The Role of Particles in Structuring the Midge (Diptera: Chironomidae) Community of Temporary Ponds (slow sand filter beds).

Submitted by
Dominic Thomas Chaloner

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Abstract.

This thesis describes an investigation of the possible role of particles in structuring a midge (Diptera: Chironomidae) community associated with slow sand filter beds. A "particle" is defined as an entity greater in size than 0.45 µm in diameter. Particle types found in filter beds include sand, schmutzdecke (detritus) and Cladophora. Larvae use particles to construct tubes and as food; particles, as substratum, can also create potential habitats. Filter beds provide an ideal model system, because they can be emulated in laboratory microcosms and support a limited number of species.

Three species were known to dominate a filter bed midge community: Cricotopus sylvestris (Fabricius) (dominant), Psectrocladius limbatellus (Holmgren) (subdominant), and Tanytarsus sp. (subdominant). Laboratory microcosms were used to discover differences in particle use, and therefore evidence of resource partitioning, between larvae of these species. Rearing experiments were also conducted to examine whether larvae of C. sylvestris, responded differently, in terms of larval growth, to different particle regimes, but also the microbial and physical regime associated with filter beds.

Differences were found between species in their preferences for certain particle types as substratum, on the basis of type but not size, reflecting the published literature. Subtle differences were also found in the tube-building behaviour of these species. Such differences could be inferred from other studies, but had not been previously considered as a possible mechanism promoting coexistence. Little evidence was found of differences in feeding, which is consistent with the literature. C. sylvestris was also found to exhibit differences in its growth on different particle and microbiological regimes. However, no evidence was found that a vertical through-flow of water, characteristic of filter beds, affected larval growth.

This study provides evidence of the importance of particles, especially through tube-building and substratum preferences, in structuring midge communities.
Acknowledgements.

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# Table of Contents

<table>
<thead>
<tr>
<th>Title Page</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>2</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>3</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>4</td>
</tr>
<tr>
<td>List of Figures</td>
<td>7</td>
</tr>
<tr>
<td>List of Tables</td>
<td>9</td>
</tr>
</tbody>
</table>

## Chapter 1: General introduction

1.1. Biology of the family Chironomidae | 10
1.2. Chironomids and community structure | 13
1.3. Chironomids and particles | 16
1.4. Objective of this study | 17

## Chapter 2: Slow sand filter beds: field site and a model system

2.1. Introduction | 19
2.2. Particle types | 21
  2.2.1. Sand | 21
  2.2.2. Schmutzdecke | 23
2.3. Light and temperature | 30
2.4. The biology of slow sand filter beds | 33
  2.4.1. The chironomids of slow sand filter beds | 33
  2.4.2. Slow sand filter beds as model systems | 35

## Chapter 3: Habitat preferences

3.1. Introduction | 39
3.2. Material and methods | 42
  3.2.1. Choice chamber design | 43
  3.2.2. Larvae | 44
  3.2.3. Substratum | 48
  3.2.4. Experimental design | 49
3.3. Results | 52
3.4. Discussion | 65
List of Figures.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Slow sand filter bed at Ashford Common Water Treatment Works and its construction.</td>
<td>20</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td><em>Cladophora</em> attached to the substratum and in the water column of filter beds at Ashford Common Water Treatment Works.</td>
<td>22</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Size fraction composition of substratum samples taken from filter beds at Ashford Common Water Treatment Works.</td>
<td>24</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Contrast between cleaned and uncleaned areas of substratum present in a filter bed at Ashford Common Water Treatment Works.</td>
<td>26</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Comparison of the number of species, in each of the major chironomid subfamilies and tribes, in some lotic and lentic communities.</td>
<td>36</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Comparison of the composition of some lotic and lentic communities, in terms of the percentage of species present that belong to one of the major chironomid subfamilies or tribes.</td>
<td>37</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Design of the choice chamber used in preference experiments.</td>
<td>45</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Results of preference experiments: <em>C. sylvestris</em> with different substratum types.</td>
<td>53</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Results of preference experiments: <em>P. limbatellus</em> with different substratum types.</td>
<td>57</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Results of preference experiments: <em>Tanytarsus</em> sp. with different substratum types.</td>
<td>59</td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>Results of preference experiments: <em>C. sylvestris</em> with different size fractions.</td>
<td>61</td>
</tr>
<tr>
<td>Figure 3.6</td>
<td>Results of preference experiments: <em>P. limbatellus</em> with different size fractions.</td>
<td>63</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Two schemes used to predict the nearest inter-tube distance.</td>
<td>82</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Photographs of the tubes built with detritus by larvae of different species.</td>
<td>87</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>Tube lengths.</td>
<td>89</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Inter-tube distance.</td>
<td>91</td>
</tr>
<tr>
<td>Figure 4.5</td>
<td>Dry weight of tubes built with detritus.</td>
<td>93</td>
</tr>
<tr>
<td>Figure 4.6</td>
<td>Dry weight of tubes built with sand.</td>
<td>95</td>
</tr>
<tr>
<td>Figure 4.7</td>
<td>Relationship between tube length and cleared area.</td>
<td>98</td>
</tr>
<tr>
<td>Figure 4.8</td>
<td>Percentage of the substratum remaining within the areas cleared by larvae after 24 hours.</td>
<td>100</td>
</tr>
<tr>
<td>Figure 4.9</td>
<td>Proportion of larvae inside or outside of tube after 24 hours.</td>
<td>102</td>
</tr>
<tr>
<td>Figure 4.10</td>
<td>Proportion of larvae building a given number of tubes after 24 hours.</td>
<td>103</td>
</tr>
<tr>
<td>Figure 5.1</td>
<td>Mentum widths.</td>
<td>126</td>
</tr>
<tr>
<td>Figure 5.2</td>
<td>Photograph of chironomid gut material, stained with DAPI and viewed with an epifluorescent microscope.</td>
<td>129</td>
</tr>
<tr>
<td>Figure 5.3</td>
<td>Results of examination of chironomid gut contents with DAPI.</td>
<td>130</td>
</tr>
<tr>
<td>Figure 5.4</td>
<td>The algal composition of chironomid gut contents.</td>
<td>131</td>
</tr>
</tbody>
</table>
Figure 5.5. Gut throughput rate. ................................................................. 134

Figure 6.1. Apparatus used to investigate the effect of through-flow of water on larval growth. ................................................................. 155
Figure 6.2. Results of rearing experiment conducted in petri dishes. .... 159
Figure 6.3. Changes in microbial ATP over time in three different microbial treatments of one substratum type. ............................... 162
Figure 6.4. The relationship between larval dry weight and length for larvae of C. sylvestris. ................................................................. 164
Figure 6.5. Results of rearing experiments conducted in model sand filter beds. ................................................................. 166
Table 2.1. Quantity of organic matter and chlorophyll a present in substratum samples taken from cleaned and uncleared areas of drained filter beds at Ashford Common Water Treatment Works ........................................... 28
Table 2.2. Light and temperature regime in filter beds at Ashford Common Water Treatment Works ................................................................. 31

Table 4.1. Readings of room temperature for tube-length and inter-tube distance experiments ................................................................. 78

Table 6.1. The body length and head capsule width of each instar of C. sylvestris ............................................................... 150
Chapter 1: General introduction.

1.1. Biology of the family Chironomidae.

Chironomids (Diptera: Chironomidae), commonly referred to as "non-biting midges", are one of the most ubiquitous and abundant groups of aquatic insects in fresh waters (Armitage et al. 1995; Pinder 1986; Williams & Feltmate 1992). Chironomids are found in a wide range of aquatic habitats (Pinder 1995b), sometimes in densities greater than 50,000 per m² (Coffman & Ferrington 1984; McLachlan 1981) and have a wide geographical distribution (Cranston 1995a). The ability of these insects to exploit all manner of aquatic habitats over a wide geographical area, probably explains why there are estimated to be 10,000 species world-wide (Cranston 1995a), within 10 currently recognised subfamilies (Cranston 1995c).

Coffman (1995) summarised four justifying arguments for the study of chironomids: ecological diversity; abundance and biomass; geographical range; and species richness. Another important reason for studying chironomids are the recurring outbreaks of "nuisance midges" (Ali 1991, 1995). Chironomids are also useful in a practical sense, as indicators of water quality and pollution (Armitage & Blackburn 1985; Beck 1977; Lindegaard 1995; Saether 1979), climate change (Walker 1995), and for the classification of water bodies (Lindegaard 1995; Saether 1975).

Despite their biological importance and usefulness, chironomids, particularly the immature stages, have been shunned or dealt with superficially in larger
studies by many aquatic biologists (Berg & Hellenthal 1992; Coffman 1995; Pinder 1986). This is probably, in part, due to an absence of sufficiently detailed taxonomic keys and the time required to identify chironomids, as well as the abundance and species diversity commonly found within chironomid communities (Pinder 1986). The publication of keys and diagnoses to larvae, pupae and adults (e.g. Coffman & Ferrington 1984; Cranston 1982; Pinder 1978; Wiederholm 1983) has helped, but such keys often only allow identification to the generic level, despite the need for ecological information about individual species (Cranston 1995a). Paradoxically, although biologists have been unwilling to study this group, a vast amount has been written about chironomids since the first review by Thienemann (1954), as shown by subsequent reviews by Oliver (1971) and Pinder (1986), and extensively in Armitage et al. (1995).

The chironomid life cycle is well characterised and consists of four stages: **egg** (see Nolte 1993; Pinder 1995a), **larva** (see Cranston 1995b; Pinder 1995a), **pupa** (see Cranston 1995b; Langton 1995) and **adult fly** (see Armitage 1995a; Cranston 1995b). The eggs are normally laid into the water, usually as a mass, or string, within a protective, gelatinous matrix, and often attached to stable objects such as stones or emergent vegetation. The larvae that hatch from these eggs progress through four instars, usually while living in, or on, the substratum. Towards the end of the final instar, the pharate pupa begins to develop within the cuticle of the larva. After a short period of time, the pupa ascends to the water surface, where the adult fly emerges via a dorsal split in the cuticle covering the thorax of the pupa. After emergence, adults, which can live from days to weeks, swarm (usually but not always
1.3. Chironomids and particles.

Recently the biological importance of particles, and the need to study them explicitly in aquatic systems, has been recognised (Wotton 1990). Defining what constitutes a particle is difficult, but it usually depends upon the physical level or the scale at which the researcher is working. In aquatic biology, by convention, a particle is regarded operationally as an entity retained by a 0.45 µm membrane filter (Wotton 1994b). However, perhaps a more realistic definition of particles is provided by Dudgeon (1994), as follows:

“Particles are discrete units of organic, or inorganic, material which can be physically sorted and biologically selected. They are used by animals in a discriminating (fine-grained) way and significantly influence the distribution of aquatic benthos.”

There are two reasons why the concept of the particle is likely to be important in any attempt to understand the biology of chironomids. Firstly, most chironomids are aquatic organisms for a large part of their existence, where they live within a medium where the particle has perhaps greater significance than in terrestrial systems. For example, water acts as an effective particle delivery system and also produces distinct particle regimes (e.g. size of sand grains or quantity of organic matter). Secondly, chironomid larvae are capable of utilising, and consequently are likely to be influenced by, particles in at least three ways: in feeding, in tube-building, and as a component of the physical habitat template (Dudgeon 1994). Those studies that have investigated the role of particles in the biology of chironomids (e.g. McLachlan & Dickinson...
1777; McLachlan *et al.* 1978; Brennan *et al.* 1978; Brennan & McLachlan 1979; Toscano & McLachlan 1980; Walentowicz & McLachlan 1980) have found that the abundance and distribution of chironomid larvae are often influenced by the quantity and/or the quality of particles present in the environment.

1.4. Objective of this study.

The objective of this study was determine whether particles could be involved in structuring a chironomid community. I was primarily interested in whether coexistence within a chironomid community could be promoted through partitioning of particle resources (i.e. resource partitioning). Therefore the primary focus of the experiments devised for this study was to discover whether larvae of different chironomid species were capable of exhibiting subtle differences in their use of particles (as substratum, in tube-building and in feeding). However, I was also interested in whether changes in the particle regime might influence and produce changes in the species composition of a given chironomid community. To investigate this, the intention was to rear chironomid larvae under different particle regimes, in conjunction with environmental conditions which might influence the quality of the particles, so as to test whether larval growth was different on contrasting particle types.

The field site used in this study was a water treatment works, where drinking water is purified commercially. A conspicuous feature of the works are slow sand filter beds in which Wotton *et al.* (1992) found a distinct chironomid
community at impressive population densities, which could, according to Armitage (1995b) reach 250,000 m\(^{-2}\). By looking at the effect that the particles present in filter beds have on chironomid larvae, it was hoped that some insight might also be gained into how chironomids larvae could affect the functioning of a filter bed. However, more importantly, these filter beds provided an ideal, model system for this study, for reasons which will be discussed in the next chapter.
Chapter 2: Slow sand filter beds: field site and a model system.

2.1. Introduction.

Materials for experiments were collected from Ashford Common Water Treatment Works, Middx., where Thames Water plc. purify some of the domestic water supplied to London. The works include 32 rectangular, concrete-lined, slow sand filter beds (30 m by 100 m and 2 m deep) (Fig. 2.1.).

The bottom of each filter bed usually consists of three layers (in ascending order): porous blocks, gravel and sand (Fig. 2.1.). Water pumped into the filter beds, usually to a depth of between 1.2 and 1.6 m, passes through the sand substratum, where dissolved organic matter (DOM) and fine particulate organic matter (FPOM), including pathogenic organisms, are removed from the water. Gradually "schmutzdecke" (which is German for "dirty layer") builds up at the surface and penetrates a few centimetres into the sand substratum. Schmutzdecke consists both of organic material developing in situ (biofilm, algae and bacteria), and, through a process which can be described as "active deposition" (Wotton et al. 1992), material from the water column (plankton, DOM and FPOM) (Brook 1954). Brook (1954) found a characteristic algal community associated with the schmutzdecke, dominated by the filamentous diatoms, Melosira and Fragilaria. Schmutzdecke, and the associated biofilm, is critical for effective water filtration, (Pescod et al. 1985; Sládecková 1991), particularly through bioadsorption and biodegradation of organic matter (Eighmy et al. 1992). Consequently, water from a recently-cleaned and refilled filter bed is not used until the schmutzdecke layer has
Figure 2.1. Slow sand filter bed at Ashford Common Water Treatment Works (Thames Water plc.) (top) and its construction (bottom).
Figure 2.4. Contrast between cleaned (left) and uncleaned (right) areas of substratum present in a filter bed at Ashford Common Water Treatment Works (Thames Water plc.).
2.3. Light and temperature.

Light and temperature are both important environmental factors that need to be taken into consideration when designing experiments, particularly when larvae are reared in the laboratory (see Chapter 6). The overlying water in a filter bed is relatively shallow (1.2 - 1.6 m), and with no overhead shading, the quantity of light penetrating to the substratum is likely to be considerable. Light meter readings (in lux) were taken at the water surface, at a depth of 1 m and then close to, without touching, the substratum at three different locations within a filter bed. Readings within the water were expressed as the percentage of the light present at the water surface, and these values are presented in Table 2.2. The light levels at the surface of the substratum could be as much as 40% of that at the water surface, and were therefore likely to be quite considerable in comparison with that found in many other aquatic systems. Therefore light, of sufficient quality and quantity, was needed during rearing experiments to enable algae to photosynthesise and grow. The temperature readings taken by Thames Water plc., in 1989, and taken during this study on one occasion during the summer of 1993, are given in Table 2.2. The range of water temperatures for the summer of 1989 were 17 to 23 °C and for one day in 1993, the water temperature ranged between 18 and 21 °C. Laboratory experiments were therefore conducted at a temperature of 20 °C whenever possible.
(ii)

<table>
<thead>
<tr>
<th>Week starting (Date)</th>
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<tr>
<td>23.7.89.</td>
<td>20.2 - 22.5</td>
</tr>
<tr>
<td>30.7.89.</td>
<td>18.0 - 21.0</td>
</tr>
<tr>
<td>6.8.89.</td>
<td>18.0 - 19.1</td>
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<tr>
<td>13.8.89.</td>
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<td>20.8.89.</td>
<td>18.5 - 20.0</td>
</tr>
<tr>
<td>27.8.89.</td>
<td>19.0 - 20.0</td>
</tr>
<tr>
<td>3.9.89.</td>
<td>18.7 - 19.0</td>
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<tr>
<td>10.9.89.</td>
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<td>17.9.89.</td>
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<tr>
<td>24.9.89.</td>
<td>17.0 - 18.0</td>
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<tr>
<td>31.9.89.</td>
<td>16.0 - 17.0</td>
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(iii)

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>20.7 ± 0.6</td>
</tr>
<tr>
<td>26</td>
<td>19.7 ± 0.6</td>
</tr>
<tr>
<td>31</td>
<td>19.2 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>19.0 ± 0.0</td>
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<tr>
<td>29</td>
<td>19.0 ± 0.5</td>
</tr>
<tr>
<td>28</td>
<td>19.0 ± 0.0</td>
</tr>
<tr>
<td>24</td>
<td>19.3 ± 0.3</td>
</tr>
<tr>
<td>18</td>
<td>18.8 ± 0.8</td>
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Table 2.2. (cont.) Light and temperature regime in filter beds at Ashford Common Water Treatment Works (Thames Water plc.): water temperature (ii) over the summer of 1989 and (iii) on one occasion during the summer of 1993 (29.6.93).
2.4. The biology of slow sand filter beds.

Research into the ecology of slow sand filter beds has been limited (Duncan 1988). In particular, the invertebrates associated with the schmutzdecke layer have been largely ignored, or any work that has been undertaken has not been published. Invertebrates that have been found in or on the substratum of filter beds include nematodes, oligochaetes and the larvae of caddisflies, mayflies, and chironomids (Brook 1954; Duncan 1988; Sládecková 1991). Such organisms are likely to influence the development, and function, of the schmutzdecke and the nature of the substratum as a whole.

2.4.1. The chironomids of slow sand filter beds.

Chironomids were mentioned in both a brief review by Sládecková (1991) of the biota associated with water supply systems and the study of the algal flora of filter beds by Brook (1954). However, Duncan (1988) while reviewing the ecology of interstitial meiofauna and flora, made no reference to chironomids. This is interesting as chironomids should be at least represented in the meiofauna as 1st instars, particularly just after a filter bed has been refilled.

In a study of filter beds at Ashford Common, Wotton et al. (1992) found a distinct chironomid community, of which samples of adult flies and larvae were dominated by three species, Cricotopus sylvestris (Fabricius); Psectrocladius limbatellus (Holmgren), and Tanytarsus fimbriatus Reiss & Fittkau. In terms of relative abundances, C. sylvestris was usually dominant, with P. limbatellus
and *T. fimbriatus* usually sub-dominant. These three species are well suited to what is a temporary habitat, as they each have a relatively short life cycle (Wotton *et al.* 1992), which results in a significant proportion of the larvae emerging as adult flies before the filter bed is drained to be cleaned. The distinct chironomid community included other species, primarily Chironomini and Tanypodinae, but these were present at much lower abundances and began to dominate the community when filter beds had been in operation for longer than the usual bed run of a month or so (Wotton *et al.* 1992).

Wotton *et al.* (1992) suggested that resource partitioning might promote the coexistence of the three dominant species, and that of the resource types proposed by Pianka (1969) (i.e. food, habitat and time), some were more, or less, likely to be partitioned. Space (i.e. habitat), or tube-building materials, were proposed as resources most likely to be partitioned. Although they suggested that food was not limiting, the quantity of food was not quantified by Wotton *et al.* (1992) and there was still a possibility, particularly at high densities, of food partitioning. Adults of the three dominant species began to emerge from filter beds at the same time of year, and all emerged at about the same time after a filter bed had been refilled. The three species were therefore temporally co-occurring, and were unlikely to be exhibiting temporal partitioning. Wotton & Armitage (1995) found that the size of adult midges varied over time, which they attributed to larval interactions (i.e. intra or interspecific competition for resources, such as space) and hence provided some evidence that some form of resource partitioning was present. Wotton *et al.* (1992) also proposed two reasons for the change in the chironomid community, from one dominated by Orthocladiinae and Tanytarsini to one
dominated by Tanypodinae and Chironomini, which they found over time. Firstly, such changes may have been a consequence of differences in the size of the larvae of the different species, resulting in the relatively small larvae of three dominant species being displaced from the substratum. However, it was also suggested that the changes in species composition might also be due to a change in the particle regime; specifically, the development of an organic coating on the surface of the substratum.

2.4.2. Slow sand filter beds as model systems.

The filter beds at Ashford Common provide a model system with which to investigate the role of particles in a chironomid community, for two reasons. Firstly, a smaller number of species are present in comparison with chironomid communities found in other freshwater systems (Fig. 2.5.), although the proportions of species present belonging to each major chironomid subfamily or tribe are similar to that found in other lentic systems (Fig. 2.6.). The low number of species also suggested that the mechanisms maintaining the structure of the community were likely to be less complicated. The chironomid community of filter beds at Ashford Common was ideal in that the larvae of the dominant species were relatively easy to distinguish from one another, while still alive and without immediate recourse to preservation, mounting and examination under a high power microscope.

The second reason was that the aquatic environment of a filter bed is more homogenous, especially in terms of particles, than most other aquatic
Figure 2.5. Comparison of the number of species, in each of the major chironomid subfamilies and tribes, in some lotic and lentic communities.
Figure 2.6. Comparison of the composition of some lotic and lentic communities, in terms of the percentage of species present that belong to one of the major chironomid subfamilies or tribes.
3.1. Introduction.

Chironomid larvae are generally, but not exclusively, benthic macroinvertebrates, so it is perhaps not surprising that the nature of the substratum is an important factor in chironomid ecology (Pinder 1986, 1995b). Four different substratum types were identified by Pinder (1986): hard rock surfaces; soft sediment (of mineral and/or organic material); submerged wood, and aquatic plants. Each of these substratum types are colonised by chironomid larvae (Pinder 1986, 1995b) and represent potential habitats. An important feature of soft sediment is the size of its constituent particles (Minshall 1984), and variations in the relative proportions of different-sized particles (i.e. the size fraction composition) can create potential micro-habitats for chironomid larvae (Rae 1985, 1987).

Schoener (1974), after evaluating studies of resource partitioning, suggested that habitat, followed by food and time, was the resource most likely to be partitioned. The term habitat, as well as micro-habitat, may be defined as a physical space, distinct from neighbouring spaces (i.e. habitats) by virtue of its particular assortment of environmental characteristics, such as the nature of the substratum. Evidence of habitat, or space partitioning, has been found in several studies of aquatic communities (e.g. Allan 1975; Brönmark & Malmqvist 1982; Hildrew & Edington 1979; Lamp & Britt 1981; Mackay & Kalff 1973), and in chironomid communities, at both the habitat and micro-habitat level (Boerger et al. 1982; Ferrington 1987; Rae 1985, 1987; Ramcharan &
Examination of past studies of chironomids of the same species and genus as considered by this study, revealed differences, and similarities, in substratum preferences.


_Psectrocladius limbatellus_ is a species characteristic of aquatic and submerged terrestrial plants (Armitage 1983; Koskenniemi & Paasivirta 1987), and Mundie (1957) suggested that this species might be inhabiting the _Cladophora_ growing in a storage reservoir. Other species of the genus _Psectrocladius_, have been found using aquatic plants, such as _Myriophyllum_ and _Potamogeton_ (Driver 1977; Bownik 1970); _Nuphar_ (Ramcharan &
Paterson 1978); *Schoenoplectus lacustris* (Drake 1983); *Typha* (Botts & Cowell 1992), and *Cladophora* (Gardarsson & Snorras 1993). However, larvae of this genus have also been found on the hard substratum of storage reservoirs (Bay 1993), where no aquatic plants were present (Bay, E.C. in pers. comm.), and in soft sediments rather than on aquatic plants (Ramcharan & Paterson 1978).

No published studies have given any indication of the types of substratum used by larvae of *Tanytarsus fimbriatus*. Larvae of the genus *Tanytarsus* have been found using soft sediments (Pinder 1980), such as mud or silt (Ali & Mulla 1978; Armitage & Blackburn 1990; Cantrell & McLachlan 1977; Ford 1962; Gardarsson & Snorras 1992; Heinis *et al.* 1994; Mundie 1957), "ooze" (Paasivirta 1972), sand (Ali & Mulla 1978; Heinis *et al.* 1994; Rae 1985, 1987), and coarser mineral substratum covered with fine silt (Armitage 1983). However, according to Coffman & Ferrington (1984), the genus *Tanytarsus* is also associated with aquatic plants (such as *Potamogeton* [Berg 1950] and Rice [Darby 1962; Way & Wallace 1989]).

There is evidence that aquatic insects can exhibit specific substratum preferences, and are able to discriminate between both subtly (e.g. particles of different sizes) and radically (e.g. plant over mineral) different substratum types (Minshall 1984; Ward 1992). However, Minshall (1984) also suggested that because aquatic insects don't always exhibit a preference consistently, or at all, that many are capable of using a range of substratum types. Furthermore, contradictions have emerged between different studies, and between laboratory and field studies (Minshall 1984) undertaken by the same
workers (e.g. Cummins & Lauff 1969). With chironomid larvae, substratum preferences have been both found consistently in the laboratory (Ferrington 1992; McLachlan 1969, 1976; McLachlan & Cantrell 1976; Wiley 1981a) and in the field (Francis & Kane 1995; Lindegaard-Peterson 1972; McLachlan & McLachlan 1975). This is despite the suggestion by Pinder (1986, 1995b) that many species are capable of colonising several different substratum types.

The objective of the experiments outlined in this chapter, was to determine whether larvae of *C. sylvestris*, *P. limbatellus* and *Tanytarsus* sp., differed in their preference for the main particle types found as substratum in slow sand filter beds. As discussed in Chapter 2, these are sand, *Cladophora* and organic material (i.e. schmutzdecke), and three size fractions of sand: < 0.5 mm, 0.5 - 1.0 mm and > 1.0 mm. Taking into account my earlier definition of habitat, each substratum type or size fraction could represent potential habitats or micro-habitats for chironomid larvae living in a filter bed.

3.2. Material and methods.

Habitat partitioning can be investigated in a number of ways. Usually, each habitat or micro-habitat is sampled, and the proportion of different species present in each are compared (e.g. Boerger *et al.* 1982; Ferrington 1987; Ramcharan & Paterson 1978). Habitat, or microhabitat, types can also be artificially created in trays or baskets (e.g. Allan 1975; Erman & Erman 1984; Rabeni & Minshall 1977; Rae 1987), which, after giving aquatic organisms time to colonise, are retrieved, and again the proportion of different species
Figure 3.1. Design of the choice chamber used in preference experiments.
sieve. This increased the survivorship of the larvae, both in transit from the field to the laboratory and while in storage. Larvae were stored in tanks at room temperature, with vigorous aeration and a small quantity of substratum taken from the field site added to each tank.

Before use, larvae were sorted into species and instar by observation under a low power dissecting microscope (cf. Storey 1986; Wiley 1981a). To sort larvae into species, a combination of features were used, including colouration, behaviour, head capsule shape, the absence, or presence, of long antennae, and the presence or absence of setal tufts. The identification of larvae was subsequently confirmed by examination with a high power microscope, once larvae were mounted and cleared on glass slides with polyvinyl lactophenol, and using the diagnostic features given by Cranston (1982) and Wiederholm (1983). Wotton et al. (1992) found two species of Tanytarsus present at the field site, T. fimbriatus and T. gracilentus, which although distinguishable as adults, cannot be separated as larvae. Throughout this study larvae are referred to as Tanytarsus sp., but which were more likely to be T. fimbriatus than T. gracilentus because the former species is more abundant during the summer when the experiments were carried out (Wotton, R.S. in pers. comm.). As insufficient larvae were available, only the preference of Tanytarsus sp. larvae for different substratum types, but not size fractions, was investigated.

4th instar larvae were recognised and picked out by virtue of the head capsule width (0.4 mm for C. sylvestris and P. limbatellus, and 0.3 mm for Tanytarsus sp.). These values were obtained by measuring the head capsule width of
larvae of different lengths (cf. McCauley 1974). Larvae with expanded thoracic segments and empty guts were not used as these were about to pupate (Kesler 1981) and pupae are unlikely to exhibit any substratum preferences.

4th instar larvae were used in preference experiments because there is no evidence that habitat preferences of chironomids change with instar (Drake 1983; Rae 1985, 1987; Ruse 1994). Throughout this study, 4th instar larvae were used because the constraints of time and resources meant that study of all instars was not possible. There were both practical and theoretical reasons for concentrating on 4th instars as opposed to any other instar. Practically, earlier instars are more difficult and time consuming to sort and identify than later instars. With rearing experiments, 2nd instars were used because egg masses of *C. sylvestris* were available, which made sorting of larvae much easier and more reliable. Theoretically, 4th instars represent the largest and longest phase of the larval stage and are characteristically benthic because larvae tend to become more negatively phototactic and positively geotactic with age (Davies 1976; Ward 1992). Also 4th instar larvae, being much bigger than other instars, probably have to expend more energy than other instars when swimming and are more conspicuous to potential predators (Ward 1992). Therefore 4th instar larvae may find it less advantageous to move into the water column to avoid competitive conditions as compared with other instars. Consequently, as competition for resources is likely to be greatest between 4th instars, which may be less able to avoid it, these larvae are therefore more likely to exhibit mechanisms, such as resource partitioning, for reducing inter-specific competition and promoting coexistence.
After sorting, larvae were left for one hour to acclimatize to the experimental temperature of 20 °C and to feed on fish food. Feeding larvae before the experiment and adding fish food suspension to choice chambers, was intended to reduce the influence of foraging activity, or differences in food quantity, on substratum preference. Previous studies of chironomid substratum preferences have found that differences in food quantity could affect substratum preferences (Ferrington 1992), or have provided a source of food (yeast suspension, McLachlan 1969; sedimentary microflora, Wiley 1981a). Tetra-Min® fish food was convenient to use and has been shown to be an adequate source of food for chironomid larvae (Rasmussen 1985).

3.2.3. Substratum.

Both "whole sand"¹ and *Cladophora* were obtained from the field site, and were repeatedly rinsed with distilled water to remove as much free organic material as possible. *Cladophora* was stored in a greenhouse at 20 °C, in tanks filled with continuously-aerated, coarse-filtered (mesh size, 53 μm) water taken from the field site. The sand size fractions were prepared by sieving whole sand through nested sieves with distilled water. Both whole sand and its size fractions were dried in an oven and stored dry. Shredded and conditioned leaf litter was used as organic material. Leaf litter, collected from Regent's Park, London, was washed with distilled water, dried and then shredded in a coffee grinder and stored in a tank under the same conditions.

¹ Unfractionated sand.
as the *Cladophora*. The leaf litter was conditioned in this way for more than 8 weeks (cf. 6 weeks used by Ward & Cummins [1979]), during which time the water was periodically changed. Before use, the leaf litter was sieved to remove coarser particles such as leaf stems, and rinsed thoroughly with distilled water. To ensure that preference was not affected by insufficient substratum (e.g. because larvae were unable burrow), large quantities were used to produce an adequate depth. Shredded, conditioned leaf litter was used because large volumes of schmutz-detritus resulted in conspicuous larval mortality and larvae migrating to the air-water interface. This is perhaps not surprising as schmutzdecke is not usually found in filter beds in the quantities required for these experiments.

3.2.4. Experimental design.

Choice chambers were prepared by first covering each half of the dishes with the same area and depth of either different (for choice dishes) or the same (for control dishes) substratum type or size fraction. In all, 4 dishes of each combination and 2 control dishes with one substratum type or size fraction, were prepared for each experimental replicate (i.e. 12 choice and 8 control dishes). Each dish was then carefully filled with 30 ml of ADC water, antibiotics (Tetracycline [final concentration 50 mg/ml] and Streptomycin sulphate [final concentration 100 mg/ml] [Aldrich Chemical Co.]), and 10 ml of

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2 Organic material derived from schmutzdecke.

3 Aerated and de-chlorinated water.
sterile fish food suspension (Tetra-Min® 1 g/100 ml; Rasmussen 1985). Stocks (0.01 g/ml) of antibiotics were made up with distilled water, filter-sterilised (Sartorius®; pore size, 0.22 µm), and stored frozen as 1 ml aliquots in sterile eppendorfs. These antibiotics were used because together they act upon a wide range of microorganisms (Brock & Madigan 1988). Also, unlike other antibiotics (e.g. Carbencillin), they have been used with Diptera larvae without any reported toxic effects (e.g. McLachlan [1969] used Tetracycline with chironomids; Ikeshoji & Mulla [1970] used Streptomycin sulphate with mosquitoes [Diptera: Culicidae], and Wotton [1978] used Streptomycin with blackflies [Diptera: Simuliidae]). The final concentrations of the antibiotics used were based on those given in McLachlan (1969) and Ikeshoji & Mulla (1970). Before addition of larvae, the perspex barriers were cleared of any material using a razor blade.

When designing these experiments, one of the main objectives was to minimise the influence of microorganisms on preference, a problem recognised by McLachlan (1969). Microorganisms, accumulating as biofilm or associated with each substratum type, might have affected the preference of larvae, either by reducing the oxygen tension or acting as a source of food. For these reasons, before use, sand, leaf litter, fish food and all possible apparatus were sterilised by either autoclaving, dry heat or washing with 70 % alcohol. However, as it was impossible and impractical to maintain complete sterility, the antibiotics were used to kill any additional microorganisms. Autoclaving and drying also killed any chironomid larvae present in the sand. Larvae present in the Cladophora after rinsing had to be removed by eye. Examination of Cladophora under the dissecting microscope showed that this
method was effective at removing larvae.

Five larvae were added to each half of the choice chamber with a plastic bulb pipette (i.e. 10 larvae in total). Larvae were placed in each half rather than on the barrier because it was difficult to add larvae consistently to the barrier. Experiments were left for 24 hours at 20 °C, in complete darkness and with dishes covered to stop loss of water through evaporation. Experiments were set up in reduced light and conducted in darkness as larvae of both *C. sylvestris* (LeSage & Harrison 1980) and *Tanytarsus* sp. (Cantrell & McLachlan 1977) have been found to be positively phototactic.

After 24 hours, dishes were taken out individually, and larvae present in the water column, or on the perspex barrier, were removed; these were recorded as exhibiting no preference. The substratum present in each half was then removed and sorted separately. The numbers of live larvae present in each half were recorded, as were the number of dead larvae. All larvae were preserved in separate, appropriately-labelled vials in 70 % alcohol for confirmation of identification.

After each experiment, choice chambers were washed thoroughly with distilled water and heat sterilised (> 160 °C) overnight. The perspex barriers were also washed thoroughly with distilled water, but sterilised by immersion in 70 % alcohol. The barriers were then rinsed with distilled water, and left overnight in a drying oven.
<table>
<thead>
<tr>
<th></th>
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<th>Organic Matter</th>
<th>No Substratum</th>
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</thead>
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<td></td>
<td></td>
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<td>32.11 ***</td>
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<td>14.23 ***</td>
<td></td>
<td></td>
</tr>
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<table>
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<th>Cladophora</th>
<th>Organic Matter</th>
<th>No Substratum</th>
</tr>
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<tbody>
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<td>26.95 ***</td>
<td></td>
</tr>
<tr>
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<td>6.74 **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic Matter</td>
<td></td>
<td>0.10 ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Substratum</td>
<td></td>
<td></td>
<td>1.10 ns</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.2. (cont.) Results of accumulative chi-squared and direction of preference. The roman numeral in brackets in each table, corresponds to the graph in which the data are illustrated; shaded boxes indicate controls; arrows indicate the direction of preference; ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
Figure 3.3. Results of individual experiments that tested the preference by larvae of *P. limbatellus* for different substratum types. For each experiment, the top row of graphs illustrate the results of larval preference in choice dishes and the bottom row of graphs those in control dishes.
Figure 3.3. (cont.) Results of accumulative chi-squared and direction of preference. The roman numeral in brackets in each table, corresponds to the graph in which the data are illustrated; shaded boxes indicate controls; arrows indicate the direction of preference; ns = P > 0.05; * = P< 0.05; ** = P<0.01; *** = P<0.001.
Figure 3.4. Results of individual experiments that tested the preference by larvae of *Tanytarsus* sp. for different substratum types. For each experiment, the top row of graphs illustrate the results of larval preference in choice dishes and the bottom row of graphs those in control dishes.
Figure 3.5. (cont.) Results of accumulative chi-squared and direction of preference. The roman numeral in brackets in each table corresponds to the graph in which the data are illustrated; shaded boxes indicate controls; arrows indicate the direction of preference; ns = P > 0.05; * = P< 0.05; ** = P<0.01; *** = P<0.001.
Figure 3.6. Results of individual experiments that tested the preference by larvae of *P. limbatellus* for different size fractions of sand. For each experiment, the top row of graphs illustrate the results of larval preference in choice dishes and the bottom row of graphs those control dishes.
Figure 3.6. (cont.) Results of accumulative chi-squared and direction of preference. The roman numeral in brackets in each table, corresponds to the graph in which the data are illustrated; shaded boxes indicate controls; arrows indicate the direction of preference; ns = P > 0.05; * = P< 0.05; ** = P<0.01; *** = P<0.001.
and the nature of the substratum (Hodkinson & Williams 1980; McLachlan & Cantrell 1976; McLachlan 1977b).

There is some information about tube-building in the genera *Cricotopus* (Brennan & McLachlan 1979; LeSage & Harrison 1980) and *Tanytarsus* (Brennan & McLachlan 1979; Walshe 1951), but nothing about *Psectrocladius*, although larvae of this genus have been found building tubes (Lindegaard-Peterson 1972; Botts, P.S. in pers. comm.). Interestingly, a table of ecological data for known chironomid genera, provided by Coffman & Ferrington (1984), refers to both *Cricotopus* and *Tanytarsus*, but not *Psectrocladius*, as tube-building genera. This table reveals other differences in the “mode of existence” of larvae of each genus. Four terms are used to describe the genera considered here: “clingers”, which possess adaptations for living on surfaces exposed to water currents; “burrowers”, which live in sediments and may build burrows; “sprawlers”, which live on the sediment surface; and “climbers”, which possess adaptations for living on the surface of aquatic vegetation (See Cummins & Merritt 1984 for greater detail). The larvae of the genus *Cricotopus* are categorised as clingers and burrowers; *Psectrocladius* as sprawlers and burrowers, and *Tanytarsus* as clingers and climbers. These descriptions suggest that larvae of the genera *Psectrocladius* and *Cricotopus* are capable of constructing tubes penetrating, or lying, on the substratum, while *Tanytarsus* larvae will limit tube-building to the surface. The tubes constructed by the Tanytarsini, which includes *Tanytarsus* sp., are robust, rigid and compact (Brennan & McLachlan 1979; Gardarsson & Snorrason 1993; Hershey 1987; Kullberg 1988; Walentowicz & McLachlan 1980; Walshe 1951), in contrast to those built by the genus *Cricotopus*, which

The objective of the experiments described in this chapter was to discover whether the three species, *C. sylvestris*, *P. limbatellus* and *Tanytarsus* sp., differed in the way their larvae built tubes. Four different aspects of tube-building were investigated and compared: firstly, the size of tubes and how close larvae will build tubes to one another (the inter-tube distance); secondly, the mass of tubes, when provided with two substratum types, so as to compare the ability of larvae of different species to use contrasting substratum types; thirdly, the relationship between the area cleared around tubes and the length of the tube, and how efficiently material is removed by larvae from these cleared areas; and finally, the propensity for larvae of different species to desert one tube and build another.

4.2. Materials and methods.

4.2.1. Larvae.

The larvae used in these experiments were obtained from the field site. The procedure employed for sorting and selecting larvae, and subsequently confirming identifications, was the same as that used given in section 3.2.2. as were the reasons why 4th instar larvae were used in all experiments. It is possible that the nature of tube-building could change with instar (cf. with
some species of caddisfly [Dudgeon 1994]), but there are no reports in the literature of such changes with chironomids, and tubes were always found in tanks where early chironomid instars were stored.

After sorting, larvae were left for one hour to acclimate to the experimental temperature and to feed on a suspension of Tetra-Min® fish food. Feeding larvae before the start of experiment was intended to reduce the influence of differences on foraging activity, which can be linked to tube-building (Wiley & Warren 1992). Larvae were added to experimental containers using fine forceps, ensuring that larvae were evenly dispersed over the bottom of the container.

4.2.2. Experimental containers.

Small plastic petri dishes (diameter, 5 cm) were used as containers for all experiments, except those that investigated the relationship between cleared areas and tube length, which used enamel pans (21.5 cm by 16.5 cm). These pans enabled larvae to produce cleared areas with unbroken, distinct perimeters. All containers were thoroughly cleaned after each experiment to prevent attached silk or biofilm from affecting subsequent experimental replicates. Petri dishes were scrubbed with distilled water, sterilised by immersion in 70 % alcohol, rinsed again with distilled water, and then left to dry overnight. Enamel pans were also scrubbed with distilled water, but were heat sterilised (> 160 °C) overnight, and then allowed to cool before use.
4.2.3. Substratum.

The substratum type used in experiments, except those that were employed to investigate differences in the mass of tubes, was detritus or organic matter derived from schmutzdecke, which is present in, and on, the substratum of filter beds (see section 2.2.). I refer to this material as schmutz-detritus and used it instead of sand, the dominant substratum type in filter beds, because measurements were easier to take from video recordings. Schmutz-detritus, when administered as a well-mixed, sieved suspension, also provided a more even layer of substratum than sand. Although use of larger quantities of schmutz-detritus resulted in larval mortality or larvae positioning themselves at the air-water interface (as discussed in section 3.2.3.), smaller quantities had no noticeable effect. Earlier studies of tube-building have provided a thin layer of detritus as substratum (e.g. LeSage & Harrison 1980) and detritus has been found to be a major constituent of the tubes produced by two of the genera investigated in these experiments (Brennan & McLachlan 1979).

Schmutz-detritus was prepared by collecting schmutzdecke from the surface of a drained sand filter. The detritus was first separated from sand, macroinvertebrates, and any aquatic vegetation by washing the schmutzdecke with distilled water, and putting the washings through a sieve (mesh size, 200 microns). The material that sedimented out was held in containers with vigorous aeration at < 5 °C. Containers were stocked regularly with fresh material obtained, and prepared, in the same way. Before use, schmutz-detritus was passed through a finer sieve (mesh size, 100 microns) to remove fine sand, and autoclaved in a sealed container to kill all organisms,
Each experiment was terminated after 24 hours. After taking a 2 minute video recording of the dishes, the tubes in each dish were checked to see whether they were occupied by larvae. All the larvae from an individual dish were removed and preserved in a single, appropriately-labelled vial for later identification. While each dish was processed, a further video recording was made, on which the position and the length of each occupied tube was made clear, using forceps as a pointer. Tubes which were either unoccupied, or not part of another tube, were ignored. A “thumbnail” sketch map was made of the dishes, on which the position, orientation and special features of tubes occupied by larvae were noted. Measurements were only taken of tubes in those dishes where there was no larval mortality and larvae were all the same species. Video recordings were made using a video camera (JVC GR-S707), mounted on a tripod and placed directly above the dishes; recordings were made directly onto videotape using a VCR (Panasonic NV-J35B). A combination of natural, and room lighting, was used to illuminate dishes.

From playback of the video recordings, measurements were taken of the size of tubes, and the distance between one occupied tube and its nearest occupied neighbour (the inter-tube distance). Measurements were taken by using pens to mark acetate sheets overlying the monitor screen. While taking measurements, a number of rules were followed to provide consistency. A tube was an area of unbroken, dense material, surrounded by a cleared area, and occupied by a larva. The length of a tube was used as an index of tube size. Tubes were never straight and therefore had to be
reduced to one, or more, straight line(s), which were measured individually with a ruler and then added together to give the total length. The inter-tube distance was the distance between the middle of one tube and the middle of the nearest occupied tube. All measurements were converted into "real" values (mm) using a conversion factor obtained from the scale recorded beneath the dishes.

Nearest neighbour analysis (Clark & Evans 1954) has been used to determine whether distributions of chironomid tubes are regular, random or contagious (Edgar & Meadows 1969; McLachlan 1977b). However, when this method was applied to data generated in this study, the results contradicted an obvious visual impression; in particular, tubes with a contagious distribution were indicated as being either randomly or regularly distributed. One explanation may be that previous studies have applied this method to a large population over a large area. In my study, a much smaller population was used in a limited area. Instead, the regular distribution of tubes was generated on paper using two possible "schemes" of how larvae could regularly distribute their tubes on the bottom of the dish (Fig. 4.1.). One scheme assumes that larvae build tubes close to the edge of the dish and at the furthest distance from two nearest neighbours (i.e. at the apex of a pentagon). The other scheme assumes that larvae build tubes in the middle of an equal portion of the dish bottom (i.e. in the centre of one of five equilateral triangles). Both schemes were equally valid as tubes were built both close to, and well away from, the edge of the bottom of the dish. From these two schemes it was possible to calculate the nearest neighbour distance (the inter-tube distance) using the appropriate geometrical formula.
4.3. Results.

Photographs of examples of the tubes built by larvae of the three species are shown in Fig. 4.2.

4.3.1. Tube length and inter-tube distance.

The tube lengths at a density of 5 larvae and 1 larva per dish are illustrated in Fig. 4.3., and the inter-tube distances in Fig. 4.4. All data were normalised by log transformation and compared using a *t*-test. For clarity, the results of these statistical tests (for this and subsequent sections), are included in the relevant figure rather than in the text.

The length of tubes built by *C. sylvestris* (9.0 ± 3.5 mm) larvae were significantly shorter than those built by larvae of *Tanytarsus* sp. (11.8 ± 3.2 mm) and by *P. limbatellus* larvae (11.8 ± 5.2 mm) at a density of 5 larvae per dish. The length of tubes built by larvae of *Tanytarsus* sp. and *P. limbatellus* were not significantly different. At a density of 1 larva per dish the tube lengths of *C. sylvestris* (10.8 ± 5.2 mm) and *Tanytarsus* sp. (14.0 ± 4.0 mm) were also significantly different, but were not significantly different from the tube lengths of *P. limbatellus* (14.2 ± 9.7 mm). Although all three species responded to the absence of conspecifics by building longer tubes, they were only significantly larger in *C. sylvestris* and *Tanytarsus* sp. The reason why no significant difference was found with *P. limbatellus*, was that, in the absence of conspecifics, the lengths of tubes built by *P. limbatellus* larvae were highly
Figure 4.3. Tube lengths: frequency histogram of tube lengths at (i) density of five larvae per dish, and (ii) density of one larva per dish.
<table>
<thead>
<tr>
<th>Comparison</th>
<th>t-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
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<td>(iii) C. sylvestris vs P. limbatellus</td>
<td>7.57 ***</td>
<td>529</td>
</tr>
<tr>
<td>P. limbatellus vs Tanytarsus sp.</td>
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</tr>
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</tr>
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</tr>
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</tr>
<tr>
<td>C. sylvestris vs Tanytarsus sp.</td>
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</tr>
<tr>
<td>(v) C. sylvestris vs C. sylvestris</td>
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<td>294</td>
</tr>
<tr>
<td>P. limbatellus vs P. limbatellus</td>
<td>0.89 ns</td>
<td>336</td>
</tr>
<tr>
<td>Tanytarsus sp. vs Tanytarsus sp.</td>
<td>4.22 ***</td>
<td>339</td>
</tr>
</tbody>
</table>

Figure 4.3. (cont.) Results of t-tests on log transformed data: (iii) five larvae per dish; (iv) one larva per dish, and (v) tubes produced by same species but at different densities; df = degrees of freedom; ns = P > 0.05; * = P< 0.05; ** = P< 0.01; *** = P<0.001.
Figure 4.4. Inter-tube distance: (i) frequency histogram of nearest inter-tube distance; (ii) results of t-tests on log transformed data; df = degrees of freedom; ns = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. 

Legend
- **C. sylvestris**
- **P. limbatellus**
- **Tanytarsus sp.**

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<tr>
<th>Comparison</th>
<th>t-value</th>
<th>df</th>
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</thead>
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<td>$18.74^{***}$</td>
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</table>
Figure 4.5. (i) The relationship between the dry weight of larvae and tubes, when detritus derived from schmutzdecke was provided as tube-building material; (ii) Spearman’s rank correlation coefficient on original data; df = degrees of freedom; ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
(iii) | $C. sylvestris$ vs $P. limbatellus$ | $t = 4.10 **$, $df = 29.$ \\
| --- | --- |
| $P. limbatellus$ vs $Tanytarsus$ sp. | $t = 2.35 ^*$, $df = 21.$ \\
| $C. sylvestris$ vs $Tanytarsus$ sp. | $t = 4.32 **$, $df = 32.$ \\

(iv) | $C. sylvestris$ vs $P. limbatellus$ | $t = 7.68 ***$, $df = 57.$ \\
| --- | --- |
| $P. limbatellus$ vs $Tanytarsus$ sp. | $t = 6.17 ***$, $df = 41.$ \\
| $C. sylvestris$ vs $Tanytarsus$ sp. | $t = 9.85 ***$, $df = 48.$ \\

(v) | $C. sylvestris$ vs $P. limbatellus$ | $t = 2.70 ^*$, $df = 17.$ \\
| --- | --- |
| $P. limbatellus$ vs $Tanytarsus$ sp. | $t = 4.66 **$, $df = 14.$ \\
| $C. sylvestris$ vs $Tanytarsus$ sp. | $t = 5.77 **$, $df = 11.$ \\

Figure 4.5. (cont.) Results of $t$-tests: (iii) larval dry weight of 0.1 mg; (iv) larval dry weight of 0.2 mg; and (v) larval dry weight of 0.3 mg; $df =$ degrees of freedom; $ns = P > 0.05$; $^* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$. 
**Figure 4.6. (cont.)** Results of *t*-tests: (iii) larval dry weight of 0.1 mg; (iv) larval dry weight of 0.2 mg; and (v) larval dry weight of 0.3 mg; df = degrees of freedom; ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
of tubes built from schmutz-detritus by larvae of the three species. No significant difference was found in the dry weight of tubes built from sand by larvae of the three species.

4.3.3. Cleared areas and efficiency of substratum utilisation.

The relationship between tube length and cleared areas is illustrated in Fig. 4.7. Pearson's product moment correlation coefficient was used to test whether there was a significant relationship between the tube length and the area cleared. To test whether the relationship for each species was significantly different, $b$ (the regression coefficient) divided by the standard error of $b$, both derived from linear regression, were compared as described by Mather (1972). The correlation coefficient and the results of this test are included in Fig. 4.7. The efficiency with which species utilise the substratum is compared in Fig. 4.8. Experimental replicates are included to show that experimenter influence on results was minimal. A $t$-test was used to compare the differences between species, the results of which are included in the relevant figure.

Although the relationship between cleared areas and tube length is significant for all species, there is no evidence to suggest that there is any significant difference in this relationship between the three species. However, significant differences were found in the efficiency with which larvae of different species clear the areas around the tube. Larvae of *C. sylvestris* left a greater percentage of material within the perimeter of a cleared area than did
(ii) Pearson's product moment correlation coefficient on data illustrated in the graph:

<table>
<thead>
<tr>
<th>Species</th>
<th>Correlation Coefficient</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sylvestris</td>
<td>r = 0.74 ***,</td>
<td>113.</td>
</tr>
<tr>
<td>P. limbatellus</td>
<td>r = 0.83 ***,</td>
<td>122.</td>
</tr>
<tr>
<td>Tanytarsus sp.</td>
<td>r = 0.84 ***,</td>
<td>171.</td>
</tr>
</tbody>
</table>

(iii) Results of analysis of the regression coefficients:

<table>
<thead>
<tr>
<th>Comparison</th>
<th>t-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. sylvestris vs P. limbatellus</td>
<td>0.54 ns</td>
<td>237.</td>
</tr>
<tr>
<td>P. limbatellus vs Tanytarsus sp.</td>
<td>0.53 ns</td>
<td>286.</td>
</tr>
<tr>
<td>C. sylvestris vs Tanytarsus sp.</td>
<td>0.05 ns</td>
<td>295.</td>
</tr>
</tbody>
</table>

Figure 4.7. (cont.) (ii) Pearson's product moment correlation coefficient on data illustrated in the graph; (iii) results of analysis of the regression coefficients; df = degrees of freedom; ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
Figure 4.8. (i) Percentage of the substratum remaining within the areas cleared by larvae after 24 hours for each individual experiment; (ii) results of t-tests for each experiment; df = degrees of freedom; ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
larvae of *P. limbatellus*.

4.3.4. Tube desertion and tube-building.

The propensity of larvae of each species to leave and move actively outside their tube is shown in Fig. 4.9., and the tendency to build more than one tube is shown in Fig. 4.10. For both sets of experiments, a $t$ - test was used to compare the results for each species, the results of which are included in each figure.

There were significantly more *P. limbatellus* larvae still inside their tubes than *C. sylvestris* larvae. Further, significantly more *P. limbatellus* larvae build a single tube than did larvae of *C. sylvestris*, while significantly more *C. sylvestris* larvae built 2 tubes than did larvae of *P. limbatellus*.

4.4. Discussion.

The tubes built by larvae of *C. sylvestris* and *P. limbatellus* clearly differed from those built by *Tanytarsus* sp. However, the tubes built by *C. sylvestris* and *P. limbatellus* larvae also differed from each other in more subtle ways. Such differences were consistent with the diversity of tube-building observed by other workers (e.g. Brennan & McLachlan 1979; Hershey 1987; Rasmussen 1984b; Walshe 1951; Wiley 1981a), but with the exception of Brennan, McLachlan and co-workers, have rarely been quantified.
C. sylvestris, because their larvae build more tubes than larvae of P. limbatellus. The tube-building of P. limbatellus appeared to lie somewhere between that of the other two species, although its tube structure was more like that of C. sylvestris than of Tanytarsus sp. P. limbatellus may therefore fit, at least in terms of tube-building, into the hypothetical "resource space" left by the other two species. Although the genus Psectrocladius is not noted for its tube-building (Coffman & Ferrington 1984), I found that P. limbatellus was a more effective tube-builder than C. sylvestris. The contrast between P. limbatellus the sedentary tube-builder, and C. sylvestris the errant tube-builder, may represent another potential mechanism by which these two species coexist: the larvae of one species remain in one place, while those of the other are relatively more mobile, opportunistic and flexible.

Although Tanytarsus sp. larvae built heavier tubes than larvae of P. limbatellus and C. sylvestris, there was some overlap in the relationship of cleared area and the tube length for the three species. There is therefore little evidence that larvae of Tanytarsus sp., despite building heavier tubes, require a greater area of substratum than the other two species. This means that either tubes do not consist of more material or larvae feed less upon the substratum and devote proportionally more substratum material to tube-building. However, Kullberg (1988) observed that the inside of Rheotanytarsus sp. tubes were covered in sheets of silk. Therefore the greater mass, as well as the general structure, of the tubes built by Tanytarsus sp. may be explained by the use of larger quantities of silk, rather than use of greater quantities of substratum. If this is the case, then why was there no difference between the three species when tubes were built with sand? The most
plausible explanation, as found by Brennan & McLachlan (1979), is that larvae of *Tanytarsus* sp. are more effective at building tubes from finer (i.e. schmutz-detritus) than coarser (i.e. sand) particles. This is consistent with the preference exhibited by *Tanytarsus* sp. larvae for detritus over sand and *Cladophora*.

The overlap of the relationship between tube length and cleared area with *C. sylvestris* and *P. limbatellus*, can be explained by the differences in the efficiency with which 4th instar larvae of different species clear the substratum. 4th instar larvae of both species are approximately the same length (4 - 6 mm, pers. observ.), which imposes an upper limit on how far larvae can stretch (or reach) without losing contact with the tube; this, in turn, determines the total cleared area (Wiley & Warren 1992). However, the difference in tube mass probably reflected differences in the quantity of material that larvae removed from that area (i.e. the “efficiency of substratum utilisation”). A significant relationship was discovered between cleared area and tube length, implying that the terms feeding (McLachlan 1977b; Wiley 1981a; Wiley & Kohler 1984; Wiley & Warren 1992) or foraging (Berg 1995) area, is not always appropriate. Substratum material from these areas is as likely to be used in tube-building as in feeding.

The results of my experiments have shown that three species found at the field site exhibit differences in their tube-building (cf. Brennan & McLachlan 1979), confirming the suggestion by Wotton *et al.* (1992) that the coexistence of larvae of different species in filter beds is likely to be promoted through partitioning of tube-building resources. These experiments also show that
different species of chironomid can be categorised into those whose larvae do not build tubes and are free-living (e.g. Tanypodinae); those that are facultative tube-builders, which may, or may not, build tubes (e.g. C. sylvestris and P. limbatellus); and those that are obligate tube-builders, which always build tubes, often in a very robust form (e.g. Tanytarsini). However, as pointed out by Brennan & McLachlan (1979), chironomid larvae also use particles in feeding. The suggestion by Wotton et al. (1992) that food is not a resource that is partitioned in filter beds will be explored in the next chapter.
Chapter 5: Feeding.

5.1. Introduction.

Chironomid larvae can use a number of mechanisms to acquire food and consume a diverse range of food types (Berg 1995). Diets can be quite flexible (Berg 1995), changing spatially and temporally (Armitage 1968; McLachlan et al. 1978; Ward & Williams 1986) and with larval instar (Williams 1981). However, there is evidence that chironomid larvae can be selective in their choice of food (Berg 1995) and are capable of having distinct diets, consisting of, for example, algae (Armitage 1968; Johnson et al. 1989; Kajak & Warda 1968; Titmus & Badcock 1981; Ward & Williams 1986; Williams 1981), detritus (Armitage 1968; Titmus & Badcock 1981; Ward & Williams 1986) or macrophytes (Darby 1962; Kangasniemi & Oliver 1983; Williams 1981), but also a specific portion of one food type, such as certain types of algae (Botts & Cowell 1992).

Some reviews have suggest that food partitioning is unlikely (Hart 1985; Hildrew & Townsend 1987), but Wotton (1994d) detailed how aquatic organisms can partition food resources (e.g suspension feeders [Stuart & Klumpp 1984]; deposit feeders [Fenchel 1975]; and predators [Reynoldson 1983]). Evidence of food partitioning in chironomid communities is also equivocal. Williams (1981) and Tokeshi (1986) found little evidence of food partitioning in epiphytic chironomid communities. However, according to Williams (1981) 4th instars could be separated into two distinct dietary groups, feeding upon either epiphytic algae and detritus or macrophytes, and both
Ramcharan & Paterson (1978) and Titmus & Badcock (1981) reported gross differences in the feeding niche of some species of chironomid. It is also worth noting that Tokeshi's methodology has been criticised (Botts & Cowell 1992), because no comparison was made of the differences in the species composition of the diatoms consumed by larvae of different species. There is also evidence that inter-specific competition could be reduced by use of different feeding tactics. For example, larvae of one species have been found as deposit feeders, ingesting benthic algae, while those of another species feed on planktonic algae as filter feeders (Johnson 1987; Rasmussen 1984a, 1985).

The morphology of the head capsule of chironomid larvae is particularly well catalogued and described (see Cranston 1995b; Saether 1980) because these features are important for identification (Coffman & Ferrington 1984; Wiederholm 1983). Structures around the anteroventral region of the head capsule show considerable variation within the family Chironomidae (Coffman & Ferrington 1984), and are all apparently involved in feeding (Cranston 1995b). Apart from the study by Olafsson (1992a), the function of these structures (i.e. the oral morphology) has rarely been studied in any depth. Berg (1995) did not explicitly discuss the role of oral morphology in feeding, but did acknowledge that there was a connection between functional feeding groups (Cummins 1973) and morphology; for example, chironomid larvae described as "scrapers" have well-developed mandibles. Berg (1995) also mentioned several species whose distinctive feeding behaviour was clearly linked to the oral morphology of their larvae. For example, larvae of *Odontomesa fulva*, use numerous labral setae to filter particles present in
water expelled out of the mouth by the gut. Also, *Constempellina* larvae use labral setae and other structures of the anteroventral region to scrape material from the substratum. Olafsson (1992a) found that species with contrasting diets (predatory, algal-detrital, and macrophyte), had contrasting oral morphologies. However, because the species considered in my study belong to genera that have been classified as algal-detrital feeders (Coffman & Ferrington 1984) and macrophytes are not dominant at the field site, the features studied by Olafsson (1992a) were unlikely to be relevant. One feature not considered by Olafsson (1992a) was the oral gape, which determines the size of particles that can be ingested (Botts & Cowell 1992; McLachlan et al. 1978; McLachlan 1981). The mentum width has been used as an index of oral gape (McLachlan et al. 1978) and Rae (1987) suggested that differences in mentum width might enable two species with similar micro-habitat preferences to coexist by consuming foods of different sizes. The mentum is an obvious feature of the head capsule, varying in size and shape throughout the Chironomidae. The shape and ventral position of the mentum means that it is ideal for scraping biofilm and detritus from surfaces (Storey 1986; Williams 1981). Darby (1962) also described how larvae used the mentum to fracture the cell walls of algal filaments, thereby releasing the cell contents.

Examination of gut contents of larvae may not necessarily give a full picture of what is ingested because of digestion (Wotton 1994d). However, examination of the contents of guts has revealed differences in the diets of chironomid larvae (Johnson 1987; Ramcharan & Paterson 1978; Rasmussen 1984a; Titmus & Badcock 1981; Williams 1981), especially when digestion is not thorough, or particles (e.g. diatom frustules or macrophyte tissue), are
studying different aspects of feeding rate for various reasons, and using different terminology (e.g. Kesler [1981] refers to grazing rate; Johnson et al. [1989] refers to gut passage time). It is therefore difficult to make generalisations.

There is evidence that larvae of different species within a chironomid community could differ in the rate at which food resources are utilised. One reason is that each species of chironomid has larvae of a characteristic size. This can, in turn, have two paradoxical consequences for the feeding rate. Firstly, as smaller larvae fill their guts far more quickly than larger larvae (Welton et al. 1991), material passes more rapidly through the guts of smaller species. However, secondly, the size of a larva influences the size of particles that it can ingest, while the speed with which particles pass through the gut is influenced by their size (McLachlan et al. 1978). Smaller larvae will therefore be predisposed to ingest smaller particles, which will tend to move more slowly than larger particles consumed by larger larvae. Thus species which have characteristically small larvae could have either faster feeding rates because their larvae fill their guts quicker or slower feeding rates if they tend to ingest predominantly smaller particles. Two large larvae could also feed at two different rates, depending upon whether they consume small or large particles; if larvae selected large diatoms, for example, they would have faster rates than larvae that ingested smaller particles, like fine detritus or small diatoms.

The objective of the experiments described in this chapter was to discover whether there were any differences in the feeding of the three species, C.
sylvestris, *P. limbatellus* and *Tanytarsus* sp. Three aspects of feeding by chironomid larvae were investigated and compared: firstly, the mentum width, as an index of oral gape; secondly, the gut contents, and finally, the rate at which material passes through the gut (i.e. the gut throughput rate).

5.2. Materials and methods.

Throughout these experiments, only 4th instar larvae were used, for the same reasons outlined in section 3.2.2.. Although some workers have advocated studying the feeding behaviour of all instars (Olafsson 1992a; Williams 1981), others have dealt with the feeding behaviour of just 3rd and/or exclusively 4th instar larvae (e.g. Johnson 1987; McLachlan *et al.* 1978; Moore 1979; Rasmussen 1984a).

5.2.1. Oral gape.

5.2.1.1. Larvae.

Olafsson (1992a) studied the morphology of different chironomid species, using sibling larvae reared from egg masses. However, in my study only the egg masses of *C. sylvestris* could be found reliably in sufficient quantities, and so samples of larvae of different species taken from the field site were used. In the past, only the menta of a small number of larvae have been measured (e.g. 26 by McLachlan *et al.* [1978]; 10 by McLachlan [1981]), but it seemed
5.2.2.1. Larvae.

The gut contents were dissected from larvae collected on one occasion from the same filter bed. Larvae, carefully separated from the substratum by sieving, were immediately preserved in formalin (5 %) at the field site and returned to the laboratory on ice in a cooler. Preserved larvae were stored in the dark at < 4 °C until larvae were dissected. Larvae were kept in chilled formalin while sorted into different species and instar under the low power dissecting microscope as described in section 3.2.2.

5.2.2.2. Dissection and mounting.

For DAPI staining, the gut contents of larvae were dissected aseptically, using sterile equipment (acid washed and autoclaved) and sterile solutions (prepared using filter-sterilised [Whatman®; pore size, 0.22 μm] double-distilled water), and by carrying out procedures in a laminar flow cabinet. Each larva was first rinsed three times in formalin (5 %), to dislodge anything loosely attached to the larval cuticle. The rinsed larva was then placed into a small volume of formalin held in the well of a cavity glass slide. Under a low power dissecting microscope, the head capsule of each larva was separated from the rest of the body, so that the gut could be withdrawn from the body of the larva using watchmakers forceps. The gut was transferred to a drop of formalin on another glass slide, where the gut contents were separated from the gut wall and peritrophic membrane. The gut contents of the larva was then transferred to 0.5 ml of formalin held in a single eppendorf. Until the gut
contents were stained with DAPI, eppendorfs were stored in the dark at < 4 °C. In order to confirm instar and species, the head capsule and eviscerated body of each larva were mounted on a slide with polyvinyl lactophenol.

The gut contents of larvae were prepared for microscopic examination using a method modified after Walker et al. (1988). The gut contents were first transferred from the eppendorf to a glass screw cap vial. The eppendorf was rinsed with sterile double distilled water, which was pipetted into a glass vial, to bring the final volume of the preparation to ca. 1.0 ml. The vial was shaken vigorously and then sonicated for 10 seconds to disperse the gut contents. DAPI was added to the vial (final concentration ca. 1μg/ml), which was then left for 15-30 minutes on ice away from light sources. The suspension of stained gut contents was pipetted into a filter holder (11 mm diameter) under a low vacuum (< 30 cm Hg) to draw the suspension through a nigrosin black-stained polycarbonate filter (Nucleopore®; 25 mm diameter; pore size, 0.22 μm). Polycarbonate filters were used so that very small particles (e.g. bacteria) do not become "lost" within the filter (cf. cellulose nitrate filters), and were also stained black to reduce background auto-fluorescence (Fry 1988). Such staining of filters meant that further examination of filters under the light microscope was difficult. Hence, the gut contents of a further set of larvae were prepared in order to compare the different types of algae. The polycarbonate filter was backed by a HA-type filter (Millipore®; 25 mm; pore size, 0.45 μm) to act as a sinter to help ensure an even distribution of material over the surface of the filter. The damp polycarbonate filter was removed from the holder, and placed on a slide on which a thin layer of Cargille type B immersion oil had been smeared. After covering the filter with an additional drop of oil and
coverslip, the slide was held at < 4 °C in darkness until examined under the microscope.

For examination of algal types, each larva was rinsed three times with distilled water to dislodge any loosely-attached material on the surface of the cuticle. The gut was then dissected from the larvae as described above, but in distilled water rather than formalin and without the use of a laminar flow cabinet. The gut was carefully transferred to 2.5 ml of distilled water, where the gut wall and peritrophic membrane were removed. After dispersing the gut contents with the forceps, the suspension of gut contents was carefully sucked up and expelled three times with an unused, disposable 2.5 ml syringe. This suspension was then filtered through a cellulose nitrate filter (Whatman®; 13 mm diameter; pore size, 0.22 μm), backed by another filter (Whatman®; 13 mm diameter; pore size, 0.45 μm) to act as a sinter, and held in a Millipore® Swinnex filter holder. After rinsing the well of the cavity glass slide three times with distilled water and passing each rinse through the filter, the filter was removed and dried on filter paper under cover in a petri dish for > 24 hours. Once dry, each membrane filter was mounted on a glass slide, smeared with immersion oil. A drop of oil was placed on top of the filter, which was then covered with a coverslip. Again the head capsule and eviscerated larval body were mounted in polyvinyl lactophenol for confirmation of species and instar.

The guts of 20 4th instar larvae of both C. sylvestris and P. limbatellus were prepared and examined using both the methods given above. Unfortunately, no 4th instars of Tanytarsus sp. were found in the samples of larvae taken for this part of the study and therefore the guts of this species were not examined.
the dimensions required to estimate the volume, when gut material was mounted on membrane filters. Area was therefore used instead of volume, calculated from the dimensions (length and width) of 20 particles of each algal type, and was repeated for each gut that was examined. The area was calculated from these dimensions using the appropriate formula for a rectangle in the case of *Melosira* and *Fragilaria*, a circle for *Cyclotella*, and two triangles for the "other" algal type. In order to take into account the "typical" area of each algal type, the total area of each type was calculated. This was done by multiplying the number of particles of one type by its typical area. This was then expressed as a percentage of the total area covered by all algal particles encountered. This was calculated by adding together the total areas for each of the four algal types.

5.2.3. Gut throughput rate.

Although Welton *et al.* (1991) criticised their use, other workers have conducted "feeding rate" experiments in the laboratory (Johnson *et al.* 1989; Kesler 1981; McLachlan *et al.* 1978; Taghon & Jumars 1984). Unlike Welton *et al.* (1991), who were quantifying the impact of chironomid grazing on field populations of algae, the aim of my experiments was to determine whether the feeding rate (i.e. gut throughput rate) of each species was different. Although increases in temperature can increase the feeding rate of some aquatic organisms (Wotton 1994d), in the case of chironomid larvae the effect is minimal over a narrow temperature range (Kesler 1981), or has no significant effect (Welton *et al.* 1991). Therefore experiments were conducted under a
variable temperature regime, but a note was made of the water temperature during each experiment.

A number of methods have been used to estimate feeding rates, such as radiotracers (Calow & Fletcher 1972; Dermott 1981; Kesler 1981), gravimetric estimations of food consumed or egested (Kesler 1981; Marchant & Hynes 1981; Mattingly 1987), and gut marker techniques (Johnson et al. 1989; Welton et al. 1991; Wotton 1978). The marker technique involves marking the gut contents of larvae of a given population (e.g. in a stream), and then measuring the movement of this marked material through the guts in larvae sampled from the population over time. This method can be used with chironomid larvae because they have simple tube-like guts (Welton et al. 1991). Gut markers used in the past have included charcoal (Welton et al. 1991) and fluorescent dyes (Johnson et al. 1989). Fluorescent dyes are useful because they can be easily distinguished from other particles by examining gut material with an epifluorescent microscope. However, charcoal was used in my study because guts have to be dissected and mounted to obtain an adequate signal with fluorescent dye (cf. Johnson et al. 1989), which takes longer than the mounting and clearing with polyvinyl lactophenol required for charcoal.

As 1,000 larvae were used for each experiment, it was not practical to sort larvae into individual species. Instead mixes of larvae, obtained from the field site in the same manner described in section 4.2.1., were used and which consisted of predominantly *C. sylvestris* and *P. limbatus* larvae; unfortunately again there were insufficient *Tanytarsus* sp. larvae to include it
in this part of the study. Larvae with swollen thoracic segments were avoided as this indicates that they are about to pupate, during which chironomids do not feed (Kesler 1981).

Each experiment was carried out in a large container (60 cm by 35 cm), with constant aeration provided by an air pump and aerator blocks. 20 weighted plastic dishes (diameter, 9 cm) were placed within this container to serve as sampling areas, and each were assigned a number. In conjunction with a random number table, this ensured that dishes were selected randomly. Schmutz-detritus, obtained from the field site in the same manner as described in section 4.2.3., was used as substratum. This was sterilised to stop microbial development and the occurrence of anoxia. Schmutz-detritus was made up as a well-mixed suspension in ADC water (75 ml of settled schmutz-detritus in 3 litres of ADC water), and then added, together with larvae, to the container. The container, covered with black plastic, was then left overnight to allow larvae to acclimate and build tubes. The container was covered to exclude light in order to encourage larvae to distribute themselves more evenly over the bottom of the container, particularly because of the phototactic behaviour pointed out in section 3.2.4.. The numbers of larvae sampled in each dish would thus be similar.

Each experiment was begun with the careful addition of 100 ml of charcoal suspension (4 g of finely ground charcoal in 200 ml of ADC water), to the container. At intervals of 5 minutes, over a period of 50 minutes, sampling dishes were randomly chosen and removed in twos. All larvae within the dishes were killed with a mixture of chloroform and alcohol (1:10), which
stopped larvae regurgitating their gut contents (Swift & Federenko 1973). All the larvae from an individual dish were sorted from the substratum and stored in a single, appropriately-labelled vial with 70% alcohol. At the beginning and the end of each experiment, the water temperature was recorded.

Larvae were mounted on glass slides with polyvinyl lactophenol, ensuring that those on one slide were taken from the same sampling dish, and left for a few days to clear. The species and instar of larvae were confirmed, using the features described in section 3.2.2. The charcoal marker extended from the mouth up to a particular point within the gut of each larva. Unmarked material posterior to this point in the gut had been consumed before the addition of charcoal. The length of the gut (the distance from mouth to anus) and the distance that the charcoal-marked material had moved within the gut was measured in each larva. The proportion of the gut filled at each time interval was calculated from these two values. This enabled an estimate to be made of the gut throughput rate.

5.3. Results.

5.3.1. Oral gape.

Frequency histograms of the mentum widths of the three species are presented in Fig. 5.1. A t - test was used to compare the mentum widths of
Figure 5.2. Photograph of chironomid gut material, stained with DAPI and viewed with an epifluorescent microscope (x250 magnification).
Figure 5.4. (cont.) (iii) relative proportions of each algal type, taking into the account the typical area that each covers; (iv) table of the results of $t$ - tests comparing the data illustrated in each individual graph ([i] refers to 1st graph; etc.); df = degrees of freedom; ns = $P > 0.05$; $^* = P < 0.05$; $^{**} = P < 0.01$; $^{***} = P < 0.001$. 

<table>
<thead>
<tr>
<th>Algal Type</th>
<th>(i)</th>
<th>(ii)</th>
<th>(iii)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melosira</td>
<td>$t = -1.40$ ns, df = 34.</td>
<td>$t = 0.53$ ns, df = 1047.</td>
<td>$t = 1.10$ ns, df = 34.</td>
</tr>
<tr>
<td>Fragilaria</td>
<td>$t = 2.87^{**}$, df = 34.</td>
<td>$t = 1.09$ ns, df = 917.</td>
<td>$t = -2.48^{*}$, df = 34.</td>
</tr>
<tr>
<td>Cyclotella</td>
<td>$t = -0.37$ ns, df = 34.</td>
<td>$t = 1.41$ ns, df = 778.</td>
<td>$t = 0.74$ ns, df = 34.</td>
</tr>
<tr>
<td>Other</td>
<td>$t = 0.10$ ns, df = 34.</td>
<td>$t = 2.48^{*}$, df = 893.</td>
<td>$t = 1.00$ ns, df = 34.</td>
</tr>
</tbody>
</table>
guts of both species. The "other" algal type constitutes a very small portion of the gut contents, both in terms of the relative proportions and the area, justifying the grouping of the individual genera together. Although the relative proportions of *Fragilaria* and the area of the "other" algal type found in the guts of the two species were significantly different, the calculated value of \( C \) was 0.997. There is therefore evidence of significant overlap in the algal component of the diets of *C. sylvestris* and *P. limbatellus* larvae.

5.3.3. Gut throughput rate.

The change in the percentage of the gut marked with charcoal over time is given in Fig. 5.5., which also includes the values of Spearman's rank correlation coefficient (Elliott 1977) used to test whether this was a significant relationship. The gut throughput rate was calculated by fitting a best fit line to this data using regression analysis; the equation of the line for each experimental replicate is included in Fig. 5.5. To test whether throughput rates were significantly different, \( b \) (the regression coefficient) divided by the standard error of \( b \), both derived from linear regression, were compared as described by Mather (1972). The results of this test are included in Fig. 5.5.

A highly significant relationship was found between time and percentage of the gut filled with charcoal. However, the two species did not differ significantly in their throughput rate, regardless of whether individual experiments or pooled data from these experiments were analysed.
Figure 5.5. Gut throughput rate as indicated by the percentage of guts filled with charcoal marker with time: (i), (ii) individual experiment replicates. The temperature regime for each experiment included in the graph (mean ± SD). Both graphs have the same horizontal axis.
Temperature = 23 ± 1 °C

Figure 5.5.(cont.) Gut throughput rate as indicated by the percentage of guts filled with charcoal marker with time: (iii) individual experiment; (iv) pooled data from the three individual experiments. The temperature regime for the experiment included in the graph (mean ± SD). Both graphs have the same horizontal axis.
by larvae of the chironomid species in this study seems unlikely.

Algae were clearly not the only materials present, but they dominated the gut contents of both *C. sylvestris* and *P. limbatellus* larvae, consistent with earlier studies of both *Cricotopus* (Mason & Bryant 1975; Wiley & Warren 1992) and *Psectrocladius* (Botts & Cowell 1992). Interestingly, the diets of *C. sylvestris* have included detritus (Mackey 1979), macrophytes (Darby 1962) and algae (this study). In addition, Brook (1954) found algae, in particular *Melosira*, in a limited examination of the guts of chironomid larvae taken from filter beds, and Soluk (1985) similarly found algae dominating in the guts of psammophilous chironomid larvae. However, in my study, a large dietary overlap was found, indicating that there was little evidence of food partitioning by *C. sylvestris* and *P. limbatellus*, at least on the basis of algal type. The absence of a significant difference is perhaps not surprising as the oral gape, which is regarded as the main mechanism of particle selection (Botts & Cowell 1992; McLachlan *et al.* 1978; McLachlan 1981), is very similar in both species. Nevertheless, an explanation is required for the differences that were found in the relative proportions of *Fragilaria* and the area covered by a typical frustule of the "other" algal type. In the case of the "other" algal type, it may be a consequence of subtle differences in the individual genera that were, for convenience, grouped together.

*C. sylvestris* and *P. limbatellus* do not differ significantly in their gut throughput rate, and therefore the rate at which food is utilised. This is perhaps not surprising, considering the conclusions of McLachlan *et al.* (1978) and Welton *et al.* (1991) and the similarity of *C. sylvestris* and *P. limbatellus* in terms of
larval size, mentum width and the dimensions of algal types found in guts. So larvae of both species were likely to take the same time to fill their guts and/or ingest particles of approximately the same size, which would in turn travel through the gut at comparable speeds. The variability in larval feeding activity (i.e. many larvae either not feeding or whose feeding rate appeared to lag behind others) found in my experiments was consistent with other studies of feeding rate (e.g. Johnson et al. 1989; Welton et al. 1991).

In conclusion, there is little evidence that food partitioning promotes the coexistence of larvae of *C. sylvestris* and *P. limbatellus*. On the other hand, *Tanytarsus* sp. may coexist with the other two species through the restriction of its diet to finer material as a result of its smaller oral gape. These results support the view that food is the resource least likely to be partitioned (Hildrew & Townsend 1987; Schoener 1974), particularly with chironomid larvae, which are often found to be omnivorous and opportunistic feeders (Berg 1995). The “active deposition” of material onto the substratum of filter beds (Wotton et al. 1992) probably also means that because there is a constant supply of food, it is unlikely to be a limiting resource for the chironomid larvae present in filter beds.
6.2. Materials and methods.

The two sets of rearing experiments were conducted using laboratory microcosms. In the first experiment, larvae were reared in petri dishes, in order to investigate the effect of different substratum types and levels of microbial biomass (i.e. biofilm) on larval growth. In the second experiment, larvae were reared in model sand filters, so that the influence of a through-flow of water on larval growth could be examined. These experiments used only *C. sylvestris* larvae because egg masses of the other species could not be found regularly in sufficient numbers. By using this dominant species the intention was to gain an impression of how conditions associated with the substratum of filter beds, might affect *P. limbatellus* and *Tanytarsus* sp..

6.2.1. Experiments using dishes.

6.2.1.1. Experimental design.

Petri dishes, incubated at suitable temperature and under appropriate diel light cycle and sufficient substratum, have been used to rear chironomid larvae (Cranston 1982; Johnson & Goedkoop 1992; Olafsson 1992a). Sterile petri dishes (Sterilin®; diameter, 9 cm; depth, 1 cm) were used in my experiments so that the presence of microbial biomass could be manipulated. Tops were also used in order to reduce water loss through evaporation. As dishes were not sealed, gaseous exchange was still possible. Experiments were conducted at 20 °C, which is similar to the temperature of the overlying
water present in filter beds during the summer (see section 2.3.). The light levels at the substratum of filter beds were found to be quite considerable (see section 2.3.), so dishes were illuminated with artificial light that enabled algae to photosynthesise and grow, with a 12 hour light: 12 hour dark diel cycle.

6.2.1.2. Substratum.

The four substratum types used were whole sand, schmutz-detritus, Cladophora, and a mix of whole sand and schmutz-detritus, which were all obtained from the field site and processed as described in sections 3.2.3. and 4.2.3. Each substratum type (except Cladophora) was prepared as three different treatments, in order to maintain, modify, or stop the development of microbial biomass.

To maintain microbial development, each substratum type (including Cladophora) was held in conical flasks, with water taken from the field site and filtered through 53 μm mesh to remove chironomid larvae. Flasks were kept in a greenhouse at 20 °C and continuously aerated with filtered air. To modify and stop microbial development, each substratum type was sterilised in sealed containers. Microorganisms were then either allowed to colonise each substratum type by exposure to the air for 24 hours (to modify microbial development), or prevented from colonisation by use of petri dish tops and antibiotics (to stop microbial development). As complete sterility cannot be maintained, my objective was to establish differences in microbial

1 Words in **bold** are used to refer to different microbial treatments.
development in the three treatments. To this end, the antibiotics, Streptomycin sulphate and Tetracycline (Aldrich Chemical Co.), were used as described and for reasons given in section 3.2.4. However, as antibiotics are not effective against all microorganisms, and will become less effective with continued microbial colonisation, measures were taken to minimise any such colonisation (e.g. water used in dishes was filter-sterilised, and aseptic technique and laminar flow cabinets were used while preparing dishes).

To produce a layer of substratum at least 2 mm deep, 13 ml of schmutz-detritus or 35 g of dry sand (the approximate mass required to displace 13 ml of water) were used. An equivalent quantity of wet sand had to be determined for maintain treatments, because the sand could not be dried for weighing. This was found to be 42 g of wet sand, once excess water had been blotted using sterile filter paper. To set up a mixture of sand and schmutz-detritus, 1 ml of schmutz-detritus was added to 35 g of dry (for modify or stop treatments) or 42 g of wet (for maintain treatment) sand and thoroughly mixed. 2.5 g of Cladophora, with excess water removed by blotting on filter paper, was found to produce the same depth. The depth of substratum used was chosen to reduce the likelihood of sediment anoxia, and left space for an overlying layer of water and an air gap between the surface of the water and the dish top. 5 ml of sterile fish food suspension (Tetra-Min®; 1 g/100 ml) was also added to each dish as a supplementary food source, together with 15 ml of filter-sterilised [Sartorius®; pore size, 0.22 μm] water taken from the field site.

The following treatments of substratum types were set up in petri dishes, each
treatment being replicated five times:

2. Sand: modify.
5. Schmutz-detritus: modify.
7. Sand & schmutz-detritus: stop.

6.2.1.3. Larvae.

*C. sylvestris* larvae were obtained from a laboratory culture, having hatched from egg masses collected from the field site and kept in large enamel dishes. My intention was initially to use 1st instar larvae hatched from individual egg masses. However, attempts to surface-sterilise egg masses failed, because the methodology is based on one (Maire 1985) normally used on mosquito egg rafts, which have a tougher, or thicker, protective shell than the gelatinous matrix of chironomid egg masses. Therefore, 2nd instar larvae were used, which were sorted under the dissecting microscope on the basis of body length (1 - 2 mm) and head capsule width (0.1 mm) (see Table 6.1.), and identified by colouration, head capsule shape and the
Table 6.1. The body length and head capsule width of each instar of *C. sylvestris*.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Body length</th>
<th>Head capsule width</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 1 mm</td>
<td>0.075 mm</td>
</tr>
<tr>
<td>2</td>
<td>1 - 2 mm</td>
<td>0.1 mm</td>
</tr>
<tr>
<td>3</td>
<td>2 - 4 mm</td>
<td>0.2 mm</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 4 mm</td>
<td>0.4 mm</td>
</tr>
</tbody>
</table>
presence of setal tufts (cf. Storey 1986). Larvae were held in a covered dish in filter-sterilised ADC water (Sartorius®; pore size, 0.22 µm), and added to each experimental dish aseptically to reduce microbial contamination (sterile pasteur pipettes were used and all procedures were carried out in a laminar flow cabinet). Many different larval densities have been used in rearing experiments in the past (e.g. a single larva of one of several species in a glass vial [Mackey 1977a]; 30 *Paratendipes albimanus* larvae in 500 ml containers [Ward & Cummins 1979]; 1 *Eukiefferiella ilkleyensis* larva in 40 ml containers [Storey 1986]). In my preliminary experiments, upwards of 70% mortality resulted when 100 larvae were used. Therefore in the experiment described in this chapter, 25 larvae were added to each dish.

After 10 days the mean larval dry weight in each dish was determined, in order to compare larval growth in each substratum type and treatment (cf. Ward & Cummins 1979). Larvae were separated from the substratum in each dish, rinsed in distilled water, counted, and then all larvae in one dish were placed into an individual, pre-weighed foil envelope. Each envelope was then dried and re-weighed as outlined in section 4.2.5.2. Hence the mean dry weight of larvae from each dish was calculated by dividing total weight by the number of larvae. The dry weight of 2nd instar larvae was estimated by extrapolation using the relationship between larval length and dry weight, and the length of 2nd instar larvae of *C. sylvestris* (i.e. 1 - 2 mm). This was used as a baseline for comparison with the results of the rearing experiment.
Figure 6.1. Apparatus used to investigate the effect of through-flow of water on larval growth: (A) Side view: (a) Frigid Units "Living Stream" system; (b) holding pipe; (c) sand filter column; (d) cooling unit and motor for propellor; (e) electric water pump; (f) small, adjustable clamps; (g) air-bleed valve; (h) propellor. (B) Individual "sand filter" column: (i) entrance hole for water from holding pipe; (j) overflow covered with fine mesh; (k) overlying head of water; (l) sand taken from filter bed; (m) coarse sand (particle size, 1 - 2 mm); (n) fine gravel (particle size, 2 - 5 mm); (o) coarser gravel/small pebbles (particle size, > 5 mm); (p) drainage hole; depth given in millimetres. Black arrows indicate direction of water flow.
The columns (see Fig. 6.1.) consisted of vertical sections of downpipe (PVC; length, 25 cm; inner diameter, 75 mm), with a square of perspex stuck on one end. Silicone sealant (Dow Corning, Michigan, USA) was used to bond the parts of the column together. Sealant was allowed to cure for at least 72 hours before columns were used. Water entered each sand filter column through a hole cut tangentially into the side of the columns, which reduced disturbance of the sand substratum. Water flowed vertically down through the substratum and out the bottom of the column through a large hole cut into the perspex square. Two rectangular pieces of perspex raised the column from the bottom of the “Living Stream”®, ensuring water left the column unimpeded. Should the water level have risen, a slot cut into the top of the column allowed any excess water to escape, while maintaining the same head of water within the column. The slot was covered with fine mesh (mesh size, 53 µm) to stop larvae escaping in the event of any such overflow.

Between the whole sand layer and the bottom of the column, three layers of graded sediment were used (coarse sand (particle diameter, 1 - 2 mm); finer gravel (particle diameter, 2 - 5 mm); coarser gravel and small pebbles (particle diameter, > 5 mm)). This reduced the loss of sand by erosion as water flowed through the column. Each sediment fraction (including whole sand) was obtained from the field site, and, before use, was thoroughly washed with distilled water, autoclaved and dried. Although the dimensions of columns were similar, the control columns were water-tight, and the water within them was kept aerated using an air pump and aerator blocks. These columns were filled with the same quantity of whole sand used in sand filter columns, but no other size fractions, and with sufficient water to produce a similar
overlying depth of water. This kept both sand and water within the column at or below the level of water surrounding all the columns (see Fig. 6.1.). In effect, the surrounding water cooled the control columns, keeping the temperature within these columns similar to that within the sand filter columns (i.e. 20 °C).

Before each experiment, the system was filled with water taken from the field site and filtered through a 53 μm mesh to remove any chironomid larvae. To provide food, 5 ml of fish food suspension (Tetra-Min®; 1 g/100 ml) and a layer of sand with established microflora, was placed on top of the whole sand. The system was left for 24 hours to equilibrate before larvae were added. When the fish food suspension was prepared, the water became discoloured. Whatever discoloured the water passed through a 0.45 μm pore size filter and so could be considered “dissolved” or the soluble element of fish food (i.e. there is a dissolved as well as a particulate element in “fish food suspension”). The soluble part was initially included in the 5 ml aliquot added to each column. However, I reasoned that this soluble element, as well as anything small enough to remain in suspension for a long period, would leach out sand filter columns as a result of the through-flow of water. As a consequence, the food conditions in sand filter columns were likely to contrast with that in control columns. In the two subsequent experimental replicates, fish food suspension was made up as usual but the soluble part was removed. This was done by repeatedly allowing the suspension to stand for one hour, decanting, and replacing the water. Repeating this process three times was sufficient to stop discoloration of the water and therefore, I assumed, to remove the most of the soluble part of the fish food.
Twenty-five 1st instar larvae, hatched from individual egg masses, were sorted under the dissecting microscope and transferred to small glass vials (length, 20 mm; inner diameter, 3 mm) using a Pasteur pipette. These vials were placed directly into each column and left. After 10 days, the larvae were separated by repeatedly washing all the whole sand in each column and sieving the rinsings. Larvae from each column were washed in distilled water, counted and placed into individual, pre-weighed foil envelopes. The larval dry weight was determined using the same method for drying and weighing outlined in section 4.2.5.2.

Once all the whole sand had been removed and larvae separated, columns with the rest of the graded sediment *in situ*, were dried in a drying oven. This killed any larvae still remaining in the column. The water in the system was also drained and the system thoroughly scrubbed and rinsed, before being used again.

6.3. Results.

6.3.1. Experiments using dishes.

The results of the rearing experiment are shown in Fig. 6.2. and the changes in microbial ATP with time in Fig. 6.3. The results of rearing experiments were compared using a *t*-test. For clarity, the results of statistical tests (for this and the subsequent section) are included in the relevant figure, rather than in the text. The relationship between mean larval dry weight and length, from which
Legend - Microbial treatments.

- ■ Maintain
- □ Modify
- □ Stop
- □ Mean dry weight of 2nd instar.

Figure 6.2. Results of rearing experiments conducted in petri dishes: (i) Dry weight of larvae reared on different substratum types. Three treatments of each substratum type were used: maintain (always some microbial biomass); modify (initially no microbial biomass, but some after time); and stop (no microbial biomass). Mean dry weight of 2nd instar obtained by extrapolation using a mass-length curve (Fig. 6.4.). Note: + indicate that no modify or stop treatments of Cladophora were used.
Table 6.2. Results of t-tests comparing maintain treatment of different substratum types; df = degrees of freedom; ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

<table>
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<tr>
<th>Comparison</th>
<th>t-value</th>
<th>df</th>
</tr>
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<tbody>
<tr>
<td>Sand vs Schmutz-detritus</td>
<td>-5.31**</td>
<td>8</td>
</tr>
<tr>
<td>Sand vs Sand &amp; schmutz-detritus</td>
<td>-2.23 ns</td>
<td>6</td>
</tr>
<tr>
<td>Sand vs Cladophora</td>
<td>5.98 **</td>
<td>8</td>
</tr>
<tr>
<td>Schmutz-detritus vs Cladophora</td>
<td>2.36 ns</td>
<td>8</td>
</tr>
<tr>
<td>Schmutz-detritus vs Sand &amp;</td>
<td>-7.82 *</td>
<td>6</td>
</tr>
<tr>
<td>schmutz-detritus</td>
<td></td>
<td></td>
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<tr>
<td>Cladophora vs Sand &amp;</td>
<td>-8.26**</td>
<td>6</td>
</tr>
<tr>
<td>schmutz-detritus</td>
<td></td>
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</table>
Figure 6.3. Changes in microbial ATP over time in three different microbial treatments of one substratum type. An arrow indicates the time when the modified substratum was exposed to the atmosphere to allow microbial colonisation. The three treatments were: maintained (always some microbial biomass); modified (initially no microbial biomass, but some after time); and stopped (no microbial biomass).
the larval dry weight of 2nd instars was determined, is illustrated in Fig. 6.4.

Sand substratum (with, and without, enrichment with schmutz-detritus) produced larvae that were significantly greater in dry weight than larvae reared on Cladophora or schmutz-detritus alone. No significant differences were found between the dry weight of larvae reared on sand with, and without schmutz-detritus, or between the dry weight of larvae reared on Cladophora and schmutz-detritus. Larvae reared on Cladophora had higher dry weights than those larvae reared on modify and stop treatments of the other substratum types. Significant differences were found in the dry weights of larvae between all microbial treatments for one substratum type. Highly significant differences were found between maintain and the other two treatments for each substratum type. The quantity of microbial ATP in different treatments indicated that the levels of microbial biomass were different in each treatment.

Therefore, sand with some established microbial development produced larvae with the highest dry weight. In addition, substratum types with some microbial biomass produced larvae with higher dry weights than substratum types with less or none. However, the stop treatment of schmutz-detritus produced a higher mean larval dry weight than the modify treatment of the same substratum type.
Figure 6.4. The relationship between larval dry weight and length for larvae of *C. sylvestris*. This was used to make an estimate of the mass of 2nd instar larvae.
6.3.2. Experiments using model sand filter beds.

When the soluble part of fish food was included in the suspension added to columns, larvae reared in the control columns were heavier than those reared in sand filter columns (Fig. 6.5.). However, when fish food suspension without the soluble component was added to columns, no significant difference was found between the dry weight of larvae reared in sand filter and control columns (Fig. 6.5.). So, in both cases, there was no evidence that a through-flow of water had a positive effect on growth of *C. sylvestris* larvae. Even though an equal number of larvae were added to each column, the number recovered at the end of the experiment was quite variable. This variability may have had a direct effect on any difference found between sand filter and control columns. Although a negative relationship was found between numbers of larvae and mean larval dry weight, the relationship was not significant (sand filter columns: $r_s = -0.35$, $P > 0.05$, df = 18; control columns: $r_s = -0.12$, $P > 0.05$, df = 17). There is then little evidence to suggest that the number of larvae present in a column at the end of the experiment had any significant effect on the mean larval dry weight.

6.4. Discussion.

Sand was the most favourable substratum for growth of *C. sylvestris* larvae, which is not surprising considering that the dominant substratum type in filter beds is sand. One habitat, that has been relatively well studied and is comparable in terms of substratum (sand, initially low in organic content and
Figure 6.5. Results of rearing experiments conducted in model sand filter beds: (i) Comparison of the dry weight of larvae reared in the presence (sand filter columns) or absence (control columns) of a through-flow of water; (ii) results of $t$-test for individual experiments; df = degrees of freedom; ns = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. 

<table>
<thead>
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<th>Individual Experiments</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Larval Dry Weight</strong> (mg, Mean ± SD)</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Legend</strong></td>
<td>Sand filter columns</td>
<td>Control columns</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$t$-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>-7.56</td>
<td>16</td>
</tr>
<tr>
<td>2nd</td>
<td>-0.22</td>
<td>15</td>
</tr>
<tr>
<td>3rd</td>
<td>-1.63</td>
<td>18</td>
</tr>
</tbody>
</table>
indicated by the smaller chironomid adults that Wotton & Armitage (1995) found emerging from filter beds which had been relaid with new sand. This sand is thoroughly washed and will not have the same quantity of established biofilm or detritus, that is associated with sand that has been in place for some time.

Despite differences in dry weight, larvae of *C. sylvestris* were able to grow on all the substratum types provided. The importance of *Cladophora*, as with macrophytes, may lie not necessarily in direct consumption, but rather with the biofilm (or epiphytes [Power 1991]) present on its filaments or the detritus that collects between its filaments (acting as “extra-organismal filters” [Power 1990]). That *C. sylvestris* larvae are capable of growing on schmutz-detritus should come as no surprise as Wotton *et al.* (1992) found adults of *C. sylvestris* emerging, albeit in reduced numbers, from filter beds which had been in operation for some time and probably therefore had a well-developed schmutzdecke layer. There is evidence that this species can tolerate mildly eutrophic habitats (Saether 1979) and has been found in polluted aquatic systems (e.g. Davies & Hawkes 1981; Murphy & Edwards 1982; Wilson 1987). However, the results of the rearing experiments also show that a substratum consisting entirely of detritus or organic matter may not provide a favourable substratum for growth of *C. sylvestris* larvae. This is consistent with the actual location of *C. sylvestris*, not at the source of pollution, where *Chironomus riparius*, a species characteristic of organic pollution (Gower & Buckland 1978), is found, but where the stream has begun to “recover”. At this point in the stream, the levels of dissolved oxygen are nearer to normal (Davies & Hawkes 1981), and the larvae probably benefit from nutrient-enhanced algal
of several studies (e.g. Cantrell & McLachlan 1977; Titmus & Badcock 1981; Wiley 1981a; Kohler 1992), but this important facet of chironomid biology has been, in many studies, taken for granted (e.g. Batzer & Resh 1991; Schmid 1992a, b) or completely ignored (e.g. Boerger et al. 1982; Rae 1985, 1987; Ramcharan & Paterson 1978; Tokeshi 1986). When summarising the biology of the different orders of aquatic insects, Williams & Feltmate (1992) failed to mention that the larvae of many chironomid species are capable of constructing tubes or burrows. The authors, however, did briefly mention chironomid tube-building in another section of their book and commented that pupae could live within tubes, or be free-living. This contrasts with their section on the Trichoptera, in which case-building behaviour was discussed in some depth. Dudgeon's (1994) excellent review illustrates the disparity in knowledge of chironomid biogenic structures (be it burrows, tubes, cases, or retreats) in comparison with the Trichoptera and the Polychaeta. Interestingly, Mackay & Wiggins (1979) proposed that the ecological diversity of the Trichoptera is, in part, a consequence of the ability of immature stages to produce silk. Wiggins & Mackay (1978) suggested this might also partly explain the equal, if not greater, ecological diversity of Nearctic Chironomidae.

The somewhat erratic exhibition of tube-building by chironomid larvae may explain this disparity of knowledge, although all Chironomidae, except the Tanypodinae, are capable of building a structure of sorts (Ashe et al. 1987). It may be that tubes and burrows are by nature cryptic, or delicate and fragile, and consequently go unobserved, even with careful sampling. The diversity of chironomid biogenic structures may also have been underestimated, especially if larvae construct something unlike the stereotypical "tube". An
example is the sheet of silk covering a fissure in the rock surface produced by *Eukiefferiella clypeata* (Brennan & McLachlan 1980).

The inconsistent exhibition of tube-building may also be a response of larvae to different environmental conditions. Cuker (1983) found larvae of *C. sylvestris* to be free-living, in contrast to other studies (e.g. Drake 1982; Hershey 1987; Mackey 1976; Menzie 1981) which described the larvae as tube-builders. This absence of tube-building or free-living mode of existence may have been a consequence of both insufficient food resources associated with the substratum of an oligotrophic lake, and the competitive effects of snail grazers and chironomid larvae of the genus *Paratanytarsus*. As a consequence food and tube-building resources were probably limiting, which might, in turn, have made a free-living mode of existence more advantageous than building tubes. A trade-off between the cost and benefit of establishing a feeding territory and building a tube, might explain why Kohler (1992) found larvae of two, apparently free-living chironomid genera, *Eukiefferiella* and *Thienemanniella*, building tubes when sufficient biofilm was present. Important in this trade-off may be the energetic cost of producing the silk used to construct tubes. Dudgeon (1987) found that if the caddisfly larva of the polycentropodid *Polycentropus flavomaculatus* was forced to build a silk net every day, then it lost weight. This cost of tube-building, in addition to that of establishing and defending a feeding territory, may help explain why larvae of *C. bicinctus* re-use previously vacated feeding territories and associated tubes (Wiley & Warren 1992). In other words, it is more cost-effective to re-occupy a vacant tube and territory, than invest resources in a new tube while establishing a new territory.
Mackay & Wiggins (1979) pointed out that the importance of silk to the Trichoptera lies not only in case-construction, but also in feeding, which is also likely to be true for chironomids. The ventromental plates, used in silk production (Cranston 1992c), are a conspicuous feature of Chironominae larvae, while those of larvae from other subfamilies tend to be less conspicuous, smaller, or vestigial. Kullberg (1988) suggested these plates, in conjunction with the maxillary plates, enabled *Rheotanytarsus* larvae to produce fine, thin strands of silk. Larvae use these strands, together with thicker strands produced by exuding silk out of the mouth, to construct nets with a mesh fine enough to catch bacteria; finer strands may also increase the surface area for adsorption of DOM (Wotton, R.S. in pers. comm.). Subtle variations in the development of the ventromental plates, and consequently the ability to produce different "types" of silk, may explain the apparent restriction of filter-feeding to the Chironominae (as shown by Coffman & Ferrington 1984). Variations in the ventromental plates may also explain the subtle differences in tube-building within the Chironomidae, including its absence in the Tanypodinae. Chironominae larvae (specifically of the genera *Microtendipes* and *Tanytarsus*) have also been observed using silk to feed upon deposited material. Larvae spread silk over the substratum, retrieve it, and then consume both silk and attached material (Walshe 1951). Clearly there must be some reason why larvae would invest the time and energy in producing silk, rather than feeding directly upon the substratum.

Lindegaard (1995) discussed how an interaction between tube-building and feeding, in concert with the availability of substratum resources, might explain the distinctive chironomid communities associated with lakes of different
trophic status (as described by Saether 1979). There are sufficient resources associated with the substratum of eutrophic lakes, such that a larva can obtain enough food from a limited area and justify the defence of a territory and construction of a tube or burrow. In contrast for oligotrophic lakes, Lindegaard suggested that species with free-living larvae predominate because of insufficient resources associated with the substratum, such that larvae have to cover a greater area than could be feasibly defended. It is probably true that the preponderance of tube-building by chironomids is affected by the availability of resources on the substratum, but species may not necessarily have larvae which are characteristically free-living or tube-builders. Rather, larvae of a majority of species are probably capable of building tubes, but they each differ in how flexible, or obligate, they are in their tube-building. The presence of *C. sylvestris* in lakes of different trophic status (Saether 1979), may be a consequence of the ability of larvae to modify their tube-building behaviour in response to changes in the particle regime. In other words, where there is sufficient material larvae are tube-builders, while in its absence larvae are free-living.

Previous studies of chironomid communities (e.g. Rae 1985, 1987; Schmid 1992a, b; Ruse 1994) suggest that differences between chironomid species, which probably includes tube-building, are likely to be subtle and complex, and consequently hard to detect. This may explain why the differences in tube-building have been observed but never considered as potential mechanisms promoting coexistence within chironomid communities. Interestingly, Rasmussen (1984b) noted clear differences in tube-building by two coexisting species, but did not comment that such differences might
The results of my study, as well as the general chironomid research literature, give the impression that chironomid larvae are capable of exhibiting differences in their use of particles. However, as Berg (1995) pointed out for feeding as well as tube-building, chironomid larvae can also be flexible in their use of particles. For example, larvae of one species could use more than one particle type as substratum, pursue both free-living and tube-building modes of existence, and consume a variety of particle types as food. Such flexibility is a striking feature of *C. sylvestris*, which may be a consequence of what has been termed its "phenotypic plasticity" (Wotton *et al.* 1992). Phenotypic plasticity is the expression of subtle differences in biology by populations of the same species, but in different locations or environments (be it at the micro or macroscale). In theory, these populations can interbreed (and hence are of the same species) but exhibit a different phenotype. However, it could be argued that different populations are in fact sub-species or cytotypes, which cannot, as yet, be taxonomically resolved. The phenotypic plasticity of *C. sylvestris* is reflected in the use by its larvae of different particle types as substratum (see Chapter 3) and food (see Chapter 5), and variations in tube-building (see Chapter 4). This species has also been found in both lentic and lotic habitats (see Chapter 6); at physiological extremes of temperature (Tuxen 1944) and salinity (Menzie 1981); undergoes cyclomorphosis (Hershey & Dodson 1987); and has a wide geographical range, including both the Nearctic (Ali & Mulla 1977; Batzer & Resh 1991; Cuker 1983; Darby 1962; Hershey 1987; LeSage & Harrison 1980; Menzie 1981) and the Palaearctic (Davies & Hawkes 1981; Drake 1982, 1983; Grzybkowska & Witczak 1990; Lindegaard 1992; Lindegaard & Jonasson 1979; Mackey 1977b; Murphy & Edwards 1982; Rossaro 1984; Titmus 1979; Tuxen 1944; Vilchez & Casas...
Another important feature of chironomid communities is the presence of species which are either obligate and facultative in their use of particles. Both Rae (1987) and Boerger et al. (1982) found such specialists (obligate) and generalists (facultative) in studies of the role of substratum in community structure. However, as Berg (1995) pointed out for feeding, any generalisation about the use of particles by a species, or genus, of chironomid should be applied with caution; closely related taxa may not necessarily use particles in the same way, especially as most species have yet to be described taxonomically, let alone ecologically. Nonetheless, there is still the possibility that as with substratum, species may be obligate or facultative in the kinds of particle types they will consume or use in tube-building.

7.2. Chironomids and community structure.

My study found evidence that coexistence of the three dominant species, *C. sylvestris*, *P. limbatellus* and *Tanytarsus* sp., may be facilitated, or promoted, through partitioning of particle resources, as substratum and in tube-building. Little evidence was found of food partitioning, which was unlikely to be important because, as pointed out by Wotton et al. (1992), food resources are probably not limiting.

Schoener (1974) suggested that spatial, or habitat, partitioning might be more important in terrestrial than aquatic systems because of a lack of habitat
because of the absence of the discrete, but ephemeral, habitat units (e.g. fallen, rotting fruit [Atkinson 1985] or sections of *Myriophyllum* [Tokeshi & Townsend 1987]) which are an important feature of this theory. By habitat units I mean "islands" or "patches" between which larvae find it difficult to move. This is not to say that patches are not present within a filter bed, although a complete layer of substratum is removed from the bottom of a filter bed during cleaning. The substratum could become patchy in two ways. Firstly, as discussed in Chapter 3, *Cladophora* often forms patches on the substratum, and in the water column. Secondly, laboratory experiments demonstrated that *Tanytarsus* sp. can form aggregations of tubes. Such patchiness or substratum heterogeneity probably helps promote coexistence, but in a way that would differ from that envisaged by Tokeshi & Townsend (1987). This is primarily because the stochastic, random element is no longer involved and any patches present probably do not represent habitat units or islands. Larvae may associate with patches in a deterministic fashion, such as those of *P. limbatellus* which, in contrast to *Tanytarsus* sp. larvae, probably dominate in patches of *Cladophora*. Similarly, larvae of *Tanytarsus* sp. may be attracted to one another and form dense aggregations, as Titmus & Badcock (1981), suggested for larvae of *Polypedilum* sp.. Furthermore, there seems no reason why larvae would be restricted to one patch because firstly, larvae of *C. sylvestris*, and *P. limbatellus* to a lesser extent, can follow, at least for a limited time period, a free-living mode of existence; secondly, larvae are capable of using more than one substratum type; and thirdly, there appears to be no obstacle that could prevent the movement of larvae between any patches that did form (such as high water velocity [Tokeshi & Townsend 1987]).
Considering the field site as a whole, the filter beds represent numerous "ponds", which resemble the temporary ponds found in rock depressions by McLachlan (1983, 1985) and could be considered as discrete, but ephemeral habitat units. However, McLachlan (1988) commented that such a model as random patch formation or its equivalent (e.g. Atkinson 1985; Shorrocks & Rosewell 1987; Shorrocks et al. 1984) was not in fact applicable. This was because although such ponds were ephemeral and the period between refilling was unpredictable (i.e. temporally unpredictable), unlike rotting fruit or Myriophyllum stems, these ponds, like filter beds, do not move or disappear permanently (i.e. spatially predictable). Hildrew & Townsend (1987) came to a similar conclusion. McLachlan (1985), however, found that midge species segregated into ponds of different duration, according to differences in the tolerance of larvae to desiccation and the duration of the larval stage. Wotton et al. (1992) found little evidence that the three dominant species segregated into different filter beds or "ponds", or that their abundance varied between filter beds. In other words, the chironomid community in each filter bed usually included all three species, usually at similar abundances (i.e. C. sylvestris dominant; P. limbatellus usually sub-dominant, and Tanytarsus fimbriatus sometimes sub-dominant) (Wotton et al. 1992).

7.3. Chironomids and the functioning of slow sand filter beds.

Chironomids are conspicuous inhabitants of slow sand filter beds (Wotton et al. 1992) where larvae, being predominantly benthic macroinvertebrates and using particles in several ways, are likely to influence the nature of the
substratum. Therefore, because the substratum is a crucial component in how a filter bed functions, so high densities of chironomid larvae are likely to have an important impact on the process of water filtration.

The construction of biogenic structures, such as tubes, burrows and cases are known to affect biofilm development in terms of diversity, biomass and activity of the microbial communities (Aller & Aller 1986; Hershey et al. 1988; Lock 1994; Pringle 1985; Sinsabaugh & Linkins 1988; Van de Bund et al. 1994). DOM is removed by adsorption and degradation by the biofilm associated with the substratum (be it sand or schmutzdecke). By increasing the quantity of biofilm, construction of tubes may enhance the ability of the substratum to remove DOM from water. Tubes may also enhance the ability of the substratum to remove FPOM as well as DOM because chironomid larvae use silk to stick particles together during tube construction. Silk is known to be a "sticky" material (Kullberg 1988; Walshe 1951) and has physico-chemical properties that may cause potential nutrients to adhere to its surface (Wotton 1994c). Larvae irrigate their tubes by sinusoidal undulations of the body, drawing in oxygen-rich water while expelling water loaded with CO₂ and excretory products. The water entering the tube will also be loaded with nutrients, so encouraging biofilm development within the tube and the surrounding substratum. Perhaps more importantly for the functioning of the filter bed, the movement of oxygen-rich water into the substratum will also help oxygenate and maintain an aerobic environment within the substratum.

Feeding by chironomid larvae will reduce the quantity of FPOM and prevent blockage of the pores between sand grains, as larvae consume and digest
material that accumulates at the surface of the substratum. However, larvae will also feed on the biofilm, including algae and bacteria, attached to sand grains or present in the schmutzdecke. This enhances the productivity and diversity of the microbial community within the biofilm (Lamberti & Resh 1984), and consequently the ability of biofilm to remove DOM from the water. Chironomid larvae are known to influence the taxonomic composition of epiphytic algal communities (Cattaneo 1983), and are capable of exhibiting some selectivity in their feeding (Berg 1995; Botts & Cowell 1992). Therefore, as discussed by Brook (1954), chironomid larvae may have an important influence on the composition of the algal community associated with the substratum, which can affect the aesthetic qualities (e.g. taste and smell) of the filtered water (Pescod et al. 1985). Larvae produce faecal pellets which, with the peritrophic membrane, represent further sites for adsorption of DOM, particularly because faecal material is a focus for enhanced microbial activity (Wotton 1994d). The formation of faecal pellets also converts DOM and FPOM into CPOM (coarse particulate organic matter), which results in the pelletisation and effective localisation of organic material at the substratum surface, rather than moving down into the substratum. Faecal pellets, as well as being fed upon, are probably also incorporated into tubes, again helping to localise organic material at the substratum surface. Organic matter will also be converted into chironomid biomass, which will eventually be removed from the filter bed when the larva emerges as a fly.

There are then many ways in which the use of particles by chironomid larvae, specifically in tube-building and feeding, could have a beneficial effect on the functioning of filter beds. Clearly, there is a need for a better understanding of
the biological communities (including those other than chironomids, such as oligochaetes) associated with filter beds. Such research seems particularly apt for what is essentially a biological process.

7.4. Concluding statement.

The coexistence of the three dominant species of chironomid larvae in filter beds at Ashford Common is likely to be promoted through differences in their use of particles, in tube-building and in habitat preferences, as suggested by Wotton et al. (1992). Although differences in substratum preference have been found and commented upon before, differences in construction of biogenic structures has not previously been considered as a mechanism promoting the coexistence of chironomid species.

Particles are important in aquatic systems, but despite the work of McLachlan, Brennan and co-workers (e.g. Brennan & McLachlan 1979; McLachlan et al. 1978; Brennan et al. 1978), and two recent review texts (Wotton 1990, 1994a), no subsequent study of chironomids has explicitly referred to, or considered, particles. The various uses of particles (i.e. food or tube-building material, and as component in an organism's habitat) may be interrelated, so different areas of particle utilisation should be investigated within one study, although this may be impossible because of the limitations of resources and time. Specifically, future studies of chironomid communities should consider all the usual resource categories of habitat (or space), food, and time, but should include some consideration of tube or burrow construction. However, it seems
likely that differences between species will be subtle and hard to detect without a combination of different experimental approaches (such as field and laboratory based experiments), involving both established and novel techniques (such as DAPI and computer image analysis).

Slow sand filter beds were used as a model system for this study. As pointed out in Chapter 2, filter beds provide an ideal system because the substratum is more homogenous, and the chironomid species diversity lower, than in other aquatic systems. Greater co-operation between the water industry and aquatic biologists to study the biology of slow sand filter beds could be extremely worthwhile. The water industry would gain important insights into how a filter bed functions, which is essentially a biological process. Aquatic biologists, on the other hand, would be given the opportunity to use a model system which may be ideal for studying many different aspects of aquatic biology.


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