REVIEW

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The multiple stressor approach in ecophysiology as exemplified by studies on temperate Collembola

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Ecophysiology, multiple stressor approach, combination effects, food shortage, water shortage, low temperature, population dynamics, Collembola

Abstract. Focussing on one stressor in ecophysiological research, such as low temperature or water shortage, may be practical but is unrealistic, and extrapolation based on these data e.g. to biogeographical distribution patterns, may fail. Ecophysiological research on temperate Collembola during estivation and overwintering serve as an example to show the importance of multiple stressor studies, including the simultaneous effects of food and water shortage and those of food shortage and low temperature. It is advocated to perform multiple stressor studies including toxic substances, such as heavy metals and organic toxicants.

INTRODUCTION

In the domain of stress physiology, focussing on one stressor (the single stressor approach) often originates from practical reasons. Studies on, for example, the effects of low temperatures on animals, performed under optimal food and water conditions, may tell us a lot about cold tolerance limits (Zachariassen, 1985) and even seasonal adaptations (Storey & Storey, 1983), but extrapolation based on these data to biogeographical distribution patterns, for example, may fail, because of this single stressor approach. There are indications that naturally occurring periodic food and water shortages greatly influence low temperature tolerance in arthropods (o.a. Cannon & Block, 1988).

Examples of multiple stressor research also derive from ecotoxicological studies (Hensbergen & van Gestel, 1995). Until recently, toxicity tests concerned single chemicals. However, in their natural environment the organisms are confronted with different chemicals, simultaneously, e.g. several heavy metals combined with polycyclic aromatic hydrocarbons (Edwards, 1983). This leads to combination effects which can be synergistic or antagonistic. Apart from these interactions between chemical stressors, combinations of chemicals with the above stressors, such as food and water shortages and temperature extremes, reflect more natural conditions. There are only a few examples of such studies: Low food quality and water shortage, for example, seem to enhance the negative effects of heavy metals on growth in isopods (M.H. Donker, pers. comm.; Joosse & Verhoef, 1987). Therefore it is advocated to perform combination stressor studies. This can be done under laboratory conditions, but only after a thorough analysis of the characteristics of the habitat of the study organisms. Preferably, these experiments should be accompanied by field studies in which the effects of the stressors can be measured in their natural situation. In

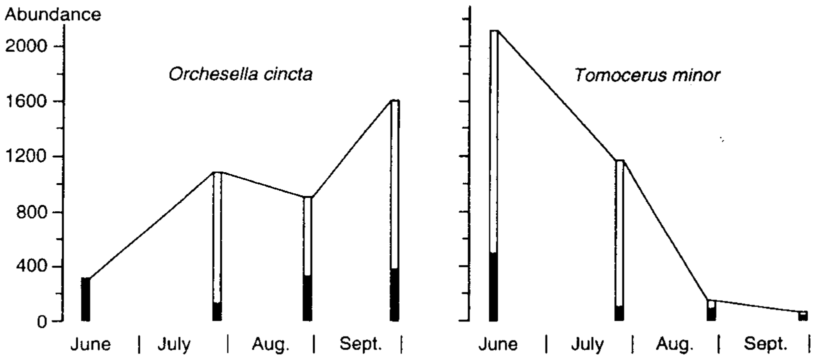

Fig. 1. Abundance (numbers m^{-2}) and population structure of *O. cincta* and *T. minor* during the summer season of 1975 in coniferous woodland. Black part of column – adults; white part of column – juveniles. (From Verhoef & van Selm, 1983.)

this paper, examples will be given of multiple stressor research on temperate Collembola, during estivation and overwintering in a forest soil and on a coastal shore, respectively.

STRESS FACTORS PLAYING A ROLE IN ESTIVATION

The population dynamics of two Collembolan species *Orchesella cincta* (L.) and *Tomocerus minor* (Lubbock), abundant in a *Pinus sylvestris* stand, planted on old sand dunes near the sea coast on the island Schiermonnikoog in the Netherlands, showed strong changes in density during the summer season (Fig. 1). Because of the sandy subsoil, periods of low precipitation led to a dry organic layer with pF-values ≥ 4.7, indicating that on the average no free water was present (Vannier, 1970; Simons, 1973). *T. minor* showed a gradual decrease, mainly in the numbers of juveniles, whereas in *O. cincta* the reverse occurred, caused by natality. This difference in abundance during the summer season can be explained by the different water economy of the two species. On average, the transpiration rate of *O. cincta* is eight times slower than that of *T. minor* (see Fig. 2), with the latter species losing water even under saturated conditions.

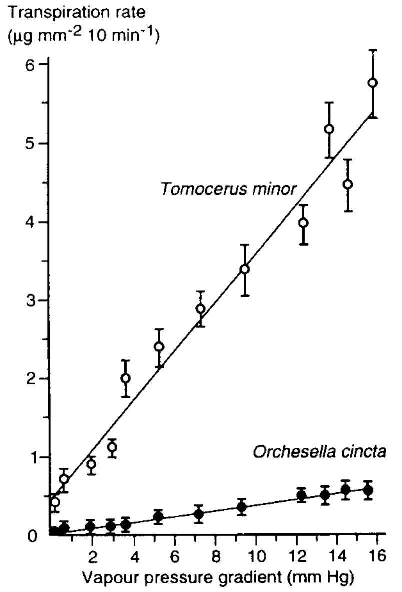

Fig. 2. Relation between transpiration rate and vapour pressure deficit in *O. cincta* and *T. minor* at 18°C in combination with a series of increasing RH values; mean ± SD. (From Verhoef & Witteveen, 1980.)

Correlations between soil moisture content and abundance for both these species were confirmed by Vegter et al. (1988) in a field study over four years in eight different

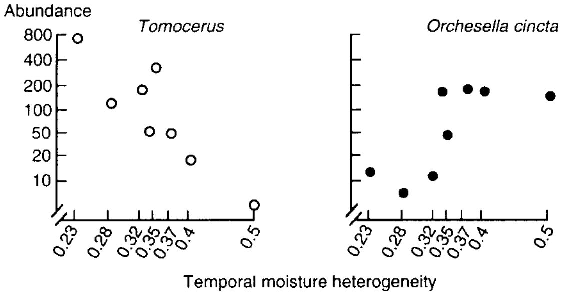

Fig. 3. Relation between abundance (numbers m^{-2}) on log-scale and temporal moisture heterogeneity, measured by an ordinary coefficient of variation: the quotient of the standard deviation of mean moisture content of soil cores from each sampling occasion and the grand mean of moisture content. Total numbers of *Tomocerus* (*T. minor* and *T. flavescens*) and *O. cincta*. (From Vegter et al., 1988.)

woodland habitats, differing in soil and litter type (Fig. 3). The abundance of *Tomocerus* (*T. minor* and *T. flavescens*) decreased with the degree of temporal moisture heterogeneity, which seems explicable from their drought sensitivity. The high densities of *O. cincta* in the heterogeneous soils can be explained by their drought resistance. Their lower abundance in more homogeneous soil, however, could not be due to the moisture factor, but may be explained by competition, probably for food, from abundant drought-sensitive species. Culturing the two species together in the laboratory in a replacement-series under relatively homogeneous conditions (in pots without structure and equally distributed algae as food) showed *T. minor* was the dominant species with a strongly negative effect on the numbers of *O. cincta* (Fig. 4).

Even when soil moisture seems to be the prime factor in determining species abundance, it is still possible that it derives from an indirect moisture effect via the food factor. This was confirmed by strong positive correlations between the biomass of fluorescein-stainable fungi in the three different horizons L, F and H of a *Pinus sylvestris* stand and the specific moisture content of each layer (Berg et al., in press). Thus, during periods of low moisture, low fungal abundance may indicate food shortage and may lead to starvation.

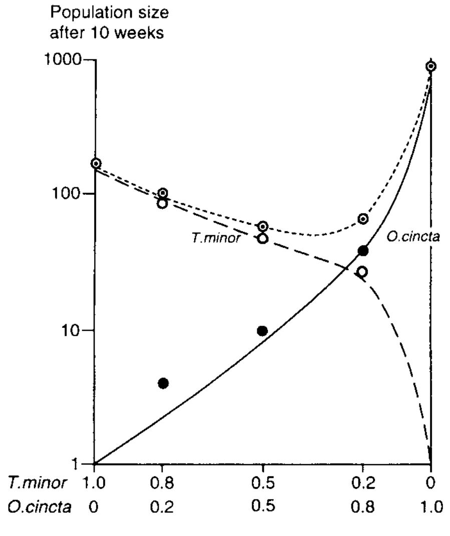

Fig. 4. Replacement diagram of *O. cincta* and *T. minor*. Initial density = 20 animals; abundance (numbers pot^{-1}) is after 10 weeks; single measurements. Dotted line represents the total numbers of both species.

Indications of starvation in the field during dry periods have been found for *O. cincta* (Testerink, 1981). At these times the percentage of full guts of these animals dropped below their normal mean values, synchronously with declines in their reserve materials, lipids and glycogen.

The reaction to food and water shortage of the two Collembolan species, however, is different (Verhoef & Li, 1983) and explains their different reactions in population numbers. If *O. cincta* is confronted with food shortage and water ad libitum, its transpiration rate increases strongly, up to 200%.

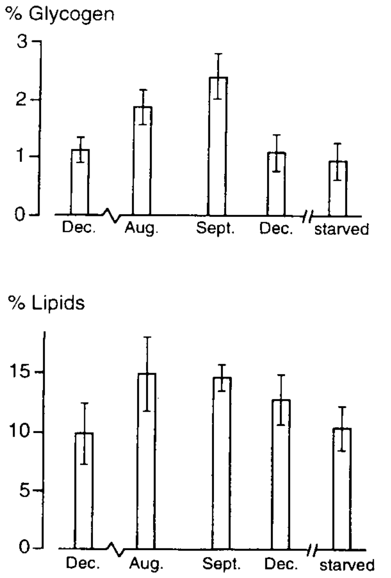

Fig. 5. Glycogen and lipid content (% of DW) of *A. maritima* collected from the Dutch Wadden Island of Texel from 1982–83. Starved – animals starved in the laboratory; mean ± SD. (From Witteveen et al., 1988.)

This may be the case when, under high moisture conditions, high numbers of *T. minor* cause a food shortage. The high transpiration rate may be a sign of a disturbed water economy in this drought-resistant species. When food shortage is combined with water shortage, the transpiration rate in *O. cincta* decreases by about 50% (Verhoef & Li, 1983). A similar effect is shown by another collembolan *Orchesella villosa* (Vannier & Verhoef, 1978). This "adaptation" may explain the success of this species in dry summer periods. The metabolic rate in *O. cincta* decreases during starvation by 60% (Verhoef & Li, 1983).

In *T. minor* food shortage as a single stress does not influence its metabolic rate. In combination with water shortage, however, there is a similar decrease as in *O. cincta* (van der Woude & Joosse, 1988). However, the transpiration rate of *T. minor* is not influenced, neither by food or by water shortage, nor by the combination stress, and remains at its high level. This may explain the strong decrease in numbers of this species during dry summer periods.

STRESS FACTORS PLAYING A ROLE IN OVERWINTERING

The population dynamics of the collembolan *Anurida maritima* (Guérin) an eulittoral species living on the mudflats of the Dutch Wadden Islands, showed a strong decrease in abundance during the winter period (Witteveen et al., 1988). This seashore scavenger is univoltine and every year in early May a new generation emerges from eggs laid in the previous summer. During the winter period nearly all the adults die. As it concerns exposed living animals, temperature was originally thought to be the main mortality factor. However, the supercooling point of the adults was −17.2 ± 1.8°C, which is slightly lower than those measured in temperate Collembola which overwinter in the adult state (van der Woude, 1987). Further, the highest mortality (90%) was observed in autumn, when severe frosts never occur. It appears, however, that the decrease in their numbers is caused by starvation. Both measurements on field-collected animals and starvation experiments in the laboratory showed that the contents of the reserve materials (lipids and glycogen) measured in late autumn resemble those of animals

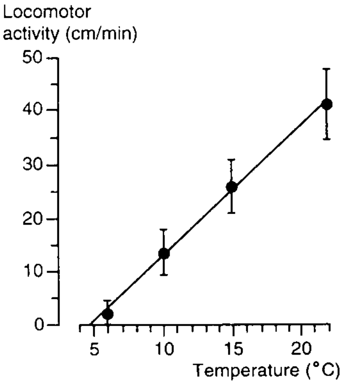

Fig. 6. Locomotor activity of *A. maritima* at different temperatures; mean ± SD. (From Witteveen et al., 1988.)

starved in the laboratory (Fig. 5). This apparent food shortage cannot be understood without taking into account the low temperatures during autumn. As the food for these Collembola are carcasses of jellyfishes, annelid worms, molluscs, crustraceans and fishes accumulated between the tidelines, this species only feeds at low tide. Thus, there is only a limited period of the day for searching and eating food. Because of the low temperatures during the autumn and the fact that the locomotor activity of this species is strongly influenced by temperature (Fig. 6), the combination of these two factors causes mortality of adult *A. maritima* and partly explains the biogeographical distribution of this species. *A. maritima* has its optimal distribution in the tropics where it has probably more generations per year and has its northernmost distribution in the south of Norway.

These two examples of research on temperate Collembola show that in invertebrate ecophysiology it deals with several stress factors operating simultaneously on the species concerned. Ecophysiological studies should therefore include combination stressor studies in the laboratory to mimick as far as possible the complicated field conditions.

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