

Assimilation efficiency of *Gammarus pseudolimnaeus* (Amphipoda) feeding on fungal mycelium or autumn-shed leaves

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Dry weight, ash-free weight, protein and energy content of food and faeces of *Gammarus pseudolimnaeus* Bousfield on different diets were compared. When feeding on elm or maple leaves, approximately 10% of the dry weight, 14-18% of the protein, and 17-19% of the energy of the ingested material was assimilated. When mycelium of various fungi which commonly decompose leaves in streams was offered, *Gammarus* assimilated 42.6-75.6% of the dry weight, 73.3-96.4% of the protein, and 67.9-83.2% of the energy of the food ingested. These results and the presumable preference of detritus-feeders for leaf areas with highest hyphal concentrations indicate that the significance of fungal substances in the diet of leaf-eating animals may be considerably higher than suggested by estimates of the average microbial biomass per unit weight of decomposing leaves.

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Сравнивали сухой вес, беззольный вес, содержание белков и калорийность пищи и экскрементов *Gammarus pseudolimnaeus* Bousfield которых кормили различной пищей. При питании лиственной вяза и клена ассимилировалось примерно 10% сухого веса, 14-18% белков и 17-19% энергии потребленного материала. При кормлении мицелием различных грибов, обычно разлагающих листья в воде, гаммарусы усваивали 42,6-75,6% сухого веса, 73,3-96,4% белка и 67,9-83,2% энергии потребленной пищи. Эти результаты и вероятное предпочтение детритофагами частей листа с наибольшей концентрацией грибного мицелия свидетельствуют о том, что значение грибов в питании животных-потребителей листвы может быть значительно выше, чем это предполагалось после определения средней биомассы микроорганизмов на единицу веса разлагающейся листвы.

1. Introduction

In small streams running through deciduous forests, autumn-shed leaves are often the single most important item at the base of the food chain (Cummins et al. 1966, Darnell 1964, Egglisshaw 1964, Fisher and Likens 1972, Hynes 1963, 1970, Minshall 1967, Nelson and Scott 1962). But sterile or freshly fallen leaves do not appear to be palatable to stream invertebrates, which much prefer to eat leaves already colonized by microorganisms (Kaushik and Hynes 1971, Mackay and Kalff 1973, Triska 1970). At early stages of leaf decay it is the fungi, and more specifically the aquatic hyphomycetes which are the dominant microorganisms (Bärlocher 1973, Bärlocher and Kendrick 1973b, Triska 1970). These fungi are easily recognized by their large, often tetradiate or sigmoid spores (Ingold 1942, 1966). When offered only mycelium of these fungi the ecological growth efficiency (i.e. percentage of food intake converted into biomass) of *Gammarus pseudolimnaeus* Bousfield was higher than when it was feeding on elm or maple leaves (Bärlocher and Kendrick 1973a), and the palatability of the leaves is decisively influenced by the specific composition of the fungal population they bear (Bärlocher and Kendrick 1973b). In the phase of the study reported here, our aim was to estimate and compare the proportions of the food assimilated (i.e. assimilation efficiency) by *G. pseudolimnaeus* when it was feeding exclusively either on leaf material or on fungal mycelium.

2. Materials and methods

Specimens of *G. pseudolimnaeus* were collected from the Credit River near Belfountain, Ontario. Leaves of elm *Ulmus americana* L. and maple *Acer saccharum* Marsh. were collected from two individual trees. They were cut into discs of approx. 1 cm diameter, leached for 4 d in running tap water at 12°C, dried for 2 d at 40°C, then stored in polyethylene bags at room temperature until needed.

The 10 fungi used in these experiment were those used in previous studies (Bärlocher and Kendrick 1973a, b). They were: *Alternaria* sp., *Fusarium* sp., *Aspergillus niger* van Tiegh., *Cladosporium* sp., *Humicola grisea* Traaen, *Tricladium angulatum* Ingold, *Tetracladium marchalianum* De Wild., *Anguillospora longissima* (Sacc. and Syd.) Ingold, *Clavariopsis aquatica* De Wild., and *Flagellospora curvula* Ingold. The first 5 fungi in the list are terrestrial hyphomycetes, the last 5 are aquatic hyphomycetes.

The purpose of the experiments was to estimate how much of the material they ingest is retained by the animals (assimilated) and how much is egested in the form of faeces (not assimilated). Faeces of *Gammarus* on leaf diets were darker in colour than faeces of animals on fungal diets (Fig. 1). Advantage of this was taken in the first experiment where the dry weight of ingested food and faeces derived from it were compared.

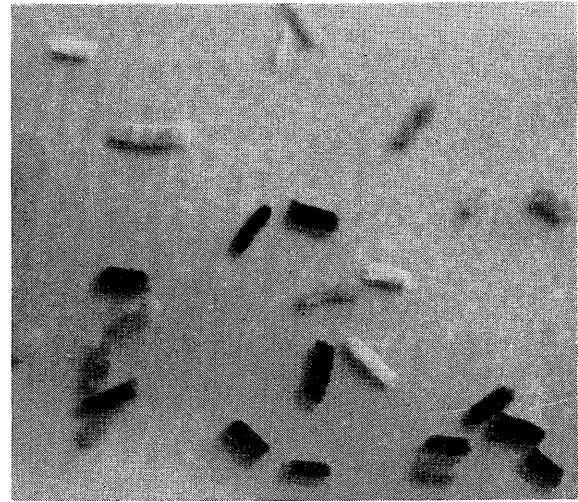


Fig. 1. Faeces of *Gammarus*. Light pellets: from animals on a fungal diet (*Tricladium*); dark pellets: from animals on a leaf diet (maple).

Animals which had been on a leaf diet were fed fungal mycelium (harvested from a liquid Malt Extract Broth) for 2 h, then put back onto a leaf diet. All faeces derived from the fungal food (recognizable by their lighter colour) were collected with a pipette at intervals no longer than 15 min, oven-dried at 40°C for 3 d, then weighed. This weight was compared with that of the ingested mycelium (estimated visually, cf. Bärlocher and Kendrick 1973a). For every set (= different source of food) 5 dishes were used, each with approximately 20 animals (= 5 replicates). All experiments were done at 17°C.

Similarly, the assimilation of dry leaf material was estimated by offering leaf discs (presoaked for 1 d at 0°C in sterilized distilled water) for 2 h to animals which had been on a fungal diet, then switching them back to a leaf diet and collecting all dark faeces. The time food particles took to pass through the gut of the animal was 45–120 min (estimation based on appearance of last dark or light pellet), provided that food was available. When no food was present the guts still contained food particles after many weeks.

In order to estimate assimilation of ash-free weight, protein and calorific contents, both food and faeces were analysed. Protein analysis and determination of ash-content were done respectively by the methods of Lowry et al. (1951) and Ward and Johnston (1962). A Phillipson oxygen calorimeter (Gentry Wiegert Instruments) and an Elektronik 19 Lab Recorder (Honeywell Inc.) were used to determine calorific values. The span of the recorder was set at 1 mv full scale deflection and the speed of the chart at 5 min/2.4 cm. Parts of tablets of benzoic acid were used for calibrating the microbomb. The calorific value of the food was estimated by using 5–10 mg pellets of mycelium which had been grown in

Tab. 1. Comparison of food and faeces of *Gammarus* on various diets. Each value based on 5 replicates, with 95% confidence limits.

	% oven-dry wt assimilated (ingestion - egestion)		% ash-free wt		% protein		% assimilated		cal g ⁻¹ of oven-dry wt		% assimilated
	food	faeces	food	faeces	food	faeces	food	faeces	food	faeces	
<i>Alternaria</i>	60.7 ± 5.2	95.4 ± 1.5	85.1 ± 2.5	65.0 ± 6.1	15.2 ± 1.2	2.5 ± 1.2	93.4 ± 7.0	5290 ± 55	4100 ± 81	69.5 ± 7.1	
<i>Aspergillus</i>	42.6 ± 6.1	62.6 ± 2.7	30.2 ± 2.6	72.4 ± 7.3	12.0 ± 0.7	5.5 ± 2.0	73.3 ± 8.7	2650 ± 60	1480 ± 70	67.9 ± 8.5	
<i>Cladosporium</i>	65.6 ± 3.1	97.8 ± 3.1	89.1 ± 3.1	68.6 ± 5.2	14.5 ± 0.9	2.0 ± 1.1	95.2 ± 5.0	4940 ± 80	3200 ± 70	77.7 ± 6.9	
<i>Fusarium</i>	71.6 ± 8.0	95.0 ± 1.0	86.7 ± 3.2	74.1 ± 4.5	13.5 ± 1.0	2.5 ± 0.7	94.8 ± 5.1	5655 ± 55	3660 ± 70	81.7 ± 9.1	
<i>Humicola</i>	68.9 ± 5.1	95.4 ± 0.6	84.2 ± 5.6	72.5 ± 6.9	20.8 ± 1.0	3.2 ± 0.8	95.2 ± 4.2	5520 ± 60	3210 ± 80	81.9 ± 8.0	
<i>Anguillospora</i>	65.2 ± 4.5	94.3 ± 0.7	79.2 ± 3.5	70.7 ± 8.0	18.2 ± 1.0	2.1 ± 1.0	96.2 ± 3.0	6240 ± 60	4110 ± 70	77.0 ± 5.4	
<i>Clavariopsis</i>	62.6 ± 4.6	94.7 ± 0.8	83.2 ± 2.1	67.2 ± 9.7	19.5 ± 0.8	1.8 ± 0.9	96.4 ± 3.6	5560 ± 50	3570 ± 70	76.0 ± 4.6	
<i>Flotellopsora</i>	60.1 ± 2.7	96.0 ± 1.5	80.1 ± 3.8	66.6 ± 8.3	15.9 ± 0.9	6.2 ± 0.6	84.3 ± 8.7	5380 ± 50	3490 ± 90	74.1 ± 6.6	
<i>Tetracladium</i>	75.6 ± 7.0	96.7 ± 1.1	90.6 ± 2.1	77.1 ± 5.4	12.1 ± 0.8	6.1 ± 0.7	87.6 ± 8.1	5970 ± 70	4110 ± 70	83.2 ± 7.1	
<i>Tricladium</i>	65.7 ± 4.2	95.7 ± 1.2	83.0 ± 5.7	70.2 ± 4.4	21.5 ± 1.2	4.1 ± 0.5	93.5 ± 7.0	4910 ± 60	2890 ± 50	79.8 ± 6.6	
Elm leaves.....	9.9 ± 4.0	87.5 ± 2.5	84.5 ± 6.1	13.0 ± 4.2	6.2 ± 0.5	5.6 ± 0.8	18.7 ± 3.2	4770 ± 60	4300 ± 80	18.6 ± 4.2	
Maple leaves.....	12.1 ± 3.8	90.6 ± 1.6	88.5 ± 2.9	14.1 ± 5.0	4.2 ± 0.5	4.1 ± 0.9	14.3 ± 4.1	4620 ± 40	4350 ± 60	17.2 ± 3.8	

Malt Extract Broth, and 1 cm diam. leaf discs. The faeces from the various diets were collected as described above, but the experiment had to be repeated several times before amounts sufficient for calorimetry could be accumulated. Preweighed faecal material was mixed with a known amount of benzoic acid and pressed into a tablet. The combined calorific value of faeces and benzoic acid was then determined.

3. Results

The percentages oven-dry weight of food assimilated by *Gammarus* on different diets are given in Tab. 1. These values and the ash-free content, protein content and calorific content of food and faeces were used to calculate the assimilation by *Gammarus* of protein, ash-free weight and energy from the various food materials (Tab. 1).

The results show that the fungi were much more profitably exploited by *Gammarus* than were the leaves. In particular, the fungal protein was very easily utilized (around 90% in most cases) while only 14-18% of the leaf protein was digested.

4. Discussion

Usually over 60%, and often as much as 80-90% of leaf material eaten by terrestrial detritus-feeders is returned to the environment in the form of faecal pellets (Bocock 1963, van der Drift 1951, Gere 1956). A recent study of the common freshwater amphipod *Hyalella azteca* yielded similar results; it was shown to assimilate only 5% when feeding on elm leaves (Hargrave 1970). Our figures (approx. 10-20% assimilation of elm or maple leaves by *Gammarus*) are of the same order of magnitude. These values have to be interpreted with some caution due to inherent shortcomings of the methods used (e.g. variations in the time different food materials take to pass through the gut, neglect of soluble substances in food and faeces, see Conover 1966, Hargrave 1970, Johannes and Satomi 1967). But there can be little doubt that much of the energy represented by the leaf cannot be directly used by most animals. On the other hand, microorganisms, with their vastly superior enzymatic equipment, are capable of decomposing and assimilating most of the leaf substances (Brock 1966, Burnett 1968, Griffin 1972, Hudson 1972, Müller and Loeffler 1971). The natural degradation of leaves is usually the result of an extended sequence of highly complex interactions between invertebrates and microflora. Microorganisms are often the first to attack freshly fallen leaves. Invertebrates will then start feeding on this modified substrate, which may consist of much unchanged leaf material, some partly broken down leaf material, microbial excretions and enzymes, and microbial cells. In this study we have shown that *Gammarus* can assimilate up to 83.2% of the energy, and up to 96.4% of the protein, of

fungi which dominate the early stages of leaf decay in streams. Similarly, Hargrave (1970) estimated that *Hyalella* utilizes 60–90% of the bacterial biomass it ingests. In other words, the effective nutritional value of microbial cells per unit weight is approximately 4–10 times higher than that of freshly fallen leaves. This fact and the probable preference of detritus-feeders for leaf areas with highest hyphal concentrations (Bärlocher 1973) indicate that the significance of fungal substances in the diet of leaf-eating animals may be considerably higher than is suggested by estimates of the average microbial biomass per unit weight of decomposing leaves. Still, there can be little doubt that a large portion of the food of detritus-feeders consists of indigestible leaf substances. Analyses of the gut contents of many stream-dwelling animals usually reveal mostly remains of vascular plants which appear to undergo little decomposition during their passage through the digestive system (Hynes 1970, Kaushik and Hynes 1971). Faeces, together with partly broken-up plant material, constitute most of the organic fraction of river sediments, which represent large reservoirs of food for many aquatic animals (Hynes 1970). For example, Minckley (1963) believes that in the stream Doe Run, Kentucky, the very abundant *Asellus bivittatus* (Isopoda) feeds almost exclusively on the faeces of the equally abundant *Gammarus minus*. There is good reason to believe that microorganisms again play an important role in the breakdown of these faeces. Nicholson et al. (1966) observed a distinct succession of various microorganisms on the pellets of the millipede *Glomeris marginata*. The snail *Hydrobia ulvae* may ingest its own faeces repeatedly, each time preferentially digesting the bacteria which decompose the organic carbon not directly usable by the animal (Newell 1965). Other observations of coprophagy have been reported by Frankenberg and Smith (1967), Frankenberg et al. (1967) and Mason and Odum (1969).

Our studies so far have been restricted to the very first step in the incorporation of autumn-shed leaves into the streams community. Undoubtedly, the further processing of the unassimilated substances in the faeces is equally important for stream life and, upon closer examination, may provide more examples of microorganisms acting as intermediaries of energy flow between plant and animal.

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