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Bacteria as food for blackfly larvae (Diptera: Simuliidae) in a lake-outlet in Finland

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Blackfly larvae (Diptera: Simuliidae) in a lake-outlet in Finland ingest large quantities of bacteria. The population density of bacteria decreases markedly from the anterior to the posterior of the mid-gut and, if all the digested bacteria are utilised in nutrition, they would provide 14 % of the measured total assimilation of larvae.

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1. Introduction

Blackfly larvae have the ability to filter, and ingest, a wide range of particle types and sizes from the river water in which they live. Almost all suspended, particulate material below a certain maximum dimension is available to the larvae (Wallace & Merritt 1980) though the extremely numerous small particles may not be filtered as efficiently as the largest ones (Kurtak 1978).

Fredeen (1964) has demonstrated that blackfly larvae can be reared to pupation on a diet solely of bacteria but Baker & Bradnam (1976) and Hansford (1978) concluded that digestion of bacteria played only a small part in the nutrition of blackfly larvae in chalk streams in southern England.

At the outlet of the river Teuronjoki from the lake Pääjärvi in southern Finland large numbers of bacterioplankton will be carried downstream and, as Carlsson et al. (1977) proposed that particles < 2 μm in diameter could be important in the nutrition of blackfly larvae in lake-outlets, one might expect bacteria to form an important component of the diet at this site. As the assimilation rate of larvae was known (Wotton 1978) this study set out to examine the part played by digested bacteria in the nutrition of larvae in Teuronjoki.

2. Material and methods

2.1. Field site

The river Teuronjoki is the major drainage channel from the lake Pääjärvi. Field samples were collected at the same site in the outlet of Teuronjoki which was used by Wotton (1978).

2.2. Methods

Four water samples were collected on 27 July 1978. A sterile syringe was used to pass four 5 ml samples of river water each through a 0.45 μm pore-size "Sartorius" membrane filter (the same pore-size as that used by Baker & Bradnam 1976). Filters were immediately removed from the holder and placed on a filter-paper pad impregnated with a solution of erythrosine in 5 % phenol. This will ensure fixing of the bacteria so that the population density of particles was as similar as possible to that found in the river water. On return to the laboratory further staining with erythrosine proceeded for several hours after which filters were de-stained on pads of filter-paper soaked in deionised water, dried, and cleared by mounting in immersion oil. The use of phase contrast microscopy then allowed counts to be made of the number of bacteria in each sample. Twenty random fields were counted on each filter, each field (of area 415 μm^2) being delimited by an eyepiece micrometer grid. In addition, 60 bacteria at random were measured for length and breadth in the case of "rods", diameter in the case of cocci, following the practice of Salonen (1977).

At the same time that water samples were taken on 27 July larvae were collected from the field into a vial containing erythrosine in 5% phenol and they were transported to the laboratory where four larvae were dissected in deionised water filtered through a 0.2 μm pore-size "Millipore" filter. In each case, the anterior 0.9 mm and posterior 0.9 mm of the mid-gut (corresponding to ten divisions of an eyepiece micrometer) were carefully cut and each section was transferred to a vial of filtered deionised water. The gut-contents were dispersed by passing the water in the vial rapidly in and out of the syringe several times (ten in almost all cases) and the whole was filtered through 0.45 μm pore-size "Sartorius" membrane filters and the same procedure for staining and clearing as that described above was applied. Counts of

bacteria were also made in the same way as that employed for the water samples.

3. Results

3.1. Bacteria in the water

The number of bacteria in the water samples are shown in Table 1. There were no significant differences between water samples ($K = 2.78$, d.f. $= 3$, $P > 0.05$ in Kruskal-Wallis one way analysis by ranks, Elliott 1977) so it can be stated that the population density of bacteria in Teuronjoki (of a size retained by 0.45 μm pore-size membrane filters) does not change markedly over the short period of collection. In all four larvae examined there was a significant decrease in the numbers of bacteria from the anterior to the posterior of the mid-gut (Table 1, $P < 0.05$ in all four cases in Mann-Whitney "U" tests).

3.2. Bacteria in the larvae

1) The four larvae which had the anterior, and posterior, of their gut-contents examined had a mean overall length of 5.4 mm and therefore a mean body tissue weight of 207 μg C (Wotton 1978). From the data presented by Wotton (1978) the G. M. regression of assimilation rate on body tissue weight gives $y = 7.37 + 0.23x$. For a larva of 207 μg mean body tissue weight the assimilation rate will therefore be 55 μg C/d.

2) From an experiment when larvae were fed charcoal in the field it is known that the first 0.9 mm of the mid-gut will be filled in c. 10 minutes by larvae of 5.4 mm overall length (Wotton 1978). Thus, as the mean biovolume of bacteria present in the anterior 0.9 mm of the mid-gut was computed at 5.9×10^5 μm^3 and, assuming feeding-rate to be maintained at approximately the same rate in larvae of this size (cf. Wotton 1978), an indication of the biovolume of bacteria ingested per day can be obtained viz. $5.9 \times 10^5 \times 6 \times 24$ $\mu m^3/d = 8.5 \times 10^7$ $\mu m^3/d$.

3) The mean biovolume of ingested bacteria was 0.153 μm^3/cell (in the water of Teuronjoki, mean individual biovolume was 0.087 μm^3). To correct for the shrinkage resulting from the staining procedure it will be necessary to multiply the estimate of ingested biovolume above by 1.85 (Straškrabová & Komárková 1979). The biovolume of live bacteria ingested per day will therefore be $8.5 \times 10^7 \times 1.85 = 1.57 \times 10^8$ $\mu m^3/d$.

4) From the anterior to the posterior of the mid-gut it is known that 46% of bacteria will "digested" and potentially available for assimilation by the larvae (this is a mean value, the range being 39—54%). The biovolume of bacteria available for nutrition will therefore be $0.46 \times 1.57 \times 10^8 = 7.2 \times 10^7$ $\mu m^3/d$.

5) Assuming bacteria to have a specific density of 1.07 and dry weight of 20% (Straškrabová & Komárková 1979) the dry weight of bacterial tissue available for assimilation can be given viz. $1.07 \times 0.2 \times 7.2 \times 10^7 \times 10^6$ μg dry weight/d = 15.4 μg dry weight/d. Assuming the carbon content of bacteria to be c. 50% of dry weight (Straškrabová & Komárková 1979) the amount of carbon potentially available to the blackfly larvae will be $0.5 \times 15.4 = 7.7$ μ C/d.

6) The contribution made by digested bacteria to the total assimilated will therefore be, on average, 7.7 of 55 = 14%.

Table 1. Numbers of bacteria in each 5 ml water sample from Teuronjoki and the number of bacteria in the anterior 0.9 mm, and posterior 0.9 mm, of the mid-gut of four blackfly larvae. All values are given with 95% confidence limits and the percentage decrease in numbers of bacteria along the gut is given for the four larvae.

Water samples	Anterior mid-gut	Posterior mid-gut	%
$4.1 \pm 0.7 \times 10^6$	$3.6 \pm 0.7 \times 10^6$	$2.2 \pm 0.3 \times 10^6$	39
$3.9 \pm 0.5 \times 10^6$	$3.7 \pm 0.6 \times 10^6$	$2.2 \pm 0.4 \times 10^6$	41
$3.5 \pm 0.7 \times 10^6$	$4.1 \pm 0.8 \times 10^6$	$1.9 \pm 0.4 \times 10^6$	54
$3.3 \pm 0.6 \times 10^6$	$4.2 \pm 0.7 \times 10^6$	$2.1 \pm 0.3 \times 10^6$	50

4. Discussion

Many of the bacteria ingested by the larvae of Teuronjoki will originate from the lake. Straškrabová & Komárková (1979) found that bacteria in agglomerates (4 cells or more) made up only 20% of the total count from the water of a reservoir in Czechoslovakia, and Salonen (1977) found that bacteria attached to detritus from lake Pääjärvi water in winter contributed only a small percentage of total bacterial numbers, so it is assumed that most bacteria ingested by larvae will either be suspended freely in the water of Teuronjoki, or present as small agglomerates. Observations of the filters of Teuronjoki water with phase contrast microscopy tend to support this assumption. It is interesting to note that the mean individual biovolume of bacteria suspended in the river water is smaller than that of bacteria ingested by the blackfly larvae and this is probably a result of the lower efficiency with which larvae can capture the smallest particle sizes available (Kurtak 1978).

The mid-gut of blackfly larvae is very large relative to the rest of the gut and roughly parallel-sided along its length, which extends for the greater part of the body. Particulate material captured by the cephalic fans, the modified head-parts used in feeding, is swept into a bolus by the mandibles and passed to the cibarium. From the very short fore-gut the bolus enters the mid-gut where it forms the latest "layer" in the gut-contents. This layering effect can be demonstrated when larvae are fed charcoal powder in suspension; the result is a distinct charcoal band across the gut-contents and this proceeds posteriorly as a discrete entity until it passes to the hind-gut and thence to the exterior. The distance a charcoal band moves along the gut, expressed as a percentage of the body length (from the posterior of the head-capsule), can be used as a measure of feeding-rate and, for larvae of 5.4 mm mean overall length, the percentages of body length travelled are 18% after 10 minutes, 37 % after 20 minutes, and 60 % after 30 minutes (Wotton 1978) which is a remarkably even mean rate of passage. The uniformity of the movement of the gut-contents along the gut allows valid comparison to be made between the anterior, and posterior, 0.9 mm of the mid-gut since these quantities will have been collected over a similar period of time. In assessing the role of digested bacteria in the nutrition of larvae it is assumed that the mid-gut is the principal site of digestion; it is also assumed that the feeding-rate measured in the field is maintained through 24 hours (cf. Kureck 1969). As Baker & Bradnam (1976) point out, however, the absolute contribution made by digested bacteria to the nutrition of blackfly larvae will require measurements of the immediate anterior, and posterior, sections of the mid-gut, not the first and last 0.9 mm as in the present study.

My results show, however, that digested bacteria can only contribute a small percentage of the measured assimilation rate of the larvae in Teuronjoki and this broadly agrees with the estimates made by Baker & Bradnam (1976) and Hansford (1978). Both the Finnish lake-outlet and the southern England chalk streams therefore contrast with the turbid rivers which Fredeen (1964) suggested had large blackfly larval populations supported mainly by digestion of bacteria. If Carlsson et al. (1977) were correct in proposing that particles less than 2 μm in diameter were the ones which were important in supporting lake-outlet assemblages of blackfly larvae then it is unlikely, from the results of the present study, that digested bacteria form a major component of the nutrition of larvae at these sites. It does not, however, rule out the possibility that bacteria which are not digested in the gut will have been "packed" adjacent to potential substrates and thus have released by-products of their own metabolism which can be absorbed and utilised by the blackfly larvae.

The discovery of mucosubstance on the cephalic fan rays (Ross & Craig, 1980) has gone a long way to providing an explanation of how larvae can capture fine particles from the river water passing over them. The question remains: What part do the abundant particles less than 2 μm in diameter (other than bacteria) found in the gut play in the nutrition of larvae?

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