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MIDGE LARVAE (DIPTERA: CHIRONOMIDAE) AS ENGINEERS IN SLOW SAND FILTER BEDS

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Abstract—Midge larvae (Diptera: Chironomidae) are found abundantly (7.4×10^4 larvae m^{-2}) in slow sand filter beds open to the atmosphere. Larvae eat the schmutzdecke accumulated at the sand surface and egest faecal pellets that are compacted and bound with mucopolysaccharides, faecal pellet size increasing with larval size. Pellets become diffuse after conditioning by microorganisms, and pellets, whether fresh or conditioned, are broken by abrasion. As up to 23% of the sand surface can be covered by fresh faecal pellets each day, midge larvae probably play an important role in the functioning of slow sand filters. © 1999 Elsevier Science Ltd. All rights reserved

Key words—midges, Chironomidae, slow sand filters, faecal pellets, engineering

INTRODUCTION

Slow sand filters are used to purify drinking water. The sand is of fairly uniform size; 85% of clean sand grains in the filter beds that we studied having a diameter of 0.25–1.00 mm. The surface area of sand grains ranges from 0.20–3.14 mm^2 (assuming sand grains to be approximately spherical) and the range of planar area (the area of substratum covered) of each grain is 0.05–0.79 mm^2. Organic matter, including pathogens, is removed as water passes between sand grains which are coated with bacterial biofilm. Some organic matter, especially bacteria, is also trapped by protists (Lloyd, 1996) and the microorganisms are grazed by invertebrates (Duncan, 1988). The biological community takes time to develop after beds are re-laid (Bellamy *et al.*, 1985a) and slow sand filters are initially "run to waste" to allow its build-up.

A layer of detritus and microorganisms, the schmutzdecke, accumulates during filtration. Schmutzdecke has a complex architecture that allows the passage of water, and it is rich in mucopolysaccharides, secreted both by organisms *in situ*, and by the impaction of fibrils that have broken away from algae (Clarke, 1988). Purification of contaminants and particulate organic carbon from water is mainly effected in the first 2–3 cm of a slow sand filter (Duncan, 1988; Graham *et al.*, 1996) and Bellamy *et al.* (1985a) have concluded that the schmutzdecke improves the removal of pathogenic bacteria from drinking water by an order of magnitude. Recently, Ojha and Graham (1996a) have used models to predict that schmutzdecke can make a significant contribution to overall particle removal from influent water.

Where filter beds are open to the atmosphere they, like all other water bodies, are colonised by aquatic insects. Among the most successful colonisers are nonbiting midge larvae (Diptera: Chironomidae) (Wotton *et al.*, 1992, 1996). First instar (life-stage) larvae hatch from eggs laid in/on the water and, after a short time spent swimming near the water surface (Pinder, 1995), they migrate to the substratum to colonise the sand in huge numbers. Larvae grow through three further stages, when pupae are produced to allow the transition from aquatic to terrestrial life. Adult flies of both sexes feed on plant sugars and, after swarming, mating occurs (Armitage, 1995). Once matured, the fertilised eggs are laid into the water of filter beds to complete the cycle.

In the filter beds we studied there is little emergence of adult midges for 15–16 days after filling with water. After this time the first mass emergence occurs (Wotton *et al.*, 1992) and the abundant production of adults is then continuous. The first midge larvae on the substratum do not have to compete with larger larvae and this may explain why they give rise to larger adults (Wotton and Armitage, 1995). In time, populations are of mixed age, and thus, size.

On the substratum, larvae of most species begin gathering particles to construct the tubes in which they live. The size and permanence of tubes varies between the species found (Chaloner and Wotton, 1996) but all tubes consist of particles bound with

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silk secreted by the insects. In addition to using particles for tube construction, many particles are also ingested. Feeding larvae appear to be nonselective of particle quality (Berg, 1995), have a rapid gut throughput time (32–120 min depending on larval size, Welton *et al.*, 1991; Chaloner, 1995), and their assimilation efficiency is probably low as a result. Larvae thus produce copious quantities of faecal pellets which are characteristically cylindrical and compact.

We measured the population density of midge larvae in filter beds and carried out laboratory experiments to investigate: (i) the size of faecal pellets produced by midge larvae of different sizes (as the midge community was of mixed size for the majority of bed runs) (ii) the number of pellets produced by larvae and (iii) the rate at which pellets broke down by biological conditioning, and by physical disruption, in sterilised, and unsterilised, tap water and water from a filter bed (to assess the role of microorganisms in conditioning). By combining data from the field, our experiments, and from the literature we indicate the importance of engineering of accumulated organic matter by midge larvae. While the microorganisms of slow sand filters have been included in models (Ojha and Graham, 1996b), we can find no record of the consideration of midge larvae in the analysis of filter performance.

MATERIALS AND METHODS

All samples of midges and organic matter were collected from freshly-drained slow sand filter beds at Ashford Common water treatment works where earlier studies had been conducted (Wotton *et al.*, 1992; Wotton and Armitage, 1995; Chaloner, 1995; Chaloner and Wotton, 1996). Samples were collected into plastic bags, returned to the laboratory in a cooler, and placed immediately into a 5°C cold room. Quantitative samples of larvae were taken from beds drained for cleaning, using petri dishes which were pushed into the sand surface, removed using a plasterer's trowel, and returned to the laboratory sealed in 70% alcohol. Six quantitative samples were taken on each of four sampling occasions from August through November.

Four enamel dishes (each 21 × 16 cm) were filled with aerated and dechlorinated (ADC) tap water and a suspension of unsterilised schmutzdecke added to produce a thin layer of organic matter on the base of the dish after settlement. Twenty-two fourth instar midge larvae were distributed between the dishes and left for 24 h. The faecal pellets produced within each feeding territory were counted into vials to assess variation in production of pellets by larvae of similar, large size. In further experiments, 21 midge larvae of a range of sizes were distributed between four new dishes prepared as above. After 24 h, the substratum around tubes had been cleared and piles of faecal pellets were visible. Pellets were collected into vials of 70% alcohol and the larvae that produced them removed and placed in further vials containing the same preservative. Measurements were then made within a week of preservation. The overall length of each larva was recorded and the length (h) and diameter ($2r$) of each faecal pellet measured. We were thus able to calculate the relationship between faecal pellet surface area ($2\pi rh + 2\pi r^2$) and larval size. Planar area of pellets was calculated from $2rh$.

Two sources of water were used in experiments on decomposition of pellets — tap water and water from the filter beds. Both contained microorganisms, but more bacteria must be present in the untreated water. Half of each water sample was sterilised in an autoclave for 1 h at >160°C. A sample of schmutzdecke was passed through a 200 mm mesh sieve and sterilised similarly in an autoclave. Sufficient of this detritus was added to five petri dishes to cover the surface of their bases. We did not attempt to replicate conditions over the substratum of a slow sand filter, but provided conditions that enabled us to follow the sequence of pellet conditioning most clearly.

One fourth instar larva of *Psectrocladius limbatellus* (Holm.), the dominant species of midge during the period of investigation, was placed into each dish. We used fourth instar larvae of roughly similar size in these experiments as we wanted faecal pellets to be of consistent size. Faecal pellets were collected on production and stored in covered petri dishes of water in the four treatments. Pellets were left in these dishes for 1–5 days. We examined the size of faecal pellets in dishes from each treatment

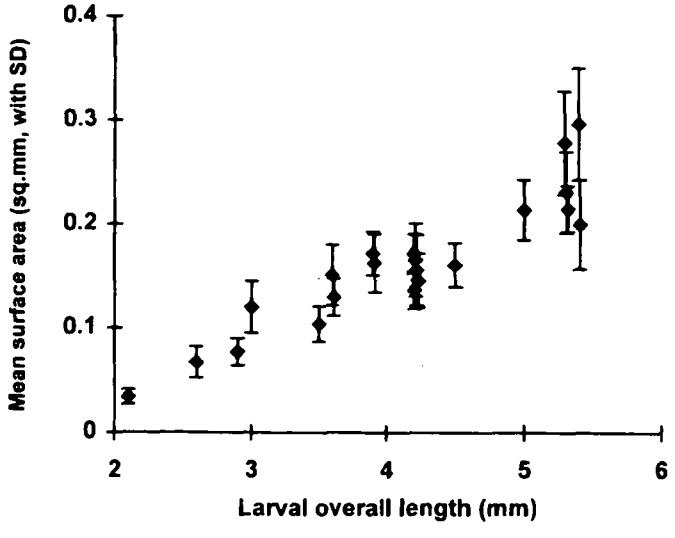

Fig. 1. The surface area (mean ± S.D.) of faecal pellets produced by midge larvae of a range of lengths.

each day and recorded their length and diameter, surface area being calculated as above.

After measurement, pellets were placed into a small vial (5 ml) and shaken vigorously by hand in 2 ml of water, inverting the vial 10 times on each occasion. This simple, but standardised procedure, was designed to test physical disruption of pellets, and thus an estimate of abrasion over time. The surface area of pellets was again calculated to assess whether pellets had changed in size and we were thus able to compare the biological and physical breakdown of pellets of different age.

Some of the schmutzdecke used, and faecal pellets produced, were stained with alcian blue on a glass microscope slide. Mucopolysaccharide is coloured a vivid blue by this stain (cf. Wetzel *et al.*, 1997) and we examined pellets under the microscope to assess the amount of this material present.

RESULTS

Population density of midge larvae from the surface of drained beds averaged $7.4 \times 10^4 \pm 1.5 \times 10^4\ m^{-2}$ (±S.D.) from August through November. Small oligochaete worms were also abundant.

Faecal pellet surface area increased ($r_s = +0.9042$, $n = 21$, Spearman rank correlation) with increasing larval length (Fig. 1). The mean surface area of faecal pellets from a larva of 2.5 mm overall length was 0.06 mm^2 (planar area 0.015 mm^2), whereas the mean surface area of faecal pellets produced by a larva of 5.0 mm overall length was 0.22 mm^2 (planar area 0.055 mm^2).

Faecal pellets showed a highly significant increase in surface area with time in unsterilised treatments (Fig. 2, $F_{5,\ 167} = 25.93$, $P < 0.001$ for tap water, $F_{5,\ 153} = 36.49$, $P < 0.001$ for filter bed water, both ANOVA). Pellets incubated in tap water showed a 97% increase in surface area over 5 days and those incubated in filter bed water an increase of 144%. When sterilised water was used, there was no significant difference in surface area with time ($F_{5,\ 145} = 0.40$, $P = 0.850$ for autoclaved tap water (a 2% increase in surface area being recorded over 5 days), $F_{5,\ 164} = 1.60$, $P = 0.162$ for autoclaved filter bed water (with an increase in surface area of 19% over 5 days, both ANOVA). This demonstrates clearly the significance of aquatic microorganisms in altering the compaction of faecal pellets.

In all treatments, and for fresh pellets, there was a highly significant difference between shaken and unshaken pellets ($P < 0.01$, Tukey–Kramer tests). Disruption of pellets therefore always occurred, but the size of pellet fragments changed significantly with time in three of the treatments ($F_{5,\ 225} = 3.81$, $P < 0.01$ for tap water, $F_{5,\ 236} = 15.55$, $P < 0.001$ for filter bed water, $F_{5,\ 218} = 5.40$, $P < 0.001$ for

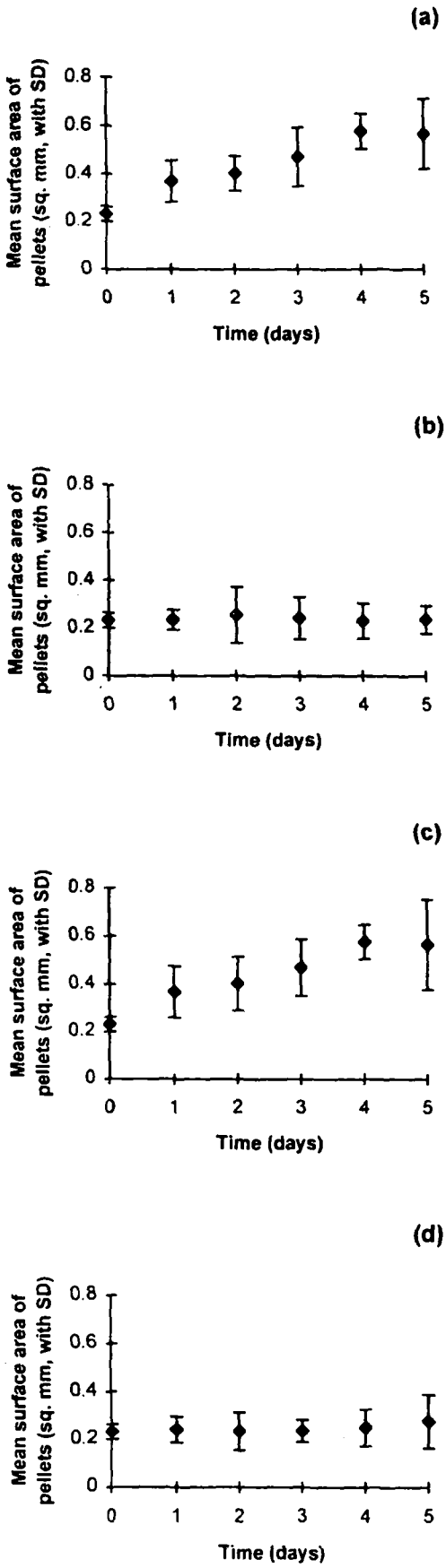

Fig. 2. Change in surface area of faecal pellets with time using four water treatments: (a) aerated and dechlorinated tap water; (b) aerated and dechlorinated tap water that has been autoclaved; (c) water from a filter bed; (d) filter bed water that has been autoclaved.

autoclaved filter bed water, all ANOVA) but not in autoclaved tap water ($F_{5,\ 197} = 1.47$, $P = 0.201$, ANOVA). When pellets were shaken to simulate abrasion, the largest remaining fragment of each faecal pellet became smaller with the duration of conditioning.

Microscopic examination of both schmutzdecke and faecal pellets showed them to be stained strongly with alcian blue. Schmutzdecke consists of cells and detritus particles embedded in a mucopolysaccharide matrix (Fig. 3a). The composition of fresh faecal pellets and schmutzdecke appear little

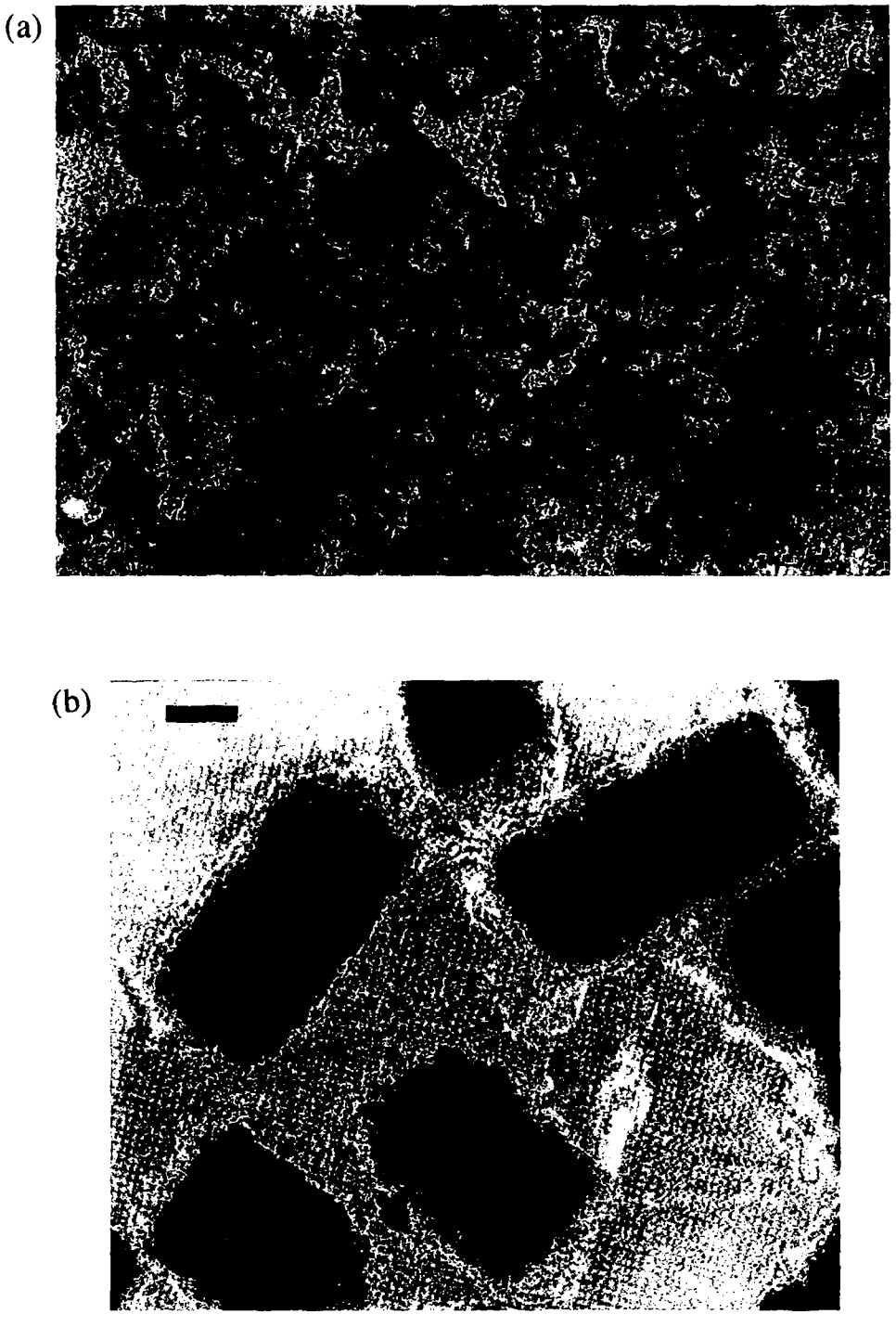

Fig. 3. (a) Schmutzdecke material with algal cells, including diatoms, clearly visible. The grey matrix shown in the photograph is heavily stained with alcian blue dye, indicating the presence of mucopolysaccharide. Scale bar is 100 μm. (b) Fresh faecal pellets produced by fourth instar larvae of *Psectrocladius limbatellus* from material in (a). Scale bar is 100 μm.

different qualitatively, although pellets are obviously compacted (Fig. 3b). After conditioning by microorganisms, pellets become diffuse.

P. limbatellus larvae produced 87 ± 49 faecal pellets per day (mean ± S.D.), showing wide variation in larvae of similar size. These pellets are added to older ones that are undergoing conditioning and disintegration. As the average planar area of faecal pellets was 0.035 mm^2 (calculated from $2rh$ for all the pellets measured) and 7.4×10^4 larvae were found on the substratum, we estimated that the total planar area of fresh faecal pellets on each m^2 of substratum was 0.23 m^2 ($7.4 \times 10^4 \times 87 \times 0.035$), showing that 23% of the filter surface was covered by fresh pellets each day. We are aware that this is an approximation, as pellets are not arranged in a uniform layer and we have observed them being eaten by larvae and incorporated into tubes. However, it does indicate the extent of the engineering of schmutzdecke by midge larvae.

The mean planar area of tubes constructed by fourth instar larvae of *P. limbatellus* has been recorded as 16.9 mm^2 (Wotton *et al.*, 1996) and 1.2 tubes (on average) were built in 24 h (Chaloner and Wotton, 1996). The total planar area covered by these tubes was therefore 1.5 m^2 over each m^2 of the sand filter for one day after larval settlement. This overestimates the real value as tubes constructed by second and third instar larvae are likely to be smaller than those of fourth instars, and it must be stressed that tubes are not likely to be constructed at this rate for the duration of larval life (Chaloner and Wotton, 1996). Nevertheless, a substantial area of the filter bed will be covered with midge tubes.

DISCUSSION

Slow sand filter beds that are open to the atmosphere are colonised by midge larvae. With time, a mixed-size community of larvae is present, and this is maintained at high population densities for the duration of a bed run, larvae feeding on the accumulating schmutzdecke. Although small larvae produce smaller faecal pellets than large larvae, the decomposition of all pellets is likely to follow a similar path. Biological conditioning results in the invasion of bacteria and fungi that digest polymer bridges binding the faecal material. When polymer bridges are broken, pellets become diffuse and this explains their increased surface area with time of conditioning. As pellets were heavily stained with alcian blue, we conclude that the binding materials are mucopolysaccharides gathered by larvae from the schmutzdecke. Our experiments show that breakdown of pellets is rapid in filter beds, with colonisation of faecal pellets by microorganisms likely to be greatest after 2–3 days (cf. Hargave, 1976), after which labile substrates are exhausted. However, midge faecal pellets can remain little altered for over a month in some habitats, depending on environmental conditions and the constituents of the diet (McLachlan *et al.*, 1979).

All pellets broke up to some degree on shaking. The more diffuse pellets from the unsterilised treatments broke apart readily as the binding polymer bridges were digested. We do not know whether physical abrasion is a significant process over the surface of sand filters but fragments of pellets clearly survived vigorous shaking even after 5 days of biological conditioning. Thus, the size and condition of faecal pellets depend on larval size, pellet age and microbial activity.

Water enters sand filters across the schmutzdecke. The numbers, and small size, of midge larval faecal pellets compared with sand grains, means that they present a large total surface area to water draining into the sand. Depending on larval size, mean pellet surface area is 0.06–0.22 mm^2 (for larvae of 2.5–5.0 mm overall length) while 85% of sand grains have surface areas between 0.20 and 3.14 mm^2 (Chaloner, 1995). It is known (Bellamy *et al.*, 1985b) that small sand grains of diameter 0.128 mm (surface area 0.05 mm^2) filter pathogenic bacteria more effectively than sand grains of diameter 0.615 mm (surface area 1.19 mm^2). Accumulations of pellets, therefore, have an effective pore size that is smaller, and potentially more retentive, than that of the sand grains and the pellets break up the "sheet-like" nature of the accumulating schmutzdecke. Grazing of schmutzdecke particles (and colloids) by midges, with subsequent transformation into faecal pellets, maintains the architecture of the layer and also keeps interstitial spaces between sand grains open (Chaloner, 1995; Wotton *et al.*, 1996).

Abundant oligochaete worms must also play a role, although these animals do not have the capacity for rapid colonisation shown by midges. However, oligochaetes are resident throughout the sand (Duncan, 1988) and some will avoid being removed during cleaning of the filter. Numbers of midge larvae, in contrast, are decimated when the surface of the sand is skimmed (Wotton *et al.*, 1992), explaining the absence of emerging adults for at least 16 days after cleaning.

In slow sand filtration, dissolved organic matter is taken up by secreted microbial exopolymer on sand grains, and bacteria are captured by protists that are also attached to the sand (Lloyd, 1996; Weber-Shirk and Dick, 1997). As chironomid faecal pellets consist of a matrix of exopolymer they, too, are likely to be adsorptive (cf. Decho and Lopez, 1993) and the considerable area of the sand filter surface that they cover (an addition of 23% each day with conditioning and break-up, proceeding over more than 5 days) means that they are likely to be significant agents of organic matter removal. The pellets may also have more adsorption sites available if organic matter previously adsorbed on to schmutzdecke polymer is removed on passage

through the insect gut (Wotton, 1996). Add to faecal pellets the numbers of silk-based chironomid tubes (which cover a large planar area, and with aquatic insect silk known to be highly adsorptive of pesticides, C. Brereton, personal communication, 1998), and there is further evidence of the importance of these insects in filtration.

There is a need to investigate the significance of *all* components of the schmutzdecke in the filtration process. This should be done with a range of different types of slow sand filters, from the most sophisticated [those with fabric coatings on the sand [Graham *et al.*, 1996] and with incorporation of activated charcoal) to the most simple (like those used by small communities in developing countries).

CONCLUSIONS

1. 7.4×10^4 midge larvae were found over the substratum of slow sand filter beds in a water treatment works.
2. Larvae fed on the accumulated schmutzdecke and egested compacted faecal pellets. Faecal pellet size increased with increasing larval size.
3. Faecal pellets were conditioned by microorganisms and became diffuse after 5 days. Pellets were also broken up by abrasion.
4. The engineering of schmutzdecke by midge larvae probably plays an important role in slow sand filtration for purification of drinking water.

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