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Role of microbial enriched detritus in the nutrition of Gammarus minus (Amphipoda)

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Microbial enriched detritus is an important food source for many stream invertebrates. Results of laboratory feeding experiments showed significant differences in survivorship of *Gammarus minus* Say on 10 different diet combinations over a 10 week period. Highest survivorship was recorded on fungus enriched leaves; intermediate rates were obtained for leaves with a viable bacterial flora and leaves on which the microflora had been killed. Low survival rates were recorded on sterile leaves and leaves with reduced microflora. The results of food preference studies showed that leaves having a viable fungus flora were most preferred while sterile leaves were least preferred. Overall, *Gammarus* showed a preference for those diets affording the best survivorship in the feeding experiments. Evidence indicates that the nutrition of *G. minus* is based on a food chain of detritus and the associated fungi.

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Детрит, обогащенный микрофлорой, представляет важный пищевой объект для многчх речных беспозвоночых. Результаты лабораторных опытов по изучению питания показали существенные различия в выживаемости *Gammarus minus* Say. на 10 различных пищевых смесях в течение 10 недель. Наиболее высокая выживаемость отмечена при содержании в листве, обогащенной грибами. Промежуточные показатели получены на листве с живой бактериальной флорой и на листьях, где флора была убита. Низкая выживаемость отмечена в стерильной листве и в листве с бедной микрофлорой. Реаультаты изучения пищевого референдума показали, что листва с живой грибной флорой является наиболее предпочитаемой пищией, а наименее предпочитаемая – стерильная листва. Гаммарусы предпочитали пищу, обеспечивающую лучшую выживаемость в экспериментальных условиях. Получены доказательства, что питание *G. minus* основано на пищевой цепи детрита и связанных с ним грибов.

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Introduction

Organic detritus, particularly allochthonous plant material, is one of the most significant factors influencing relationships within woodland streams. Utilization of this material by detritivores as a basic food source has long been recognized (Slack 1939, Jones 1950, Curry 1954, Edmondson 1957, Brown 1961, Nelson and Scott 1962, Chapman and Demory 1963, Odum and DeLa Cruz 1963, Hynes 1963, Minshall 1967, 1968, McDiffit 1970), but only recently have attempts been made to determine the functional role that the microflora associated with detritus degradation and transformation plays in secondary productivity.

Information obtained from laboratory rearing experiments (Fredeen 1960, 1964, Marzolf 1966, McConnell 1968) have shown that many invertebrate species as well as some fish, are capable of subsisting on bacterial diets for long periods of time. Of particular interest, but heretofore largely unexplored, is the relationship between detritus-inhabiting fungi and consumer nutrition. Recent studies by Kaushik and Hynes (1968, 1971) indicate a correlation between fungal growth and protein enrichment of submerged leaves and other decaying plant material thus suggesting at least an indirect contribution to detritus feeders. The reports of Ivanova (1958), Cummins et al. (1966), Coffman (1967), and Triska (1970) provide at least presumptive evidence that the mycoflora constitutes a potentially direct food source, whereas Clemens (1949) earlier described rearing young *Gammarus* through the first few instars on a diet of yeast. Recent work by Bärlocher and Kendrick (1973a, b, 1975) indicates that fungi are an important food item in the diet of *Gammarus pseudolimnaeus* Bousfield, but that different species of aquatic fungi vary considerably in their ability to serve as the sole source of food.

Considering the general paucity of information on the microbial contributions to higher trophic levels, the present study was designed to elucidate the relative importance of bacteria and fungi in the nutrition of *Gammarus minus* – a common inhabitant of woodland streams.

Materials and methods

Ten diets comprising different combinations of the detritus-microflora complex were tested and assayed on the basis of survival and reproduction of *Gammarus minus*. Details of their preperation are described below and summarized in Tab. 1.

Diets containing detritus were prepared from newly fallen elm *Ulmus americana* L. leaves. After collection, the leaves were strung on monofilament line in groups of 6 and dried for 2 wk at 85°C. The leaf packets were then sealed in a heavy guage plastic bag, sterilized with ethylene oxide (Cryoxide, Amer. Sterilizer Co.), and stored at room temperature until needed. Two weeks prior to each feeding experiment, a leaf packet (LP) was transferred to a 2–1 erlenmyer flask containing 1 l of sterile distilled water and processed according to the diet involved. Three replicates were prepared for each diet and unless otherwise specified, the flasks were maintained at 12°C throughout the 2 wk incubation period.

Microbial infested detritus, comprising the L – B, L – F, and L – BF diets, was obtained by adding freshly collected stream leaves as inoculum to each flask. Antimicrobial agents specified in Tab. 1 were added prior to inoculation.

In the detritus dead microflora diet (LDM) the LP's were submerged directly in the stream for two weeks, returned to the laboratory in stream water, and resterilized with ethylene oxide.

Fungus-enriched leaves (FEL) were prepared by placing sterile elm leaves (presoaked in distilled water until pliable) directly on a week-old colony of *Tetrachaetum elegans* Ing., previously isolated from submerged stream leaves. Cultures of the fungus were propagated on malt extract agar (12.5 g malt extract, 20 g agar, 1 l distilled water) in petri dishes (20 × 100 mm) at 18°C. After infestation (usually 3–4 d) 3 leaves were removed and added to each flask. Bacteria-enriched leaves (BEL) were similarly prepared, except 3–4 day-old colonies (isolated directly from submerged stream leaves on nutrient agar) were used as inoculum. No attempt was made to distinguish or select species of bacteria for use in this diet. In order to distinguish between the microflora and the leaf substrate, treatments B (bacteria) and F (fungus) were used. In these treatments pure cultures of *Bacillus subtilis* and *T. elegans* were grown in liquid media, filtered, and fed to the experimental animals. No leaf substrate was used.

Following incubation, the diet being tested was placed in a plastic box (30 × 15 cm) containing 1 l of sterile distilled water, and 20 adult *Gammarus* were added. The animals, all in mature size classes, were divided

Tab. 1. Summary of experimental diets and treatments used in their preparation (for further explanation, see text).

Diet	Treatment
Leaves + live microflora (LLM)	Leaf packet (LP) + submerged stream leaves (SL).
Leaves – fungal flora (L – F)	LP + SL + 50 mg Nystatin
Leaves – bacterial flora (L – B)	LP + SL + 25 mg each of penicillin G and streptomycin.
Leaves – fungi and bacteria (L – BF)	LP + SL + 50 mg Nystatin + 25 mg each of penicillin G and streptomycin.
Bacteria-enriched leaves (BEL)	LP + stream bacteria
Fungus-enriched leaves (FEL)	LP + *Tetrachaetum elegans*
Leaves with a dead microflora (LDM)	LP submerged in stream and resterilized
Leaves only (L)	LP
Bacteria only (B)	*Bacillus subtilis*
Fungi only (F)	*T. elegans*

evenly among the treatments by size and sex. These organisms were selected from stock cultures, which had been maintained in the laboratory for at least 2 wk. All feeding experiments were conducted at 12.0 ± 0.5°C in a walk-in environmental room having a 12:12 L:D cycle. The number of survivors, condition of the diet material, and ancillary observations were recorded weekly. Young, released between observation periods, were counted and removed to reduce cannibalism. Samples of water and diet materials were periodically plated out on both malt extract and nutrient agar to check the effectiveness of the antimicrobial populations during the feeding experiment. The leaves and water were changed weekly to avoid any accumulation of waste products.

In addition to the feeding experiments, a series of laboratory and field tests were conducted to determine (1) if *Gammarus* could distinguish between different diets, (2) whether or not they exhibited a preference for individuals diets, and (3) whether or not there was a relationship between diet preference and survival as shown in the feeding experiments. The laboratory tests were conducted using LLM and one test substrate placed at opposite ends of the plastic container. Twenty *Gammarus* were then released in the center of the container, and the number of individuals on each substrate was recorded daily for a period of 5 d. To correlate the laboratory tests with *Gammarus* behavior in the stream, LLM and one test substrate were placed in large mesh bags and submerged in pairs in the stream. After 48 h, the bags were carefully removed and the number of individuals within each bag recorded.

Results of the feeding experiments were analyzed by comparing survival on each diet within a given experiment to each other diet by setting up contingency tables and applying the χ^2 test for goodness of fit. The null hypothesis was that there was no difference in survival among the treatments. The level of significance was $P = 0.05$ in all cases. The preference studies were similarly analyzed by comparing LLM to the test substrate.

Results and discussion

Three feeding experiments were conducted comparing various diets (Tabs 2, 3). The LLM treatment containing a microflora similar to that of fresh stream leaves served as a control. This diet was used in each series and the other diets compared to it within each experiment. In the first experiment the diets tested were LLM, L − B (leaves minus bacteria), L − BF (leaves minus bacteria and fungi), and L (sterile leaves). At the end of the 10 wk experimental period, significant differences in survivorship were apparent among the treatments. *Gammarus* on the LLM diet showed 45% survival over the 10 wk. A total of 77 juveniles were released. On the L − B leaves which had a high fungal growth, survival was higher (60%) but not significantly higher than LLM. In the L − B treatment 116 juveniles were released. The above treatments showed significantly higher survivorship than the L − BF and L treatments, both of which had greatly reduced microbial populations. Survival on both of these diets was 3%; no juveniles were released in either treatment.

In the second feeding experiment, LLM was compared to bacteria-enriched leaves (BEL), fungus-enriched leaves (FEL), and leaves with nonliving microflora (LDM). The L − F treatment was found to be unsatisfactory. Presumably, the concentrations of nystatin sufficient to inhibit fungal growth were lethal to the adults. Bacteria-enriched leaves (BEL) were therefore substituted for this treatment. Survivorship on LLM was 83% with 165 juveniles released. The FEL treatment showed 88% survival and 187 juveniles for the 10 wk period. Both of these treatments showed significantly higher survival than BEL and LDM which had, respectively, 63% survival and 87 juveniles and 62% survival and 124 juveniles. In the third experiment the diets tested were LLM, B, and F. In this series the *Gammarus* on the LLM diet showed 57% survival. Juvenile production was 201 individuals. On the F (fungus) diet survivorship was 67%, not significantly different than the control. A total of 231 juveniles were released. Survival on the B (bacteria) treatment was significantly lower than the above diets, with 36% survival; however, juvenile production was high (273).

Based on the above results, the diets can be divided into 3 groups based on survival of the adults:

1. High survival, those treatments which included living fungi; LLM, F, FEL, and L − B.
2. Intermediate group, treatments which had a bacterial flora or nonliving microflora; BEL, L − F, and B.
3. Low survival, treatments in which the microflora was greatly reduced or absent; L − BF and L.

Differences in survivorship and juvenile production among the three LLM diets seem to be largely due to natural fluctuation in the life cycle of *G. minus*. Laboratory data correlate very well with the life cycle in the stream; i.e. the rather low survival among all treat-

Tab. 2. Results of the feeding experiments showing survivorship for each diet.

	Diet	Survivors (%)	Deaths
Experiment 1	LLM	27 (45%)	33
	L − B	36 (60%)	24
	L − BF	2 (3%)	58
	L	2 (3%)	58
Experiment 2	LLM	50 (83%)	10
	FEL	53 (88%)	7
	BEL	38 (63%)	22
	LDM	37 (62%)	23
Experiment 3	LLM	34 (57%)	26
	F	40 (67%)	20
	B	16 (36%)	44

Tab. 3. Analysis of the results of the feeding experiments comparing the diets within each experiment using contingency table and χ^2.

Experiment 1			
Diet 1		Diet 2	
	L – B	L – BF	L
LLM	N.S.	P = 0.001	P = 0.001
L – B		P = 0.001	P = 0.001
L – BF			N.S.
Experiment 2			
Diet 1		Diet 2	
	FEL	BEL	LDM
LLM	N.S.	P = 0.05	0 = 0.05
FEL		P = 0.05	P = 0.01
BEL			N.S.
Experiment 3			
Diet 1		Diet 2	
	F	B	
LLM	N.S.	P = 0.01	
F		P = 0.001	

ments in series 1 is reflected in declining numbers in the stream population at the time experiment was conducted. The high juvenile production in experiment 3 reflected peak juvenile production in the stream population. Juvenile production was not analyzed statistically because the eggs are carried in the brood pouch of the female for several weeks before release. Thus, young released in the first 5 wk were already in the brood pouches of the females at the start of the experiment and were not affected by the dietof the female. Cannibalism is another factor which may have influenced the number of juveniles counted.

Data from the laboratory and field preference tests showed a general correlation between nutritional characteristics of the substrate and its colonization by *G. minus* (Tab. 4). Fungus-enriched leaves and conditioned leaves with a reduced bacterial flora were significantly preferred over control (LLM). The control, on the other hand, was significantly preferred to both unconditioned and bacteria-enriched leaves. The fact that conditioned leaves were generally preferred over *T. elegans* may be attributed to mechanical problems associated with feeding on the colony between layers of plastic screen.

The most obvious point to be made from the foregoing studies is that the foliicolous mycoflora constitutes an important nutritive element in the food supply of *G. minus*. This conclusion is based largely on the fact that the presence or absence of elm leaves did not affect survivorship, while the presence of a microbial flora, especially the fungi, was critical. Leaves appeared to be important primarily as a substrate for the microbial communities. This hypothesis is further strengthened by evidence from the preference studies in which the amphipods showed a definite preference for fungus-enriched substrate. While not conclusive, it appears as though the mycoflora is a major factor underlying the food habits of *G. minus*.

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Tab. 4. Results of laboratory and field preference tests showing numbers of individuals recorded on each substrate.

Substrate	Laboratory Days 1	2	3	4	5	Total	*P*	% LLM	Field Replicates 1	2	3	Total[1]	*P*	% LLM
1. LLM	15	15	16	16	11	73	0.001	27.4	18	52	48	118	0.001	47.4
L	3	3	3	3	8	20			0	18	7	25		
2. LLM	8	10	13	15	15	61	0.001	46.0	29	27	22	78	0.001	36.0
BEL	8	7	5	4	4	28			12	12	4	28		
3. LLM	11	6	3	2	7	29	0.001	241.0	23	24	3	50	0.001	210.0
L – B	9	14	16	18	13	70			70	16	21	106		
4. LLM	5	8	10	5	10	38	0.05	150.0	23	7	27	57	0.001	193.0
FEL	13	10	10	15	9	57			30	43	37	110		
5. LLM	9	13	12	3	12	49	N.S.	70.0	80	54	43	177	0.001	49.1
F	8	5	6	15	5	39			28	33	26	87		

[1] After 48 h.

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Erratum

Thomas, W. A. and Grigal, D. F. 1976. Phosphorus conservation by evergreenness of mountain laurel – Oikos 27: 19–26.

Two values in Tab. 3 (p. 24) in this article are unfortunately incorrect. The correct values are given below.

	Value ($month^{-1}$)
A 13	$0.6 \times 0.48^{(T-7.5)2}$
A 24	$0.105 \times 0.94^{(T-6.0)2}$