

The Role of Bacteria in the Nutrition of Aquatic Detritivores

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Summary. Bacteriological analyses of the guts of four common lotic invertebrates are described. The results from these analyses suggest that *Simulium* and *Chironomus* digest at least half of the bacteria that they ingest in situ, but no evidence has been found for the digestion of bacteria by *Baëtis* or by *Ephemerella*. Moreover, *Simulium* and *Chironomus* do not appear to be selective, with regard to bacterial type, in their digestion. The limitations of the method are discussed and the relative importance of bacteria compared with other components of the insects' diet is assessed. Evidence is presented which supports the hypothesis that bacteria are not as quantitatively important as other components of the detrital food material.

Introduction

In freshwater ecosystems detritus is the major foodstuff of a large number of invertebrate animals including many oligochaetes, crustaceans, chironomids, Trichoptera, Ephemeroptera, Sphaeriidae and Simuliidae (Ladle, 1972). In this context detritus may be defined as non-living particulate organic matter along with its associated microbes. The micro-organisms appear to be necessary to make organic matter palatable to some invertebrates (Kaushik and Hynes, 1971; Mackay and Kalff, 1973) and in at least two situations the microbes appear to be more important as a food themselves than the organic matter on which they are situated (Bärlocher and Kendrick, 1975; Calow, 1974). However, a hyphomycete mycelium by itself has been shown to be insufficient to support growth of a mayfly nymph (Cummins et al., 1973), but Fredeen (1964) has reared *Simulium* in the laboratory on nothing but bacteria. To date investigations have centred on the qualitative importance of microbes and little attention has been paid to their quantitative value in the field. The work presented here was aimed at obtaining some preliminary quantitative information on

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the relative importance of bacteria in the natural diet of selected detritus feeding invertebrate larvae.

The animals studied were *Simulium* spp., *Chironomus thummi* (Kieffer), *Ephemerella ignita* (Poda), and *Baëtis* spp., which were all abundant at least during the early summer. They were all found either in the River Frome or an associated ditch (*Chironomus*) in close proximity to the River Laboratory of the Freshwater Biological Association (Nat. grid ref. SY 870867). The River Frome is rich in all the major plant nutrients (Casey and Newton, 1973) and at the sampling site had a width of about 11 m. It has a mean discharge of $5 \text{ m}^3 \text{ s}^{-1}$, pH 8.1 and temperature range of 3–21°C (Westlake et al., 1972). The ditch was only a few centimetres deep, very slow flowing and fed by land drains.

Materials and Methods

a) *Sampling.* *Simulium* larvae were found in large numbers attached to the stems and finely divided leaves of *Ranunculus penicillatus* (water crowfoot), the dominant submerged macrophyte in the River Frome. Three species were present, namely *S. ornatum* Meigen, *S. lineatum* L., and *S. equinum* L., but no distinction was made between them. Only the larger (5–8 mm long) larvae were used. *Chironomus* specimens were found in the ditch and they were obtained by sifting the surface sediment with a sieve of 1 mm pore size. The mayfly nymphs were caught in a pond net passed through a bed of *Ranunculus* in the direction of the current. The small nymphs and those with fully developed wing buds were discarded, the former because their small size made dissection difficult and the latter for fear they had stopped feeding. All the animals were immediately transported to the laboratory in river water with minimum disturbance. No distinction was made between *Baëtis rhodani* (Pict.), *B. scambus* Etn., and *B. niger* (L.) which are all known to occur in the River Frome.

b) *Bacteriology.* In the laboratory each animal was washed four times in separate aliquots of sterile water to reduce contamination by the animal's natural surface flora. The mid-gut was then aseptically removed from the larva and divided into three equal lengths. The fore and hind thirds were next placed separately into 20 mls of sterile distilled water and shaken mechanically until the gut contents were seen to have detached from the gut wall. Spread plates on casein-peptone-starch (CPS) medium (Jones, 1970) were prepared from 0.1 ml aliquots of the suspensions, five replicates for each portion of gut. The plates were incubated at 25°C for seven days before counting. 16 *Simulium*, 13 *Chironomus*, 16 *Ephemerella*, and 12 *Baëtis* juveniles were treated in this way.

In addition to the plate counts above, direct microscopic counts were also made on similar gut portions of the *Simulium* and *Chironomus*. For this technique four gut portions were suspended together in 20 ml of filter-sterilised distilled water and filtered through a 0.45 µm pore size black membrane filter. The bacteria were then stained with acridine orange according to the method of Bell and Dukta (1972) and examined with incident ultra violet illumination from an HBO200 mercury discharge lamp fitted to a Leitz Orthoplan microscope. A 3 mm BG 12 exciting filter and a 530 nm barrier filter were used with setting 3 on the Ploem illuminator. Twelve such determinations using 48 animals were made on *Simulium* and four on *Chironomus*.

Fifty bacterial colonies were subcultured from the spread plates of two *Simulium* fore gut preparations and fifty more were obtained from the corresponding hind guts. 'Fore' and 'Hind' guts in this paper refer to the anterior and posterior thirds of the mid gut respectively. The one hundred bacterial cultures were purified by repeated streaking and the following diagnostic tests applied: Gram stain (Kopeloff and Beerman's modification), morphology, oxidase, catalase, and motility by the hanging drop method. One hundred isolates were also made in similar fashion from *Chironomus* and two hundred from *Ephemerella* spread plates, but only the Gram stain was applied to these.

c) *Chemical Analysis.* The suspended solids were obtained by centrifuging 50 l aliquots of river water at approximately 18,000 g in the continuous flow head of an MSE 18 refrigerated centrifuge. Proximate analyses were carried out according to the methods of Allen et al. (1974) except that nitrogen was determined on a Hewlett Packard CHN analyser.

The results have been analysed with non-parametric statistics following the helpful review of Jones (1973).

Results

The plate count results are summarised graphically in Figure 1 but it should be noted that the results are given per unit gut in each case and differences in the size of guts of different individuals or genera have not been allowed for. The hypothesis that high numbers of bacteria in the fore gut were generally associated with high numbers in the hind gut has been tested statistically by Spearman's rank correlation method (Table 1). It can be seen that there is a highly significant correlation in *Simulium* and *Chironomus*, less so in *Baëtis* and not at all in *Ephemerella*. To determine whether or not numbers of bacteria were significantly higher in the fore gut of each genus compared with the hind gut Wilcoxon's signed rank test (Siegel, 1956) was used. The values in Table 1 show that in *Simulium* there was a very highly significant difference between fore and hind guts, *Chironomus* also exhibited a significant difference, but no departure from the null hypothesis has been shown for either *Baëtis* or *Ephemerella*. In Table 1 column 5 the average bacterial count in the hind guts is expressed as a percentage of the bacterial count in the fore gut.

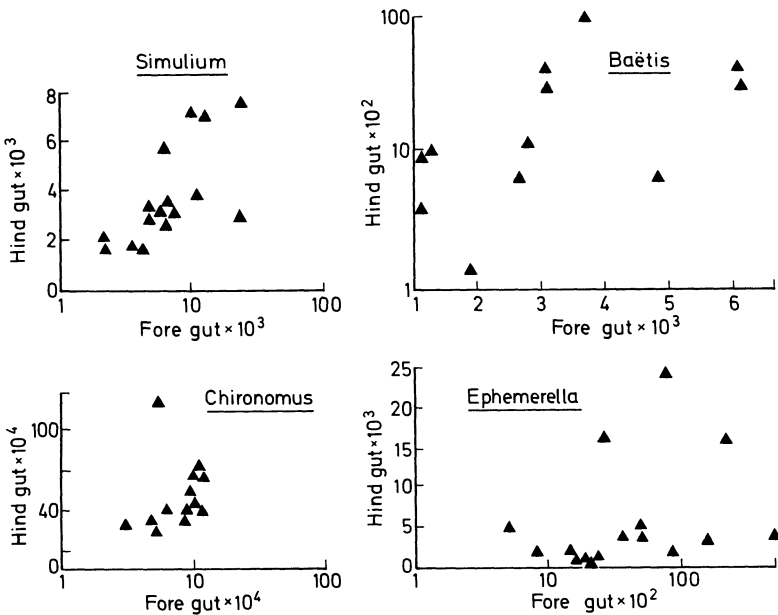


Fig. 1. Colony counts of bacteria isolated from the fore guts of juvenile insects compared with similar counts from the hind guts of the same individuals

Table 1. The relationship between plate counts of bacteria in the fore and hind guts of the genera studied

Genus	No. of samples (<i>n</i>)	Spearman's coefficients (r_s)	Wilcoxon's values (<i>T</i>)	Hind as % of fore
<i>Simulium</i>	16	0.757**	0***	55.1
<i>Chironomus</i>	13	0.791**	12*	73.0
<i>Baëtis</i>	12	0.643**	17	75.5
<i>Ephemerella</i>	16	0.379	46.5	165.0

Asterisks denote statistical significance thus *** indicates $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$

The direct count results, illustrated in Figure 2, are very similar in nature to the plate counts. Thus the number of bacteria in the fore guts of both genera are significantly correlated by Spearman's test with the number in the hind guts (Table 2) and there are also significantly more bacteria in the fore guts compared with the hind guts. Moreover, although the absolute values of the direct counts are an order of magnitude higher than the plate counts of bacteria (cf. Figs. 1 and 2) it is possible to compare the two by calculating the number in the hind gut as a percentage of the number in the fore gut. If this is done for each animal (or group of four animals in the direct counts) then one can test for a significant difference between the two by Mann and Whitney's *U* test (Elliott, 1971). It can be seen from Table 2 that no such significant difference was discernible despite the apparently large difference between the mean percentages for *Chironomus*.

The results of the diagnostic tests on isolates from *Simulium* are given in Table 3 and from *Chironomus* and *Ephemerella* in Table 4. The tests were not intended to enable even tentative identifications to be made, but rather to enable groups of organisms with some properties in common to be formed. Ten such groups were formed for *Simulium* (Table 3).

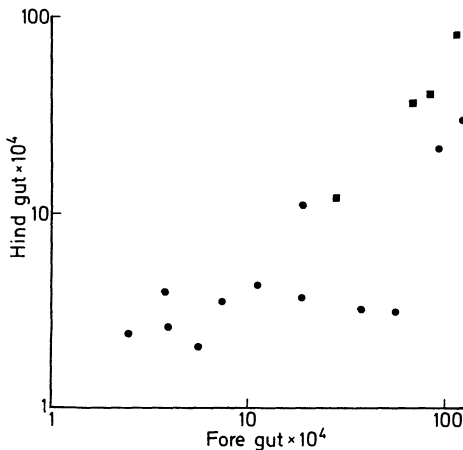


Fig. 2. Direct counts of bacteria obtained by fluorescence microscopy correlating numbers in the fore guts of larvae with those in the corresponding hind guts. Circles represent *Simulium*, squares *Chironomus*

Table 2. The relationship between direct counts of bacteria in the fore and hind guts of *Simulium* and *Chironomus*. Symbols as for Table 1 with the addition of *U* which is the lower value computed by Mann and Whitney's test. There was insufficient data to determine *T* for *Chironomus*

Genus	<i>n</i>	<i>r_s</i>	<i>T</i>	<i>U</i>	%
<i>Simulium</i>	12	0.699*	1***	66.5	43.1
<i>Chironomus</i>	4	1.000*		20.0	55.1

Table 3. Results of diagnostic tests on isolates from *Simulium* guts. The columns labelled fore and hind refer to the number of isolates from the fore and hind gut with the reactions in the other columns. The group numbers are arbitrary and the order is not intended to be significant

Group No.	Gram	Motility	Oxidase	Catalase	Fore	Hind
1	-	-	-	-	3	3
2	-	+	-	-	0	1
3	-	-	-	+	16	17
4	-	+	+	+	1	2
5	-	+	+	-	0	1
6	-	+	-	+	3	0
7	-	-	+	+	7	5
8	+	-	-	-	12	11
9	+	+	-	-	1	0
10	+	-	-	+	6	9

Table 4. Results of Gram staining on isolates from the guts of *Chironomus* and *Ephemerella*. Fore and hind columns have the same meaning as in Table 3. *Ephemerella* decreasing indicates that the organisms were taken from an organism where the numbers of bacteria decreased from fore to hind gut. The converse applies to *Ephemerella* increasing

	Gram	Fore	Hind
<i>Chironomus</i>	-	46	43
	+	4	7
<i>Ephemerella</i> decreasing	-	22	41
	+	27	8
<i>Ephemerella</i> increasing	-	43	44
	+	6	5

Discussion and Conclusions

Plate counts of bacteria detect only those organisms that will grow on the medium provided and hence one might infer from the analysis of the results

by Wilcoxon's signed rank test for *Simulium* and *Chironomus* (Table 1) that the bacteria were killed during their passage through these animals' guts, but not digested. However, the direct microscopic counts (Table 2) give similar results in terms of percentage change from fore to hind gut which indicates that the bacteria are not only killed, but at least partly broken up. Therefore it is tempting to conclude that in *Simulium* and *Chironomus* bacteria provide part of the assimilated food, but this conclusion is only true if the following assumptions are valid:

- a) that no appreciable mixing of the gut contents occurs,
- b) that the gut contents have a short retention time,
- c) that the gut has no caeca and is of constant diameter,
- d) that the gut is kept continuously full,
- e) that the food source is of constant composition.

If the gut contents are retained for more than a few h (i.e. assumption b) is invalid) then the bacteria would have time to multiply within the gut which would make sensible interpretation of this type of experiment impossible. Ladle et al. (1972) by feeding easily identifiable charcoal to *Simulium* larvae in situ have shown that these animals have food retention times of 20–30 min for small animals and up to 1 h for the larger individuals. The same experiment also shows that assumption a) is correct. A similar experiment in the laboratory on *Chironomus thummi* gives a food retention time of less than 2 h (L.C.V. Pinder, pers. comm.). None of the animals studied has caeca between the fore and hind thirds of the mid gut which was the only part concerned. Also the dimensions of the gut seemed more or less constant in all cases. Retention times of food by the Ephemeroptera were not determined, but Brown (1961) states that the turnover time in *Baëtis* is about 30 min. All of the animals dissected had full guts and this appears to be a general phenomenon of lotic invertebrates (Jones, 1950). Hence assumptions (a) to (d) inclusive are probably valid for all the animals studied but assumption (e) is not so easily ratified because three different feeding strategies are involved. Thus the *Simulium* spp. are sedentary on vegetation continuously filtering suspended solids whereas the *Chironomus* live in tubes in the sediment and feed frequently, but not continuously by ingesting the surface of the sediment which they can reach without completely leaving their tubes (Miall, 1895). The Ephemeroptera on the other hand swim or creep along periodically scraping the aufwuchs from the surface of stones, etc. ... Hence assumption e) is probably correct for *Simulium* and *Chironomus* for periods of a few hours at least, but may not be for *Baëtis* and *Ephemerella* which may eat algae at one time and moribund higher plant fragments a few moments later. The significant correlations, r_s , at high and low levels between numbers of bacteria in the fore and hind guts (Table 1) indicate that during the time it took to fill the guts the bacterial content of the food remained relatively constant. Had the composition of the food varied significantly during that same period no correlation would have been observed. Hence the analysis by Spearman's rank correlation test confirms the above hypotheses on the composition of food ingested by *Simulium* and *Chironomus*, and also indicates

that *Baëtis* is similar. Thus from these initial investigations only the food of *Ephemerella* seems to vary in its bacterial content.

The inclusion of *Baëtis* is provocative because they were observed to swim rapidly from place to place and to feed erratically. It seems unlikely that the bacterial content of the surface films on which *Baëtis* feeds is constant although it may be just this factor which is important in the choice of substrate by *Baëtis*. Hence an experiment was devised in which the substrate was likely to be more uniform. A perspex tank was left in a stream for 6 days so that a natural bacterial film could develop on its surfaces. It was covered to prevent the growth of algae and after this period *Baëtis* were introduced and left to feed for 6 h while river water was passed through constantly. Bacterial numbers in the fore and hind guts were then determined as before by plate counts. Regrettably only five individuals were examined in this way, but there was no significant correlation between numbers in the fore and hind guts ($r_s=0$) and 4 out of 5 showed an increase in numbers in the hind gut relative to the fore gut. Hence it must be concluded that we have been unable to demonstrate the utilization of bacteria by *Baëtis*. However, it must be emphasised that these results assume that equal portions of gut were taken in each dissection and a more accurate picture would have been obtained if the results had been determined per unit weight.

The results of the diagnostic tests on bacteria isolated from the insect guts (Tables 3 and 4) enable us to determine whether or not those animals which digest bacteria (*Simulium* and *Chironomus*) are selective in that digestion. It may be postulated that as Gram positive bacteria have much thicker cell walls than Gram negative organisms the latter may be more susceptible to digestion. Stuart et al. (1931) found such selectivity in the feeding of the planktonic cladoceran *Moina*, but *Simulium venustum* grew equally well on Gram positive and Gram negative bacteria (Fredeen, 1964). Our results (Tables 3 and 4) support those of Fredeen and moreover, within the Gram negative bacteria, which are much more common than the Gram positive, there appears to be no differentiation by *Simulium* either (Table 3). Nevertheless, as stated above, the tests performed are not sufficient to enable proper identification to be made and would not distinguish, for example, between *Pseudomonas* and *Aeromonas*. These two bacterial genera have been shown by Brinkhurst and Chua (1969) to have different resistance to digestion by two species of tubificid worms living in the same habitat. Each survives passage through the gut of one, but not the other. Thus although the evidence presented here suggests that *Simulium* and *Chironomus* do not selectively digest bacteria, it does not entirely preclude the possibility.

The quantity of bacteria digested, determined above, is a minimum level only as it represents the difference between the fore and hind thirds of the mid gut. Had it been possible to compare the fore and hind tenths, for example, a considerably higher value might be anticipated. Nevertheless assuming an average weight for each ingested bacterium some calculations on the contribution made by the bacteria to the total diet are possible. The dry weight of the bacteria is not easy to ascertain because bacteria vary considerably in size, laboratory grown specimens differ markedly from in situ organisms and moisture

contents are unknown. However, Zvyagintsev and Rogachevskii (1973) found the average bacterial density to be 1.1 and a moisture content of 90% seems reasonable so that the dry weight of a single bacterium from the river is probably of the order of 2×10^{-13} g. Using the above assumptions and our counts of bacteria by direct microscopy one may calculate that a *Simulium* larva ingests some 2.8×10^7 bacteria per day which is equivalent to 5.6 μg dry weight. If the whole of the bacterial community digested is absorbed then the bacteria contribute some $3.2 \mu\text{g day}^{-1}$ or 1.7×10^{-2} cal day^{-1} (Prochazka et al., 1973).

The number of bacteria ingested by *Simulium* per day can also be determined by another quite independent method. The average quantity of suspended solids and bacteria (from plate counts) during April to July inclusive in the River Frome on two successive years were $4.75 \mu\text{g ml}^{-1}$ and 3.7×10^4 cells ml^{-1} respectively (Baker and Farr, unpubl.) and the weight of the gut contents is approximately 50 μg (Ladle et al., 1972). Hence assuming the complete gut contents are exchanged 30 times a day the number of bacteria ingested by *Simulium* determined by this indirect method is 1.1×10^7 bacteria per day which is remarkably close to the figure computed from direct measurement.

The energy requirement of *Simulium* is not known so the relative importance of the 1.7×10^{-2} cal day^{-1} supplied by bacteria is difficult to assess accurately. However, the assimilation efficiencies of a number of aquatic animals are known and these have been tabulated according to taxonomic affinities by Ladle (1974) and food preference by Berrie (1976). From these tables assimilation efficiencies of detritivores vary from 2% for *Daphnia* to 85% for *Cypridopsis*. Hence any efficiency ascribed to *Simulium* must be speculative, but even if it had the relatively low value of 10% then the quantity assimilated from an average daily food intake of 1.5 mg, i.e. 150 μg , would still be very much higher than the 3.2 μg shown to be supplied by bacteria. This is not to say that bacteria cannot support the growth of *Simulium*, indeed Fredeen (1964) grew three Canadian species very successfully in the laboratory on a diet of bacteria alone. Rather we are saying that there appears to be insufficient bacteria in suspension in the River Frome to support growth of *Simulium* by themselves. Fredeen (1964) obtained maximum survival of *S. venustum* at bacterial concentrations of 1.3×10^7 cells ml^{-1} , several orders of magnitude higher than the normal number in the River Frome.

If the bacteria are unable to supply sufficient energy to *Simulium* what other component(s) of suspended solids are its principal food material? Suspended solids are generally regarded as relatively refractory and therefore not easily digested. Hence, contrary to observation, one would expect the material to pass relatively slowly through the gut if it was being utilised (Hynes, 1970). Nevertheless, suspended detritus probably varies in composition from river to river and embodies components from plants, animals and micro-organisms. In southern English chalk streams such as the River Frome the plant derived detritus is mainly from the 'softer' plants such as autochthonous *Ranunculus penicillatus* and *Rorippa nasturtium-aquaticum*, and allochthonous *Alnus glutinosa* and *Salix viminalis* (Dawson, 1976) rather than conifers, mosses, etc. which decompose more slowly. A proximate analysis of bulked samples of the suspended solids is given in Table 5. No analysis was made for soluble carbohy-

Table 5. Proximate analyses of food materials of *Simulium* (suspended solids) and *Chironomus* (ditch sediment)

	Suspended solids	Ditch sediment
Crude protein	10.6%	3.9%
Crude fat	6.5%	3.2%
Holocellulose	9.5%	4.5%
Lignin	7.8%	6.6%
Ash	67.2%	82.6%
Total	101.6%	100.8%

drate, but one would not expect that fraction to be important because it is likely to be rapidly leached away (Kaushik and Hynes, 1971). According to Allen et al. (1974) the result for lignin is likely to be high and the holocellulose fraction probably still contained 2–4% of the lignin. Nevertheless crude protein and crude fat, both of which are generally regarded as relatively easily assimilable materials, together constitute 17% of the total suspended solids. Comparable analyses for other rivers appear to be lacking, but these results suggest that although *Simulium* digests at least 50% of the bacteria it ingests, non-living detrital materials still account for the major proportion of its energy intake.

The situation for *Chironomus* is slightly different, it has twice as many bacteria in its gut compared with *Simulium*, but it is also twice the size (mean dry weight 0.8 mg). So even with a comparable food retention time the energy from bacteria alone is unlikely to be sufficient for all its needs. However, the average number of bacteria in the sediment ingested by the *Chironomus* was $(8.2 \pm 2.7) 10^7 \text{ g}^{-1}$. Thus unlike the suspended bacteria this benthic population appears in theory to be an adequate source of food for *Chironomus* by itself. Therefore in contrast to *Simulium* which may feed preferentially on bacteria but cannot obtain a sufficient quantity in the River Frome, *Chironomus* appears to feed preferentially on some component of its diet other than bacteria. The analysis of the sediment on which *Chironomus* lives is given in Table 5 from which it appears that about 7% is easily assimilable. However, these data should be treated with caution because sampling the surface few millimetres of sediment was found to be extremely difficult and some deeper sediment was inevitably included.

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