days, even though the majority of worms had recovered within the first 2 days.

#### *Microclimate*

Two four-channel Grant Squirrel data loggers were deployed in the field to monitor the microclimates of the field sites over the fieldwork period (see Smith, 1988). On each logger, three channels utilized thermistor probes to monitor temperature at various levels within the substrate. The fourth channel was used during the summer months to log relative humidity just above the substrate surface. During 1986 one logger monitored the microclimate of a frost boil, but was redeployed to an *Andreaea* carpet during 1987–8. The other logger was used in an *Usnea* community over the entire period. A third logger, with just two thermistors to record temperature, was used in a *Drepanocladus* turf over the 1987 winter.

## RESULTS

*Cold-hardiness*

This work provided the first data on the variation of cold-hardiness in nematodes throughout the year and has shown that they may exhibit a wide range of seasonal responses. *Ditylenchus* was the most cold-hardy species studied, maintaining a mean SCP of around  $-28^{\circ}\text{C}$  throughout the whole year. In 1986 this species exhibited a seasonal trend with a mean of  $-31^{\circ}\text{C}$  in May rising to a mean of  $-25.5^{\circ}\text{C}$  in November. However, 1987 showed no such trend with the mean SCP remaining around  $-28^{\circ}\text{C}$  throughout the year.

In contrast to *Ditylenchus*, species inhabiting the moss communities showed bimodal SCP distributions similar to those found in many microarthropods (Cannon and Block, 1988). Seasonal variation in mean SCPs in these species occurred mainly through changes in the relative frequencies of the number of worms in the high and low groups. In this respect *Coomansus* showed the greatest variation, with the number of animals in the low group fluctuating from 20 to 90% between summer and winter. In *Teratocephalus* the incidence of high group nematodes was always low, even in summer. *Plectus* showed a much higher frequency of high group individuals with up to 60% present in summer. The *Eudorylaimus* spp. studied also varied in this respect with low group *E. pseudocarteri* being rare, even in winter. By contrast, *E. spaulli* and *E. coniceps* showed considerable change from high group to low group between summer and winter. Low group mean SCP temperatures for all these species were around  $-22^{\circ}\text{C}$  with high group means between  $-8$  and  $-6^{\circ}\text{C}$ .

None of the species studied were found to be capable of tolerating freezing at low temperatures (below  $-15^{\circ}\text{C}$ ). However, nematodes with high group SCPs, especially *Coomansus* and *Eudorylaimus* spp. (in particular *E. pseudocarteri*) showed a high incidence of survival of freezing when this occurred at high temperatures (above  $-10^{\circ}\text{C}$ ).

*Water loss and dehydration survival*

The ability of *Ditylenchus* and *Teratocephalus* to resist water loss differed greatly. Typically it took 2.5 min to reduce the water content of *Ditylenchus* to 50% of its fully hydrated value when exposed to an atmosphere of 0% RH at  $20^{\circ}\text{C}$ , and 11 min at  $5^{\circ}\text{C}$  with the same humidity. Similar water loss in *Teratocephalus* took 1.5 min at  $5^{\circ}\text{C}$ , but at 60% RH. In contrast, *Ditylenchus* took 40 min to incur comparable water loss under these conditions. Loss of entire body water took approximately four times longer than the time required to reduce the water content by 50%, irrespective of temperature, humidity and species.

In *Ditylenchus* the recent history of water stress may affect the rate at which the worm will resist future water loss. This decreases with the time over which the worm is exposed to free water following a period of dehydration.

Survival of dehydration in both species is extensive. Despite relatively rapid water loss, *Teratocephalus* can achieve a 50% survival rate after 50 days of dehydration at 60% RH and  $5^{\circ}\text{C}$ , although survival of animals obtained during late summer and early winter is better than that of animals collected during the rest of the year. *Ditylenchus*, which has much greater resistance to water loss than *Teratocephalus*, shows 50% survival after 100 days under the same conditions. At 0% RH this is reduced to 40 days suggesting that the rate at which this species loses its body water strongly influences the time over which it will survive. The temperature at which the

nematodes are maintained following dehydration also has a profound effect on survival, as 50 % mortality after 40 days is increased to 100 % by 20 days if they are maintained at 20°C rather than 5°C.

### CONCLUSIONS

Examination of the microclimate data enables the ecophysiological work to be set in the context of the stresses encountered in the field. The lowest temperature recorded from the *Usnea* community was -21.6°C in contrast to -11.8°C within the *Andreaea* carpet. The supercooling abilities of the nematode species from these habitats reflects this difference in stress with *Ditylenchus* showing winter mean SCPs 6-8°C lower than the species from the moss communities. SCPs should not be viewed as directly representing the lower lethal temperatures in the field, as freezing may occur at slightly higher temperatures if nematodes are maintained at such temperatures for any significant period. They do, however, provide a good index for comparative studies on cold-hardiness.

Microclimate data also provide a possible explanation for the absence of a high group in summer populations of *Ditylenchus*. The *Usnea* thalli, in which this species is found, may often experience overnight temperatures down to -6°C throughout the summer months, whereas the temperature 1 cm below the surface of the *Andreaea* carpet never experienced temperatures lower than -2°C between November and March. With high groups supercooling only as low as -7°C, a summer 'high group strategy' in *Ditylenchus* would be excessively risky, whereas the likelihood of high group nematodes within *Andreaea* carpets freezing during summer is minimal.

The ability to withstand water loss in the field is more difficult to interpret from laboratory studies. Microclimate data indicate that low relative humidities (down to 15.5% RH) at high temperatures (over 30°C) do occur in *Usnea* communities. Such conditions are unlikely to affect hydrated nematodes, as water loss will commence as soon as interstitial water has evaporated, when the atmospheric relative humidity is still quite high. Temperatures at this stage are also relatively low. It therefore seems unlikely that the rates of dehydration encountered by *Ditylenchus* will cause a reduction in the period over which it can survive desiccation in the field. Exposure to high temperatures when worms are dehydrated may present a greater threat to survival.

Water loss in *Teratocephalus* occurs at much greater relative humidities than with *Ditylenchus*, since *Andreaea* will lose extracellular water slower than *Usnea* by virtue of the much greater resistance to air circulation presented by a moss as opposed to a foliate lichen. Therefore, survival of the periods of dehydration likely to be encountered within *Andreaea* carpets may be very high in *Teratocephalus*.

The results obtained during the fieldwork period of this project generally support the hypothesis that water availability is as much limiting to survival as are the direct effects of temperature for the nematodes investigated at Signy Island. The species studied, whilst showing extensive cold-hardiness, clearly exhibit a well developed strategy to survive water loss.

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