EXPERIMENTAL STUDY ON THE ROLE OF AUTUMN-SHED LEAVES IN AQUATIC ENVIRONMENTS

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I. INTRODUCTION

The importance of imported organic matter and debris from terrestrial plants as food at the lowest trophic level in running water has been stressed by several authors (Ivlev & Ivassik 1961; Hynes 1963; Darnell 1964; Minshall 1967). Nelson & Scott (1962), after a thorough study of a rocky outcrop in a Piedmont stream, concluded that, although this was an area of exceptionally high primary production because of the presence of *Podostemum*, 66% of the net production of primary consumers was derived from allochthonous organic matter. A substantial part of this material is known to be the autumn-shed leaves of riparian trees. Although exact estimates of the quantity of these leaves entering the streams are not available, the fact that woodland leaf litter production in different parts of the world ranges from 2·5 to 6·8 metric tons/ha/year (Bray & Gorham 1964) indicates that their contribution is likely to be quite high. It is known that members of almost all the important groups of benthic organisms feed on the leaves or on the plant detritus. Egglishaw (1964) has conclusively demonstrated that the distribution of stream bottom fauna is significantly correlated with distribution of plant detritus. Nevertheless, despite their obvious importance in stream ecology, autumn-shed leaves have received little attention from aquatic biologists.

Nelson & Scott (1962) found that the ratio between the weights of detritus feeders and detritus was higher than that between herbivores and plants. This they believed indicates that detritus feeders obtain a portion of their food in the form of bacteria or some bacterial metabolic product. This would seem to be the only way in which dead leaf material is converted into animal protein. We should perhaps here admit that as a pair of zoologists we were surprised to find how much protein remains in leaves when they fall from the trees.

II. MATERIALS AND METHODS

Autumn-shed leaves of elm, *Ulmus americana* L., alder, *Alnus rugosa* (Du Roi) Spreng. and oak, *Quercus alba* L., were collected soon after their fall during the second and third weeks of October 1965. All the elm leaves used in this study were collected from one tree and those of oak from two. Alder leaves were obtained from a number of trees growing in the same area. Leaves were air-dried in the laboratory and were stored at room temperature in polyethylene bags. *Hyalella azteca* (Saussure) (Amphipoda), *Asellus communis* sensu Racovitza (1920) (Isopoda) and *Paraleptophlebia mollis* (Eaton) (Ephemeroptera) were used for conducting feeding experiments. In the laboratory, specimens were maintained in enamel trays, kept in a constant temperature room at 10° C and were fed on elm leaves. The level of illumination in the constant temperature room generally ranged from zero to 60 ft-candle h/day and was too low for algal growth.

In all the experiments, filtered Speed River water was used in glass bowls with a capacity of about 1 litre. Prior to their use in the experiments leaves were rinsed in cold tap water, to remove attached debris, and were soaked for about 10 min. Leaf-discs about 1 cm in diameter were then punched out with a cork borer and were dried again at room temperature.

For estimation of insoluble protein, about 100 mg of leaf-discs, powdered in a mortar, and 10 ml of 0·1 N NaOH were placed in a 50 ml conical flask and kept overnight at room temperature in a mechanical shaker. The extract containing protein and other substances was separated by centrifuging. To 1 ml of the extract, 2 ml of 10% trichloroacetic acid (TCA) was added and the mixture was kept overnight in a centrifuge tube at 5° C for the complete precipitation of protein. The precipitate was then separated by centrifuging and redissolved in 1 ml of 0·1 N NaOH. From this solution two aliquots of 0·1 ml were taken for determination of the protein content by Lowry's method (Lowry *et al.* 1951). Bovine serum albumin, fraction V, was used for preparing standard curves. Membrane filtration was used for concentrating faecal pellets. The filters were then air-dried, weighed and protein estimated by the same procedure.

Estimation of protein in the animals was done by a slightly different method. The organisms were dried in a desiccator and were hand-ground in a cold room (5° C) in 2 ml of cold (5° C) 20% TCA. To this, 8 ml of 20% TCA was added and the material was kept overnight at 5° C in a 50 ml conical flask. Precipitated protein along with the other debris was separated by centrifuging in a cold room. To the precipitate, 10 ml of 0·1 N NaOH was added and kept overnight in a mechanical shaker at room temperature. The supernatant containing dissolved protein was again separated and estimation of protein was done by Lowry's method as for the leaves.

Enumeration of microbial densities in leaf litter has rarely been done by the serial dilution plate technique, mostly because of the difficulty of obtaining a homogeneous suspension for dilution (Witkamp 1963). This difficulty was overcome by using an omni-mixer homogenizer (Ivan Sorval, Inc.). Each leaf-disc was homogenized in a sterilized chamber of stainless steel, containing 5 ml of sterilized distilled water. At every sampling time, a bacterial count was made on five discs each of elm, alder and oak. Each disc was homogenized separately and dilution plates were made in triplicate using nutrient agar. Plate counts were made after 96 h of incubation at 30° C.

For preliminary observations on the type of fungal flora present, twenty-five discs of each type of leaf were plated at every sampling time on Czapek agar (Oxoid) containing 30 ml/l of streptomycin, and fungi were allowed to grow at room temperature. To check whether the fungal propagules were confined only to the surface, surface sterilization was effected by immersing the discs in 0·1% mercuric chloride for 1–5 and for 10 min.

To inhibit the growth of fungi Williams & Davies (1965) used 50 μg/ml each of nystatin and actidione. In the present study only nystatin (Squibb, Ltd) was employed. Bacterial growth was suppressed by using 1·5 μg/ml each of streptomycin and penicillin. No attempt was made to assay the effectiveness of the antibiotics used, as the results obtained were significant. The use of both the antifungal antibiotics would perhaps have given an even better contrast.

III. RESULTS

1. *Feeding experiments*

Since most of the soluble proteins of the leaves are likely to get washed out in the streams, only the insoluble proteins become available to the organisms and only this

fraction was estimated. The percentages of protein in the leaves and percentage of moisture and protein in the organisms used are shown in Table 1. All percentages relate to the air-dried weights.

Leaves were fed to the three types of organism and a total of twelve feeding experiments, six with elm and three each with alder and oak, were conducted. Each experiment was set up with five replicates. Since the percentages of insoluble protein in the leaves and the organisms were known, the total quantity of protein at the beginning of each experiment could be estimated. At the end of the experiment, the unused leaves, the surviving organisms, the faeces and other debris were analysed for insoluble protein to check if there was any change in the protein content of the system. The mean values obtained for

Table 1. *Insoluble protein content of the leaves and moisture and protein content of organisms based on eight samples of each*

	% protein (air dry weight)			% moisture			% protein (air dry weight)		
	Elm	Alder	Oak	*Hyalella azteca*	*Asellus*	*Paraleptophlebia mollis*	*Hyalella azteca*	*Asellus*	*Paraleptophlebia mollis*
Mean value	6·5	10·1	7·7	79·5	81·4	82·3	29·2	27·2	51·6
Standard deviation	0·5	0·5	0·4	1·2	1·1	0·9	1·8	1·6	1·7

the unused leaves were compared by Student's t test with those given in Table 1 for the respective type of leaf.

(a) *Elm leaves*

Four experiments were conducted with *Hyalella azteca* and the results are given in Table 2. A significant ($P<0·05$) increase in the mean total protein was recorded in experiments No. 1 and No. 2. In the other two experiments there was a slight decrease but in neither was it significant ($P>0·05$). In most of the replicates the insoluble protein in the leaves which had not been consumed by the organisms showed an increase over the original value of 6·5% (Table 1), and the maximum percentage recorded was 12·3 in experiments No. 1. The mean percentage of protein in the unconsumed leaves in all the four experiments showed a highly significant ($P<0·005$) increase over the normal value.

Only one experiment each with *Asellus* and *Paraleptophlebia mollis* was conducted (Table 3). The mean total protein content at the end of the experiment with *Asellus*, when compared with that at the beginning, showed a slight, but not significant, decrease ($P>0·05$). In contrast the mean quantity of total protein at the end of the experiment with *Paraleptophlebia mollis* was observed to be significantly ($P<0·05$) more than that estimated at the initiation of the experiment. A highly significant ($P<0·005$) increase in protein content in the unconsumed leaves was, however, recorded in both the experiments.

(b) *Alder leaves*

One experiment was conducted with each of *Hyalella azteca*, *Asellus* and *Paraleptophlebia mollis* (Table 4). In all the experiments a decrease in the total protein as well as in the percentage of protein in the unconsumed alder leaves was noticed ($P<0·05$).

(c) *Oak leaves*

With the oak leaves also only one experiment was conducted with each of the three types of organism (Table 5). In all the experiments a significant ($P<0·05$) reduction in the

Table 2. *Results of feeding elm leaves to* Hyalella azteca

Experiment no.	Replicate no.	No. of days	Temperature (°C)	No. of organisms introduced	No. of organisms recovered	Total protein at the beginning (organisms + leaves) (mg)	Total protein at the end (organisms + leaves + faeces and debris) (mg)	% protein in the unused leaves
1	1	14	Room temp.	40	11	10·5	10·4	12·1
	2				9	10·5	11·3	12·3
	3				14	9·9	10·2	11·6
	4				17	11·0	11·4	11·4
	5				17	10·5	11·4	11·6
2	1	21	15	20	11	8·2	8·6	8·7
	2				11	7·8	8·5	9·0
	3				14	8·4	8·8	9·6
	4				10	8·3	8·1	8·7
	5				14	8·4	9·2	9·0
3	1	30	10	20	14	9·4	8·3	7·0
	2				8	8·7	7·7	9·1
	3				13	10·5	9·4	8·7
	4				14	9·1	9·7	9·8
	5				14	9·3	10·4	9·8
4	1	42	10	20	11	8·4	6·0	4·6
	2				20	8·6	8·2	9·3
	3				20	8·2	7·9	7·5
	4				20	8·5	9·6	8·3
	5				20	9·0	10·7	11·0

Table 3. *Results of feeding elm leaves to* Asellus *and* Paraleptophlebia mollis

Replicate no.	No. of days	Temperature (°C)	No. of organisms introduced	No. of organisms recovered	Total protein at the beginning (organisms + leaves) (mg)	Total protein at the end (organisms + leaves + faeces) (mg)	% protein in the unused leaves
Asellus							
1	38	10	10	10	12·8	12·4	8·4
2				9	13·8	13·7	8·1
3				10	14·2	14·5	8·4
4				10	12·6	11·6	7·4
5				10	11·4	11·3	8·2
Paraleptophlebia mollis							
1	30	5	10	10	10·9	11·2	8·3
2				9	10·8	11·6	9·0
3				10	10·9	10·0	7·1
4				10	11·9	13·2	9·5
5				9	11·6	12·8	8·8

Table 4. *Results of feeding alder leaves to* Hyalella azteca, Asellus *and* Paraleptophlebia mollis

Replicate no.	No. of days	Temperature (°C)	No. of organisms introduced	No. of organisms recovered	Total protein at the beginning (leaves + organisms) (mg)	Total protein at the end (leaves + organisms + faeces) (mg)	% protein in the unused leaves
Hyalella azteca							
1	42	10	20	16	13·1	8·9	7·2
2				20	13·8	10·6	7·4
3				17	13·1	11·9	9·7
4				16	14·1	10·5	7·6
5				19	13·2	9·3	6·4
Asellus							
1	45	5	10	7	15·1	13·0	10·3
2				7	14·7	14·0	10·7
3				7	16·8	12·0	7·8
4				9	14·9	10·8	7·8
5				9	14·3	11·0	7·8
Paraleptophlebia mollis							
1	35	10	10	10	15·0	9·0	5·8
2				10	14·3	9·8	7·0
3				10	14·8	9·7	6·3
4				10	16·5	12·6	7·8
5				10	15·5	9·3	5·6

Table 5. *Results of feeding oak leaves to* Hyalella azteca, Asellus *and* Paraleptophlebia mollis

Replicate no.	No. of days	Temperature (°C)	No. of organisms introduced	No. of organisms recovered	Total protein at the beginning (leaves + organisms) (mg)	Total protein at the end (leaves + organisms + faeces) (mg)	% protein in the unused leaves
Hyalella azteca							
1	42	10	20	18	9·4	7·8	7·3
2				16	10·8	9·2	7·7
3				17	9·1	7·6	6·9
4				20	9·1	8·4	6·7
5				18	9·7	7·4	6·5
Asellus							
1	30	10	10	7	10·9	8·6	8·7
2				7	11·8	10·6	10·0
3				7	10·4	8·7	8·1
4				6	14·5	10·1	9·3
5				5	11·7	7·9	9·3
Paraleptophlebia mollis							
1	30	10	10	8	10·8	7·4	6·3
2				9	9·9	7·1	6·3
3				8	10·7	8·9	7·6
4				9	9·9	7·3	6·8
5				9	11·2	8·0	7·2

total protein was recorded. The percentage of protein in the unconsumed leaves in the experiment with *Asellus* showed a highly significant increase ($P<0{\cdot}005$). In the other two experiments, however, the unconsumed leaves showed a significant decrease in protein content ($P<0{\cdot}05$).

2. *Insoluble protein content and bacterial count of the leaves kept in the running stream water*

To observe whether this phenomenon of increase or decrease in the protein content of leaves also occurs when they enter streams, some experiments were conducted by placing the leaves in stream water in an artificial stream. Leaf discs placed in bags of about 17×12 cm size made from nylon netting with a mesh size of about 2 mm, were left in running stream water kept mostly at 10° C. Five bags were used for each type of leaf and samples taken after 10, 35 and 50 days were air-dried and analysed for protein content. The results, given in Table 6, show that the mean percentage of protein in elm leaves after

Table 6. *Mean protein content of the leaves left in the running stream water*

No. of days leaves kept in water	% of protein		
	Elm	Alder	Oak
10	8·4	10·3	7·4
35	7·9	7·8	6·7
50	8·6	7·9	6·8

Table 7. *Means of five triplicate bacterial counts in the leaves left in the running stream water*

No. of days leaves kept in water	No. of bacterial colonies $\times 10^{-5}$/leaf disc		
	Elm	Alder	Oak
0	139	–	–
10	537	893	689
20	151	240	84
35	134	219	90
50	0·56	0·24	42

10, 35 and 50 days remained considerably higher than the value of 6·5 (Table 1). The other two types of leaf showed reductions in protein content, the reduction being more pronounced in alder leaves. In the elm and oak leaves the mean values of percentage protein after 10, 35 and 50 days of exposure, when tested by an analysis of variance, did not show any significant difference but those for alder differed significantly ($P<0{\cdot}005$).

The results of bacterial analysis are summarized in Table 7. The highest mean bacterial count obtained for any sample of alder leaves was 1321×10^5, the corresponding figures for oak and elm being 1002×10^5 and 990×10^5 respectively. In all the three types of leaf-disc the bacterial count recorded after 20 days was less than that noted after 10 days. There was almost no difference between the bacterial densities recorded after 20 and 35 days, but the count obtained after 50 days showed further reduction. As these results tend to indicate that increase in the protein percentage in the elm leaves is not attributable to the bacterial population, experiments were conducted by selective inhibition of bacteria and fungi to elucidate this point.

3. *Effects of antibiotics*

Discs of elm leaves were left in glass bowls containing 500 ml of filtered stream water. For each of the three experiments conducted six sets of bowls were used and five replicates comprised each set. Three sets were kept at room temperature and the other three in the constant temperature room maintained at 5° C in the first experiment and at 10° C in the

Table 8. *Mean protein content of elm leaves treated with the antibiotics and of 'control' elm leaves*

	% protein					
	Room temperature			Constant temperature room		
Experiment no.	Control	Leaves with antifungal antibiotic	Leaves with antibacterial antibiotics	Control	Leaves with antifungal antibiotic	Leaves with antibacterial antibiotics
1	5·1	4·4	5·7	6·8	5·2	7·0
2	4·1	4·7	5·2	6·8	6·0	7·2
3	5·1	3·5	5·7	6·9	6·5	8·2

other two. The first experiment was run for 45 days and the second and third for 50 and 45 days respectively. One set of bowls, the control, contained only elm leaves and no antibiotics. One contained antifungal antibiotic and the third, the antibacterial antibiotics.

Table 9. F *values obtained for the treatments in the three experiments with antibiotics*

	F values for experiment no.		
Treatments	1	2	3
Total	12·0****	14·1****	30·6****
Temperature	28·5****	57·0****	103·8****
Antibiotics	14·3****	4·1*	22·3****
Temperature × antibiotics (interaction)	$0{\cdot}14^{n.s.}$	$2{\cdot}5^{n.s.}$	$2{\cdot}4^{n.s.}$

n.s. = not significant, **** = $P<0{\cdot}005$, * = $P = 0{\cdot}05$–$0{\cdot}025$.

The results given in Table 8 show that the percentage of protein in the leaves treated with the antifungal antibiotic, both at room temperature and in the constant temperature room, was less than that in the leaves of the other two sets, except in experiment No. 2 at room temperature. In contrast, the percentage of protein in the leaves treated with antibacterial antibiotics had increased, and that of the control leaves generally assumed values between those of the other two sets.

The six means obtained in each of the three experiments showed overall significant differences (Table 9) when statistically tested by the method used for factorial arrangements of treatments (Snedecor 1956). In none of the experiments were the values obtained for interaction between the temperature and the antibiotics significant at 5% level. In experiment No. 2, the value for the effect of antibiotics was significant only at 5% level. Barring this value all the other *F* ratios obtained for the total effect of treatments and for the effect of temperature and of antibiotics were highly significant ($P<0{\cdot}005$).

The results of orthogonal comparisons (Table 10) show that in all the experiments conducted in the constant temperature room, the mean percentages of the protein in the

leaves treated with the antifungal antibiotic were significantly different ($P<0{\cdot}025$ to $P<0{\cdot}005$) from the corresponding values of the other two sets. This difference, however, was not prominent at room temperature. Only in two experiments at room temperature did the mean percentage of protein content in the leaves treated with the antifungal

Table 10. F *values for the orthogonal comparisons made in the three experiments with antibiotics*

Temperature	Comparison	*F* value for experiment no. 1	2	3
Constant temperature room	Antifungal antibiotic v. control and antibacterial antibiotics	21·8****	6·6**	8·1***
	Control v. antibacterial antibiotics	$0{\cdot}08^{n.s.}$	$0{\cdot}6^{n.s.}$	11·4****
Room temperature	Antifungal antibiotic v. control and antibacterial antibiotics	7·1**	$0{\cdot}01^{n.s.}$	28·8****
	Control v. antibacterial antibiotics	$2{\cdot}3^{n.s.}$	5·4*	$2{\cdot}2^{n.s.}$

n.s. = not significant, **** = $P<0{\cdot}005$, *** = $P = 0{\cdot}005$–$0{\cdot}01$, ** = $P = 0{\cdot}01$–$0{\cdot}025$, * = $P = 0{\cdot}025$–$0{\cdot}05$.

antibiotic show significant differences from that in the other two sets. Comparison of the mean percentage values obtained for the untreated (control) leaves and for the leaves treated with antibacterial antibiotics showed significant differences in two experiments, in one at room temperature and in the other at 10° C.

4. *Preliminary observations on the fungal flora of the leaves*

Leaf-discs placed in the running stream water were removed from the bags after 10, 20, 35 and 50 days and were plated for fungal growth. Types of fungi observed are listed in Table 11. Fourteen genera were isolated and except for the group Mucorales, all

Table 11. *Fungi recovered from the leaf-discs of elm, alder and oak left in the running stream water*

1 *Alternaria* sp.
2 *Fusarium* spp.
3 *Epicoccum nigrum* Link
4 *Coniothyrium* spp.
5 *Gonatobotrys* sp.
6 *Acremoniella* sp.
7 *Trichothecium roseum* Link
8 *Cladosporium* spp.
9 *Trichoderma* viride Pers. ex Fr.
10 *Helminthosporium* sp.
11 *Penicillium* sp.
12 *Phoma* sp.
13 *Aureobasidium* sp.
14 *Mucorales*

belonged to the Fungi Imperfecti. *Alternaria* sp., *Fusarium* sp. and *Epicoccum nigrum* were observed in high percentages in elm, alder and oak throughout the period of 50 days. Similarly *Gonatobotrys* sp. in elm and *Coniothyrium* sp. in oak were also recorded on all the sampling occasions, while *Trichoderma viride* and *Cladosporium* spp. respectively occurred in alder and oak leaves up to the thirty-fifth day. The occurrence of the other genera was rather sporadic with many of them appearing only up to the twentieth day. Fungi were also observed in the leaves which had been surface sterilized.

IV. DISCUSSION

Leaves are known to vary in composition with differences of site, in successive years, at different positions in the crown and also during the period during which they remain on

trees (Ovington 1956). Since the leaves used in the present study were collected during a short period and from restricted situations, it seems probable that these variations were minimized in our material.

Kjeldahl nitrogen in some species of alder, oak and elm leaves collected in late summer or fall has been reported by, among others, McHargue & Roy (1932), Chandler (1941), Ovington (1956), Goldman (1961), Nykvist (1962) and Carlisle, Brown & White (1966). The values reported by different authors for alder leaves range between 2·31 and 2·8%, for oak leaves between 0·93 and 2·9% and for elm leaves between 0·77 and 2·13%. A sample of fallen European elm leaves has been reported to contain 7·5% of air-dried weight of extractable protein (Hynes 1963). The percentages of insoluble protein in alder, oak and elm leaves used in the present study were 10·1, 7·7 and 6·5 respectively on the basis of air-dried weight. The corresponding values for insoluble protein nitrogen work out to be 1·61, 1·23 and 1·04. These values would have been higher if the calculations had been made on the basis of oven-dried weight. Although the results obtained by different authors are not strictly comparable, the values obtained in this study are in general agreement with published results.

One striking result of our work was the finding that the unconsumed leaves in all six feeding experiments with elm leaves showed a highly significant ($P<0·005$) increase in protein content. In contrast, except for the one experiment with oak and *Asellus*, no increase in protein content was observed in alder and oak leaves, and in some experiments there was a significant decrease. It is clear therefore that leaves of different species behave differently. Changes in the nitrogen content of the decomposing leaves have been reported by several woodland ecologists. An increase was recorded in partially decomposed leaves of beech and birch, but maple and poplar leaves exhibited decreases (Coldwell & DeLong 1950). Definite uptake of exogenous nitrogen has been observed in beech (Saitô 1957) and oak leaves (Gilbert & Bocock 1960). Similarly, of the twenty-six species of woodland leaf litter and also filter paper, placed on moder, eight species including oak and also filter paper showed increase in the absolute amount of nitrogen (Bocock 1964). From another study Bocock (1963) concluded that the additional nitrogen in the decomposing oak leaves was derived mainly from atmospheric precipitation, insect frass and plant material from the trees. In the present study, however, it cannot be stated whether the increase in percentage protein in the elm leaves represents an absolute gain in nitrogen. It is perhaps reasonable to assume that it was so in those cases where the protein content of elm leaves increased to more than 11%. The source of additional nitrogen, if any, must have been the water in which the leaves were kept for feeding.

Although an increase in the protein content of the unconsumed elm leaves has been observed even in replicates in which mortality of organisms did not occur, the increase was generally observed to be greater in those experiments in which a larger number of organisms had died (Table 2). Richards & Norman (1931) noted that the nitrogen content of willow peelings increased from 1·58 to 3·34% during decomposition with some added source of nitrogen whereas without the added nitrogen source the increase was only up to 2·21%. In one of our simple experiments, not mentioned earlier, elm leaves were kept in five glass bowls each with 500 ml of filtered stream water and 5–6 mg of dried and crushed *Hyalella azteca*. The protein content of the leaves, determined after 45 days, went up to 11·3%. It would therefore appear that the nitrogen content of the water into which the leaves fall is an important factor. The increase in the protein content of the elm leaves left in the running stream water was not as pronounced as was observed in the feeding experiments. This may be because no organisms were kept

in the stream and thus the changes in the nitrogen content of water brought about by dead or metabolizing animals were not possible.

On an average, air-dried alder leaf-discs weigh about 25 mg and those of oak and elm about 16 and 20 mg respectively. On the basis of these figures and those given in Table 7, the bacterial counts for 1g of air-dried leaf material of alder, oak and elm, after 10 days of exposure to stream water, work out as 357×10^7, 413×10^7 and 268×10^7. These values are considerably higher than the maximum count reported for different leaf litter by Saitô (1957) and Ivarson & Sowden (1959) and most of the counts reported by Witkamp (1963, 1966). This perhaps indicates that the technique used in the present study for making leaf suspensions yields better results.

In the three types of leaf studied a general decline in the bacterial count was observed (Table 7). Saitô (1956) reported an increase in the bacterial count of decomposing beech leaves. Witkamp (1963, 1966) observed that easily decomposable leaf species like red mulberry (*Morus rubra* L.) show a decline in the bacterial population whereas in initially inert leaves, such as pine needles, the population increases. Increase in the bacterial count in oak (*Quercus alba*) and no significant change in red bud (*Cercis canadensis* L.) were also recorded by Witkamp (1966). Although quantitative estimates of bacteria do not necessarily reflect their importance in biological breakdown of organic matter (Witkamp 1963) it appears that the bacterial population is not a causal factor in increasing the protein content of the elm leaves. However, no great reliance can be placed on these results as only one type of medium was used.

Since in all the experiments, except for experiment No. 2 at room temperature (Table 10), use of an antifungal antibiotic significantly ($P<0{\cdot}025$ to $P<0{\cdot}005$) lowered the protein content of the leaves, it is clear that growth of fungi is associated with the increase in protein content. That bacteria are not related to the increase in protein content is obvious from the fact that in all the experiments their inhibition did not cause decreases in the protein content of the leaves. In fact an increase was invariably observed (Table 8) which, however, was not significant except in experiment No. 2 at room temperature ($P<0{\cdot}05$) and in experiment No. 3 at 10° C ($P<0{\cdot}005$). The effect of temperature in changing the protein content of the leaves was highly significant ($P<0{\cdot}005$, Table 9). The results also suggest that there is some sort of competition between the bacterial population and mycoflora. When the growth of the former is inhibited the activity of the latter increases and results in higher percentages of protein. In contrast, when the growth of fungi is suppressed, the bacterial population builds up and results in decreased protein content of the leaves. When both bacteria and fungi are allowed to grow (control) the protein content of the leaves generally assumed values between those of the other two sets (Table 8). The use of an antifungal antibiotic resulted in increased numbers of bacteria and actinomycetes in decomposing coniferous litter and when antibacterial antibiotics were used the number of fungi increased (Ivarson & Sowden 1959). Inhibitory effects between bacteria and fungi and between fungi themselves have also been demonstrated by Saitô (1958, 1960).

Since fungi could be recovered from the leaves which had been surface sterilized, the fungal propagules are not restricted to the surface of leaves. The list of fungi recorded (Table 11), however, is not likely to include all the fungi present on the leaves because only one medium was used and because of the probable suppression of some fungi caused by the growth and early proliferation of others. The fungi observed are common soil fungi and of the fourteen genera listed eight have been recorded by Saitô (1956) from beech leaves at different stages of decomposition in soil. Most of them have also

been reported from aquatic habitats (Cooke 1961). Cooke has strongly suggested that these fungi should be regarded as definite members of the population in aquatic systems and that their role in that habitat is clearly of fundamental importance.

It is difficult to state whether the organisms feed on the leaves because of the microbial population supported by them. Perhaps, as suggested by Hynes (1963), in view of the high protein content of the leaves, much of the feeding is direct. The importance of bacteria and fungi in the nutrition cannot, however, be overlooked. Ivlev (1945) has mentioned the successful use of filter paper as food by chironomid larvae with the help of bacteria, and the importance of bacteria is also evident from the work of Fredeen (1964) and Newell (1965). Similarly, large quantities of fungal hyphae have been recorded from the guts of the spring tail *Tomocerus (Pogongnathus) flavescens* Tullberg from bogs and fens (Smirnov 1958). Kevan (1965) has also mentioned that some springtails rarely eat anything but fungi. In any event, as pointed out by Cooke (1961), intentionally or accidently, the organisms feeding on the dead leaves must be eating the microbial population. This, as is evident from the work of Nelson & Scott (1962), is extremely important from the point of view of calculating biological efficiencies.

In conclusion, on the basis of preliminary observations made in the present study it may be stated that the composition of the fallen leaves entering an aquatic environment, the quality and temperature of water and the type of microflora involved in the leaf decomposition are some of the factors that are likely to dictate the degree of importance of autumn-shed leaves in a water body. In any study of the trophic structure of a stream or a body of water in which autumn-shed leaves are used as food, the microbial population deserves more consideration than it has received. Work on certain other leaf species and on some other aspects is in progress.

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SUMMARY

Preliminary observations were made on the possible role of autumn-shed leaves of *Ulmus americana, Alnus rugosa* and *Quercus alba* in aquatic environment.

The leaves were fed to *Hyalella azteca, Asellus communis* and *Paraleptophlebia mollis*. In all but one experiment there was a significant decrease in the percentage of protein in the unconsumed alder and oak leaves. The unconsumed elm leaves in all the experiments showed a highly significant increase in their protein content. Elm leaves left in an artificial stream also showed an increase, but alder and oak leaves showed a slight decrease.

In some cases the increase in protein content perhaps represents an increase in the absolute quantity of nitrogen, as has been observed by some woodland ecologists in the decomposing leaf litter in forests.

The results tend to indicate that the increase in the protein percentage in the elm leaves is not attributable to the bacterial population but is associated with the growth of the fungi. There is some sort of competition between the bacteria and the mycoflora developing on the leaves. The effect of temperature was highly significant in altering the protein content of the leaves.

Some of the factors that are likely to influence the importance of autumn-shed leaves in aquatic environment are their composition, the type of microflora involved in their decomposition and the quality and temperature of water. In any study of the trophic structure of a body of water in which autumn-shed leaves are used as food, the microbial population deserves more consideration than it has received.

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