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Ber. nat.-med. Verein Innsbruck	Suppl. 10	S. 305 – 311	Innsbruck, April 1992

8th International Congress of Myriapodology, Innsbruck, Austria, July 15 - 20, 1990

Feeding Biology of the Millipede *Glomeris hexasticha*

(Glomeridae, Diplopoda)

by

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Abstract: Several aspects of the feeding biology of *Glomeris hexasticha* BRANDT (Diplopoda) were studied. Individuals fed mainly on old oak leaf litter in the F-layer of the oak forest under study. Food preferences changed during postembryonic development, as did the microbial communities in consumed food during passage through the digestive tract. Numbers of microorganisms in faecal pellets were twice those of ingested food, and exhibited a substantial increase in respiration.

1. Introduction:

Millipedes are primary decomposers of dead plant material on the soil surface, and are therefore useful indicators of decomposition, as well as of relations between invertebrates and microorganisms during decomposition and humification in soil (McBRAYER 1973, VAN DER DRIFT 1975, ANDERSON & BIGNELL 1980, HANLON & ANDERSON 1980). Representatives of the genus *Glomeris* LATREILLE are widespread inhabitants of the deciduous forest litter ecosystems (VAN DER DRIFT 1975, BERTRAND et al. 1987, IATROU & STAMOU 1989). In Czechoslovakia *G. hexasticha* BRANDT, 1833 is widely distributed and often abundant in forest and grassland biotopes (TAJOVSKY 1989).

Only limited information is available about the feeding biology of *G. hexasticha*, including its consumption of food and its humificative function (GERE 1956). More recent information is now available on the composition of its gut microflora (CHU et al. 1987, 1988), and the decomposition of its faecal pellets (TAJOVSKY et al., *in press*).

The present contribution, which is part of a wider study on the biology and ecology of *G. hexasticha*, provides additional information on its feeding biology and on the influence of microbial communities on its ingested food.

2. Material and Methods:

Millipedes and plant litter were collected in a mixed deciduous forest (*Abieto-Quercetum*) near Netolice in South Bohemia (485 m a.s.l.). Mean air temperature at that location is 7,3° C, mean annual precipitation is 650 mm. Its soil is classified as "brown forest" soil. Leaf litter used for all feeding experiments was 10 - 11 months old, microbially attacked but entire and well defined.

2.1. Feeding Activity:

Gut contents of freshly caught animals were examined under a dissecting microscope. The following materials (containing 70 - 75 % water) were used for the food preference tests: leaf litter of oak (QR *Quercus robur*), beech (FS *Fagus silvatica*), maple (AP *Acer pseudoplatanus*), spruce (PA *Picea abies*); a mixture of leaf litter from the F-layer (MF); spruce needle from the F-layer (PF), grass (CA *Calamagrostis arundinacea*) litter; and root litter

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(RL). These materials were distributed as isolated units in Petri dishes (20 cm in diameter). Millipedes and litter material came from the deciduous forest noted above. After collection in September and October 1988 the millipedes were treated under laboratory conditions 5 - 7 days in glass vessels with natural food ressources. Millipedes (both males and females) were then placed in these dishes and after 1, 3, 5, 22, 30 hours and 2, 3, 4, 5, and 6 days their activity (consumption of food, defecation) was noted. Altogether, 40 animals were tested and evaluated within the framework of four weight groups (each group with 10 animals, weight ranges see Table 1).

Table 1: Food preference of *Glomeris hexasticha*, size classes of millipedes and proportional rankings of preferred food types (in %). **QR** oak; **FS** beech; **AP** maple; **PA** spruce; **MF** mixture of leaf litter of F-layer; **PF** spruce needle of F-layer; **CA** grass; **RL** roots. Preference trends: **full line,** increasing consumption of oak; **broken lines,** decreasing consumption of grass and root litter.

Rank	Live body weight (mg)			
	9,5-19,5	19,6-35,4	35,5-55,4	55,5-126,2
1	MF 23	AP 25	MF 29	QR 34
2	RL 20	PA 19	QR 15	MF 15
3	CA 14	MF 14	PA 14	AP 14
4	PF 12	QR 13	FS 13	PA 11
5	QR 11	RL 12	RL 11	FS 9
6	PA 8	PF 9	PF 8	PF 9
7	AP 7	CA 7	AP 7	RL 5
8	FS 5	FS 1	CA 3	CA 3

Food consumption (C, dry weight in mg · day^{-1} · ind^{-1}), production of faecal pellets (FU, dry weight in mg · day^{-1} · ind^{-1}) and percent assimilation of food (A = [C - FU]/C 100) were determined for oak, maple and beech leaf litter which predominated in the L-layer of the locality under study at the time of material collection. Individual animals were treated as described above for 3 or 5 days at 21° C and 12-hours daily photoperiod. Simultaneously, Petri dishes containing only food were used as controls under identical conditions. The weights of animals starved for 24 h both before and after the experiments were recorded, as were dry weights of food consumed and faeces produced. Dry weights were determined after heating at 105° C for 5 hours. The results of food consumption were tested by analysis of variance (ANOVA).

2.2. Influence of Millipedes on Microbial Communities:

Millipedes were kept in glass vessels under laboratory conditions with a mixture of leaf litter. Microbial communities and respiration activity in the initial litter and released faecal pellets were analysed from materials in the vessels. Direct counts of microorganisms were established by epifluorescent microscopy (membran filter method, KRISTUFEK et al. 1987). Physiological groups of microorganisms were established using the dilution plate count method on various media: nutrient broth agar No. 2 (IMUNA Sarisské Michalany, heterotrophic bacteria), water agar (SZEGI 1983; oligotrophic bacteria), mineral agar with filter paper discs (POKORNA-KOZOVA 1965; cellulolytic bacteria), Ashby agar (oligonitrophil bacteria), starch and chitin agars (SZEGI 1983, LINGAPPA & LOCKWOOD 1961; actimomycetes) and soil extract agar with bengal rose (FASSATIOVA 1979, micromycetes).

Respiration (CO_2 production) was measured in moistened samples (70 %) after 10 days of incubation at 15° C in 100 ml air-tight bottles, each containing a beaker with 2 ml 0,1 N NaOH. CO_2-C absorbed in NaOH was determined by automatic titration with 0,1 N HCl. All results obtained were recalculated per 1 g of dry weight of substrates (TAJOVSKY et al., in press).

Transmission (TEM) and scanning electron microscopy (SEM) was used to examine interactions between millipedes and the microflora under study. Materials for TEM investigation (litter, gut and pellets) were prefixed in 2,5 % phosphate-buffered glutaraldehyde (16 h), fixed in 2 % phosphate-buffered OsO_4 (1 h), dehydrated through a graded series of acetone and embedded in POLARBED 812 resin. Ultrathin sections were coloured with uracyl acetate and then lead citrate, and examined using a CARL ZEISS EM 9 S-2 electron microscope. For SEM

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studies the gut was freeze dried immediately after dissection, then broken, coated with gold and examined using TESLA BS 300 electron microscope.

3. Results:

3.1. Feeding Activity:

The gut contents of G. *hexasticha* contained mostly dead plant material, especially leaf litter in different decomposition stages. The fragments of oak leaves were most obvious. Fragments of fungal mycelia, spores, mineral grains, soil algae and testate amoebae were also present.

The results of food preference tests with the proportional rankings of preferred food types are shown in Table 1. The lowest weight group of animals preferred the mixture of leaf litter from F-layer but also grass and root litter. With the increase of body weight, leaf litter was more preferred and on the contrary grass litter and root litter preference decreased. Generally the most preferable food was a mixture of leaf litter from the F-layer, then oak and maple leaves. The preference trends are marked in Table 1 for oak as the full line, for decreasing consumption of grass and root litter as the broken lines.

The characteristics of feeding activity of *G. hexasticha* (Table 2) show, that this millipede consumed mostly oak leaves, fewer maple leaves and still fewer beech leaves. The values of consumed food obtained were significantly different only for oak and beech leaf litter (ANOVA, LSD 0.05 = 4.8879). No significant differences were established for oak and maple and maple and beech leaf litter. Consumption and faecal production of beech litter was only about half that of the oak and maple litter. Assimilability of litter decreased in the following order: oak > beech > maple. The amount of dry weight leaf litter consumed corresponded to 19,6, 18,0 and 10,9 % of the live body weight, for oak, maple and beech, respectively. At the same time millipedes released an average of 70,6 % oak, 85 % maple and 84,3 % beech material to their faecal pellets.

Table 2: Daily consumption of food (**C**), production of faecal pellets (**FU**) and assimilability of food (**A**) for *Glomeris hexasticha*. **d** days of experiment, **n** number of replications, w_i w_f mean initial and final live body weight.

	d	n	$w_i \pm SE$ [mg]	$w_f \pm SE$ [mg]	$C \pm SE$ [mg d^{-1} ind^{-1}]	$FU \pm SE$ [mg d^{-1} ind^{-1}]	A [%]
oak	5	25	72,05 ± 11,01	73,60 ± 11,43	14,13 ± 2,49	9,97 ± 2,09	43,6
maple	3	25	69,01 ± 10,39	71,60 ± 10,46	12,43 ± 2,03	10,57 ± 2,44	29,0
beech	3	25	52,57 ± 10,49	56,49 ± 10,90	5,72 ± 0,98	4,82 ± 0,87	20,4

3.2. Influence of Millipedes on Microbial Communities:

The direct counts of microorganisms in litter and pellets showed a double enhancement of microbial activity in faecal pellets ($0,93 \cdot 10^9 \cdot g^{-1}$ and $1,85 \cdot 10^9 \cdot g^{-1}$, respectively). On the agar media only a small increase of the majority of physiological bacterial groups, such as for example heterotrophic, oligonitrophil and oligotrophic bacteria, was noted (Table 3). Micromycetes slightly decreased, the decrease of actinomycetes was more outstanding.

After its passage through the digestive tract the CO_2 production of ingested materials increased nearly five times, from 0.24 µg CO_2 - C · g^{-1} · day^{-1} in litter to 1,16 µg CO_2 - C · g^{-1} · day^{-1} in pellets.

In all animals examined the food was present only in the midgut and in the hindgut (Fig. 1). TEM demonstrated, that in comparison with leaf litter (Fig. 2) in plant fragments, in the midgut there were relatively few microorganisms. Lysed bacterial cells and empty dead fungal hyphae were

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Table 3: Physiological groups of microorganisms in litter and faecal pellets of *Glomeris hexasticha* (number · 10^5 · g^{-1}, mean valus from 4 repetitions).

Physiological groups	Litter	Pellets
bacteria heterotrophic	39,35	48,59
bacteria oligotrophic	47,74	87,90
bacteria oligonitrophil	77,65	98,96
bacteria cellulolytic	0,0013	0,0005
actinomycetes	2,10	0,77
micromycetes	2,83	2,39

observed here (Fig. 3). In the first part of the hindgut (ileum) some bacteria along or near the intima of epidermal gut cells was noted (Fig. 4). The presence of these apparently symbiotic microorganisms was confirmed by SEM investigations (Fig. 5). Ultrathin sections of the colon showed that these bacteria gradually penetrated into the gut contents (Fig. 6) and grew as constituents of the produced pellets.

4. Discussion:

4.1. Feeding Activity:

As analyses of the gut contents and preference tests showed, this species consumed mostly the type of litter predominant in the locality, although oak litter is considered difficult to digest. MARCUZZI (1970) and WOOTEN & CRAWFORD (1975) noted that food selection by millipedes appears to be a function of habitat. If this idea is valid, habitat may play a more fundamental role in food preference relationships by millipedes than many other factors (BOCOCK 1964, SAKWA 1974).

Consumption of leaf litter and decrease in grass and above all root litter consumption parallel with increasing body weight of the tested animals can mean the changes in the composition of ingested food during postembryonal development of this millipede.

GERE (1956), in long-term experiments (14 - 59 days) determined that a single *G. hexasticha* has a mean daily consumption of oak litter of approximately 2.6 mg. He noted also that, owing to long captivity, the metabolism of the animals under study, decreased and that they therefore consumed less food than they would have under natural conditions. This can explain the higher values of consumed food of our shorter 3 - 5 day experiments. STRIGANOVA (1975) also established, in short-term experiments with *Megaphyllum projectum* (VERHOEFF) and *Unciger foetidus* (C.L. KOCH) high daily consumption values of hornbeam litter (24.8 mg and 26.8 mg, respectively).

Assimilability of the food established for *G. hexasticha* in laboratory conditions is higher than the 6 - 15 % range reported for the most millipede studies in this regard (GERE 1956, BOCOCK 1963, McBRAYER 1973) but corresponds to levels reported by WOOTEN & CRAWFORD (1975) and STRIGANOVA (1977). The present results may have been influenced by the short duration of the experiment, by the condition of the food (10 - 11 month-old leaf litter) as well as by temperature used in the feeding study.

4.2. Influence of Millipedes on Microbial Communities:

Interactions between invertebrates and microorganisms are a very important aspect of the decomposition processes. It is known, for example, that the comminution of plant material by millipedes as well as by other soil feeding animals increases the surface area available for microbial colonization (HASSALL et al. 1987), and that the intestines of these animals act as a favourable envi-

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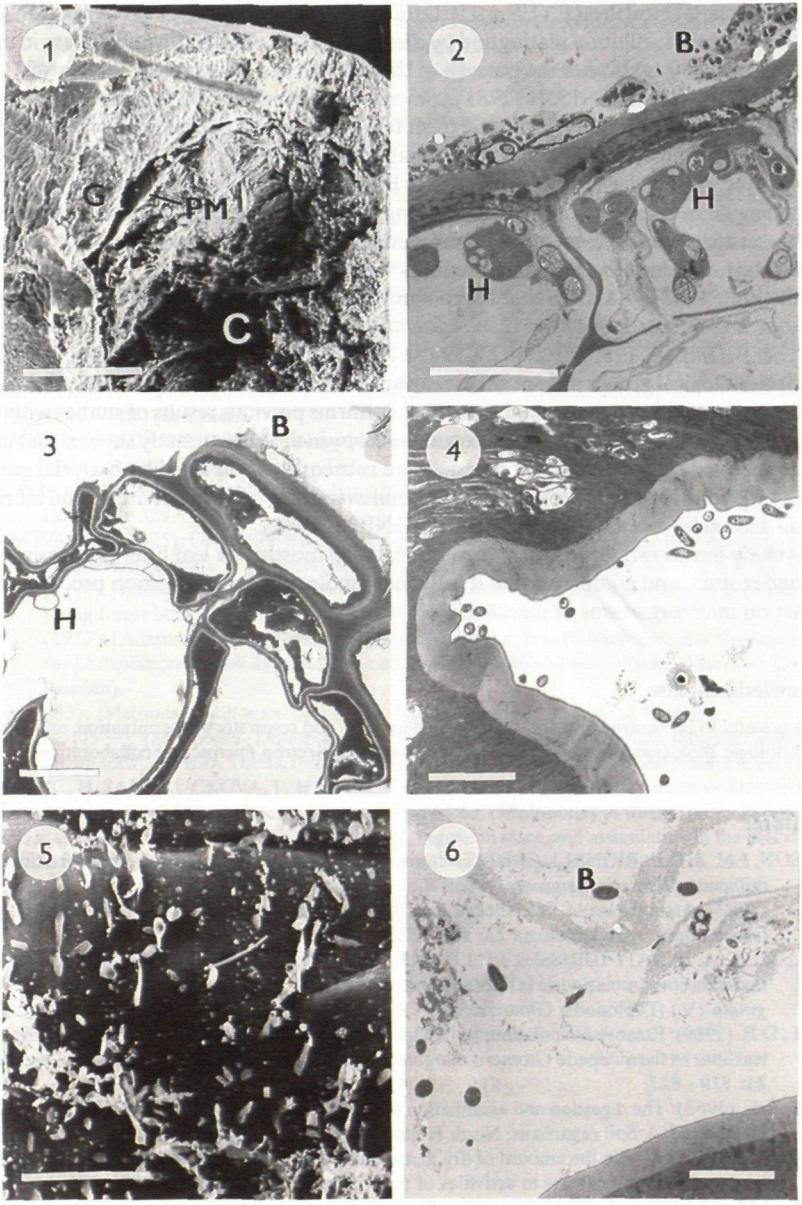

Fig. 1: Scanning electron micrograph of the gut of *Glomeris hexasticha*. – G gut wall, PM peritrophic membrane, C gut content. Bar = 250 µm.

Fig. 2: Presence of fungal hyphae (H) and bacteria (B) in the oak leaf litter. Bar = 5 µm.

Fig. 3: Lysed bacterial cells (B) and empty dead fungal hyphae (H) in the midgut content. Bar = 5 µm.

Fig. 4: Bacteria along the intima of epidermal gut cells (ileum). Bar = 5 µm.

Fig. 5: Presence of microbial particles on the intima surface of the hindgut. Bar = 25 µm.

Fig. 6: Penetration of symbiotic bacteria (B) into gut content (colon). Bar = 5 µm.

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ronment for bacterial growth (REYES & TIEDJE 1976, HANLON 1981). ANDERSON & BIGNELL (1980) found by dilution plating and by direct observation a significant increase in bacterial populations (10 - 100 fold) after the passage of the oak-beech litter through the gut of *Glomeris marginata* (VILLERS). HANLON (1981) compared the bacterial and fungal standing crops on leaf litter and pellets of *G. marginata*. The fresh faeces contained approximately double the bacterial standing crop, but only half the fungal standing crop of the litter ingested. McBRAYER (1973) observed that the faecal pellets of another litter-feeding millipede contained more bacteria and fewer fungi than food. Thus, the present finding of twice the numbers of microorganisms in pellets of *G. hexasticha* is in agreement with published results. However, in contradiction to the above mentioned data, the decrease of fungal numbers was not significant.

At the same time it is known that microorganisms colonizing leaf litter are a valuable nutritional resource to millipedes such as *G. marginata* and are assimilated with high efficiency (ANDERSON & BIGNELL 1982, BIGNELL 1989). After the gut passage of food the composition of microbial physiological groups did not change, although the TEM results indicated definite changes in microbial communities in consumed food. This confirms previous results of studies with *G. hexasticha* by CHU et al. (1987, 1988). Electron microscopy in the present study showed that microorganisms in ingested litter were largely digested and subsequently replaced by bacterial gut microflora. Results given here also agree with the microbial changes in food, gut contents and faeces of *G. marginata* fed under laboratory conditions (BIGNELL 1989).

This study has shown that *G. hexasticha* consumed mostly oak leaf litter predominant in the locality under study and confirmed that it influences indirectly decomposition processes through the impact on microorganisms in ingested food.

5. Acknowledgements:

I am grateful to H. Santruckova for the microbial analysis and respiratory determination, and G. Villemin (Centre Pédologie Biologique, Nancy, France) for transmission electron microscopy collaboration.

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Zeitschrift/Journal: Berichte des naturwissenschaftlichen-medizinischen Verein Innsbruck

Jahr/Year: 1992

Band/Volume: S10

Autor(en)/Author(s): Tajovsky Karel

Artikel/Article: Feeding Biology of the Millipede Glomeris hexasticha (Glomeridae, Diplopoda). 305-311