THE UNIVERSITY OF HULL

Bacteria in Contrasting Headwater Streams

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by

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Summary of Thesis submitted for Ph.D. degree

by Carrie Ann Rimes, B.Sc.,

on

Bacteria in Contrasting Headwater Streams.

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Mean concentrations of suspended bacteria were marginally greater in the calcareous streams, while heterotrophic activity was substantially greater. Mean cell volume was also greater. The concentration and activity of suspended bacteria in the calcareous streams usually showed linear downstream increase, while in the acid streams, the downstream increase was less, and was frequently not observed.

In Mill Beck (a calcareous stream) it was found that the population of epiphytic bacteria near the source was easily sufficient to sustain the observed downstream increase in suspended bacteria. In Dungeon Burn (an acid stream) a substantial population of epiphytic bacteria was also found, but there was no downstream change in concentration of suspended bacteria; reasons are suggested for the apparent non-release of epiphytes in the Galloway stream.

The mean volume of suspended bacteria in Mill Beck changed between the source and downstream limit of a vegetated section, to resemble that of epiphytic bacteria, suggesting that suspended bacteria were dislodged epiphytes.

Estimates were made of the attachment rate of suspended bacteria to submerged vegetation in Mill Beck; daily attachment represented only a small proportion of the total standing crop of epiphytic bacteria.

A further study in Mill Beck, over a Spring growing period, demonstrated a temporal change in the density of epiphytic bacteria, which was related to change in discharge and temperature. The results supported the suggestion that epiphytic bacteria might largely be the source of suspended bacteria in this headstream.

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CONTENTS

CHAPTER 1

Introduction.

1.1 Role and origins of bacteria in running water.

Bacteria are fundamental participants in the flow of energy and cycling of nutrients in streams and rivers. Most aquatic bacteria are heterotrophic (Rheinheimer, 1974) and thus degrade (or mineralize) both dissolved and particulate organic matter. Bacteria also provide a food source for aquatic fauna (Fry, 1982).

The running water environment is, by definition, a transitory one. There is a constant throughput of water and suspended material, thus in any section of stream, the suspended bacterial population is the net result of upstream inputs, and downstream losses from the system. Potential inputs and losses of suspended bacteria are shown in Fig. 1.1. Bacteria may originate from within the stream by release of attached bacteria from submerged surfaces such as plants, rocks and stream sediments, or with inputs of surface water, groundwater and effluents. Within-the-plankton growth and cell division may also occur, dependent upon the activity of the population, which may vary, in part, according to inputs of both autochthonous and allochthonous organic material. Losses of suspended bacteria can result from attachment of cells to within-stream surfaces and from predation by invertebrates and protozoa.

The conventional view of suspended bacteria in running water was that the majority of bacteria originated outside the river system, in soil or sewage effluent, while relatively few were autochthonous in origin (Wuhrmann, 1964; Rheinheimer, 1974). Changes in concentration or heterotrophic activity of bacteria were thought to be related principally

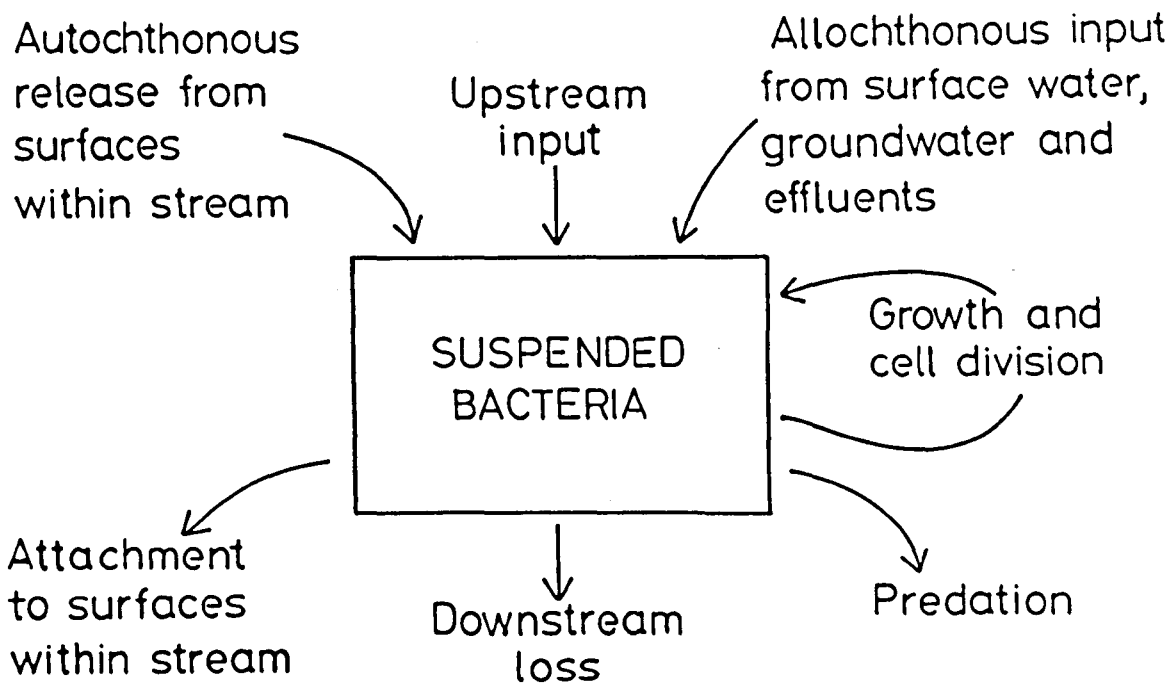


Figure 1.1 Diagramatic representation of sources and losses of suspended bacteria in running waters.

to variation in organic-pollution load. More recent studies of clean waters, however, have shown that substantial populations of suspended bacteria (generally 10^4–10^6 ml^{-1}) may be found in unpolluted streams, for example, c.0.4-1.0 x 10^5 ml^{-1} in three mountain streams (Geesey et al, 1978), 0.4-9.2 x 10^6 ml^{-1} in two small streams in woodland and grassland (Marxsen, 1980a), 0.3-8.9 x 10^5 ml^{-1} in a small, acidic woodland stream (McDowell, 1984) and 0.3-4.4 x 10^6 ml^{-1} in a small calcareous river (Goulder, 1980). Moreover, in some rivers and streams, except at times of sustained high flow, suspended populations may vary in size and activity (as determined by incorporation of radio-labelled substrates), independently of allochthonous input of bacteria and organic matter, and may thus be largely autochthonous in origin (Baker & Farr, 1977; Goulder,1980; Marxsen, 1980a,b).

1.1.i Autochthonous sources of suspended bacteria.

The study of naturally-occuring, autochthonous bacteria in mature rivers, is likely to be hindered by more or less inevitable interference from, for example, purified sewage effluents, storm sewers and run-off from roads and agricultural land. In the unpolluted headwaters of streams, such pollution may, however, be minimal.

Marxsen (1980b) studied two headwater streams in eastern Hesse (F.R.G.) and found that activity per suspended bacterium was higher in the grassland stream than the woodland stream. It was assumed that the grassland stream bacteria were better adapted to the low substrate concentrations available and thus may have included a higher proportion of cells of autochthonous origin. McDowell (1984) suggested that the rapidity of increase in suspended bacterial numbers in a small woodland stream during intense rainstorms, indicated flushing of the stream channel rather than runoff from the soil.

Goulder (1984) investigated the headwaters of a small, spring-fed, calcareous stream in the Yorkshire Wolds (Mill Beck, 7.2.i), and found that abundance and heterotrophic activity of suspended bacteria, from March 1981 to March 1982, generally showed parallel, gradual downstream increase. The increment per unit length of stream was greatest in the summer when aquatic vegetation was most abundant and discharge was low. It was suggested that release from within-stream surfaces, and from vegetation in particular, may have been an important cause of downstream increase in abundance and heterotrophic activity of suspended bacteria.

Epiphytic bacteria on the surface of submerged macrophytes may be abundant, for example, densities of 0.04-26.0 x 10^6 cm^{-2} were found on branches of Ranunculus penicillatus (Hossell & Baker, 1979b) and 0.1-23.0 x 10^6 cm^{-2} on surfaces of Nasturtium officinale and Lemna minor (Hossell & Baker, 1979a). Kudryavtsev (1984) reported densities of epiphytic bacteria of 0.3-39.9 x 10^6 cm^{-2} on submerged macrophytes in the littoral of a reservoir, and Baker & Orr (1986) found densities of 0.3-10.5 x 10^6 cells cm^{-2} on leaf surfaces of six aquatic plants in calcareous streams.

Epilithic aquatic bacteria, on surfaces of stones and boulders may also be important, particularly in more extreme environments, such as oligotrophic waters of some upland streams (Haack & McFeters, 1982). The epilithic mat may consist of bacteria, fungi, algae, protozoa and metazoa surrounded by a polymer slime matrix (Lock, 1981). Epilithic bacteria have been found to range in density from 0.7- > 400 x 10^6 cm^{-2} in sub-alpine and sub-arctic streams (Geesey et al, 1978; Albright et al, 1980; Haack & McFeters, 1982).

Bacteria are also found associated with stream sediments. Studies of such bacteria are few. However, Bott & Kaplan (1985) estimated 9.4 x 10^8 -2.35 x 10^{10} cells g^{-1} dry weight of sediment from streams in woodland

and on agricultural land, somewhat lower than values of c.2-15 x 10^{10} cells g^{-1} for lake sediments (Jones, Orlandi & Simon, 1979).

1.1.ii Inputs of allochthonous bacteria.

Input of allochthonous bacteria may be important when streams receive a large proportion of surface and shallow groundwater, particularly during rainy weather. Marxsen (1981) and Ladd et al (1982) found that shallow unpolluted groundwater had a higher bacterial concentration than adjacent streams. McDowell (1984) concluded that both allochthonous and autochthonous sources of bacteria were important in a small woodland stream. During periods of sustained high discharge, bacteria were probably washed into the stream from adjacent soil as well as from the stream channel and banks. Baker & Farr (1977) found a similar situation in the River Frome (southern England) during the winter months.

1.1.iii Cell division in the water column.

Increase in concentration of suspended bacteria through cell division in the water column is dependent upon (1) the rate of cell division, and (2) the retention time of water within the stream.

Measurement of cell division rates within rivers and streams is complicated by other factors, apart from growth, which cause changes in population size (Fig.1.1). Baker (1981) measured growth rates of suspended bacteria in the virtually cell-free borehole water which supplies the River Frome, using a large recirculating experimental channel, where input of bacteria from other sources was eliminated. A doubling time of 42 h was calculated. Bott (1975) monitored increase in density of attached cells on glass slides in situ in a small rural stream. The attachment component was determined by increase in density on ultra-violet-irradiated slides. Total increase in density minus attachment gave a potential maximum rate, because

no account was taken of detachment or predation. Doubling times of 2.8-51h were found over a temperature range of 0-21°C. Campbell & Baker (1979) used ^{35}S to determine the uptake of sulphate by suspended bacteria taken from the River Frome. This was combined with counts of suspended bacteria, and estimates of dry weight of a bacterium and percentage S content, to yield a doubling time of 42 h (Hossell & Baker 1979c).

More recently, the incorporation of ^{3}H-labelled thymidine into bacterial DNA has been monitored, to give an estimate of bacterial production. The resulting doubling times for suspended bacteria in lakes include, 168-888 h (Riemann, Fuhrman & Azam, 1982), 17-91 h (Riemann, 1984), 288 h (Bell, 1984) and 8-288 h (Lovell & Konopka, 1985).

1.2 The effect of physico-chemical variables.

Physico-chemical variables may affect the suspended bacterial population by influencing: (1) the heterotrophic activity and growth rate of the suspended population; (2) the heterotrophic activity and growth rate of the source population, hence the supply of cells available for release to the stream water; (3) the process of release from the source population to the stream water. Variables such as temperature, pH, conductivity and turbidity may influence (1) and (2), whereas, discharge and current velocity may be partly responsible for (3).

1.2.i Temperature and discharge.

It has been suggested that temperature influences both the abundance (Baker & Farr, 1977; Albright et al, 1980; Goulder, 1980; Marxsen, 1980a; Goulder, 1986) and the activity (Albright et al, 1980; Bell, Holder-Franklin & Franklin, 1980; Goulder, 1980; Marxsen, 1980b; Nuttall, 1982; Goulder, 1986) of suspended bacteria in rivers and streams. Discharge may

also be important. McDowell (1984) found a strong positive correlation between concentration of suspended bacteria and discharge in a small woodland stream. At high discharge, however, Geesey & Costerton (1979) observed a dilution effect in the Athabasca River, Northern Canada. In the calcareous River Frome (Baker & Farr, 1977), there was a positive correlation between bacterial numbers and discharge from December to March, with high discharge and flooding, but a strong negative correlation between the variables from April to July. Goulder (1980) found an overall negative correlation between both abundance and activity of suspended bacteria and discharge in the calcareous River Hull.

1.2.ii pH.

The effect of low pH on the chemistry and ecology of poorly buffered freshwaters, is attracting considerable interest, due to the current concern about acid rain or deposition (Henriksen, 1982; Sutcliffe, 1983; Burns et al, 1984; Fowler, Cape & Leith, 1985; Harriman & Wells, 1985).

Acidic conditions in streams are often associated with biological impoverishment, indicated by depletion in abundance and diversity of fish and invertebrates (Hall et al, 1980; Harriman & Morrison, 1982; Townsend, Hildrew & Francis, 1983; Stoner, Gee & Wade, 1984; Simpson, Bode & Colquhoun, 1985). Birds dependent on aquatic invertebrates may also be less abundant where streams are acidic (Omerod, Tyler & Lewis, 1985).

Microbial activity may be reduced at low pH, since there is evidence of reduced leaf litter degradation (Burton, Stanford & Allan, 1985; Mackay & Kersey, 1985), reduced decomposition of algal detritus (Hendry, 1982) and reduced cellulolytic decomposition (Hildrew et al, 1984). In lakes, however, some authors have found no evidence of reduced microbial decomposition rates at low pH (Hoeniger, 1985; Schindler et al, 1985), possibly because the microflora at the sediment-water interface maintained

a microenvironment with a higher pH (Kelly et al, 1984). In contrast, Rao, Jurkovic & Nriagu (1984), using in situ sediment pH measurements in Canadian lakes found that microbial respiration rates in acid lake sediments were much reduced.

Bacteria amongst micro-organisms in freshwaters, may apparently be inhibited at low pH. Rao and Dutka (1983) found that numbers of total suspended bacteria were nearly an order of magnitude lower in acid lakes, moreover, the proportion of actively-respiring bacteria and concentration of colony-forming-units (CFU's) were a 100 fold less below a critical pH of 5.5 (Rao, Jurkovic & Nriagu, 1984). Ferroni, Leduc & Choquet (1983) found that bacterial activity was reduced by c.7 times in a Canadian lake between the in situ pH 7.5 and an experimental pH of 5.0. In a polluted English stream, mean bacterial activity and abundance of CFU's were much reduced (7-142 times) within the pH range 2.7-5.7 when compared to pH 6.3-7.6 (Milner & Goulder, 1986).

1.3 Outline of thesis.

The first section of this thesis extends the work of Goulder (1984) by investigating downstream change in abundance and heterotrophic activity of suspended bacteria in four further Yorkshire Wolds streams, and by way of contrast, in four oligotrophic, acidic, upland streams in the Galloway Hills. This is followed in the second section by studies of the relationship between epiphytic and suspended bacteria; firstly an investigation of the potential ability of epiphytic bacteria to contribute to suspended bacterial populations in both a Wolds and a Galloway stream, and secondly, similar studies in the Wolds stream over a five month period. The mean cell dimensions and volume of epiphytic and suspended bacteria in the Wolds stream are compared, and the attachment rate of suspended bacteria to macrophytes within the stream is also considered.

Nomenclature throughout the thesis follows Clapham, Tutin & Warburg (1981) for vascular plants; Smith (1980) and MacVicar (1926) for bryophytes.

SECTION 1

SUSPENDED BACTERIA IN HEADSTREAMS OF THE YORKSHIRE WOLDS AND GALLOWAY HILLS

The suspended bacteria in headstreams of the Yorkshire Wolds and Galloway Hills were studied, to investigate: (1) differences in abundance, heterotrophic activity and cell volume of suspended bacteria in calcareous and acidic streams; and (2) patterns of downstream change in abundance and heterotrophic activity of suspended bacteria in the two stream types.

CHAPTER 2

Description of sites

2.1 Study areas.

The Yorkshire Wolds and Galloway Hills (Fig. 2.1.a) are areas with contrasting hydrology, vegetation and land use, resulting chiefly from differences in geology, rainfall and altitude.

2.1.i Yorkshire Wolds.

The Yorkshire Wolds are of Cretaceous chalk, extending in a curve from the Humber to Flamborough Head (Fig. 2.1.b), gaining maximum height of over 200 m in the north west. The escarpment to the north and west, slopes steeply down to the Vales of Pickering and York, while to the south east, the Wolds decline gently to the hummocky plain of Holderness, where the chalk is buried under glacial deposits. Rainfall for the area is fairly low. Averages for the years 1982-1984 were 682 mm at South Dalton (central Wolds, SE 965 452, altitude 34 m) and 705 mm at High Mowthorpe (north Wolds, SE 888 685, altitude 175 m; from data supplied by the Yorkshire Water Authority). The water percolates rapidly through the permeable chalk, with little surface run-off. It accumulates in vast aquifers and may take many years to return to the surface (Westlake et al, 1972).

Most of the streams rise below 150 m, those on the north and west rise below the scarp and flow westwards to the Derwent, and southwards to the Humber, whereas the dip slope streams flow eastwards to the Gypsy race or River Hull, over gravel and boulder clay of recent origin. The slopes of the streams are gentle and the beds are principally of gravel and silt (Plate 2.1). The scarp slope streams generally have permanent spring

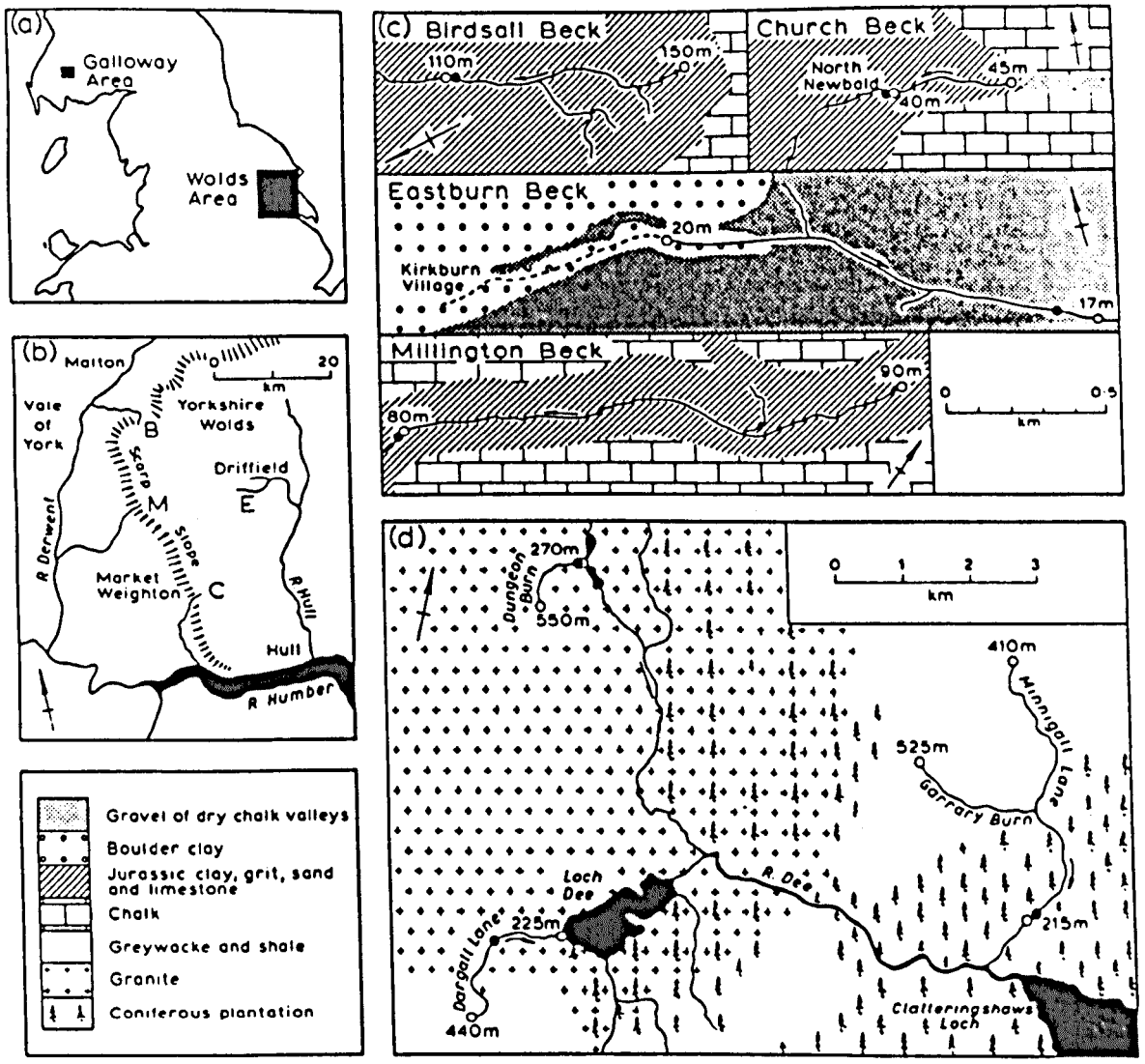


Figure 2.1 Yorkshire Wolds and Galloway Hills. Maps to show: (a) Location of study areas. (b) The Wolds area; the labelled headstreams are B, Birdsall Beck; C, Church Beck; E, Eastburn Beck; M, Millington Beck. (c) Detail of the Wolds streams. (d) The Galloway area; the headstreams studied are labelled. In (c) and (d) The upstream and farthest downstream sampling stations are indicated (open circles) and their approximate altitude is given; points at which discharge was measured are also shown (solid circles). Crossed arrows in (b), (c) and (d) indicate north, simple arrows show direction of stream flow. The key applies to (c) and (d).

sources, whereas the dip slope streams are often intermittent in their upper reaches. Both dip slope and scarp slope streams are fed principally from the chalk aquifers and usually maintain a fairly constant day to day flow, although seasonally there may be marked differences in discharge, with high flow during the winter and spring, gradually falling during the summer and early autumn. Chalk stream water is very hard, relatively rich in most plant nutrients (Westlake et al, 1979) with a pH of c.7.0-8.0.

The Wolds support extensive arable cultivation, with pasture on the steeper slopes and heavier soils beyond the chalk margins. The streams are sometimes shaded by belts of trees and shrubs, and here aquatic vegetation is sparce (Plate 2.2), while in other unshaded areas, submerged and emergent vascular plants are often abundant (Plates 2.3, 2.4), particularly Nasturtium officinale, Apium nodiflorum and Ranunculus fluitans.

2.1.ii Galloway Hills.

The Galloway Hills in the Loch Dee area (Fig. 2.1.d) are dominated by a granite intrusion surrounded by metamorphosed Ordivician greywackes and shales, forming hills of over 800 m in parts. The bed rock weathers slowly, and supports thin, nutrient-poor soils. The average yearly rainfall for Loch Dee during 1982-1984 was 2539 mm (from data supplied by the Solway River Purification Board); more than three times higher than on the Wolds. The rocks are relatively impermeable, thus most rainfall drains as surface run-off. There is a dense stream network, draining northwards to the River Doon, south-westwards to the River Cree, and south-eastwards to the River Dee. The streams rise in boggy areas, and descend, often steeply over beds of boulders, rocks, gravel and peat (Plates 2.5 - 2.8). Water levels in the streams tend to respond quite rapidly to heavy rainfall, often producing marked fluctuations of discharge over short time periods. The water generally has a low base cation and nutrient content

Plate 2.1 Church Beck, c.400 m below the source, showing chalk gravel and silt stream bed. The photograph was taken in May 1986, when aquatic vegetation was sparce. During 1982-1983, however, this section of stream supported a dense covering of submerged and emergent macrophytes. The vegetation may have suffered during the severe winter of 1985-1986.

Plate 2.2 Eastburn Beck, May 1985, c.400m below the permanent source. Shaded section with sparce aquatic vegetation.

Plate 2.3 Eastburn Beck, May 1985, c.1 km below the permanent source, where stream flows through open pasture and arable land. The photograph shows submerged Ranunculus fluitans.

Plate 2.4 Mill Beck, showing beds of submerged and partly emergent macrophytes, where the stream flows through open pasture. (a) May 1986, c.1 km below the source, where the stream flows fairly swiftly over a chalk gravel bed, supporting mainly Nasturtium officinale and Apium nodiflorum. (b) May 1984, 0.3 km below the source, where the stream flows slowly through dense beds of Nasturtium officinale, Apium nodiflorum and Glyceria fluitans, with Caltha palustris, Agrostis stolonifera and Juncus inflexus at the stream margin.

(a)

(b)

Plate 2.5 Minnigall Lane, April 1985, c.0.5 km below the source, where the stream flows in a peat channel, through Calluna heath and Molinia grassland.

Plate 2.6 Garrary Burn, April 1985, c.4.1 km below the source, where the stream flows over a stone and gravel bed with peat banks, through Molinia grassland and forestry plantation.

Plate 2.7 Dargall Lane, May 1985, c.1.5 km below the source, where the stream flows over a bed of granite and metamorphic stones and boulders, through Calluna heath and Molinia grassland.

Plate 2.8 Dargall Lane, May 1985, c.1.5 km below the source, where the stream descends steeply over granite slabs and boulders.

(Burns et al, 1984).

The Galloway catchments are upland heath, blanket bog and grassland with some young sitka spruce (Picea sitchensis) plantation. Angiosperm aquatic vegetation is sparce, but bryophytes may be locally abundant, principally Nardia compressa and Scapania undulata. Algal films sometimes cover the stream beds, particularly during the summer months.

In the Galloway area, the combined effects of acid precipitation, low neutralization capacity of the soils and coniferous afforestation have been implicated in the acidification of streams and lochs (Burns et al, 1984; Harriman & Wells, 1985; Tervet, unpublished). Although 80% of the rainfall has a pH greater than 4.5 (Tervet, unpublished), the total mass of deposited hydrogen ions is large because of the high precipitation. Inputs of acidity with rainfall have been estimated to exceed 1 kg H^+ha^{-1} annually, in comparison with 0.3-0.6 kg $H^+ha^{-1}a^{-1}$ for east coast regions of Britain such as the Wolds (Fowler, Cape & Leith, 1985). The mean pH of streams entering Loch Dee during 1981 was 5.0-5.3 (Burns et al, 1984). Many of the lochs in the area have pH values below 5.5 (Tervet, unpublished) and some have shown a pH decline in the last 100 years by as much as 1 pH unit (Battarbee et al, 1985).

2.2 Selection of streams.

The aim was to find headstreams with a discrete source and with a reasonable downstream length which was free from human interference. About 30 likely headstreams rising at the margins of the Wolds, and about 10 in the Galloway Hills, were selected from O.S. maps and examined on the ground. The major problems in the Wolds area were organic inputs from ditches and field drains, road drainage and impoundments for fish ponds, often close to the source. On the Galloway Hills the problems were run-off

from land recently ploughed for afforestation, and the practice of dumping limestone into streams in an attempt to raise pH and improve fishing in the recipient lochs (Burns et al, 1984).

The four streams chosen for study in each area are shown in Fig. 2.1.c and d), and listed with their major features in Table 2.1

Of the Wolds streams, Birdsall Beck, Church Beck and Millington Beck are scarp slope streams with near-permanent, point-source springs. The water upwells through chalk gravel at Church Beck, issues through a pipe at Birdsall Beck, and a culvert at Millington Beck. Eastburn Beck is a dip slope stream, with a diffuse source, where the water seeps gradually through chalk gravel, collecting initially in small pools. The position of the source at Eastburn Beck varies according to the height of the water table. Birdsall Beck and Millington Beck flow through damp pasture, Church Beck is bordered by arable land and Eastburn Beck by arable and pasture. All four streams are flanked in some sections by trees and shrubs.

The four Galloway streams flow into the River Dee system. Dargall Lane, however, originally flowed westwards to the River Cree, but was diverted eastwards, into a smaller stream during the 1930's to augment the water supply to Loch Dee, for the Galloway Hydro-Electric scheme (Hill, 1984). The stream channel in the lower portion of the stream (from the discharge measurement site to Loch Dee, Fig. 2.1.d), thus may still be undersized for the volume of water. Garrary Burn and Minnigall Lane merge, hence the lower 2.3 km is common to both streams. Dargall Lane, Dungeon Burn and the upper portions of Garrary Burn and Minnigall Lane flow through upland heath, blanket bog and grassland dominated chiefly by Calluna vulgaris, Molinia caerulea and Nardus stricta. The area is grazed by red and roe deer, and feral goats, with sheep in parts of the Garrary Burn and Minnigall Lane catchments. The lower reaches of Garrary Burn and Minnigall Lane flow through coniferous forestry plantation (Fig. 2.1.d). Part of

Table 2.1 Yorkshire Wolds and Galloway Hills. Major features of selected streams.

Stream	Grid reference (the most upstream sampling station)	Length of sampled headstream (km)	Average slope over sampled section (m km^{-1})
Wolds streams			
Birdsall Beck	SE 813 634	0.81	49
Church Beck	SE 917 367	0.40	13
Eastburn Beck	SE 980 552	1.80	2
Millington Beck	SE 843 532	1.70	6
Galloway streams			
Dargall Lane	NX 446 775	3.20	67
Dungeon Burn	NX 458 837	1.37	204
Garrary Burn *	NX 513 816	4.68	66
Minnigall Lane *	NX 528 831	5.42	36

* Garrary Burn and Minnigall Lane merge, hence the lower 2.30 km is common to both streams.

this area was drained and planted during the early part of the sampling period.

2.3 Vegetation of the selected streams.

The aquatic vegetation in the streams from the two areas was almost totally dissimilar. Only one true hydrophyte (Equisetum palustre) and two marginal species (Deschampsia cespitosa and Cirsium palustre) were common to both areas.

Aquatic vegetation may provide a good indication of the trophic status of streams (Haslam, 1978). Newbold & Palmer (1979) ranked 150 species of British aquatic vascular plants, according to the trophic conditions in which the plants occur. Each species was given a trophic ranking number; low numbers corresponding to plants of oligotrophic conditions, high numbers to rich conditions. The species in the Wolds and Galloway streams that are included in the Newbold & Palmer list are given in Table 2.2 together with their trophic ranking number. Mean trophic ranks for each stream were calculated, and gave values of 68-78 for the Wolds streams compared to 23-32 for the Galloway streams. Even though both groups of streams fell within the mesotrophic category (defined by Newbold & Palmer to comprise plants within the 22-103 trophic ranking number range), the mean trophic ranks indicate that the Wolds streams were substantially richer than the Galloway streams. Only 44% (Wolds) and 28% (Galloway) of the total riparian species of vascular plants found in these streams (38% and 18% including bryophytes) were included in the Newbold & Palmer list. Full species lists, together with an assessment of percentage cover, are given in Appendix 1.

Holmes (1983) gives a key to river types based on river and bank species. Using the full species lists, the Wolds streams all keyed out

Table 2.2 Wolds and Galloway streams, 1982-1983. Composition and relative trophic status of aquatic vegetation.

Species (and trophic ranking number)	Wolds streams				Galloway streams			
	BB	CB	EB	MB	DL	DB	GB	ML
Drosera rotundifolia (1)	-	-	-	-	-	+	-	-
Potamogeton polygonifolius (4)	-	-	-	-	+	-	+	+
Lobelia dortmanna (11)	-	-	-	-	+	-	-	-
Carex nigra (14)	-	-	-	-	-	-	+	+
Eriophorum angustifolium (17)	-	-	-	-	+	-	+	+
Myriophyllum alterniflorum (18)	-	-	-	-	+	-	-	-
Carex rostrata (22)	-	-	-	-	+	-	-	+
Ranunculus flammula (26)	-	-	-	-	+	-	+	+
Juncus bulbosus (44)	-	-	-	-	+	+	+	+
Ranunculus fluitans (45)	-	-	+	-	-	-	-	-
Glyceria fluitans (47)	+	+	+	+	-	-	-	-
Juncus effusus (51)	+	-	-	+	-	-	-	-
Agrostis stolonifera (53)	+	+	+	+	-	-	-	-
Caltha palustris (54)	-	-	-	+	-	-	-	-
Equisetum fluviatile (57)	-	-	-	-	-	-	+	+
Equisetum palustre (58)	-	+	-	+	+	-	-	-
Iris pseudacorus (60)	-	-	+	+	-	-	-	-
Littorella uniflora (61)	-	-	-	-	-	-	+	+
Myosotis scorpioides (62)	-	-	+	+	-	-	-	-
Veronica anagallis-aquatica (66)	-	-	+	-	-	-	-	-
Veronica beccabunga (76)	+	+	+	+	-	-	-	-
Mentha aquatica (77)	+	+	+	+	-	-	-	-
Berula erecta (81)	-	-	+	+	-	-	-	-
Catabrosa aquatica (86)	-	-	-	+	-	-	-	-
Nasturtium officinale (97)	-	+	+	+	-	-	-	-
Sparganium erectum (103)	-	-	+	-	-	-	-	-
Apium nodiflorum (106)	+	+	+	+	-	-	-	-
Carex acutiformis (110)	-	-	-	+	-	-	-	-
Ranunculus sceleratus (111)	-	-	-	+	-	-	-	-
Carex riparia (114)	-	-	-	+	-	-	-	-
Groenlandia densa (117)	-	-	+	-	-	-	-	-
Sum of trophic ranks	410	514	990	1243	200	45	223	245
Mean trophic rank	68	73	76	78	25	23	32	31

Only those vascular aquatic plants categorized by Newbold & Palmer (1979) are included, their trophic ranking numbers are given in brackets. Presence (+) or no record (-) of each species encountered is indicated for Wolds streams, Birdsall Beck (BB), Church Beck (CB), Eastburn Beck (EB) and Millington Beck (MB) and for Galloway Streams, Dargall Lane (DL), Dungeon Burn (DB), Garrary Burn (GB) and Minnigall Lane (ML).

within his group A, which includes lowland and nutrient-rich rivers. Further subdivision put Birdsall Beck into category A4ii, clay ditch; Church Beck and Eastburn Beck into category A4iii, spring-fed streams in clay catchments; and Millington Beck into category A3iii, small silted, enriched chalk river. However, these subdivisions were based on presence or absence of single species, and may be of questionable value. The Galloway streams all fell into category D3ii, mountain rivers with boulders and adjacent peat, with filamentous algae, Nardia compressa, Sphagnum spp. and Nardus stricta as dominant indicators.

2.4 Sampling sites.

For each of the eight streams, 10 equidistant points were marked on 1:10560 O.S. maps, and located on the ground. For the Wolds streams, the positions were checked using measuring tapes. The uppermost site was at the spring source (Wolds, scarp slope streams), or at about the most upstream location where there was permanent water flow (Eastburn Beck), or at the point where water draining from a bog first formed an obvious stream channel with distinct banks (Galloway streams). The downstream limit was generally set by an obvious potential pollution source, or by merger with a lake or larger water course. This approach maximized the study length of stream, although it produced a wide range of sampled lengths, from 0.40-5.41 km (Table 2.1).

CHAPTER 3

Methods used in the study of suspended bacteria.

3.1 Sampling programme.

Water samples were taken for analysis of suspended bacteria and physico-chemical variables from the selected Wolds and Galloway streams between July 1982 and December 1983. Intervals between sampling were irregular, but when possible, most streams were sampled in the same calendar month. Streams were generally sampled regardless of weather conditions, although with the Galloway streams, this sometimes proved impractical due to spate flow; sampling was then postponed for 1-2 days. Millington Beck was not sampled after December 1982 because of fish-pond construction close to the source.

3.2 Collection of water samples.

On each sampling occasion, water samples were taken from all 10 sampling sites, starting with the downstream stations. Water was collected in sterile 300 ml medical flats, in midstream, at about 10 cm depth, after initially rinsing the bottles, slightly downstream of the sampling point. Samples were returned to the laboratory as quickly as possible (c.1-4 h) and allowed to equilibriate at 10°C before laboratory procedures.

3.3 Physico-chemical variables.

Discharge was determined at one point on each stream (Fig. 2.1), from velocity measurements, made at 0.6 x depth at intervals across the stream,

using an Ott small current meter (A. Ott, Kempten, F.R.G.). pH, temperature, conductivity and turbidity were measured for all 10 sampling sites on each stream. pH in the Wolds streams was measured in the field using an EIL (Model 30C) portable pH meter, or in the laboratory with an EIL (Model 7065) bench pH meter; both meters had a combination electrode with automatic temperature compensation. The electrode was initially swirled in a beaker of water sample, then allowed to equilibriate and the steady reading was recorded with the sample quiescent. pH in the Galloway streams was measured in the field. Water temperature was measured directly in the stream using a mercury-in-glass thermometer; conductivity was measured at 10°C using a laboratory meter and turbidity, relative to an arbitrary standard, was obtained by nephelometry.

3.4 Acridine-orange direct counts.

The concentration of total suspended bacteria was determined by epifluorescence microscopy using the acridine-orange direct-count (AODC) method (Daley, 1979).

Subsamples of 10 ml were preserved with 0.2 µm membrane-filtered neutral formaldehyde at 2% (w/v) final concentration (Daley & Hobbie 1975), for up to two weeks prior to counting. They were then stained with acridine orange at 10 mg l^{-1} for 10 min and, according to cell concentration, 0.2-2 ml (together with 5 ml of 0.2 µm membrane-filtered water) was filtered using gentle suction through a 0.2 µm polycarbonate (Nuclepore) membrane filter (filter area 189 mm^2). Before use, the membranes were stained black by immersion in Irgalan Black (Ciba-Geigy; 2 g l^{-1} in 2% acetic acid; Hobbie, Daley & Jasper 1977), for at least five min, then rinsed several times in distilled water. After filtration, the wet membrane was transfered to a microscope slide, with a drop of low-

fluorescence immersion oil (Cargille Type LF, R.P. Cargille Laboratories, N.J. 07009, U.S.A.) directly on the membrane surface.

The microscope was a Zeiss Photomicroscope fitted with a 3RS epi-condenser. The light source was a 200 W mercury super-pressure lamp. Excitation was through a 3 mm BG 18 red-suppression filter and a 3 mm BG12 (transmission peak 405 nm) exciter filter. A chromatic beam splitter LP510 nm was used, and the image was viewed through a 2 mm barrier filter 50 (500 nm cut off). A x100 plano-chromat (N.A. 1.3) oil immersion objective was used and counting was at a total magnification of x1600 (field area 0.0064 mm^2).

Bacteria (which fluoresced green) were counted in 30 fields (c.15-20 cells in each field), and 95% confidence limits were fixed using central-limit theorem ($\pm\ t_{0.05(n-1)}\ \sqrt{(s^2/n)}$, where $t_{0.05(n-1)}$ = appropriate Student's t-value for 0.05 level of significance with n-1 degrees of freedom, s^2 = sample variance, n = number of values; Elliott, 1977). Free-living bacteria always greatly outnumbered particle-bound suspended bacteria, hence no distinction was made between these categories.

3.5 Heterotrophic activity: (1) glucose assimilation by a trace addition approach.

The routine measure of heterotrophic activity was turnover rate for glucose assimilation. This is the fraction of natural, dissolved glucose which is assimilated by bacteria in unit time (Wright, 1978).

Samples were incubated with ^{3}H-glucose at a presumed-trace concentration, which should not alter the assimilation rate of the natural glucose (Wright & Burnison, 1979), hence the fraction of isotope assimilated (f), during time (t(h)), equals the fraction of natural glucose

assimilated in an unmodified sample. Therefore f/t (h) equals turnover rate. This is the inverse function of natural turnover time, which is the number of hours required for the population to take up a quantity of substrate equal in concentration to the existing concentration.

For each water sample, 0.1 ml sterile ^{3}H-glucose solution (c.37 kBq ml^{-1}, D-[2-^{3}H]-glucose, specific activity c.3 GBq mg^{-1}) was added to three sterile universal bottles, and to a further control bottle containing 0.25 ml neutral formaldyhyde. Stream water (10 ml) was then added to each universal bottle, giving an added glucose concentration of c.0.1 µg l^{-1}, and in the controls, a formaldyhyde concentration of 1%. The bottles were incubated in darkness at 10°C for 2 h (Wolds streams) or 4-5 h (Galloway streams). Incubations were stopped by filtration through 25 mm diameter, 0.2 µm, Oxoid (cellulose acetate) membrane filters. The filters were washed with 5 ml distilled water, transfered to vials containing toluene-based fluor, allowed to clear, and their radioactivity was determined by liquid scintillation counting. Radioactivity assimilated (CPM) equalled the mean of the three replicates minus the mean control value. Radioactivity added to each bottle was obtained by adding 0.1 ml of the original ^{3}H-glucose solution to three scintillation vials and determining mean CPM. The fraction of isotope assimilated (f) equalled radioactivity assimilated (CPM) / radioactivity added to each bottle (CPM). Turnover rate equalled f / incubation time (h).

All incubations were carried out on the afternoon-evening of the sampling day.

3.6 Heterotrophic activity: (2) glucose mineralization by a kinetic approach.

Further assessments of heterotrophic activity were made on samples from three representative sites per stream (upstream, intermediate and downstream) during April-May 1985.

Glucose-mineralization potential (Vmax) and turnover time were determined using the Harrison, Wright & Morita (1971) modification of the Wright & Hobbie (1966) kinetic method, in which substrate mineralization rather than assimilation is measured.

The procedure was to add 0.1 ml of sterile ^{14}C-glucose solution (c.37 kBq ml^{-1}, D-[U-^{14}C]-glucose, specific activity c.48 MBq ml^{-1}, at a concentration of c.0.7 mg l^{-1}), to each of 20, 120-ml Clinbritic brown glass serum bottles (Malcolm Britton Co. Ltd., London). These bottles were divided into five sets of four replicates. A volume of 1.63 mg l^{-1} sterile, unlabelled glucose solution in the range 0-0.2 ml was added to the replicates in each set, and 2 ml of 2.5 M H_2SO_4 was added to one bottle in each set to provide a blank. A 20 ml sub-sample of stream water was then added to each bottle, to give five concentrations of added glucose in the range 4-21 µg l^{-1}. Each bottle was then closed with a rubber serum cap which formed a gas tight seal. A glass rod with a glass cup fused to its end passed through each serum cap, and held a concertina-folded square (c.4 x 3 cm) of Whatman No. 1 filter paper above the surface of the water in the serum bottle. This arrangement is illustrated in Fry & Humphrey (1978). The bottles were incubated for 2 h (Wolds streams) or 5 h (Galloway streams) in darkness at 10°C. Incubations were stopped by injecting 2 ml 2.5 M H_2SO_4 through each serum cap (except blanks), into the sub-sample, to kill bacteria and release $^{14}CO_2$. 2-phenylethylamine (0.25 ml) was injected onto the

paper wicks, which were left overnight for $^{14}CO_2$ absorbtion, then transfered to vials for liquid scintillation counting.

Interpretation of results depends upon glucose mineralization rate increasing hyperbolically with increase in glucose concentration, when the transport kinetics equation of Wright & Hobbie (1966) applies:

$$t/f = (K+S)/Vmax + A/Vmax$$

where t = incubation time (h), f = fraction of added ^{14}C-glucose which is mineralized to $^{14}CO_2$, K = an uptake constant equivalent to the Michaelis constant ($\mu g\ l^{-1}$), S = natural glucose concentration ($\mu g\ l^{-1}$), Vmax = glucose-mineralization potential or the rate when glucose concentration is non-limiting ($\mu g\ l^{-1}h^{-1}$), and A = concentration of added glucose ($\mu g\ l^{-1}$). Straight line plots of t/f against A were fitted by linear regression using the Statistical Package for the Social Sciences (SPSS; Kim & Kohout, 1975a). An F-test was used to confirm significance and a data set was rejected when $P > 0.05$. Each plot (Fig. 3.1) yielded (1) Vmax (the reciprocal of the regression coefficient); (2) (K+S) / Vmax (the intercept on the t/f axis) which represents the turnover time (h) for the natural glucose in the sample. The reciprocal of this gave turnover rate, which should be equivalent to values obtained by the trace-addition approach (Wright, 1978); (3) K+S (the intercept on the A axis) which sets an upper limit for the natural glucose concentration.

3.7 Heterotrophic activity per bacterium.

A measure of the average activity per bacterial cell or specific activity (Wright, 1978) was obtained by dividing turnover rate (for both glucose assimilation and mineralization) and Vmax (for glucose

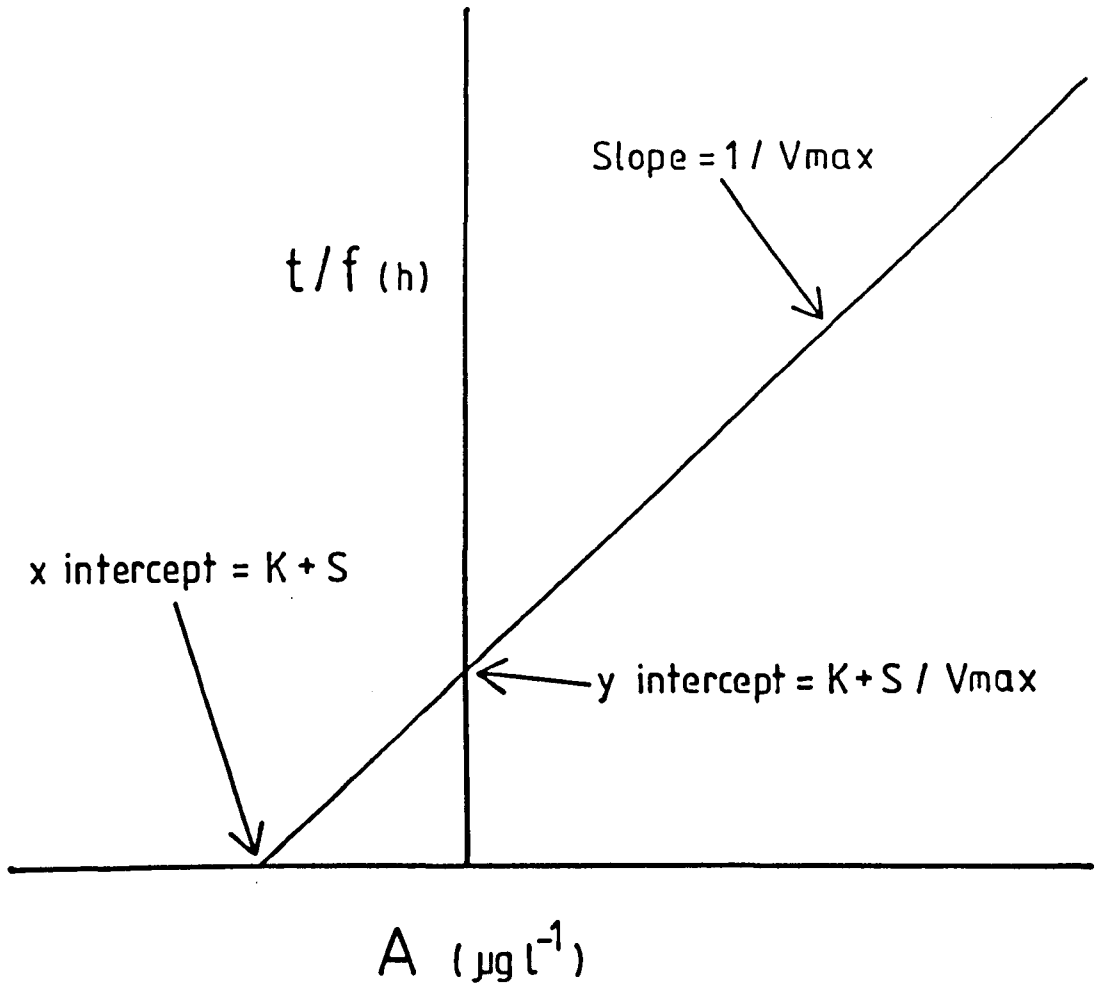


Figure 3.1 Plot of transport kinetics equation showing derived uptake data. t/f = turnover time (h), A = added glucose (µg l^{-1}), Vmax = glucose mineralization potential, K = an uptake constant (µg l^{-1}) and S = natural glucose concentration (µg l^{-1}). (From Wright & Burnison, 1979).

mineralization) by the concentration of total bacteria. Turnover rate per bacterium represented the average water volume that each individual clears of glucose in unit time. Vmax per bacterium is the average potential rate of glucose mineralization for each individual bacterium (Goulder, 1979).

3.8 Cell volume.

The cell volumes of suspended bacteria, in occasional samples, were determined by transmission electron microscopy (TEM) following negative staining (Montesinos, Esteve and Guerrero, 1983). The bacteria in a 200-500 ml sub-sample (and a replicate) were concentrated on 45 mm diameter, 0.2 µm cellulose acetate (Oxoid) membrane filters. Each filter was vigorously agitated (Fisons Whirlimixer) to loosen bacteria into 5 ml of 0.2 µm membrane-filtered, 0.1 M ammonium acetate solution. One or two drops of this concentrate were dried at 70°C on a formvar-coated copper grid. To stain bacteria, a drop of 0.5% uranyl acetate solution was placed on the grid and, after 1 min, excess stain was removed by blotting and the preparation allowed to air dry. The grids were viewed using a Jeol JEM 100 C electron microscope, at magnification x10 000 or x20 000, and the lengths and widths of the first 100 bacteria (50 x two replicate preparations) encountered were measured. Cell volumes were calculated assuming that rod-shaped cells were cylinders and that cocci were spheres.

3.9 Data analysis.

The pattern of downstream change in concentration and activity of suspended bacteria was investigated by regression analysis using SPSS (Kim & Kohout, 1975a,b). Forward-entry stepwise multiple-regression analysis was performed between the bacterial variable and distance downstream, with

an additional polynomial term in the equation at each step; an F-test was used to establish whether a second- or higher-degree polynomial equation was a significantly better fit ($P = < 0.05$) than the first-degree (straight line) equation (Kim & Kohout, 1975b). The overall significance of the best-fit regression equation was also determined using an appropriate F-test (Kim & Kohout, 1979a). Regression analysis was not applied to data where the concentration and activity of bacteria increased abruptly between sites, since it was a procedure intended to describe gradual downstream change.

CHAPTER 4

Abundance, heterotrophic activity, and cell volume of suspended bacteria.

4.1 Results.

4.1.i Physico-chemical variables.

The results of physico-chemical determinations, in the Wolds and Galloway streams, between July 1982 and December 1983 are summarized in Table 4.1 (full data in Appendix 2). The ranges of discharge in the Wolds and Galloway streams mostly overlapped, thus on this criterion the streams were of similar size. The Galloway streams were, however, more liable to sudden fluctuations of flow according to rainfall, whereas the Wolds streams showed more gradual seasonal change, hence the ranges and coefficients of variation were much wider for the Galloway streams. The Wolds streams were alkaline or circumneutral (mean pH 7.5-7.8) whereas the Galloway streams were distinctly acid (mean pH 4.6-5.3), and more prone to rapid change in pH with fluctuation in discharge. The pH of Dungeon Burn (mean 4.6) was generally lower than the other Galloway streams (means 5.0-5.3). Conductivity of the Wolds streams (means 314-454 $\mu S\ cm^{-1}$) was also markedly greater than the Galloway streams (means 31-35 $\mu S\ cm^{-1}$). Mean temperatures were similar although the Wolds streams showed less variation because of their high proportion of constant-temperature (c.10°C) spring water. All the streams were usually transparent but the Wolds streams had slightly higher turbidity, indicating greater amounts of suspended solids. Birdsall Beck in particular showed a higher relative turbidity (mean 7.2%) than the other Wolds streams (means 0.8-3.1%).

Table 4.1 Wolds and Galloway streams, 1982-1983. Summary of physico-chemical variables. Values are means, ranges and coefficients of variation (%), n = number of determinations.

Stream	pH				Conductivity ($\mu S\ cm^{-1}$)				Temperature (^{o}C)				Relative turbidity (%)				Discharge ($x10^{-3} m^{3}\ s^{-1}$)			
	Mean	Range	CV	n	Mean	Range	CV	n	Mean	Range	CV	n	Mean	Range	CV	n	Mean	Range	CV	n
Wolds streams																				
Birdsall Beck	7.8	7.3-8.2	2	60	397	323-475	9	60	8.6	5.5-12.0	17	60	7.2	0-59.5	151	60	10	3-27	93	6
Church Beck	7.5	6.6-7.9	3	69	454	403-535	9	69	9.0	6.0-12.3	20	70	0.8	0-2.3	71	69	6	4-10	40	7
Eastburn Beck	7.5	7.1-7.8	3	55	326	318-423	9	55	10.0	7.0-12.5	11	47	3.1	0-44.0	216	55	249	59-690	95	7
Millington Beck	7.6	6.0-8.0	6	40	314	237-378	11	40	9.2	7.3-12.3	18	40	1.2	0-3.8	75	40	14	6-34	99	4
Galloway streams																				
Dargall Lane	5.2	4.4-6.3	10	70	34	22-62	33	70	7.3	0.3-11.0	97	70	0.1	0-1.3	174	70	408	12-1104	110	7
Dungeon Burn	4.6	4.1-5.3	8	73	35	22-53	23	80	8.4	1.0-24.0	65	80	0.1	0-1.0	179	80	57	4-200	115	8
Garrary Burn	5.3	4.1-7.5	17	63	31	22-39	17	80	9.8	2.3-18.5	40	80	0.3	0-1.0	135	80	243	18-1024	137	8
Minnigall Lane	5.0	4.2-7.1	16	76	33	22-64	31	90	9.0	0.5-18.0	57	90	0.4	0-2.5	138	90	512	22-1270	81	9

4.1.ii Abundance and heterotrophic activity of suspended bacteria.

The results of determinations of total bacteria, turnover rate (for glucose assimilation) and turnover rate per bacterium, in the Wolds and Galloway streams, between July 1982 and December 1983, are summarized in Table 4.2. Mean concentrations of total bacteria in the Wolds streams ($5.1-10.2 \times 10^5$ ml^{-1}) were mostly marginally greater than in the Galloway streams ($3.3-6.8 \times 10^5$ ml^{-1}). However, mean turnover rates and turnover rates per bacterium in the Wolds streams were, respectively, c.17 and c.13 times greater than in the Galloway streams ($6.3-10.8 \times 10^{-3}$ h^{-1} compared to $0.2-0.9 \times 10^{-3}$ h^{-1} and $1.0-1.5 \times 10^{-8}$ ml h^{-1} compared to $0.07-0.13 \times 10^{-8}$ ml h^{-1}). Total bacteria and turnover rate were generally higher for Birdsall Beck than for the other Wolds streams, although turnover rates per bacterium were similar (Table 4.2). Of the Galloway streams, total bacteria, turnover rate and turnover rate per bacterium were usually higher in Garrary Burn and Minnigall Lane than Dargall Lane and Dungeon Burn (Table 4.2).

The results of heterotrophic activity determinations using the kinetic approach, carried out during April-May 1985, are summarized in Table 4.3 (full data in Appendix 3). The associated concentrations of total bacteria in the Wolds streams were fairly similar to those of the Galloway streams (means $1.5-18.8 \times 10^5$ ml^{-1} compared to $1.9-3.4 \times 10^5$ ml^{-1}). Representative plots of t/f against A are shown in Figs. 4.1.a and b. For the Wolds streams, all nine regressions were significant, and for the Galloway streams, 10 out of 12 plots produced significant regressions ($P < 0.05$). Turnover rates (for glucose mineralization) and turnover rates per bacterium were respectively c.29 and c.12 times greater in the Wolds streams than in the Galloway streams (means $4.1-43.5 \times 10^{-3}$ h^{-1} compared to $0.1-1.2 \times 10^{-3}$ h^{-1} and $2.2-2.6 \times 10^{-8}$ ml h^{-1} compared to $0.1-0.3 \times 10^{-8}$ ml h^{-1}). Vmax and Vmax per bacterium were

Table 4.2 Wolds and Galloway streams, 1982-1983. Summary of concentration of total bacteria, turnover rate for glucose assimilation (by the trace-addition approach) and turnover rate per bacterium. Values are means, ranges and coefficients of variation (%), n = number of samples.

Stream	Total bacteria ($x10^5 ml^{-1}$)				Turnover rate ($x10^{-3} h^{-1}$)				Turnover rate per bacterium ($x10^{-8} ml\ h^{-1}$)			
	Mean	Range	CV	n	Mean	Range	CV	n	Mean	Range	CV	n
Wolds streams												
Birdsall Beck	10.2	0.1-59.5	119	60	10.8	0-29.4	73	60	1.4	0-3.7	69	60
Church Beck	5.1	0.3-15.3	67	69	6.5	0.1-16.1	59	68	1.5	0.32-3.4	46	68
Eastburn Beck	7.2	1.0-56.4	121	55	6.3	0.5-33.4	124	55	1.0	0.10-3.0	69	55
Millington Beck	7.1	0.1-12.4	50	39	9.4	0-19.7	47	38	1.5	0-5.2	62	38
Galloway streams												
Dargall Lane	4.3	1.0-8.7	48	70	0.3	0.02-1.1	75	70	0.08	0.01-0.22	60	70
Dungeon Burn	3.3	1.2-6.8	36	80	0.2	0.01-0.5	52	80	0.07	0.01-0.23	60	80
Garrary Burn	4.8	1.1-12.6	56	79	0.5	0.07-2.0	81	80	0.12	0.02-0.74	96	79
Minnigall Lane	6.8	1.1-40.9	88	90	0.9	0.02-10.9	172	90	0.13	0.003-1.4	130	90

Table 4.3 Wolds and Galloway streams, April-May 1985. Summary of concentration of total bacteria, turnover rate, turnover rate per bacterium, Vmax, Vmax per bacterium and K + S, for glucose mineralization (by the kinetic approach). Values are means, ranges and coefficients of variation (%), number of samples (n) = 3, except for Dargall Lane where n = 1, (-) indicates not applicable.

Stream	Total bacteria ($x10^5 ml^{-1}$)			Turnover rate ($x10^{-3} h^{-1}$)			Turnover rate per bacterium ($x10^{-8} ml\ h^{-1}$)		
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV
Wolds streams									
Birdsall Beck	18.8	7.8-25.9	51	43.5	13.3-60.2	60	2.2	1.7-2.5	19
Church Beck	2.7	1.9-3.4	28	6.7	4.6-8.1	28	2.6	2.2-3.0	16
Eastburn Beck	1.5	1.2-1.9	23	4.1	2.5-6.1	45	2.6	2.0-3.2	23
Galloway streams									
Dargall Lane	1.9	-	-	0.1	-	-	0.1	-	-
Dungeon Burn	2.9	2.7-3.2	9	0.7	0.4-1.1	54	0.2	0.1-0.4	87
Garrary Burn	3.1	2.5-4.2	31	1.2	0.3-2.5	96	0.3	0.1-0.6	84
Minnigall Lane	3.4	2.0-5.5	55	0.5	0.3-0.8	53	0.2	0.1-0.2	29

Stream	Vmax ($x10^{-3} \mu g\ l^{-1} h^{-1}$)			Vmax per bacterium ($x10^{-11} \mu g\ h^{-1}$)			K + S ($\mu g\ l^{-1}$)		
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV
Wolds streams									
Birdsall Beck	347	84.5-516	66	16.8	10.9-19.9	30	7.6	6.4-8.6	15
Church Beck	90.0	34.5-122	54	32.9	18.6-45.5	41	12.8	7.6-15.7	35
Eastburn Beck	18.3	10.6-28.5	50	12.7	5.5-19.7	56	5.2	1.7-7.5	59
Galloway streams									
Dargall Lane	1.5	-	-	0.8	-	-	12.6	-	-
Dungeon Burn	3.7	2.7-5.0	32	1.3	1.0-1.9	38	6.3	4.4-7.6	26
Garrary Burn	10.3	3.5-19.7	82	3.1	1.4-4.7	53	10.4	7.8-13.8	30
Minnigall Lane	7.8	1.5-20.4	139	1.7	0.6-3.7	102	10.9	4.1-24.3	106

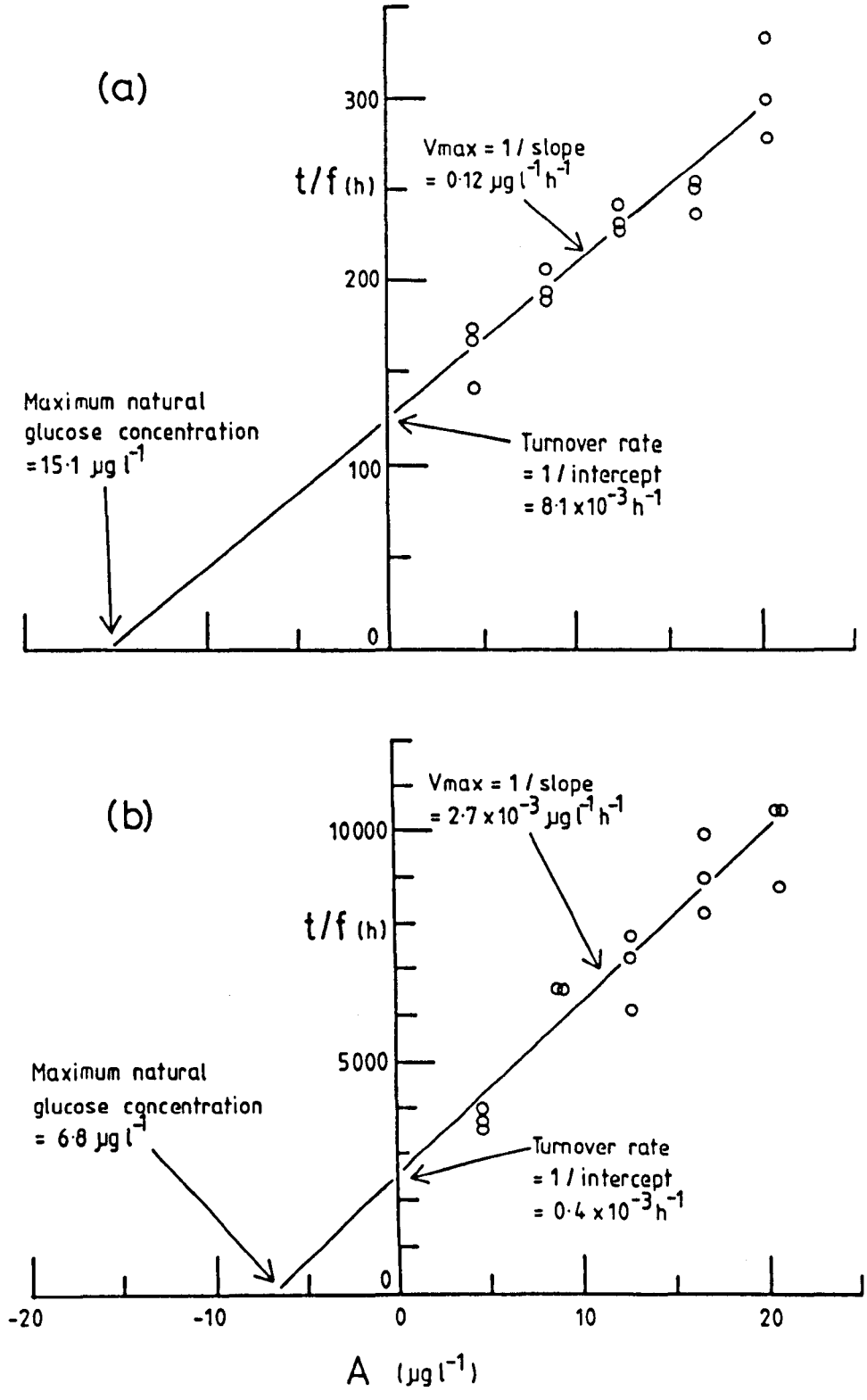


Figure 4.1 Kinetic plots of mineralization of ^{14}C-labelled glucose to $^{14}CO_2$ by suspended bacteria from (a) Church Beck, c.250 m below the source, 13 May 1985 and (b) Dungeon Burn, c.600 m below the source, 29 April 1985. The plots are of t/f (turnover time against A (added glucose concentration). The regression equations are (a) $y = 8.20x + 123.8$ ($P < 0.001$) and (b) $y = 369.2x + 2509$ ($P < 0.001$).

respectively c.26 and c.12 times greater in the Wolds streams (means 18.3-347 x 10^{-3} $\mu g\ l^{-1}h^{-1}$ compared to 1.5-10.3 x 10^{-3} $\mu g\ l^{-1}h^{-1}$ and 12.7-32.9 x 10^{-11} $\mu g\ h^{-1}$ compared to 0.8-3.1 x 10^{-11} $\mu g\ h^{-1}$). The upper limit for natural glucose concentration (K+S) was similar in both Wolds (5.2-12.8 $\mu g\ l^{-1}$) and Galloway (6.3-12.6 $\mu g\ l^{-1}$) streams.

4.1.iii Cell volume.

The results of bacterial cell volume determinations are summarized in Table 4.4 (full data in Appendix 4). Mean cell volumes in the Wolds streams (0.07-0.11 μm^3) were c.2.3 times greater than in the Galloway streams (0.04-0.05 μm^3).

4.2 Discussion.

Initial considerations of geology, topography, climate, land use and vegetation suggested that the Wolds amd Galloway streams may show contrasts in terms of physico-chemical, and thus, possibly bacterial variables.

Of the physico-chemical data, pH and conductivity provided the greatest contrast between the two areas (Table 4.1). There might be some doubt over the accuracy of the individual pH determinations, particularly from the Galloway streams, since the measurement of pH in poorly buffered water is potentially subject to considerable error (Covington, Whalley & Davison, 1985a,b; Neal & Thomas, 1985). It is reassuring, however, that pH values for Dargall Lane (mean 5.2, range 4.4-6.3, Table 4.1) were very similar to those obtained independently by Burns et al (1984) for the same stream during 1981 (mean 5.1, range 4.6-7.1).

In terms of the abundance of suspended bacteria, the Wolds and Galloway streams were fairly similar, however the c.17-fold difference in

Table 4.4 Wolds and Galloway streams, 1982-1983. Summary of bacterial cell volumes determined by transmission electron microscopy. Samples from representative stations were taken in February and October 1983 from Wolds streams and in November 1982 and March and July 1983 from Galloway streams. Values are means, ranges and coefficients of variation (%), n = number of samples (100 cells per sample were measured), (-) indicates not applicable.

Stream	Cell volume (μm^3)			
	Mean	Range	CV	n
Wolds streams				
Birdsall Beck	0.11	0.07-0.15	34	5
Church Beck	0.10	0.08-0.13	12	5
Eastburn Beck	0.11	0.09-0.13	16	3
Millington Beck	0.07	0.06-0.07	-	2
Galloway streams				
Dargall Lane	0.04	0.02-0.08	50	7
Dungeon Burn	0.05	0.05-0.06	12	4
Garrary Burn	0.04	0.02-0.06	50	4
Minnigall Lane	0.04	0.01-0.05	44	7

turnover rate for glucose assimilation and the c.13-fold difference in turnover rate per bacterium suggested a real difference between bacteria of the two stream groups (Table 4.2). The comparison of turnover rate per bacterium is potentially more valuable, since this variable is independent of population size (Wright, 1978). It is interesting to note, in this respect, that the mean turnover rate per bacterium for Birdsall Beck, was similar to the other Wolds streams, despite higher mean total counts and turnover rates (Table 4.2). This was associated with consistently higher turbidity (Table 4.1), and it is possible that Birdsall Beck received a higher proportion of shallow ground water than the other Wolds streams. In the Galloway area, mean turnover rates per bacterium, as well as total counts and turnover rates were higher for Garrary Burn and Minnigall Lane than for Dargall Lane and Dungeon Burn (Table 4.2). This may reflect the longer length of stream sampled (Table 2.1). The mean turnover rates per bacterium in the Wolds streams (means 1.0-1.5 x 10^{-8} ml h^{-1}, Table 4.2) were similar to the mean value of 1.3 x 10^{-8} ml h^{-1} (range = 0-3.24 x 10^{-8} ml h^{-1}, CV = 55%, n = 119) obtained by Goulder (1984) for Mill Beck, a further Wolds stream.

There were, however, several potential drawbacks to turnover rate as a measure of heterotrophic activity, and the use of the trace-addition method for its determination. (1) Turnover rate is a function of both substrate-utilization rate and substrate concentration, thus a high rate indicated either a high assimilation rate or a low glucose concentration. (2) The method relies on the added ^{3}H-glucose (0.1 µg l^{-1}) being a small proportion (< 10%) of the natural concentration. If the added glucose was a significant increment then the activity measured would be a response to the added substrate. No analytical information is available on glucose concentration in these headstreams, although K+S values from the kinetic approach, which set an upper limit for glucose concentration, were

similar for Wolds and Galloway streams and were substantially greater than 0.1 μg l^{-1} (Table 4.3). It is arguable, however, that K+S is more a reflection of the half-saturation concentration (K) than of substrate concentration (S) (Jost & Ballin, 1984). It nevertheless seems unlikely that glucose concentrations in the higher-trophic-status Wolds streams were lower than in the Galloway streams, hence, despite (1), above, higher turnover rates were probably not a result of lower substrate concentration.

The values of turnover rate and turnover rate per bacterium for glucose mineralization using the kinetic approach (Table 4.3) were again greater in the Wolds streams and were reasonably similar to rates obtained by the trace-addition approach (Table 4.2). Values of Vmax and Vmax per bacterium were substantially higher in the Wolds streams (Table 4.3) and these measurements were independent of substrate concentration.

It has been suggested that the kinetic approach is sometimes unreliable at low pH. In acid-stressed Canadian lakes, regressions of t/f against A were often not sufficiently significant to justify calculation of turnover times and Vmax (Ferroni & Leduc, 1984; Leduc & Ferroni; 1984). This was apparently not a serious problem with the Galloway streams, however, since 10 out of 12 regressions were significant ($P < 0.05$).

Thus it is reasonable to conclude that suspended bacteria in the Wolds streams, although not markedly more abundant, had greater heterotrophic activity, and activity per bacterium than in the Galloway streams.

The difference in heterotrophic activity could have been partly due to difference in cell size. Electron microscopy offers a much higher resolution than light microscopy, and the size of bacteria can accordingly be measured very precisely. However, shrinkage of cells during sample preparation has sometimes been found to cause errors in size estimation (Bratbak, 1985; Fry & Davies, 1985). Montesinos, Esteve & Guerrero (1983)

however, found that shrinkage was less for TEM than for scanning electron microscopy (SEM). If shrinkage did occur, and was assumed to be consistent, although absolute cell volumes would be underestimates, comparisons of relative sizes between sites would still be valid. In the Wolds streams, the overall mean cell volume (0.10 μm^3, n = 1500 cells from 15 samples) was c.2.5 times greater than for the Galloway streams (0.04 μm^3, n = 2200 cells from 22 samples), but the overall turnover rate per bacterium for glucose assimilation and mineralization, and Vmax per bacterium were from c.12-13 times greater than in the Galloway streams (Tables 4.2; 4.3). Hence the greater activity in the Wolds streams cannot entirely be explained by greater cell size. The bacteria in the Wolds streams must have been physiologically more active per unit biomass (as a result of either a higher percentage of active cells or higher activity per bacterium), or there might have been a greater proportion of cells capable of utilizing glucose.

Acidic streams are often characterized by reduced abundance of fauna and species diversity, and possibly lower microbial activity (1.2.ii). The results from the Wolds and Galloway streams provide further evidence of markedly lower bacterial activity in acid conditions. Changes in pH, however, are often associated with parallel changes in a range of physico-chemical and biotic variables. For example, low pH often occurs in conjunction with oligotrophic conditions, where low levels of major nutrients and dissolved organic material may also limit bacterial activity. Hessen (1985) found that bacterial biomass in an oligotrophic lake showed a strong positive correlation with the dissolved humic content of the water, as well as pH. Low pH can be associated with elevated aluminium levels, moreover, at least in relation to fish, aluminium may become more toxic at low pH (Odonnell, Mance & Norton, 1984), although, stream aluminium levels may also vary according to discharge (Hooper & Shoemaker, 1985),

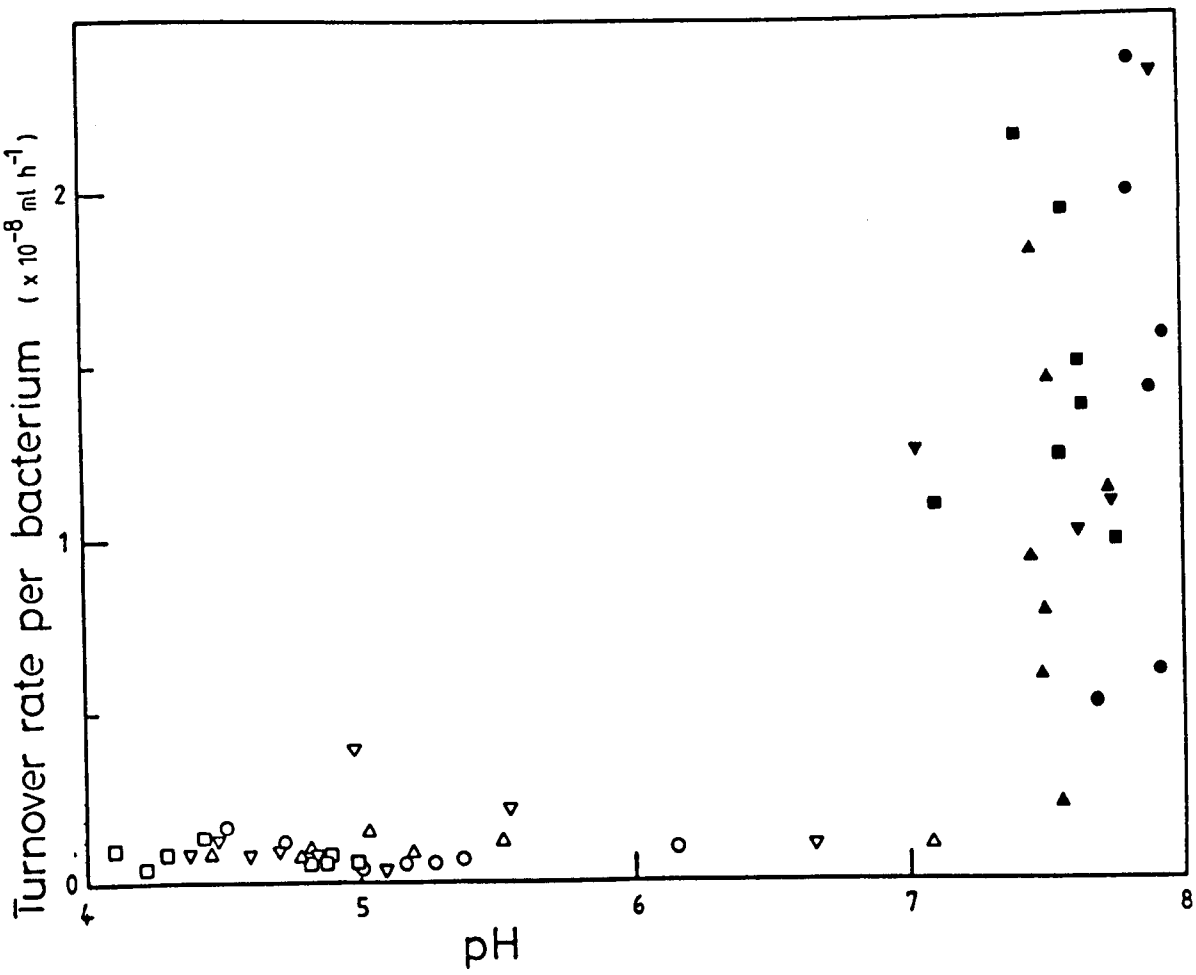


Figure 4.2 Suspended bacteria in Wolds and Galloway streams, 1982-1983, Scatterplot of mean turnover rate per bacterium for glucose assimilation against mean pH for each sampling day; each point represents 8-10 samples. Solid symbols indicate Wolds streams; (circles) Birdsall Beck, (squares) Church Beck, (triangles) Eastburn Beck and (inverted triangles) Millington Beck. Open symbols indicate Galloway streams; (circles) Dargall Lane, (squares) Dungeon Burn, (triangles) Garrary Burn and (inverted triangles) Minnigall Lane.

independently of pH.

Fig. 4.2 shows the mean turnover rate per bacterium for glucose assimilation for each sampling day, plotted against mean pH. The Wolds and Galloway streams clearly formed two distinct groups, but within each group there was no direct relationship between activity per bacterium and pH (for Wolds streams $r = 0.14$, $n = 24$, $P > 0.1$; for Galloway streams $r = 0.04$, $n = 31$, $P > 0.1$). Thus, provided this was not due to inadequacies in pH measurement, the difference between bacterial activity in the Wolds and Galloway streams was perhaps the result of the combined effects of many variables rather than pH alone.

CHAPTER 5

Downstream change in abundance and heterotrophic activity of suspended bacteria

5.1 Results.

The full results from each sampling occasion, from July 1982 to December 1983, showing downstream change in total bacteria and turnover rate for glucose assimilation are given in Figs. 5.1-5.4 (Wolds streams) and Figs. 5.5-5.8 (Galloway streams). On four occasions, total bacteria and turnover rate increased abruptly between sampling sites (Birdsall Beck 27 October 1983, Eastburn Beck 12 November 1982, Garrary Burn 20 July 1982 and Minnigall Lane 21 July 1982) due to obvious point-source inputs, thus regression analyses were not applied.

In the Wolds streams there was a gradual downstream increase in total bacteria and turnover rate on 63% and 71% of sampling occasions (n = 24), which was best described by a first-degree (straight line) equation. Second- or third-degree polynomial equations provided a better fit on only seven occasions for total bacteria and four occasions for turnover rate. In one instance there was no significant regression (turnover rate, Eastburn Beck, 25 October 1983).

Downstream change was less consistant in the Galloway streams. A straight line gradual downstream increase in total bacteria and turnover rate occured on 50% and 31% of sampling occasions (n = 32). Curvilinear increases occured on one occasion for total bacteria, and on four occasions for turnover rate. Straight-line downstream decrease occured once for total bacteria, and four times for turnover rate. Relationships best

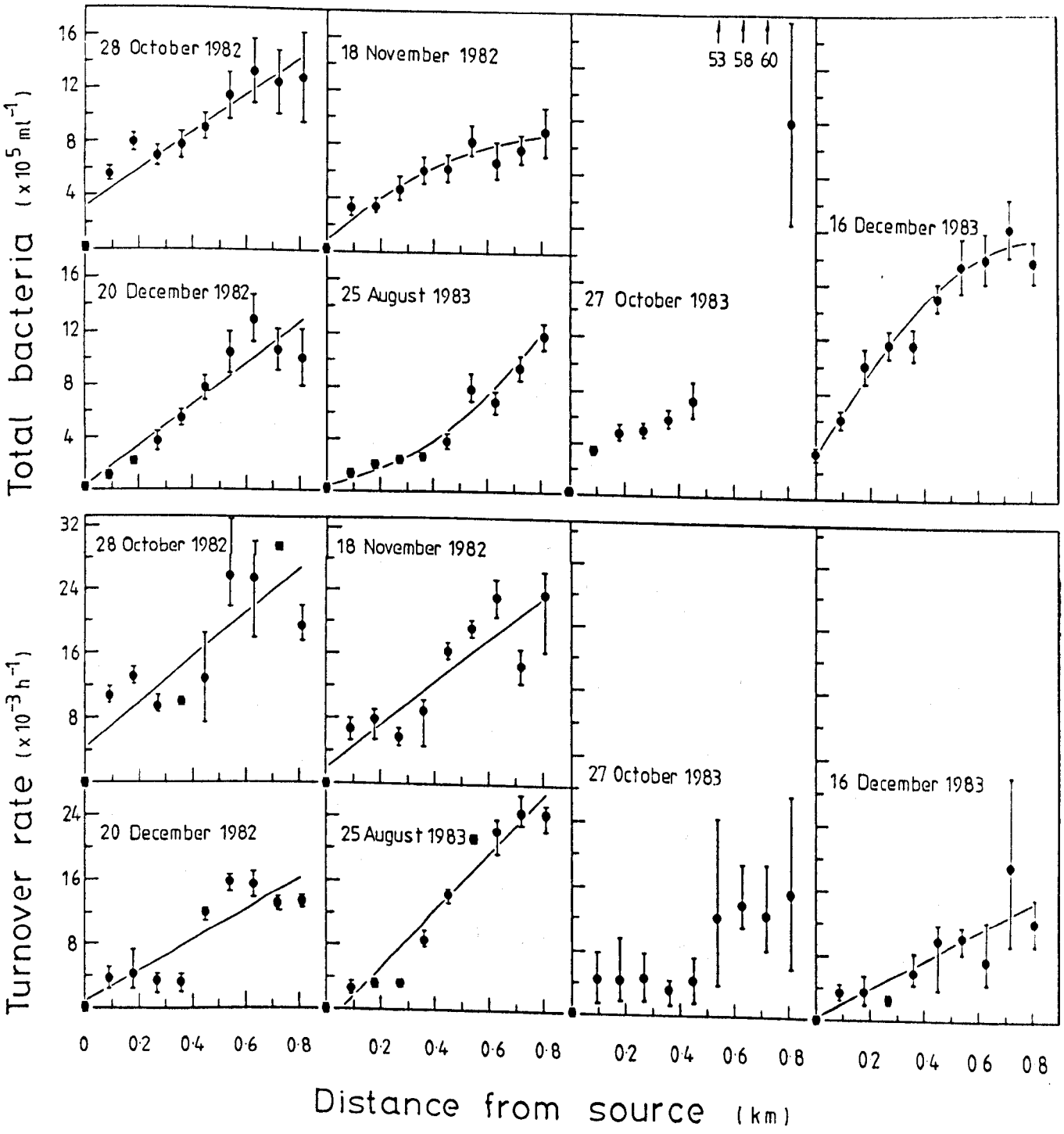


Figure 5.1 Suspended bacteria in Wolds streams, downstream change in Birdsall Beck, 1982-1983. (Top) total bacteria (with 95% confidence limits) and (bottom) turnover rate for glucose assimilation (mean and range from three incubations). Continuous lines indicate regression significant at $P < 0.05$. No regressions were fitted to results of 27 October 1983 because of an explicable discontinuity.

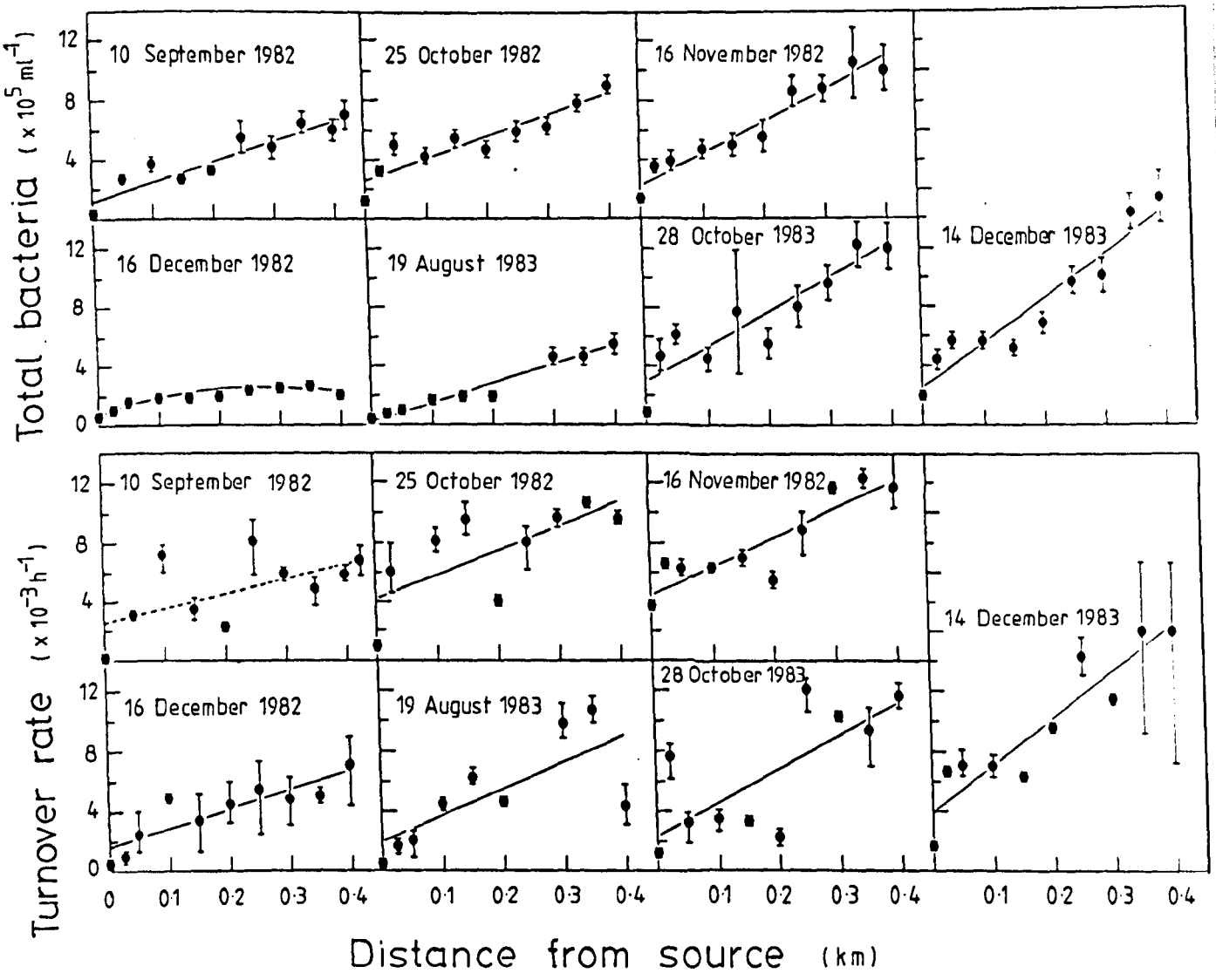


Figure 5.2 Suspended bacteria in Wolds streams, downstream change in Church Beck, 1982-1983. Layout and symbols as in Fig. 5.1 apart from broken line which indicates regression significant at $P < 0.1$.

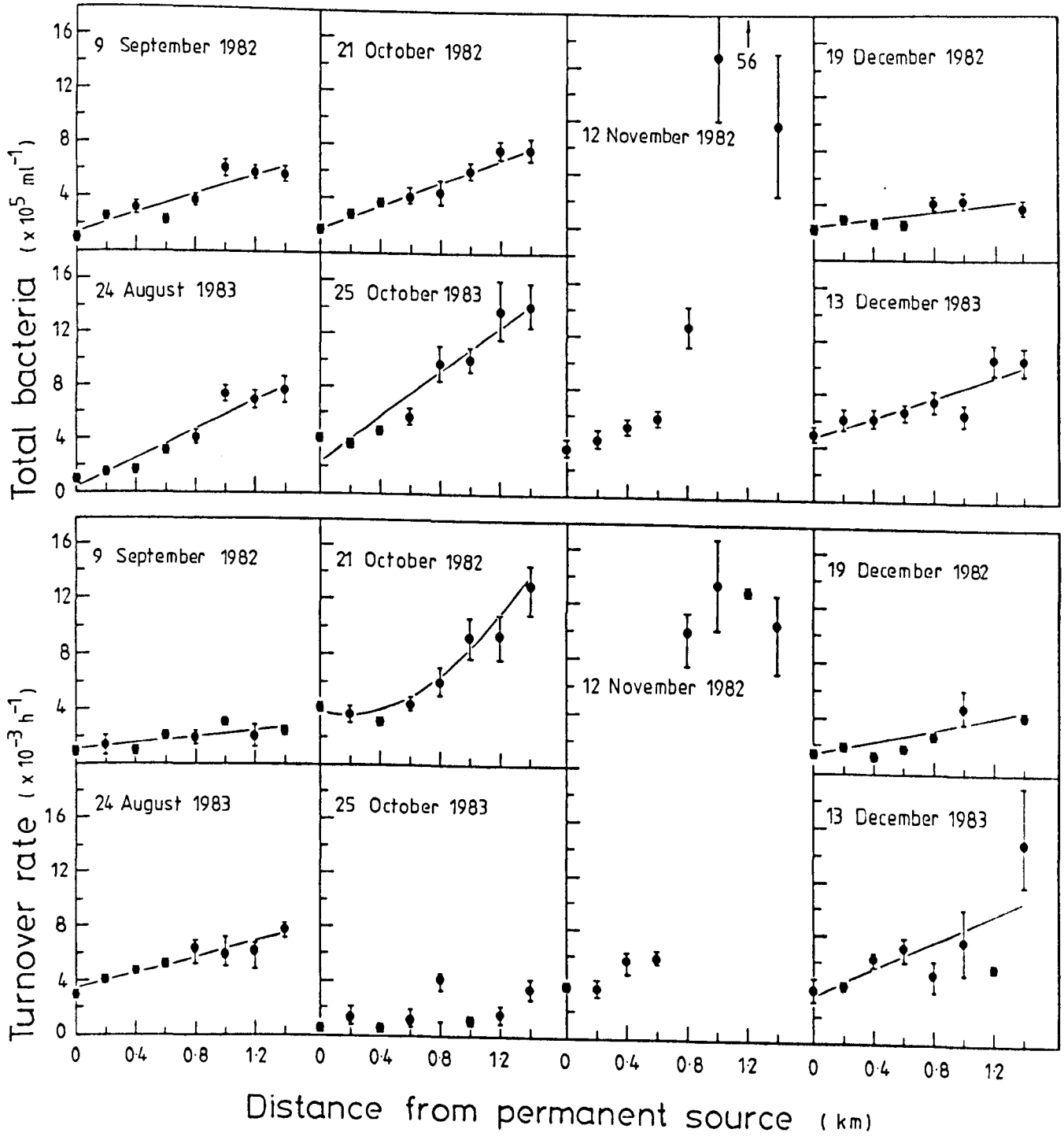


Figure 5.3 Suspended bacteria in Wolds streams, downstream change in Eastburn Beck, 1982-1983. Layout and symbols as in Fig. 5.1 apart from omission of regression line when P > 0.1, and results of 12 November 1982 when no regression lines were fitted because of an explicable discontinuity.

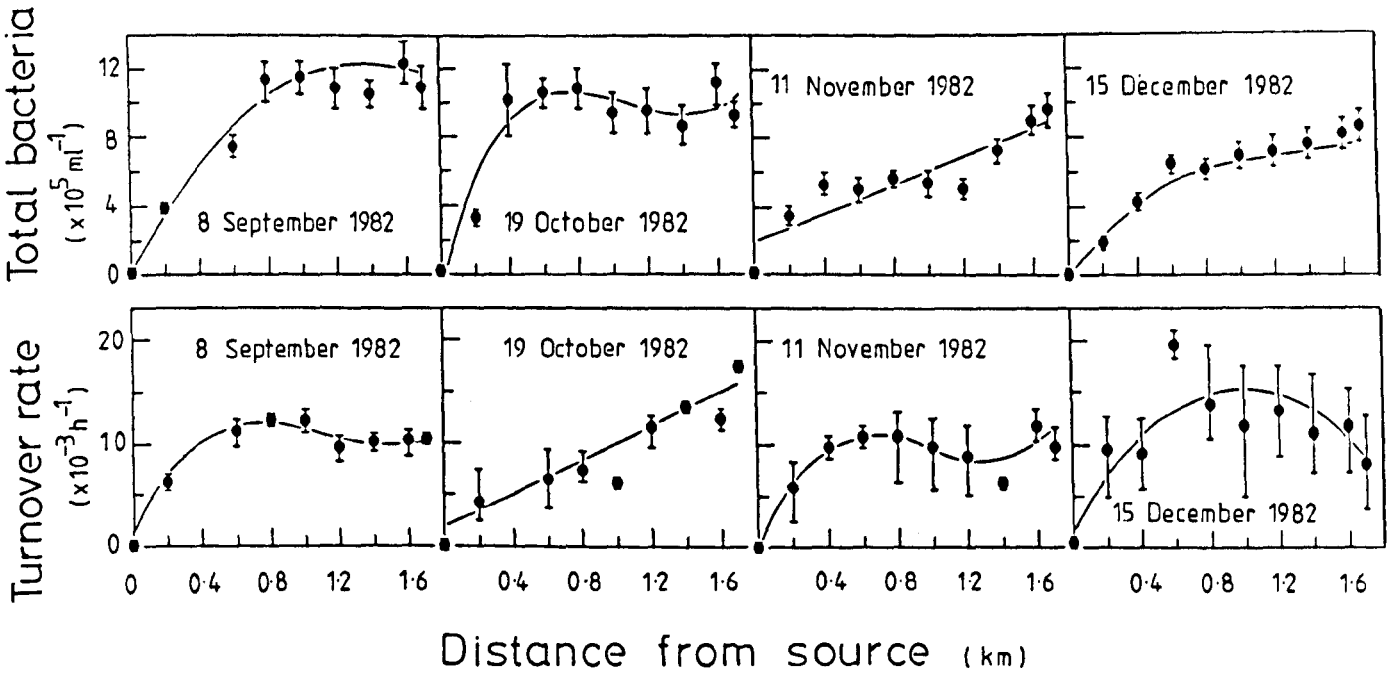


Figure 5.4 Suspended bacteria in Wolds streams, downstream change in Millington Beck, 1982. Layout and symbols as in Fig. 5.1.

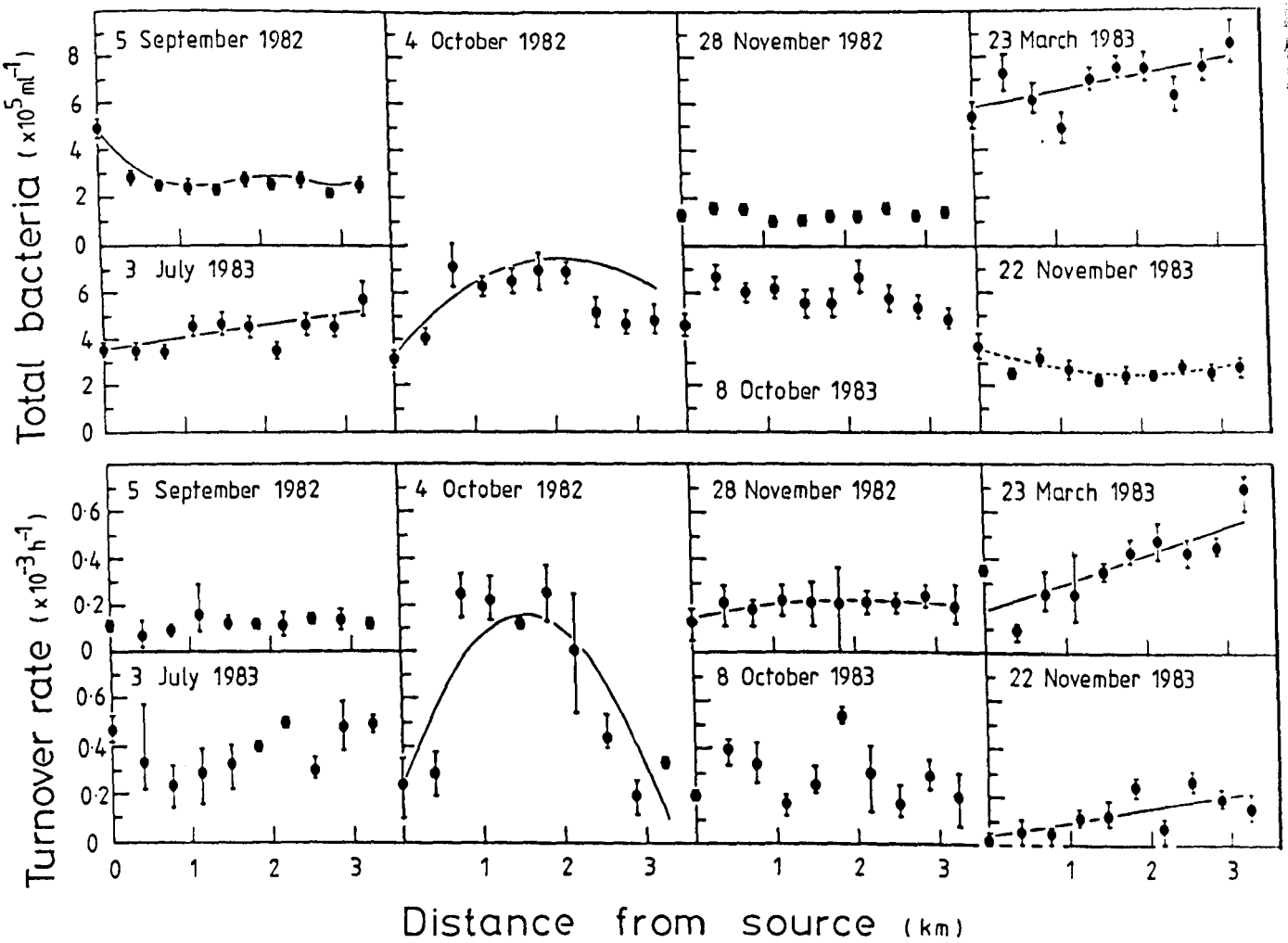


Figure 5.5 Suspended bacteria in Galloway streams, downstream change in Dargall Lane, 1982-1983. (Top) Total bacteria (with 95% confidence limits) and (bottom) turnover rate for glucose assimilation (mean and range from three incubations). Continuous lines indicate regression significant at $P < 0.05$, broken line $P < 0.1$, omission of regression line signifies $P > 0.1$.

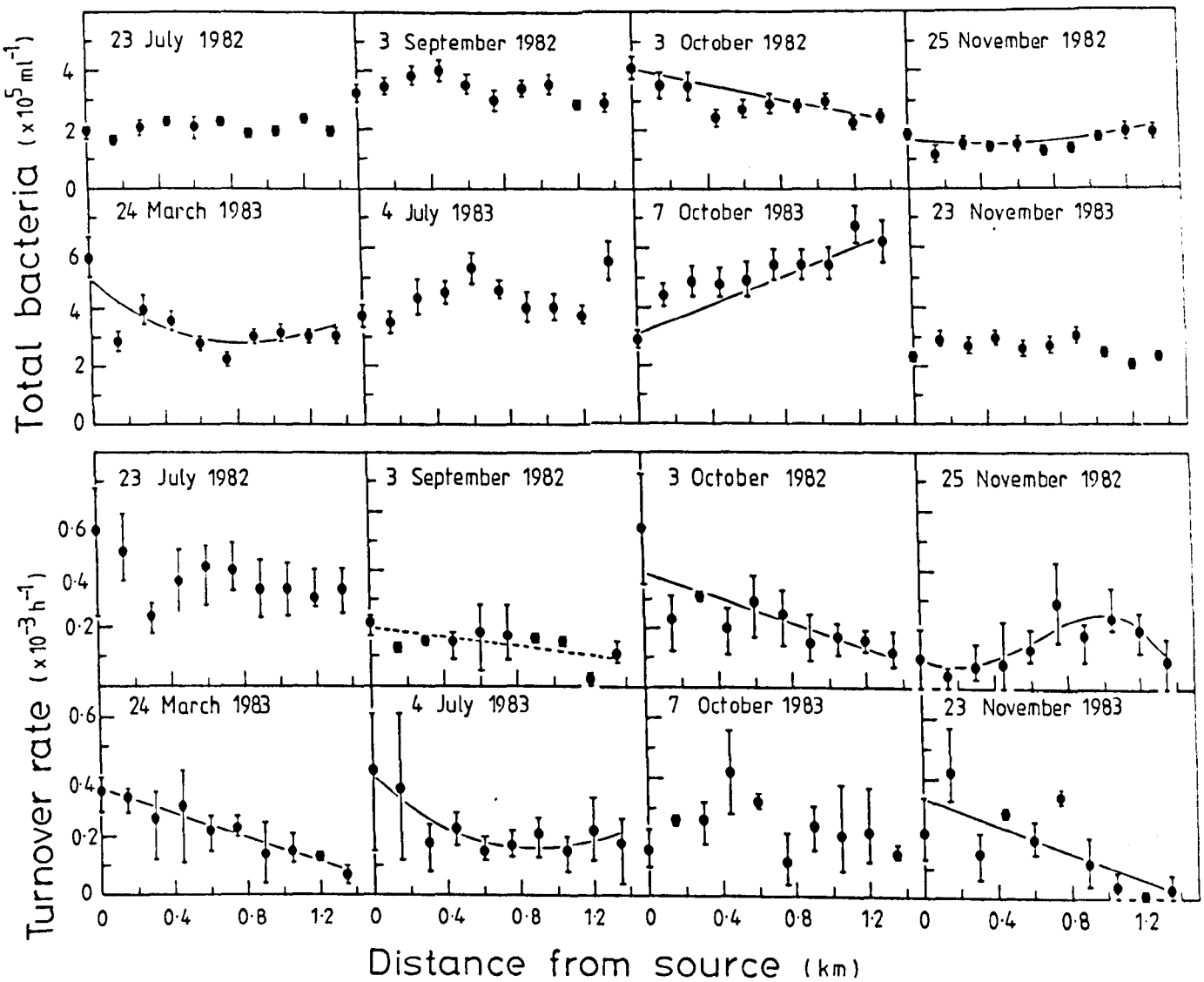


Figure 5.6 Suspended bacteria in Galloway streams, downstream change in Dungeon Burn, 1982-1983. Layout and symbols as in Fig. 5.5.

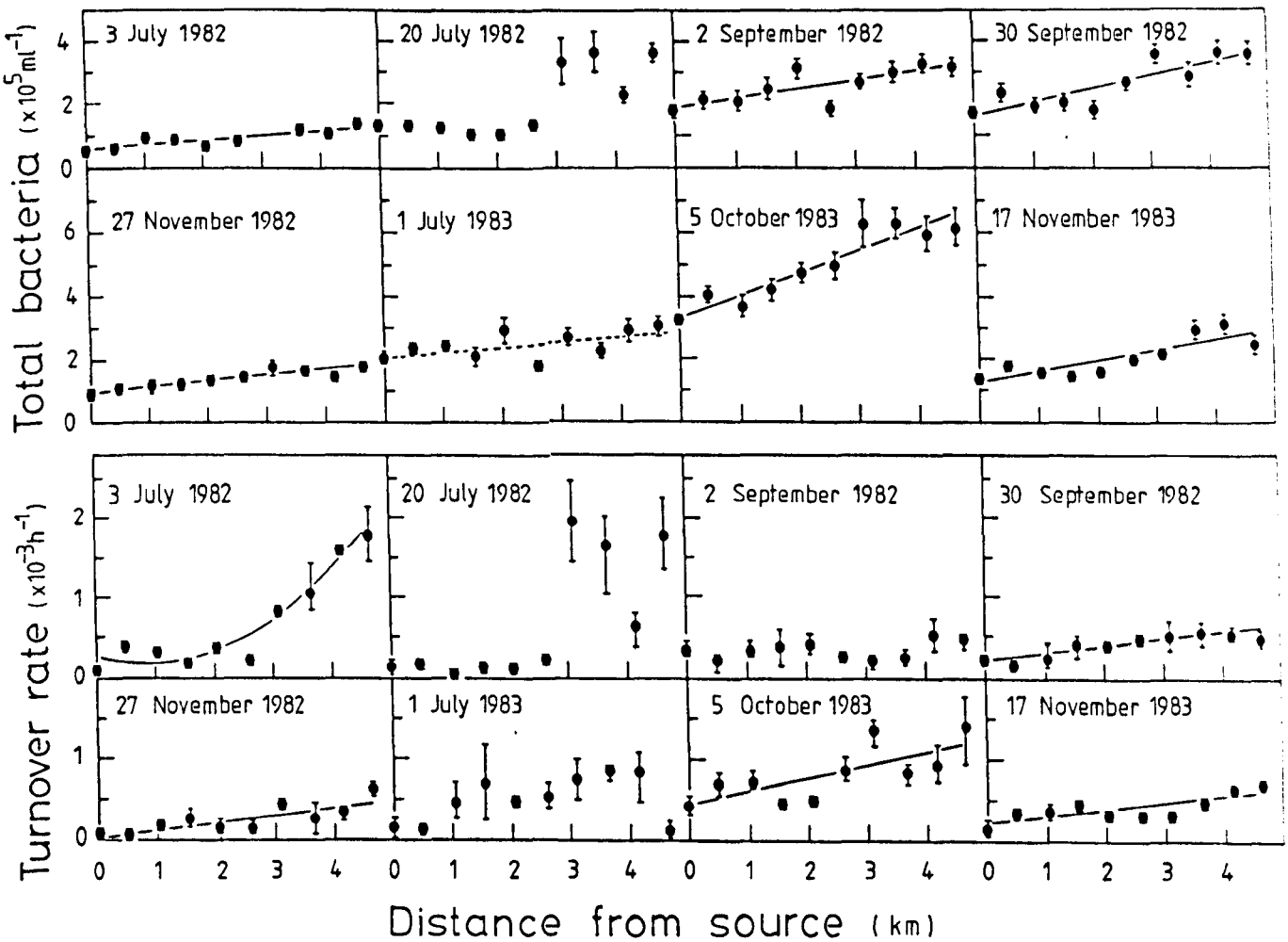


Figure 5.7 Suspended bacteria in Galloway streams, downstream change in Garrary Burn, 1982-1983. Layout and symbols as in Fig. 5.5 apart from results of 20 July 1982 when no regressions were fitted because of an explicable discontinuity.

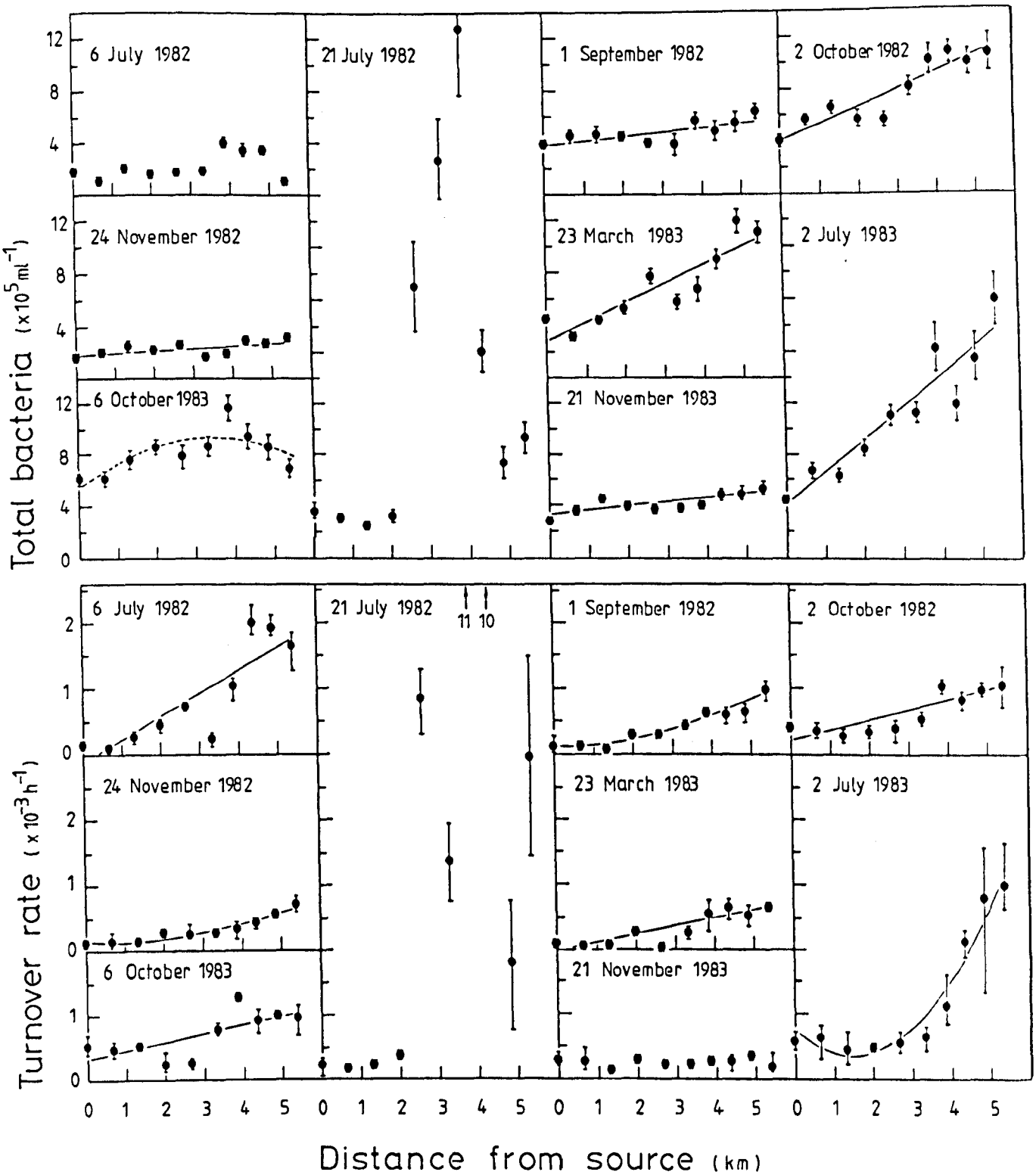


Figure 5.8 Suspended bacteria in Galloway streams, downstream change in Minnigall Lane, 1982-1983. Layout and symbols as in Fig. 5.5 apart from results of 21 July 1982 when no regressions were fitted because of an explicable discontinuity.

described by higher-degree polynomial equations, involving downstream change occured on five and four occasions, respectively. There was no significant regression on seven and eight occasions, respectively.

Values for the rate of downstream increase in total bacteria and turnover rate, per unit length of stream, for all sampling days when a straight-line relationship applied, are summarized in Table 5.1 (full data in Appendix 5). The mean rate of increase of total bacteria was c.16 times greater in the Wolds streams, ranging from 4.1-19.1 x 10^5 $ml^{-1}km^{-1}$, compared with 0.4-1.0 x $10^5 ml^{-1}km^{-1}$ in the Galloway streams. For turnover rate, the mean rate of increase was more than 250 times greater in the Wolds streams, ranging from 2.9-25.4 x $10^{-3}h^{-1}km^{-1}$ in the Wolds streams compared to -0.2-0.2 x $10^{-3}h^{-1}km^{-1}$ in the Galloway streams.

5.2 Discussion.

The suspended bacteria of the Wolds and Galloway streams, as well as showing contrasts in heterotrophic activity (Chapter 4), showed very different patterns and rates of downstream change in total bacterial numbers and turnover rate for glucose assimilation.

The abrupt downstream increases observed on two occasions in the Wolds streams were explained by intermittent, discrete, turbid inflows during rainy weather. A field ditch discharged into Birdsall Beck on 27 October 1983 (Fig. 5.1) and a subsurface drain into Eastburn Beck on 12 November 1982 (Fig. 5.3). Otherwise, results from the Wolds streams demonstrate that a gradual, straight-line downstream increase in abundance and activity of suspended bacteria, as found in Mill Beck by Goulder (1984), is the usual pattern for these calcareous headstreams.

The possible sources of suspended bacteria are outlined in 1.1. Input of allochthonous bacteria to the Wolds streams may have occured,

Table 5.1 Wolds and Galloway streams, 1982-1983. Summary of rate of downstream increase in concentration of total bacteria and turnover rate for glucose assimilation per unit length of stream. Results are only from those sampling days when there was a straight-line relationship with distance; values are means, ranges and coefficients of variation (%), n = number of sampling days, (-) indicates not applicable.

Stream	Increase in total bacteria ($x10^5 ml^{-1} km^{-1}$)				Increase in turnover rate ($x10^{-3} h^{-1} km^{-1}$)			
	Mean	Range	CV	n	Mean	Range	CV	n
Wolds streams								
Birdsall Beck	14.8	13.8-15.7	-	2	25.4	17.9-35.2	28	5
Church Beck	19.1	12.7-29.6	35	6	18.6	10.2-31.4	37	7
Eastburn Beck	4.5	1.4-5.5	49	6	2.9	1.2-5.1	57	4
Millington Beck	4.1	-	-	1	8.6	-	-	1
Galloway streams								
Dargall Lane	0.6	0.5-0.7	-	2	0.1	0.06-0.1	-	2
Dungeon Burn	0.4	-1.0-1.7	-	2	-0.2	-0.07- -0.2	40	4
Garrary Burn	0.6	0.3-1.4	62	7	0.1	0.08-0.2	35	4
Minnigall Lane	1.0	0.2-2.5	89	6	0.2	0.1-0.4	59	4

particularly in Birdsall Beck, where suspended solids (as indicated by turbidity) were consistently higher than in the other streams (Table 4.1), however, no information on volume or bacterial content of groundwater entering these streams is available.

The gradual downstream increase in abundance and heterotrophic activity of suspended bacteria in the Wolds streams is however compatible with a largely autochthonous source, that is release of bacteria from surfaces and sediments within the stream. The submerged aquatic vegetation was often luxurient, particularly near the source of the streams and potentially could have supported large populations of epiphytic bacteria (1.1.i). The downstream increase in abundance and heterotrophic activity of suspended bacteria was often greatest where the submerged vegetation was particularly dense, for example, the first 0.4-0.6 km of Millington Beck (Fig. 5.4). In Eastburn Beck (Fig. 5.3), the greatest increase appeared to occur in the downstream section (c.0.8-1.4 km), where there were dense beds of Ranunculus fluitans.

Cell division within the suspended population may have occured. A wide range of doubling times (< 10 to > 500 h) have been reported for suspended bacteria in freshwaters (1.1.iii). At a low flow rate with a short doubling time, cell division could potentially cause a measurable increase in the suspended bacterial population. Marxsen (1980a), however, considered that cell division of suspended bacteria was probably unimportant in small headstreams.

If most bacteria originated within the streams, then straight-line downstream increase might be explained by approximately equal release relative to discharge along the distance studied. The rates of increase per unit length of stream (Table 5.1) were broadly similar to those obtained by Goulder (1984) for Mill Beck (1.5×10^5 $ml^{-1}km^{-1}$ for total bacteria and 2.1×10^{-3} $h^{-1}km^{-1}$ for turnover rate).

The curvilinear downstream increases which were sometimes observed in the Wolds streams may have been related to: (1) variation along the stream in biomass of submerged vegetation and numbers of epiphytic bacteria available for release to the water column; (2) change in rate of attachment of cells to surfaces, since this can depend on the suspended-population concentration (Chapter 9); or (3) variation along the stream in volume and bacterial content of groundwater input, which could cause dilution or or increase in concentration of suspended bacteria.

In the Galloway streams, downstream increase of total bacteria and turnover rate was less frequent than in the Wolds streams, and was often associated with spate flow conditions, where sampling occured either during (Dargall Lane 4 October 1982, Fig. 5.5) or after heavy rainfall (Dungeon Burn 7 October 1983, Fig. 5.6; Garrary Burn 5 October 1983, Fig. 5.7; Minnigall Lane 2 October 1982, 24 November 1982, 23 March 1983, 2 July 1983, Fig.5.8). The abrupt downstream increases in Garrary Burn and Minnigall Lane in July 1982 (Figs. 5.7 and 5.8) occured where two newly-cut forestry ditch networks discharged into the streams.

Overall, however, the increase per unit length of stream in the Galloway streams was much lower than in the Wolds streams. In terms of bacterial numbers, this was partly due to the difference in initial abundance at the uppermost sampling site. The Wolds streams were fed by deep spring water which was almost devoid of bacteria, whereas input to the Galloway streams was principally shallow ground water which already contained substantial bacterial populations.

The potential for downstream increase in the Galloway streams through release of bacteria from within-stream surfaces may have been limited, since there was little fine sediment, and submerged vegetation was not usually prolific. However, even in areas where submerged leafy liverworts were abundant, such as dense mats of Nardia compressa in parts

of Dungeon Burn, there was very little downstream increase in bacterial numbers and a mean downstream decrease in turnover rate (Table 5.1). An epilithic algal mat was also extensive at times, particularly during the summer months, which potentially could have supported a large bacterial population (1.1.i). Release of epilithic bacteria may have contributed to downstream increase in numbers and activity, particularly after heavy rain, when the algal film was often scoured from the stream bed (for example, Minnigall Lane 2 July 1983, Fig. 5.8). It could also explain the very rapid increase in numbers and activity, that occured with sudden increase in discharge during collection of water samples along Dargall Lane, 4 October 1982 (Fig. 5.5). However, in general, most downstream increases and decreases observed in the Galloway streams were probably sufficiently small to be explained by input along the course of the stream of groundwater either richer or poorer in bacteria than the existing stream water.

Groundwater may also have contributed to the downstream increases of total counts and heterotrophic activity in the Wolds streams, however, the very rapid increases associated with areas of luxurient vegetation suggest a strong autochthonous component.

This raises the question of whether there really were sufficient epiphytic bacteria associated with the submerged vegetation in the Wolds streams to account for the observed increase in the suspended population? Conversely, in the Galloway streams, in areas such as parts of Dungeon Burn, where submerged vegetation was abundant, why was there no observable release of epiphytic bacteria?

The second section of this thesis attempts to answer these questions, with a closer examination of the populations of epiphytic bacteria.

SECTION 2

RELATIONSHIPS BETWEEN EPIPHYTIC AND SUSPENDED BACTERIA

CHAPTER 6

Methods used in the study of epiphytic bacteria.

In order to study a population of epiphytic bacteria within a given area of stream bed, it is necessary to determine (1) a measure of bacteria per unit vegetation and (2) the density and extent of vegetation present. Such studies were carried out in both Mill Beck, a Wolds stream (7.2.i), in a section with dense, species-rich beds of submerged and partly emergent angiosperms, and Dungeon Burn, a Galloway stream, where the vegetation consisted almost entirely of discrete, submerged mats of the leafy liverwort Nardia compressa.

6.1 Total counts and heterotrophic activity of epiphytic bacteria per unit vegetation.

6.1.i Use of stomaching for removal of epiphytic bacteria.

The spatial distribution of epiphytic bacteria on the leaf and stem surfaces of submerged macrophytes is often very uneven (Baker and Orr, 1986), consequently it is often preferable to remove the epiphytes from the plant material and examine them in suspension. This was achieved by stomaching, a procedure which can remove even deep seated bacteria with a negligible temperature rise (Fry and Humphrey, 1978).

Submerged macrophyte shoots were cut at the stream bed, after removing any emergent foliage and placed directly into sterile polythene bags. A known volume of membrane-filtered (0.22 μm) water (10-150 ml, depending on the amount of plant material) was added to the bag, which was then placed in the stomacher (Colworth Stomacher-400, A.J.Seward Ltd., London) and

vigorously pounded on its outer surface by the paddles inside the machine. With suspensions of 50 ml or less, a buffer bag containing 200 ml of water was also placed in the Stomacher during treatment (Fry and Humphrey, 1978). When a delay between collection and treatment was unavoidable, plant material was stored in 2% neutral formaldyhyde solution. With N.compressa, clumps of shoots were collected into sterile polythene bags. The shoot bases which were encrusted with gravel were cut off and discarded and any further gravel was also removed from the shoots. Stomaching was then carried out in double or triple layer bags to safeguard against puncturing. After stomaching, the epiphyte suspensions were strained through a 400 µm nylon screen to remove larger macrophyte debris. Macrophyte material remaining in the bag was rinsed by addition of an equal volume of diluent water. This was stomached for a further 15 s, then combined with the first suspension. Rinsing was omitted when shoots were stomached in 50 ml or less. Bacteria in these epiphyte suspensions (stomachates), were then counted, after suitable dilution, using the AODC method (3.4), or used for heterotrophic activity determination (3.6). When epiphyte suspensions were used for heterotrophic activity determination, stream water was used as a diluent during stomaching, in order to maintain, as far as possible, the in situ chemical conditions of the surrounding water. Distilled water was used, however, if only total counts of epiphytic bacteria were required.

When using a quantitative approach, information is required about the efficiency of stomaching for epiphyte removal. Investigations were carried out to determine (1) the optimum duration of stomaching which should ideally be short enough to minimize cell damage but long enough to remove most bacteria, and (2) the proportion of total bacteria released.

6.1.ii Optimum duration of stomaching.

Submerged shoots of Nasturtium officinale were collected near the

source of Millington Beck on 17 March 1983 and 25 April 1983. On each occasion a batch of 20 matched leaflets was treated by stomaching with 50 ml diluent for periods of 2-15 min, and AODC's were carried out on the resulting suspensions.

Stomaching beyond 5 min released few additional bacteria (Fig. 6.1), whereas 2 min was less effective. Very similar results were obtained for Callitriche sp. (Fry, Goulder & Rimes, 1985), and 3-5 min was also found to be optimum stomaching time for the removal of algal epiphytes from Cladophora glomerata (Bowker, Teutem & Fry, 1986). Therefore a standard 5 min stomaching time was used throughout the following experiments.

6.1.iii Proportion of total bacteria released by stomaching.

Comparisons were made between in situ counts on leaf surfaces using the phenolic aniline blue (PAB) method (Hossell & Baker, 1979a) and AODC's on epiphyte suspensions after stomaching. Submerged shoots of N.officinale and Apium nodiflorum were collected irregularly during 1983 and 1984 from Millington Beck, Church Beck and Mill Beck (Table 6.1). Leaflets were stained with PAB for 1-2 min. The PAB stain comprised phenol (37.5 g l^{-1}) and aniline blue (0.5 g l^{-1}) in 20% acetic acid (Hossell & Baker, 1979a). Portions of leaflets were mounted in a drop of PAB stain and viewed with bright-field microscopy (magnification x1000-x1600). The density of epiphytic bacteria was determined by counting 400-600 bacteria, in a known area, on the upper and lower surfaces of five leaflets. A further 50 leaflets were stomached with 50 ml diluent, and AODC's were carried out on the resulting suspensions. Mean leaf area was determined by tracing the outline of 10 leaflets onto graph paper.

For N.compressa, 2 methods were used: (1) comparison of in situ counts on leaf surfaces made by the PAB method with AODC's from epiphyte suspensions (as above), (2) comparison of PAB counts before and after

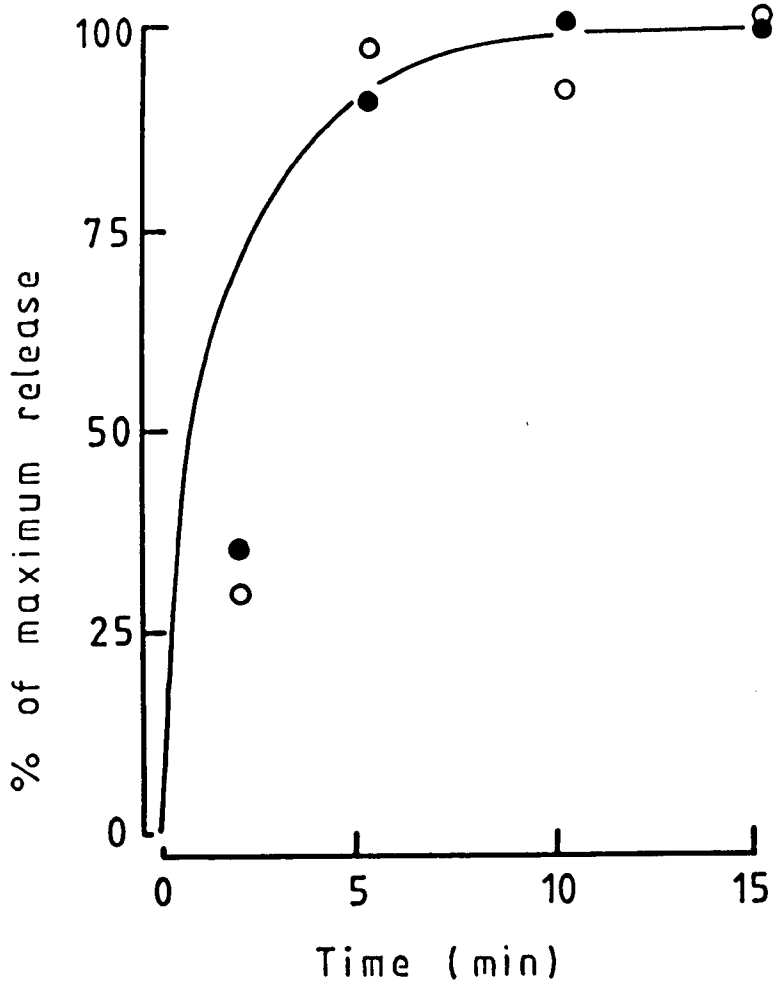


Figure 6.1 The effect of stomaching time on the removal (as percentage of maximum release achieved) of epiphytic bacteria from submerged leaves of Nasturtium officinale. Two experiments; (open circles) 17 March 1983, (closed circles) 25 April 1983. Curve fitted by eye.

treatment in a stomacher. The second method was feasible because shoots remained relatively intact during stomaching. Clumps of N.compressa were collected from Dungeon Burn in November 1983 and July 1984. Leafy shoots were detached from the clumps, and apices and stem bases were removed. The remaining central portion of 30 shoots, with a known number of leaflet pairs were stomached in 50 ml diluent. AODC's were carried out on the epiphyte suspension. PAB counts were made on 10 leaflets from the stomached shoots and on 10 leaflets from five further non-stomached shoots. 400-600 cells were counted on each leaflet. The mean leaflet area of 30 leaflets (five from each of six shoots) was determined by counting grid-square graticule units at magnification x64.

The density of epiphytic bacteria found on submerged leaves of N.officinale, A.nodiflorum and N.compressa, and the proportion removed by stomaching, is shown in Table 6.1. The mean proportion of total bacteria released by stomaching was similar for all three species (0.52-0.65). This compares reasonably with the proportion of 0.36 obtained for Callitriche sp. (Fry, Goulder & Rimes, 1985). There was considerable variation, however, between determinations made using plants collected at different sites or dates. The proportion released was not related to density of epiphytic bacteria; Spearman's rank correlation coefficient, $r = -0.25$ ($n = 23$, $P > 0.05$). Other factors such as current velocity, age of plant and season may control the ease with which bacteria can be detached by stomaching. Therefore, ideally, the proportion of epiphytic bacteria released by stomaching should be determined on each sampling occasion. This proved impractical during the following investigations, because of the extremely time-consuming nature of the PAB method. Therefore the overall mean value of 0.62 for N.officinale and A.nodiflorum (Table 6.1) was applied as a correction factor for stomaching efficiency to numbers of epiphytic bacteria removed from all species collected from the Wolds

Table 6.1 Density of epiphytic bacteria on submerged leaves of aquatic plants and the proportion of total removed by treatment for five minutes in a stomacher.

Date	Stream	Distance below source (m)	Mean density of epiphytic bacteria on leaves ($\times 10^7 cm^{-2}$) * and proportion removed by stomaching (in brackets)					
			Nasturtium officinale		Apium nodiflorum		Nardia compressa	
6 March 1983	Millington Beck	50-100	2.38	(0.30)	-	-	-	-
16 March 1983	Millington Beck	50-100	2.67	(0.24)	-	-	-	-
24 April 1983	Mill Beck	0-48	1.78	(0.37)	-	-	-	-
16 September 1983	Mill Beck	0-48	2.09	(0.68)	1.49	(0.37)	-	-
28 September 1983	Church Beck	200-300	4.71	(0.28)	3.45	(0.20)	-	-
18 October 1983	Millington Beck	400-600	2.89	(0.72)	5.31	(0.75)	-	-
20 November 1983	Dungeon Burn	500-590	-	-	-	-	1.67	(0.51)(a)
20 November 1983	Dungeon Burn	500-590	-	-	-	-	1.67	(0.50)(b)
9 February 1984	Mill Beck	225-360	0.82	(0.76)	0.93	(0.44)	-	-
29 February 1984	Mill Beck	225-360	1.24	(0.72)	0.85	(1.00)	-	-
15 March 1984	Mill Beck	225-360	0.86	(0.57)	0.51	(0.40)	-	-
12 April 1984	Mill Beck	225-360	2.51	(1.00)	1.19	(1.00)	-	-
3 July 1984	Mill Beck	225-360	1.50	(1.00)	1.90	(1.00)	-	-
9 July 1984	Dungeon Burn	500-590	-	-	-	-	1.12	(0.64)(a)
9 July 1984	Dungeon Burn	500-590	-	-	-	-	1.12	(0.41)(b)
Mean			2.10	(0.60)	1.95	(0.65)	1.40	(0.52)

(*) From counts made directly on leaf surfaces using PAB method.
(a) Method 1, comparison of PAB counts with AODC's of stomachates.
(b) Method 2, comparison of PAB counts before and after stomaching.
(-) Indicates no measurement was made.

streams and the mean proportion of 0.52 (Table 6.1) was used to correct numbers of epiphytic bacteria released from N.compressa in Dungeon Burn.

6.1.iv Preparation of stomachate for heterotrophic activity determinations.

Epiphyte suspensions produced by stomaching are unsuitable for direct determination of Vmax because of release of additional substrate from lysed macrophyte cells (Fry and Humphrey, 1978). Hence a "washing" process was carried out, by centrifuging 250 ml of epiphyte suspension for 15 min at 8000 RCF, then resuspending the pellet in 250 ml of sterile, filtered (0.22 µm) stream water. Centrifuging and resuspension were carried out a total of three times. Fry and Humphrey (1978) found this procedure reduced the total carbohydrate concentration in the final supernatant to about 1% of the original concentration. Glucose-mineralization capacity (Vmax) was then determined using the final suspension after further dilution (x4-x40) with sterile stream water. AODC's of the original and final epiphyte suspensions demonstrated a loss of bacteria of up to 52% (mean 23%, n=9) during centrifuging and resuspension. It was assumed that reduction in Vmax was proportional to the loss of bacteria, so Vmax values were adjusted accordingly. AODC's were carried out for each investigation, therefore individual correction factors were applied.

Centrifugation facilities were unavailable for the Scottish samples, therfore, an alternative, filtering, procedure was used. To test the technique, a trial comparison between centrifuging and filtering was carried out using submerged N.officinale collected from West Beck, a Wolds Stream near Great Driffield (Grid Ref. TA 064 561), described by Goulder (1980). Stomaching yielded 300 ml stomachate, 250 ml of which was centrifuged, as above, diluted 10 times with sterile stream water, and used for standard Vmax determination (3.6) with 20 ml of diluted stomachate in

each serum bottle. Of the remaining 50 ml non-centrifuged stomachate, 8 ml was filtered with 72 ml sterile stream water through a 45 mm diameter, 0.22 µm Oxoid membrane filter. The filter was cut into four quarters and each quarter placed in a serum bottle. This was repeated five times, for the five glucose concentrations. Sterile stream water (20 ml) was added to the bottles, which were then vigorously agitated (Fisons Whirlimixer) for 30 s, and used for standard Vmax determination. Each bottle, therefore, both in the centrifuged and filtered sets, received the equivalent of 2 ml of original stomachate. Both plots of t/f against A were highly significant ($P < 0.001$), yielding a Vmax value (with 95% confidence limits) of 6.98 (5.50-9.57) x 10^{-3} µg $l^{-1}h^{-1}$ for the stomachate subjected to filtering which was very similar to the Vmax value (after correction for 35% loss of cells during centrifuging) of 5.51 (4.35-7.49) x 10^{-3} µg $l^{-1}h^{-1}$ for the centrifuged stomachate. The confidence limits here were calculated using the equation for small sample numbers: $\pm$ 1 / ($t_{0.05(n-2)}$ x SE b), where $t_{0.05(n-2)}$ = appropriate Student's t value for 0.05 level of significance with n-2 degrees of freedom, SE b = standard error of the regression coefficient and n = number of values (Kim & Kohout, 1975a).

6.1.v Expression of results.

Results describing either the number or activity of epiphytic bacteria can be expressed relative to the amount of the original macrophyte material, usually on a weight or plant-surface area basis. Plant-surface area determination before stomaching was not desirable because of possible loss of epiphytic bacteria during measurement. After stomaching, the plant material was not usually sufficiently intact to distinguish individual leaves and stems. The fresh weight of plant material is difficult to determine because of a variable amount of water retained between leaves

and stems (Fry and Humphrey, 1978), therefore results were expressed on a dry weight basis.

A 200 ml aliquot of the strained epiphyte suspension, and the macrophyte debris retained on the nylon screen, were dried separately at 70-80°C and weighed, thus the total dry weight of plant material treated by stomaching was obtained. The concentration of bacteria (cells ml^{-1}) and Vmax ($\mu g\ l^{-1}h^{-1}$) in the epiphyte suspension could thus be expressed in terms of bacteria per unit dry weight of submerged vegetation (cells g^{-1}) and glucose-mineralization potential per unit dry weight of plant material per unit time ($\mu g\ g^{-1}h^{-1}$).

It proved important to include the epiphyte suspension in dry weight calculations, since this was found to contain a mean of 45% (range 16-80%, n = 74) of total dry weight of stomached material of macrophytes from the Wolds streams, and a mean of 32% (range 23-51%, n = 7) of total dry weight of N.compressa from Dungeon Burn.

When stream water was used as a diluent during stomaching (Chapter 7), no account was taken of the weight of salts in the water, thus the stomachate dry weights (particularly from Mill Beck, where the water is calcareous) may have been overestimated. Stream water epiphyte suspensions from Mill Beck had a mean dry weight of 0.25 g 100 ml^{-1} (range 0.14-0.35g 100 ml^{-1}, n = 9). Water collected from the source of Mill Beck on 3 March 1986 had a mineral dry weight of 0.030 g 100 ml^{-1}. Assuming the mineral content of the spring water to remain constant, the stomachate component of dry weights may thus have been overestimated by 9-23%.

6.2 Density and extent of vegetation.

The density of vegetation was assessed principally by determining the dry weight of macrophyte material per unit area of stream bed. When

attachment of suspended bacteria to submerged plant surfaces was being considered (Chapters 9 & 10), it was also found necessary to express the density of vegetation in terms of surface area of macrophyte material per unit area of stream bed.

6.2.i Dry weight of vegetation.

The density of vegetation was determined by harvesting quadrats placed at 18 points, located using co-ordinates of random numbers, over the section of stream in question. The few points located in non-vegetated areas were ignored and substitute random points were located. For Mill Beck vegetation, 25 cm x 25 cm quadrats were used. Any emergent foliage was cut at the water surface and discarded, then submerged leaves and stems were collected into polythene bags and later sorted into species, dried at 70-80°C and weighed.

In Dungeon Burn, N.compressa was harvested within 15 cm x 15 cm quadrats. The samples usually consisted of a dense mat which often contained much gravel, therefore, after drying, the mats were broken up and stirred vigorously in a large volume of water. Plant material floated and was skimmed off, then the remaining mineral residue was dried and reweighed. This weight was then subtracted from the original dry weight. To confirm that adequate separation was achieved, six quadrats of N.compressa were collected from Dungeon Burn, during October 1983. Each sample was dried and split into two roughly equal portions and the percentage dry weight of plant material in each portion was determined, (1) by floating-off plant material as above and (2) by ashing at 450°C for 4 h. The mean percentages of plant material obtained by the different procedures were very similar (Table 6.2); 34% by method 1 and 33% by method 2.

On all sampling occasions, the 18 sample weights did not deviate

Table 6.2 Dungeon Burn, October 1983. Comparison of floating and ashing methods for separation of plant material and gravel in clumps of Nardia compressa.

Quadrat no. (15cm x 15cm)	Dry weight of harvested material (g)	Plant material in quadrats (%) Method 1 (a)	Method 2 (b)
1	19.60	62	65
2	14.50	30	40
3	27.56	30	16
4	79.00	17	16
5	25.73	53	48
6	109.29	13	12
Mean		34	33

(a) Method 1, floating-off plant material in a large volume of water and reweighing gravel residue.
(b) Method 2, loss on ashing at 450°C for 4 h.

significantly from a normal distribution ($P > 0.05$, Kolmogorov-Smirnov test, using SPSS; Hull & Nie, 1981), hence 95% confidence limits were calculated using the following equation: mean $\pm t_{0.05(n-1)} \times s / n$, where $t_{0.05(n-1)}$ = appropriate Student's t value for 0.05 level of significance with n-1 degrees of freedom, s = standard deviation of the sample, n = number of values (Sokal & Rohlf, 1969, p146).

6.2.ii Surface area of vegetation.

Measurement of vegetation surface area (i.e. area of leaves and stems) was achieved indirectly by calculating surface area to dry weight conversion factors for different species. Shoots were collected into polythene bags after discarding any emergent foliage, and later separated into (i) leaves and (ii) stems plus petioles, which were spread flat and photocopied onto gridded paper. Their surface areas were determined by regarding leaves as two-sided laminae and stems and petioles as cylinders. Both (i) leaves and (ii) stems plus petioles were then dried at 70-80°C and weighed. Plant-surface area for both categories could thus be determined and overall area to weight ratios calculated for each species.

6.2.iii Extent of vegetation.

The spatial area occupied by aquatic vegetation in the stream sections under investigation was assessed using two methods.

Method 1 was used at Mill Beck, where the areas of vegetation were sufficiently large to be directly mapped. The positions of margins and transitions between submerged vegetation, emergent vegetation and vegetation-free areas were recorded at 1 or 2 m intervals, using a tape measure placed across the stream between two parallel base lines. A map was drawn in the field at a scale of 1:100, from which the total areas of

stream bed and aquatic vegetation (submerged plus emergent) were determined.

Method 2 was used for Dungeon Burn, where the patchy distribution of N.compressa made a vegetation map difficult to draw. It proved less time consuming than method 1, and subsequently was also used for Mill Beck. The area of stream bed was obtained from a map drawn in the field at 1.100, as in method 1. Presence or absence of vegetation was recorded at 150 randomly located points (50 in each of three equal-length segments) and percentage cover of vegetation was calculated. The area occupied by vegetation was then determined using percentage cover and total stream-bed area.

CHAPTER 7

Epiphytic bacteria as a potential source of suspended bacteria in Mill Beck and Dungeon Burn.

7.1 Introduction.

The suggestion that suspended bacteria in streams, at least during non-spate conditions, may consist principally of cells sloughed from within-stream surfaces and in particular from the surface of plants, has been put forward by several authors (1.1.i). The frequent pattern of linear downstream increase in concentration and heterotrophic activity of suspended bacteria in Wolds streams (Chapter 5) perhaps supported this idea, although no quantitative evidence was available to confirm that the populations of epiphytic bacteria were capable of sustaining the observed increases.

The investigations described within this chapter examined (1) downstream loss, as number and activity of suspended bacteria, from a short (48 m), richly vegetated length of stream, immediately below the source of Mill Beck (a Wolds stream), and (2) the total number and activity of epiphytic bacteria in this length of stream. The aim was to establish whether the magnitude of the population of epiphytic bacteria was sufficient for it to sustain the observed downstream drift of suspended bacteria.

In Dungeon Burn by contrast, downstream increase in total numbers and heterotrophic activity of suspended bacteria rarely occured (Chapter 5), despite an extensive cover of submerged Nardia compressa with associated algal film. Studies similar to (1) and (2) above, but not including activity determinations, were carried out to investigate the apparent

non-release of epiphytic bacteria.

7.2 Description of sites.

7.2.i Mill Beck.

Mill Beck rises near Market Weighton (Fig. 2.1.b), below the Cretaceous chalk scarp of the Yorkshire Wolds and initially flows westwards over Jurassic Lias (Fig. 7.1). The principal source (Grid. Ref. SE 899 426) is a brickwork culvert emerging from beneath a disused railway. The railway embankment straddles the spring line and neighbourhood springs were presumably channelled into the culvert during railway construction in the 1860's (Goulder, 1984). A subsidiary spring joins at 150 m (Fig. 7.1) and several lesser springs and spring fed ditches enter between 950 m and 1250 m. The principal source is intermittent, flowing from mid-winter to late-summer, according to the height of the water table in the chalk.

The stream flows for about 1100 m through pasture, where submerged and emergent vegetation is often abundant; chiefly Nasturtium officinale, Apium nodiflorum, Glyceria fluitans, Sparganium erectum and Epilobium hirsutum, and then for about 300m through a narrow strip of dense woodland with heavy shading and virtually no aquatic vegetation. The stream bed is mostly silt and chalk gravel with some accumulation of fallen leaves and woody debris beneath trees.

The current is generally rapid when the principal source is flowing; for example on 15 July 1983, mean and range of velocity at nine open-water locations over 1600 m downstream from the source was 0.18 (0.10–0.24) m s^{-1}, which suggests a retention time of about 2.5 h over the length of stream (Goulder, 1984). This may, however, be an underestimate, since water velocity may be much reduced within dense stands of submerged and emergent macrophytes. The mean and range of stream width and midstream

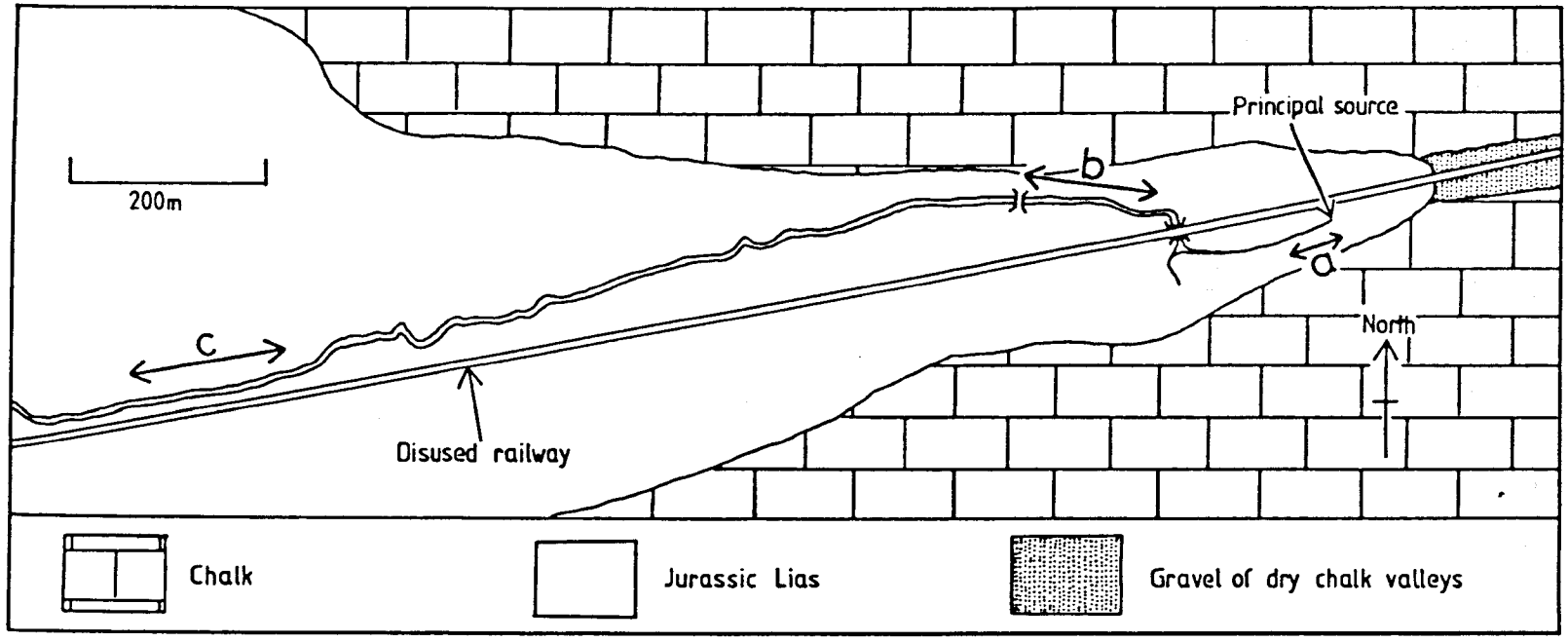


Figure 7.1 Map of Mill Beck, 0- c.1400 m (direction of flow is east to west). Double-headed arrows indicate study sections; (a) 0-48 m, vegetated section in open pasture, (b) c.225-360 m, vegetated section in open pasture, (c) c.1100-1250 m, vegetation-free section in woodland. (Sections (b) and (c) are considered in Chapter 10.)

depth, at nine locations on 15 July 1983 equalled 2.8 (0.9-4.6) m and 32 (23-43) cm, respectively.

The study described in this chapter was confined to a 48 m, richly vegetated section immediately below the principal source (Fig. 7.1; Plate 7.1). Spring-water temperature was constant at c.10°C during May and June 1983 and pH at the source and 48 m ranged from 7.2-7.5.

7.2.ii Dungeon Burn.

Dungeon Burn is described in Chapter 2. The first 500 m stretch was unsuitable for this study, because the stream bed was indistinct and hidden beneath boulders for much of its course, so the area studied was a 90 m length of stream (Plate 7.2) which began about 500 m downstream of the source at altitude c.410 m (Grid Ref. NX 459 842). The stream bed varied from granite slabs to coarse gravel and supported discrete mats, up to 10 cm thick, of the fibrous perennial leafy liverwort Nardia compressa, often with a covering of algal growth. Stream temperature ranged from 3.0-4.0°C in November 1983 and 15.0-15.5°C in July 1984. The range of pH was 4.3-4.4 (November) and 5.2-5.5 (July).

7.3 Methods.

7.3.i Mill Beck.

Field work was carried out during three replicate five-day blocks in May-June 1983.

On day 1, the pattern of downstream change in concentration of suspended bacteria was examined. Water samples (10 ml) were taken, downstream samples first, at 5 m intervals from mid-stream at about 10 cm depth, using a displacement pipette. Samples were transfered to sterile universal bottles, for determination of total bacteria by the AODC method

Plate 7.1 Mill Beck, May 1983. Richly vegetated section, 0-48 m. View from culvert-source looking downstream.

Plate 7.2 Dungeon Burn, April 1985, c.530 m below the source, where the stream descends steeply over granite slabs, with submerged mats of Nardia compressa.

(3.4). Significant downstream increase in concentration of suspended bacteria was demonstrated by linear regression of concentration against distance (SPSS New Regression; Hull & Nie, 1981).

On each of days 2-4, the total number and glucose-mineralization capacity of bacteria lost through drift, from the 48 m of stream, were estimated. Water samples (2 litre) were collected at 0 m and 48 m from about 10 cm depth, in 2 sterile 1 litre reagent bottles. The 48 m samples were combinations of smaller volumes (300 ml) collected at intervals across the stream. AODC's were carried out and Vmax was determined, as outlined in 3.6, however, five replicate 25 ml sub-samples were used at each glucose concentration and incubation was for 5 h. Discharge (m d^{-1}) was determined from velocity measurements made with an Ott small current meter, at 0.6 of the depth, at 10 cm intervals across the 92 cm wide source culvert. Stream depth in the 48 m section was measured at 72 random points over the 5-day period. Drift loss of bacteria (cells d^{-1}) and of glucose -mineralization capacity (mg $h^{-1}d^{-1}$) equalled the increase in bacterial concentration or Vmax over 48 m times the discharge.

The number of epiphytic bacteria and their glucose-mineralization capacity per unit dry weight of submerged vegetation, were also estimated on each of days 2-4, using the procedure described in Chapter 6. Submerged shoots were collected from points located using random co-ordinates to ensure that they represented the entire aquatic macrophyte community. The length of stream was divided into three 16 m segments and one shoot at, or closest to each of six random locations within each segment was collected and transfered to a sterile stomacher bag (three shoots per bag). The species composition over the whole study was: Nasturtium officinale (72 shoots), Apium nodiflorum (39), Veronica anagallis-aquatica (28), Mentha aquatica (8), Veronica beccabunga (6), Myosotis scorpioides (6), Glyceria maxima (1), Ranunculus repens (1), Rumex longifolius (1).

Epiphytic bacteria were removed from the plant material by stomaching as described in 6.1.i-ii. Each bag was stomached with 150 ml of sterile, membrane-filtered (0.22 µm), stream water and a further 150 ml water was used for rinsing. The stomachates from all six bags were combined to yield a total volume of 1800 ml. AODC's were carried out and 250 ml of stomachate was prepared for heterotrophic activity determination by centrifuging and resuspension (6.1.iv). After further dilution (x4-x40) with sterile stream water, Vmax was measured, using five replicates of 25 ml subsamples at each glucose concentration. Incubation was for 2 h. Results were expressed per gram dry weight of vegetation (6.1.v), and corrected for stomaching efficiency of 62% (6.1.iii).

Submerged vegetation was harvested on day 5, for estimation of dry weight of plant material per unit area of stream bed (6.2.i), and the area of stream bed occupied by aquatic vegetation was determined from a map drawn at a scale of 1:100, on 13 May 1983 (method 1; 6.2.iii).

The density and glucose mineralization capacity of epiphytic bacteria per unit dry weight of vegetation (cells g^{-1} and $\mu g\ g^{-1}h^{-1}$) were combined with the mean dry weight of vegetation per unit area of stream bed ($g\ m^{-2}$) to yield estimates of the mean density and glucose mineralization capacity of epiphytic bacteria per unit area of stream bed (cells m^{-2} and $mg\ m^{-2}h^{-1}$). These values were then multiplied by the area of stream bed occupied by vegetation (m^{-2}) to give estimates of the total number and glucose-mineralization capacity of epiphytic bacteria (cells and $mg\ h^{-1}$) within the 48 m section of stream. This information together with the drift loss data, allowed drift loss per day to be expressed as a percentage of total number and glucose-mineralization capacity of the standing crop of epiphytic bacteria.

7.3.ii Dungeon Burn.

Field work was carried out during two replicate three-day blocks, in November 1983 and July 1984.

Water samples (10 ml) were taken on days 1-3, over the 90 m section, in mid-stream at 10m intervals, downstream samples first, for determination of suspended bacteria by AODC. Discharge was also obtained on days 1-3, at a point with uniform cross-section, from velocity readings at intervals of 5 cm over a width of 70 cm in November and 2 cm intervals over a 24 cm width in July. Stream depth in the 90 m section, was measured at 186 points located using random co-ordinates, over each 3-day period.

The mean concentration of suspended bacteria over the 90 m section (cells ml^{-1}) times the discharge (m^3 d^{-1}) equalled the daily throughput of bacteria (cells d^{-1}).

The number of epiphytic bacteria per unit dry weight of submerged N.compressa was estimated on day 3. The length of stream was divided into three 30 m segments and clumps of shoots (dry weight 0.3-1.0 g) were collected from each of six randomly located points within each section and placed in sterile stomacher bags (three clumps in each bag). The clumps were prepared for stomaching (6.1.i), which was then carried out using 150 ml diluent and rinsing water for each bag (6.1.i-ii). In November, total counts were carried out on the combined stomachate from the six bags (total volume 1800 ml), whereas in July, separate determinations were made on the six epiphyte suspensions. Results were expressed per gram dry weight of vegetation (6.1.v), and corrected for 52% stomaching efficiency (6.1.iii).

Submerged N.compressa was harvested on day 3, for determination of dry weight per unit area (6.2.i). The area of stream bed occupied by vegetation was estimated by surveying the area of stream bed on day 1 and determining the percentage cover of vegetation on day 2 (method 2;

6.2.iii).

Results from stomaching, harvesting and surveying were combined (as for Mill Beck) to give an estimate of the total number of epiphytic bacteria in the 90 m length of stream.

A single determination of glucose-mineralization capacity (Vmax) of both suspended and epiphytic bacteria was made in August 1984, The water sample (500 ml) was collected from the downstream end of the 90 m length of stream. Subsamples of 20 ml were used for each of three replicates and a blank, at each glucose concentration. Shoots from four clumps of N.compressa were both stomached and rinsed in 150 ml of filtered (0.22 µm) Dungeon Burn water and the resulting epiphyte suspension was prepared for Vmax determination using the filtering procedure (6.1.iv). The equivalent of 1.25 ml of stomachate was added to each serum bottle using three replicates plus control at each glucose concentration. Incubation time for both water and stomachate was for 5 h at 10°C.

7.4 Results.

7.4.i Mill Beck.

Concentration of suspended bacteria in Mill Beck on day 1, showed a gradual linear increase over 0-48 m (Fig. 7.2), with highly significant linear regressions ($P < 0.001$), on all three occasions. Thus, it was probably justified to consider the difference in concentration and glucose-mineralization capacity of suspended bacteria between water samples taken at 0 m and 48 m on days 2-4, to be a measure of the increase over that section.

The concentration of suspended bacteria at the source was generally very low ($< 2.0 \times 10^4$ ml^{-1}; Table 7.1, Figure 7.2). The comparatively high 0 m concentration on 9 May (9.6×10^4 ml^{-1}) occured during a

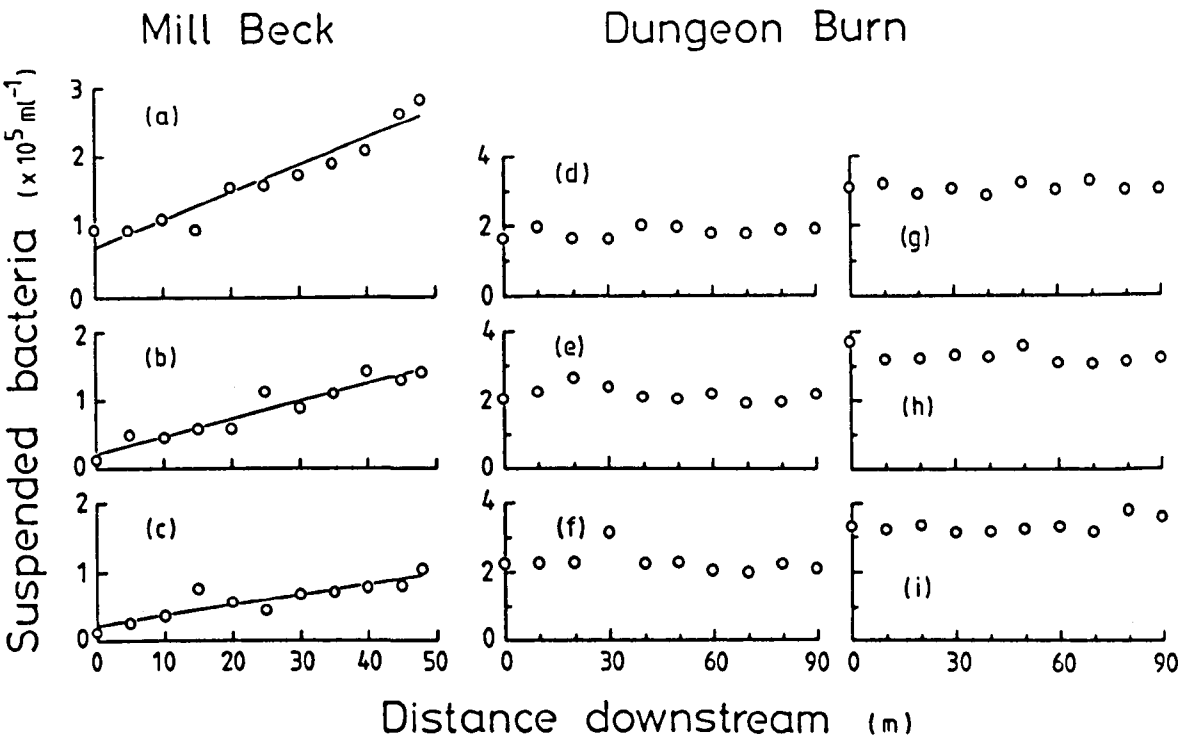


Figure 7.2 Concentration of suspended bacteria from 0-48 m in Mill Beck, (a) 9 May, (b) 23 May, and (c) 6 June 1983, and over 90 m in Dungeon Burn, (d-f) 18-20 November 1983, (g-i) 7-9 July 1984. 95% confidence limits were plus or minus c.8-10% of these estimates. In Mill Beck there were significant linear regressions, (a) $F = 100.9$, (b) $F = 87.0$, (c) $F = 35.0$; $n = 11$, $P < 0.001$. In Dungeon Burn there were no significant regressions, $F = 0.01$-3.1, $n = 10$, $P = 0.12$-0.95.

Table 7.1 Mill Beck, source to 48 m, May-June 1983. Determination of loss of bacteria and glucose-mineralization capacity (Vmax) through downstream drift. Values are means (n = 3), ranges and coefficients of variation (%).

	10, 11 and 12 May			24, 25 and 26 May			7, 8 and 9 June		
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV
Concentration of bacteria at source ($x10^4 ml^{-1}$)	1.0	0.7-1.3	30	1.3	0.9-1.6	28	0.8	0.7-1.0	22
Concentration of bacteria at 48 m ($x10^4 ml^{-1}$)	10.5	9.2-12.9	20	10.1	8.1-11.2	17	12.1	8.9-15.2	26
Increase in concentration ($x10^4 ml^{-1}$)	9.5	8.1-12.2	25	8.8	6.5-10.3	23	11.3	8.2-14.2	27
Vmax at 48 m ($ng\ l^{-1}h^{-1}$) *	5.8	3.4-10.2	67	4.1	2.8-5.3	31	6.6	5.6-7.3	14
Discharge (m^3d^{-1})	4536	4224-4752	6	5817	5779-5866	1	5922	5729-6178	4
Daily drift loss of bacteria ($x10^{14}d^{-1}$)	4.3	3.8-5.2	18	5.1	3.8-6.0	23	6.7	4.7-8.8	30
Daily drift loss of glucose-mineralization capacity ($mg\ h^{-1}d^{-1}$)	25.6	16.2-43.3	60	24.0	16.0-30.5	31	39.2	32.0-42.9	16

(*) These values also represented increase in Vmax over 48 m because Vmax at source was considered to be zero (see text).

downpour, when obvious overland flow was entering near the source. Twenty four hours later the concentration (at 1.3×10^4 ml^{-1}) had declined to its usual low level. Heterotrophic activity at 0 m proved too low to measure effectively using the kinetic approach. The $^{14}CO_2$ released during incubations was scarcely more than release from the controls, and on only one occasion (10 May) was there a significant regression between t/f and A, yielding a negligible mineralization capacity of 0.04 ng $l^{-1}h^{-1}$. Thus no heterotrophic activity measurements were made from 0 m samples of 7-9 June, and Vmax on all nine occasions was taken as indistinguishable from zero, which was reasonable considering the very low concentration of bacteria in these samples.

The increase in suspended cell concentration over 0-48 m on sampling days 2-4 (means 8.8-11.3 x 10^4 ml^{-1}), combined with discharge results (means 4536-5922 m^3d^{-1}), gave mean daily drift loss of bacteria of 4.3-6.7 x 10^{14} d^{-1} (Table 7.1). Vmax in 48 m samples (means 4.1-6.6 ng $l^{-1}h^{-1}$), which also represented increase over 0-48 m, was combined with discharge to yield mean daily drift loss for glucose-mineralization capacity of 24.0-39.2 mg $h^{-1}d^{-1}$ (Table 7.1).

Total number and Vmax of epiphytic bacteria per unit dry weight of vegetation (means 4.4-6.2 x 10^{10} g^{-1} and 3.5-10.7 µg $g^{-1}h^{-1}$) are included in Table 7.2. There was an overall increase during the study period from mid-May to mid-June, despite some day to day variability, particularly of Vmax. This may have been due to considerable scatter shown by many of the plots of t/f against A from which Vmax values were derived.

The dry weight and species composition of harvested vegetation within the 48 m section are given in Table 7.3. The principal species were Nasturtium officinale, Veronica anagallis-aquatica and Apium nodiflorum. The mean dry weight per unit area increased over the study period (from 264 to 325 g m^{-2}) despite removal of emergent foliage before collection.

Table 7.2 Mill Beck, source to 48 m, May-June 1983. Number and glucose-mineralization capacity (Vmax) of epiphytic bacteria. Values are means (n = 3), ranges and coefficients of variation (%).

	10, 11 and 12 May			24, 25 and 26 May			7, 8 and 9 June		
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV
Bacteria per unit dry weight of vegetation ($x10^{10}g^{-1}$)	4.4	3.8-5.5	22	4.8	4.6-5.1	5	6.2	6.0-6.4	4
Vmax per unit dry weight of vegetation ($\mu g\ g^{-1}h^{-1}$)	3.5*	1.7-5.3	73	7.2	1.7-13.5	83	10.7	2.8-25.2	119
Epiphytic bacteria per unit area of stream bed ($x10^{12}m^{-2}$)	11.4	9.7-14.3	22	13.2	12.6-13.8	5	20.0	19.1-20.5	4
Vmax per unit area of stream bed ($mg\ m^{-2}h^{-1}$)	0.91*	0.44-1.38	73	1.94	0.45-3.65	83	3.40	0.90-8.05	119
Total epiphytic bacteria ($x10^{15}$)	3.6	3.1-4.5	22	4.1	3.9-4.3	5	6.2	6.0-6.4	4
Total glucose-mineralization capacity ($mg\ h^{-1}$)	284*	138-430	73	605	140-1138	83	1060	282-2510	119

(*) Indicates mean of only two values.

Table 7.3 Mill Beck, source to 48 m, May-June 1983. Dry weight and species composition of harvested vegetation. Values are means (n = 18), figures in brackets are 95% confidence limits. Total area of vegetation = 306m^2

	13 May	27 May	10 June
Nasturtium officinale (% total dry weight)	50	48	51
Veronica anagallis-aquatica (% total dry weight)	35	24	17
Apium nodiflorum (% total dry weight)	5	11	12
Other species* (% total dry weight)	10	17	20
Dry weight per unit area for all species (g m^{-2})	264 (209-320)	276 (227-325)	325 (266-383)
Total dry weight (kg)	81	85	100

(*) Comprised of Mentha aquatica, Veronica beccabunga, Myosotis scorpioides, Glyceria maxima, Ranunculus repens, Rumex longifolius, Glyceria fluitans, Agrostis stolonifera and Rhynchostegium riparioides.

Fig. 7.3 shows the results of the survey. There was an extensive semi-submerged grassy margin, but as water did not flow over this area, it was not considered to be part of the aquatic vegetation. Apart from the margin, the area of stream bed from 0-48 m was 312 m^2, of which aquatic vegetation occupied 306 m^2 (98%), comprising 226 m^2 of emergent vegetation and 80 m^2 of submerged vegetation.

The area of stream bed occupied by vegetation (Fig. 7.3) times the mean dry weight of harvested vegetation (Table 7.3) gave an estimate of total dry weight of vegetation within the 48 m section (81-100 kg; Table 7.3).

Stream depth (means 29-36 cm) and discharge for each sampling day were combined with stream-bed area to give an estimate of retention time (means 27-28 min for 48 m) and velocity of water (means 2.9-3.0 cm s^{-1}) over the 48 m section (Table 7.4).

Combination of total number and Vmax of epiphytic bacteria per unit dry weight of vegetation (Table 7.2) with results of vegetation harvesting (Table 7.3) and stream survey (Fig. 7.3) yielded estimates of (1) the number and glucose-mineralization capacity of epiphytic bacteria per unit area of stream bed (means 11.4-20.0 x 10^{12} m^{-2} and 0.91-3.40 mg $m^{-2}h^{-1}$; Table 7.2) and (2) total number and Vmax of epiphytic bacteria in the 48 m section (means 3.6-6.2 x 10^{15} and 284-1060 mg h^{-1}; Table 7.2).

The daily drift loss of bacteria through downstream drift (Table 7.1) therefore represented, on average, 10.7-12.4% of total epiphytic bacteria and 8.0-17.6% of the glucose-mineralization capacity of epiphytic bacteria (Table 7.5). If downstream drift of bacteria was due entirely to release of epiphytic bacteria from submerged vegetation, the population of epiphytic bacteria would have needed to double every 199-236 h to maintain constant population number and 357-612 h to maintain constant

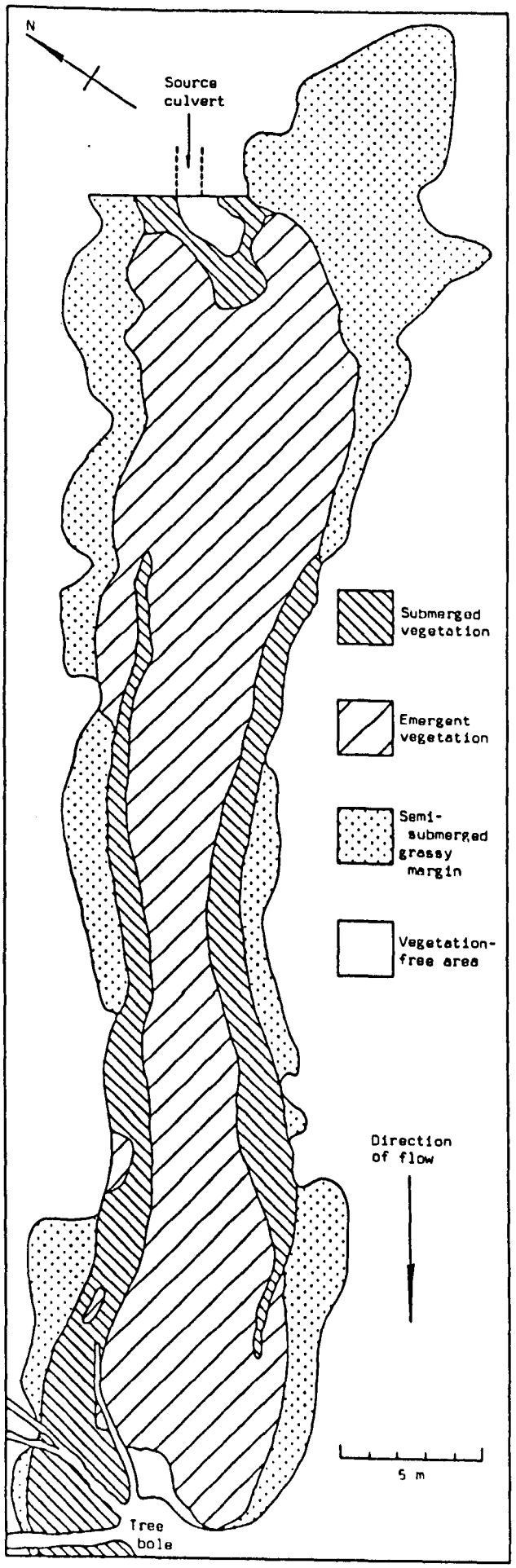


Figure 7.3 Map of Mill Beck, 0-48 m, from survey of 13 May 1983.

Table 7.4 Mill Beck, source to 48 m, May-June 1983. Stream depth, retention time and mean velocity. Values are means, ranges and coefficients of variation (%), n = number of values.

	10, 11 and 12 May				24, 25 and 26 May				7, 8 and 9 June			
	Mean	Range	CV	n	Mean	Range	CV	n	Mean	Range	CV	n
Stream depth (cm)	29	10-43	26	72	36	15-47	19	72	36	20-46	19	72
Retention time * (min)	28	25-32	13	3	28	27-29	3	3	27	27-28	3	3
Mean velocity (cm s^{-1})	2.9	2.5-3.2	12	3	2.9	2.8-2.9	2	3	3.0	2.9-3.0	2	3

(*) Retention time of water over 48 m.

Table 7.5 Mill Beck, source to 48 m, May-June 1983. Daily drift loss as percentage of total number and glucose-mineralization capacity of epiphytic bacteria, and doubling times required to maintain a constant epiphyte population. Values are means (n = 3), ranges and coefficients of variation (%).

	10, 11 and 12 May			24, 25 and 26 May			7, 8 and 9 June		
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV
% of number (d^{-1})	12.0	11.6–12.3	3	12.4	9.6–14.5	21	10.7	7.9–13.8	28
% of glucose-mineralization capacity (d^{-1})	17.6*	3.8–31.4	111	8.0	2.7–18.3	112	8.0	1.7–11.4	68
Doubling time for constant number (h)	200	195–207	3	199	166–250	22	236	174–304	28
Doubling time for constant glucose-mineralization capacity (h)	357*	77–637	111	612	131–896	68	611	212–1404	112

(*) Indicates mean of only two values.

glucose-mineralization capacity.

Specific activity was calculated for suspended and epiphytic cells (Table 7.7). Vmax per epiphytic bacterium (means 8.2-16.7 x 10^{-11} µg h^{-1}) was greater than Vmax per suspended bacterium (means 4.3-5.6 x 10^{-11} µg h^{-1}), although the range of values showed considerable overlap.

7.4.ii Dungeon Burn.

Concentrations of suspended bacteria in Dungeon Burn (means 2.1 and 3.3 x 10^5 ml^{-1}; Table 7.6) were higher than close to the Mill Beck source but showed no downstream increase over the 90 m studied (Fig. 7.2). Discharge was about 60-150 times lower than in Mill Beck, with means of 90 and 36 m^3d^{-1} in November and July (Table 7.5). Both sampling occasions were during spells of dry weather and discharge values were below the range of discharges recorded during the 1982-1983 routine sampling (Table 4.1), although in this study, the measurement site was c.800 m further upstream. The mean stream depth of 5 cm was also much less than in Mill Beck, and together with discharge, gave estimates of water retention time of 76 and 146 min over 90 m. Thus, mean velocities (2.0 and 1.0 cm s^{-1}) were only slightly less than in Mill Beck (Table 7.6).

The number of epiphytic bacteria per unit dry weight of vegetation (1.8 and 2.5 x 10^{10} g^{-1}; Table 7.6) was marginally less than in Mill Beck, however, the mean dry weight of vegetation per unit area (776 and 956 g m^{-2}; Table 7.6) was greater in Dungeon Burn. The results of the surveys are shown in Fig. 7.4 and gave stream bed areas of 89.8 m^2 in November and 67.8 m^2 in July. The percentage vegetation cover (73% and 79%; Table 7.6) was lower than in Mill Beck, however, the number of epiphytic bacteria per unit area of vegetated stream bed (10.2 and 18.7 x 10^{12} m^{-2}; Table 7.6) was fairly similar.

Table 7.6 Dungeon Burn, 90 m length of stream, November 1983 and July 1984. Determination of daily throughput of suspended bacteria, epiphytic bacteria per unit dry weight of Nardia compressa, total dry weight of submerged N.compressa, total epiphytic bacteria, and throughput of bacteria as percentage of epiphyte standing crop.

	18, 19 and 20 November	7, 8 and 9 July
Discharge (m^3d^{-1}) (a)	90 (85-98) 7	36 (30-45) 22
Stream depth (cm) (d)	5 (0.5-50) 70	5 (0.5-58) 181
Retention time (min) (e)	76	146
Mean velocity (cm s^{-1})	2.0	1.0
Mean concentration of suspended bacteria ($x10^5ml^{-1}$) (a)	2.1 (1.8-2.3) 12	3.3 (3.1-3.4) 5
Daily throughput ($x10^{13}d^{-1}$) (a)	1.9 (1.6-2.2) 16	1.2 (1.0-1.4) 17
Epiphytic bacteria per unit dry weight of N.compressa ($x10^{10}g^{-1}$)	1.8	(b) 2.5 (1.6-4.6) 44
Dry weight of N.compressa per unit area (g m^{-2}) (c)	776 (531-1021)	956 (628-1284)
% cover of N.compressa	73	79
Area of stream bed (m^2)	89.8	67.8
Area of submerged N.compressa (m^2)	65	53
Total dry weight of N.compressa (kg)	51	51
Epiphytic bacteria per unit area of stream bed ($x10^{12}m^{-2}$)	10.2	18.7
Total epiphytic bacteria ($x10^{15}$)	0.9	1.3
Daily throughput as % of epiphyte standing crop (d^{-1}) (a)	2.1 (1.8-2.5) 16	0.9 (0.8-1.1) 17

(a) Mean of three values with range and coefficient of variation (%).
(b) Mean of six values with range and coefficient of variation (%).
(c) Mean of 18 values with 95% confidence limit.
(d) Mean of 150 values with range and coefficient of variation (%).
(e) Retention time of water over 90 m.

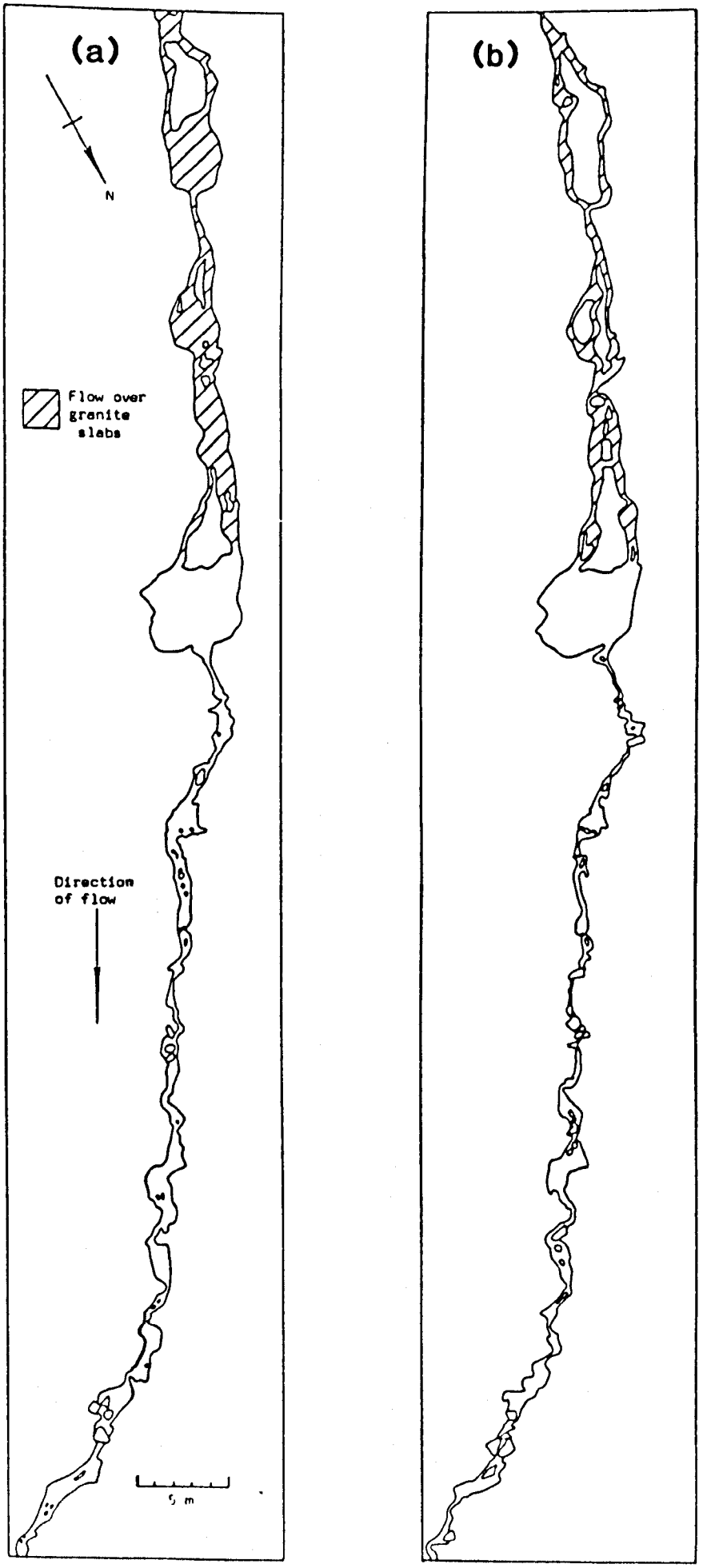


Figure 7.4 Maps of Dungeon Burn, 90 m length of stream, from surveys of (a) 18 November 1982 and (b) 8 July 1983.

Table 7.7 Mill Beck, source to 48 m, May-June 1983 and Dungeon Burn, 90 m length of stream, August 1984. Vmax per bacterium for suspended and epiphytic populations. Mill Beck; values are means, ranges and coefficients of variation (%). Dungeon Burn, n = 1.

	Mill Beck									Dungeon Burn
	10, 11 and 12 May			24, 25 and 26 May			7, 8 and 9 June			1 and 3 August
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV	
Vmax per suspended [a] bacterium ($x10^{-11}\mu g\ h^{-1}$)	5.2	3.7-7.9	46	4.3	2.5-6.5	47	5.6	4.5-6.3	17	1.0
Vmax per epiphytic bacterium ($x10^{-11}\mu g\ h^{-1}$)	8.2 *	3.0-13.3	-	15.2	3.3-29.2	86	16.7	4.7-39.3	117	4.6

(a) Mill Beck, values at 48 m; Dungeon Burn, value at c.590 m.
(*) Indicates mean of only two values.
(-) Indicates not applicable.

Values of concentration of suspended bacteria and discharge were combined to give a mean daily throughput of cells of 1.9×10^{13} d^{-1} in November and 1.2×10^{13} d^{-1} in July (Table 7.5). Combining results from AODC's on epiphyte suspensions, harvesting, percentage cover estimation and survey gave total numbers of epiphytic bacteria over 90 m as 0.9 and 1.3×10^{15} (Table 7.6). Hence, daily throughput of suspended bacteria represented only a small percentage (means 2.1% and 0.9%; Table 7.6) of standing crop of epiphytic bacteria.

Glucose-mineralization capacity determinations during August 1984, gave Vmax values of 5.5 ng $l^{-1}h^{-1}$ for suspended bacteria and 1.1 µg $g^{-1}h^{-1}$ for epiphytic bacteria per unit dry weight of vegetation. Vmax per bacterium (Table 7.7) was more than four times greater for epiphytic bacteria (4.6×10^{-11} µg h^{-1}) than suspended bacteria (1.0×10^{-11} µg h^{-1}). Both values were lower than the equivalent specific activities in Mill Beck.

7.5 Discussion.

The results of this study do not conclusively prove that epiphytic bacteria were the principal source of suspended bacteria in the first 48 m of Mill Beck, but they strongly suggest that the population of epiphytic bacteria was sufficient to sustain the downstream increase in total suspended bacteria and glucose-mineralization capacity (Table 7.5).

Published doubling times for epiphytic bacteria include 61 h for bacteria attached to Lemna minor in the R. Frome, determined by increase in total bacterial numbers, net of estimates of both attachment and detachment (Hossell & Baker, 1979c), and 6 h for marine epiphytes of Zostera capricorni, where the incorporation of ^{3}H-labelled thymidine into bacterial DNA was measured and combined with AODC's to give an estimate of

total production (Moriarty & Pollard, 1982). A wide range of doubling times (< 10 to > 500 h) have been reported for suspended bacteria in freshwaters (1.1.iii). Thus the doubling times required in Mill Beck to maintain constant epiphyte numbers and glucose mineralization capacity, net of observed drift loss (199-612 h; Table 7.5), should be readily achievable. The actual doubling times of epiphytic bacteria were probably shorter, because of (1) losses through predation and (2) an increase in total population over the sampling period (Table 7.2).

The range of values of Vmax per suspended and per epiphytic bacterium for Mill Beck overlapped, however mean Vmax per suspended bacterium was c.2.6 times lower (Table 7.7). One possibility is that this may reflect differential detachment of bacteria, for example, moribund and small daughter cells may be less securely attached than the active parent population. Fry & Humphrey (1978) found that Vmax per bacterium for mineralization of glucose and acetate was greater for epiphytic than for suspended bacteria in a South Wales pond. Strzelczyk & Mielczarek (1971) cultured suspended and epiphytic bacteria from a eutrophic lake and found that epiphytic bacteria were more active, as measured by oxygen uptake.

In Dungeon Burn, there was no downstream change in concentration of suspended bacteria (Fig. 7.2), although the size of the population of epiphytic bacteria in terms of total numbers per unit area of stream bed was very similar to Mill Beck (Tables 7.2 and 7.6). The loss of only 2.1% (November) and 0.9% (July) per day would have produced a doubling in concentration of suspended bacteria over the 90 m studied. The mean velocity in Dungeon Burn was lower than in Mill Beck (Table 7.6), thus the shear stress exerted on the epiphytes may have been less (Silvester & Sleigh, 1985). The difference, however, was only slight, moreover, at times of higher discharge during 1982-1983, marked downstream increases were still not frequent (Fig. 5.6). The growth form of the vegetation may

have been important, since water in Dungeon Burn flows over dense mats of N.compressa and there may be limited exchange with sloughed bacteria trapped within the mat. In Mill Beck, by contrast, the current flows through the vegetation and will readily entrain any sloughed cells.

The supply of cells available for release is ultimately related to growth rate rather than population size, thus it could be argued that the observed non-release of epiphytic bacteria in Dungeon Burn may have been due to low activity of N.compressa epiphytes under oligotrophic and acidic conditions. The result of the single heterotrophic activity determination for epiphyte suspensions from N. compressa, to some extent supported this suggestion, since specific activity for epiphytic bacteria was lower than in Mill Beck (Table 7.7). The epiphytic bacteria associated with N.compressa, however, seem to have been more active than the suspended bacteria in Dungeon Burn (Table 7.7). Ladd, Costerton & Geesey (1979) also found that suspensions of epilithic bacteria from mountain streams were as much as 26 times more active than suspended bacteria. There may have been micro-environmental advantages for attached bacteria within the N.compressa mat, which potentially could include: (1) Increased concentration of nutrients that may occur at the interface between any submerged surface and the overlying water (Marshall, 1980). Aquatic liverworts may also constitute efficient filters for particulate material (Caines, Watt & Wells, 1985). (2) Supply of photosynthetically produced organic compounds from the macrophytes (Wetzel, 1975) and the associated algal film (Geesey et al, 1978). (3) Possible modification of water chemistry within the liverwort mat, for example, there is some evidence that liverworts such as N.compressa may remove or release trace metals into the surrounding water, according to the prevailing pH (Caines, Watt & Wells, 1985). The Vmax per epiphytic bacterium in Dungeon Burn, however, was obtained from incubation of epiphyte suspension and may have been an overestimate of the in situ

value, since Ladd, Costerton & Geesey (1979) found that dispersed samples of sessile bacteria from algal films in mountain streams were c.3 times more active per cell than in situ samples, and suggested that the polysaccharide matrix of the intact biofilm may limit diffusion of dissolved organic compounds.

Results from other studies of epiphytic bacteria have generally been expressed in terms of bacteria per unit leaf area. Comparisons can be made, however, by applying a conversion factor for mean surface area of plant material per unit dry weight. Conversion factors were obtained for four macrophyte species categories, on seven occasions during the Spring and early Summer of 1984 at a downstream location of Mill Beck (Chapter 10). The mean value for all species during May and June 1984 was 475 cm^2g^{-1} (n=12; Table 10.1). The resulting numbers of bacteria per unit surface area of macrophyte at the source of Mill Beck (9.3-13.1 x 10^7 cm^{-2}) were somewhat higher than from other studies on similar species, using PAB counts, for example, 0.8-2.3 x 10^7 cm^{-2} for submerged leaflets of N.officinale (Rorippa) (Hossell & Baker, 1979a), and 0.2-1.0 x 10^7 cm^{-2} for A.nodiflorum, G.maxima, N.nodiflorum and V.beccabunga (Baker & Orr, 1986). Kidd-Haack, Bitton & Laabes (1985) reported a density of epiphytic bacteria as low as 0.06 x $10^7 cm^{-2}$ for Sphagnum sp. using a gelatin impression technique.

The variation in density of epiphytic bacteria reported in the literature may be partly methodological, for example, Hossell & Baker (1979a) found that an impression technique (cellulose ester film) failed to remove all the epiphytes from N. officinale leaflets. In situ PAB counts may also underestimate population size, particularly where bacteria are densely clustered, as shown by, for example, Lorsch & Ottow (1985) with scanning electron micrographs of epiphytic bacteria concentrated in surface grooves on leaves of Berula erecta. With older and senescing plant

material, bacteria may have invaded macrophyte epidermal cells as illustrated by Rogers & Breen (1981) with transmission electron micrographs of Potomogeton crispus leaves. Such cells may not be enumerated by the PAB method, although they would probably be released during stomaching. Conversely, the results from Mill Beck, expressed on a surface area basis, are necessarily approximations, since the surface area to dry weight ratio was calculated using shoots collected 12 months later at a more downstream location.

Vmax values for epiphytic bacteria per unit area of macrophyte in Mill Beck (0.07-0.23 ug $cm^{-2}h^{-1}$) were lower than values for macrophytes in the littoral of a lake (0.44-2.26 µg $l^{-1}cm^{-2}h^{-1}$, Allen & Ocevski, 1982), however, discs of plant material rather than stomachates were used, and their results include an unexplained volume dimension.

Nevertheless, some between-site and between-species variation in density and activity of epiphytic bacteria is to be expected, dependent upon a wide range of variables, including species, age and physiology of the host plant, and the physico-chemical nature of the surrounding water body.

In Mill Beck, at least during May-June 1983, the population of epiphytic bacteria was probably easily large enough to sustain the observed downstream drift of suspended bacteria. The extent to which this situation is maintained over a growing season, is examined in Chapter 10.

CHAPTER 8

The dimensions and volume of epiphytic and suspended bacteria from Mill Beck, determined by Image Analysis.

8.1 Introduction.

The gradual downstream increase in numbers and heterotrophic activity of suspended bacteria in the Wolds streams (Chapter 5), suggested a diffuse source of cells. By estimating the magnitude and glucose-mineralization capacity of the total population of epiphytic bacteria over a section of Mill Beck (Chapter 7), it was demonstrated that this was probably easily large enough to account for the increase in concentration and glucose-mineralization capacity of suspended bacteria over the same length of stream. The range of values for glucose-mineralization capacity per bacterium for epiphytic and suspended bacteria overlapped, however mean values for epiphytic bacteria were more than two times higher than for suspended cells, suggesting a difference in either activity per unit biomass or size of cell.

A further investigation was therefore carried out to determine the mean dimensions and volume of epiphytic and suspended bacteria from Mill Beck.

The cell volume measurements carried out during 1982-1983 by TEM (Chapters 3 and 4) may have been underestimates due to possible shrinkage of cells during sample preparation (4.2), therefore in this case, widths, lengths and thus volumes of cells were measured using television image analysis of photographs taken with epifluorescence microscopy, as recommended by Fry & Davies (1985).

8.2 Methods.

Water and macrophyte samples were collected from Mill Beck on 17 March 1985. Water samples were taken in sterile 300 ml medical flats, from the principal source and at c.360 m downstream, this being below a densely vegetated section. Submerged shoots of Nasturtium officinale and Apium nodiflorum were collected from the vegetated section (c.225-360 m below the source) into sterile stomacher bags (3 x 3 shoots per bag for each species), and stomached with 150 ml diluent per bag (6.1.i-ii). Sub-samples (10 ml) of water and epiphyte suspensions were preserved overnight with formaldehyde at 2% final comcentration.

Measurements followed a procedure of Fry & Davies (1985). Samples were stained with acridine orange (final concentration 5 g l^{-1}) for 3 min, then filtered through 0.2 µm black-stained membranes (3.4). Filtered volumes were as follows: source water 1 x 90 ml; 360 m water 3 x 25-30 ml; stomachates 3 x 5 ml. Membranes were then rinsed with 0.2 µm filtered water, mounted with a drop of paraffin oil under a coverslip and viewed with a Zeiss epifluorescence microscope, similar to the one described in 3.4. Photographs were taken using Kodak Tri-X pan film (speed = 400 ASA) with an Olympus OM2 camera with automatic exposure facility, at final magnification of x800.

The negatives were illuminated, and viewed with a Newvicon scanner of a Quantimet 800 image analyser (Cambridge Instruments, Rustat Rd., Cambridge), equipped with shading corrector, 1-D autodetector, image editor and feature measurement modules. Illuminated features were accepted or rejected by the measurement modules of the system on the basis of their optical density - a process termed detection (Bradbury, 1979). Thus, the shading corrector, autodetector and image editor were used to ensure that only bacteria were measured and irrelevant debris ignored. The

shading corrector was set using an area of exposed film without bacteria or debris. Negatives of bacteria were then placed under the scanner, and viewed on the visual display unit. The detected areas of the negative were indicated by brightly illuminated spots, which were enlarged by increasing the level of detection until the brightly illuminated areas were the same size and shape as the images of bacteria, with minumum background detection. Clearly defined and detected cells were then selected using the accept option on the image editor.

About 200 cells were detected for most samples, yielding a total of at least 600 cells from the 360 m water and both stomachates. Only 129 cells were detected from the source water sample, because of the very low initial cell concentration (Fig 7.2). The area and perimeter (in pixels) of each bacterium in the edited image was measured using the feature measurement modules, and the data transfered to a digital VAX computer. Calculation of length, width and volume was done with the Minitab Statistical Package (Ryan, Jointer & Ryan, 1976), assuming bacteria to be paralled-sided rods with hemispherical ends.

Statistical analyses were carried out using SPSS (Nie et al, 1975; Hull & Nie, 1981). Untransformed data were used to calculate 95% confidence limits (± 2 standard errors). In order to compare volumes, one-way analysis of variance was attempted, however, homogeneity of variance was not achieved (as indicated by Cochran's and Bartlett's tests) for both untransformed and log-transformed data. Further analyses were therefore abandoned.

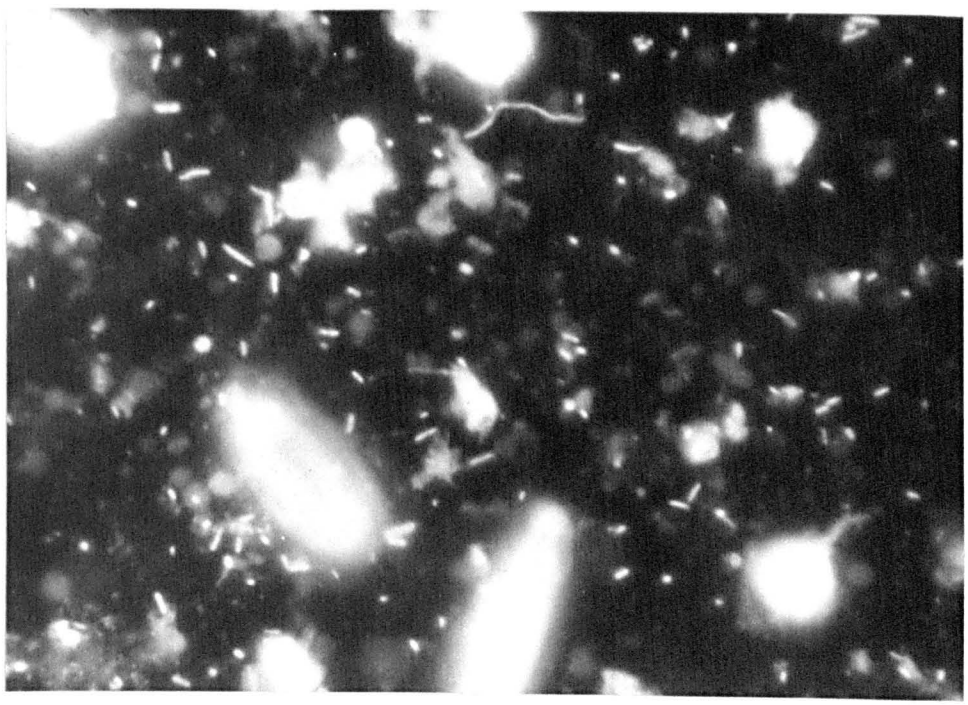
8.3 Results.

Plates 8.1.a and b show examples of photomicrographs of (a) suspended bacteria from 360 m, and (b) epiphytic bacteria from N.officinale. Mean

Plate 8.1 Photomicrographs of bacteria from Mill Beck (March 1985), taken with epifluorescence microscopy. (a) Suspended bacteria from c.360 m below the source. (b) Epiphytic bacteria (and algae) released by stomaching from submerged Nasturtium officinale, collected between 225 m and 360 m below the source.

(a)

(b)



dimensions and volumes of bacterial cells together with 95% confidence limits are shown in Table 8.1. Mean lengths, breadths and volumes of suspended bacteria at the source (2.24 μm, 0.229 μm and 0.108 μm^3, respectively) were less than mean values for suspended bacteria at 360 m (2.79 μm, 0.284 μm and 0.218 μm^3) and for epiphytic bacteria of N.officinale (2.67 μm, 0.330 μm and 0.246 μm^3) and A.nodiflorum (2.90 μm, 0.278 μm and 0.187 μm^3). Figure 8.1 shows how the frequency of different cell volumes was also dissimilar in the source water. The bacterial community at the source had a higher frequency (59%) of small (<0.08 μm^3) cells than at 360 m (44%) and on N.officinale (26%) and A.nodiflorum (35%). There appeared to be some variation in length, width and volume between epiphytic bacteria from the different species. Epiphytic bacteria on A.nodiflorum had a smaller mean volume (0.187 μm^3) than those on the surface of N.officinale (0.246 μm^3), with no overlap of confidence limits. However, the mean length, width and volume for epiphytic bacteria of both N.officinale and A.nodiflorum (2.79 μm, 0.304 μm and 0.217 μm^3) were very similar to the dimensions of suspended bacteria at 360 m (2.79 μm, 0.284 μm and 0.218 μm^3).

8.4 Discussion.

The change in mean dimensions and cell-volume frequency of suspended bacteria in Mill Beck between the spring-fed source and the downstream limit of a densly vegetated section, so as to resemble epiphytic bacteria (Table 8.1; Fig. 8.1), supports the hypothesis that the suspended bacteria might largely be released epiphytes.

The mean cell volumes from Mill Beck (0.108-0.246 μm^3, Table 8.1) were similar to those from a South Wales lake (0.22-0.41 μm^3) and reservoir (0.153 μm^3) obtained by Fry and Davies (1985), using the same

Table 8.1 Mill Beck, 17 March 1985. Mean dimensions and volumes of bacterial cells, determined by image analysis. 95% confidence limits (± 2 SE) are in brackets, n = number of cells measured.

Origin of bacteria	n	Length (μm)	Width (μm)	Volume (μm^3)
Water from spring-fed source	129	2.24 (1.98–2.50)	0.229 (0.207–0.251)	0.108 (0.085–0.130)
Water from downstream limit of vegetated section	648	2.79 (2.60–2.98)	0.284 (0.271–0.296)	0.218 (0.191–0.246)
Epiphytes of *Nasturtium officinale*	715	2.67 (2.50–2.84)	0.330 (0.318–0.341)	0.246 (0.223–0.269)
Epiphytes of *Apium nodiflorum*	624	2.90 (2.74–3.06)	0.278 (0.267–0.290)	0.187 (0.170–0.203)

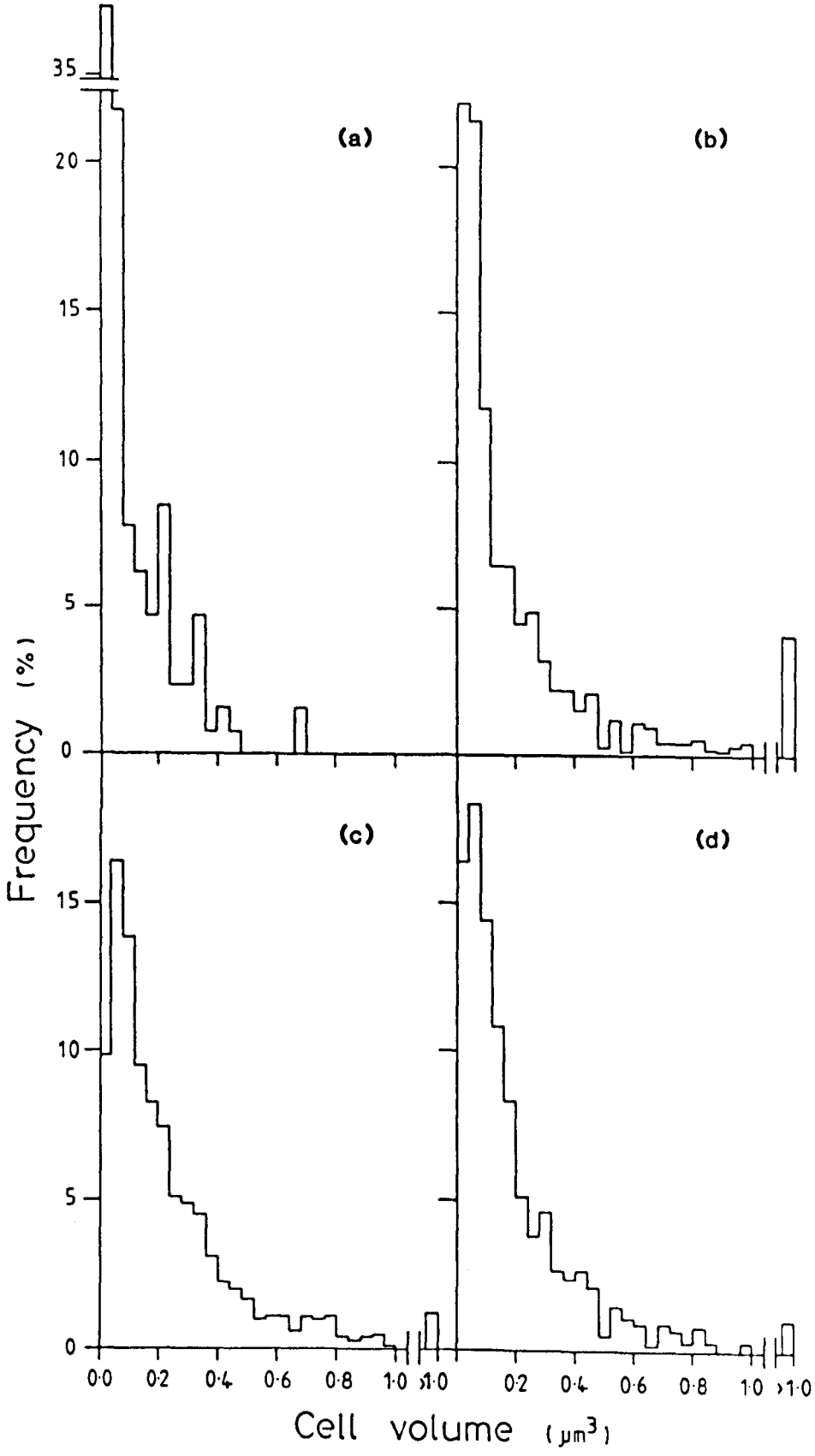


Figure 8.1 Mill Beck, 17 March 1985. Percentage frequency of different bacterial cell volumes; (a) suspended bacteria at spring-fed source, (b) suspended bacteria at lower limit of vegetated section (c.360 m), (c) epiphytic bacteria of Nasturtium officinale and (d) epiphytic bacteria of Apium nodiflorum.

method of image analysis as used here. Smaller cell volumes have been reported using optical microscope methods, for example, 0.042 μm^3 and 0.048 μm^3 (Riemann, Fuhrman & Azam, 1982) and 0.09 μm^3 (Riemann, 1983) for suspended lake bacteria, 0.13 μm^3 for stream sediments (Bott & Kaplan, 1985) and a range of 0.076-0.21 μm^3 for groundwater (Marxsen, 1981).

Fry & Zia (1982), measuring projected photographs of cells, found no difference in the mean volumes of bacteria from spring water (0.51 μm^3) and a canal, channel and lake with stands of macrophytes (means 0.32-0.55 μm^3), however, the proportion of small cells (<0.2 μm^3) was 60.1% in the spring water compared with 37.7-45.5% in the water bodies with macrophytes. Although mean cell volumes were smaller in Mill Beck, there was a similar shift in frequency distribution, with 77.8% of cells <0.2 μm^3 at the source of Mill Beck, compared with 68.4% at 360 m and 57.7% and 68.2% for epiphytic bacteria of N.officinale and A.nodiflorum (Fig. 8.1).

The similarity in volume between epiphytic and suspended bacteria at 360 m suggests that the difference in Vmax per bacterium (Table 7.7) between populations of suspended and epiphytic bacteria in Mill Beck may have been due to lower activity per unit biomass in the suspended population, rather than a smaller mean cell volume. It is possible that the less active of the epiphytic bacterial cells are more liable to detachment. This is because the physiological state of a bacterium may affect its adherence to surfaces (Fletcher, 1977, 1979, 1980) which may, in part, be due to the ability to produce and maintain an adhesive, extracellular polysacharide matrix (Costerton, Geesey & Cheng, 1978; McEldowney & Fletcher 1986).

CHAPTER 9

The attachment rate of suspended bacteria to submerged vegetation

9.1 Introduction.

The downstream increase in total number and heterotrophic capacity of suspended bacteria over 48 m at the source of Mill Beck may have been due to detachment of epiphytic bacteria (Chapter 7), although the actual detachment rate may have been larger than the observed downstream increase, because of possible losses of suspended bacteria through reattachment to plant surfaces within the section.

Attachment rates may be assessed using artificial substrata, but such rates depend upon the physical and chemical nature of the surface used (Mills & Maubrey, 1981; Baker, 1984), and may differ from attachment rates to plant surfaces. The use of real plants as a colonization substratum is attractive but may be complicated by cell division of the epiphytic population already present, and changes in the colonizable area brought about by leaf growth. Problems of detachment and predation by protozoa, invertebrates and possibly algae (Bird & Kalff, 1986), are common to methods using both plants and artificial substrata. Equations describing simultaneous growth and attachment have been used by Hossell & Baker (1979c) and Caldwell et al (1981,1983) to separate the two components, but neither method allowed for predation of attached populations.

A method for assessing attachment rates was developed, using laboratory-grown plants initially having a low level of colonization, which allowed detection of small changes in population size. Problems due to predation, cell division and leaf growth were minimized by using short (maximum 10 h) colonization periods. Three exercises were carried out with

laboratory-grown plants as follows: (1) A laboratory investigation to determine whether a measurable increase in epiphytic bacteria, ascribable to attachment is obtained during submergence in stream water over times up to 10 h. (2) An investigation to confirm that measurable attachment of bacteria is also obtainable in the field. (3) The determination of attachment rate in the field over a range of concentrations of suspended bacteria in the surrounding water.

9.2 Methods.

9.2.i Growth of minimally-colonized plants.

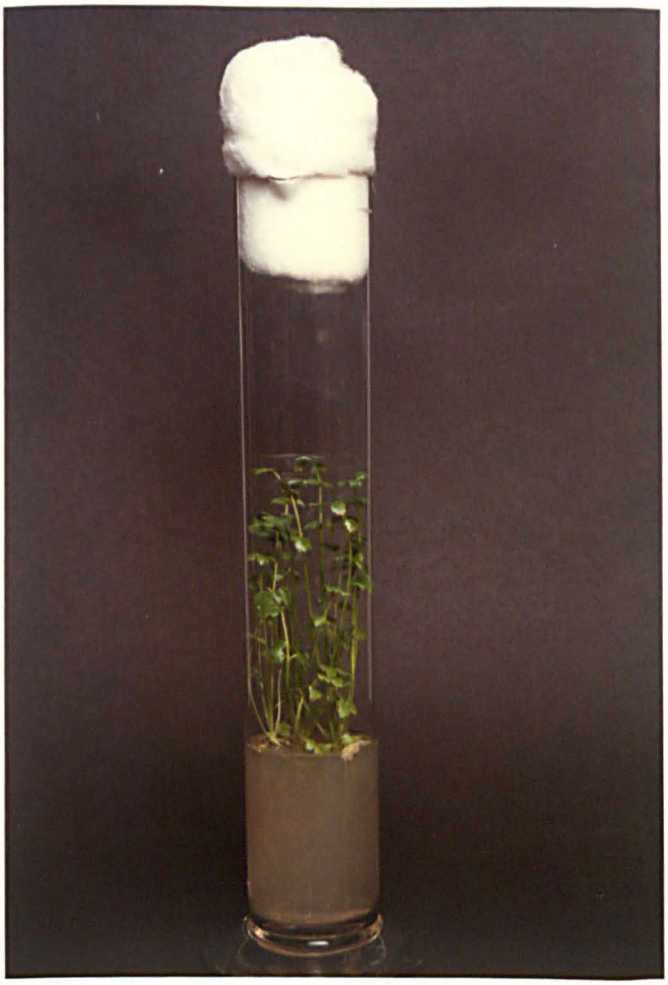
Seeds of Apium nodiflorum (supplied by the University of London Botanical Supply Unit) were placed in a small gauze packet to facilitate handling, then washed for 2 min in 1.0 g l^{-1} mercuric chloride solution and rinsed thoroughly with sterile water. The seeds were transfered to moist sterile filter paper, and germination occured after c.1 week at 20°C, under low light conditions. Seedlings were grown on inorganic-nutrient agar (4 g l^{-1} in double-strength Long Ashton solution; Hewitt, 1966) firstly in sterile 50 ml wide-necked conical flasks (Plate 9.1.a) and, after 2-3 weeks in 1 litre measuring cylinders (Plate 9.1.b), both stoppered with cotton wool. The plants were irrigated with sterile nutrient solution, at c.3-4 week intervals. Growth for 10-15 weeks at 15-20°C under white fluorescent lamps yielded plants with c.150-200 leaflets and dry weight of c.0.5-1.0 g.

Plants with visible bacterial or fungal colonies on their rooting agar were discarded, but nevertheless the plants which were used had some epiphytic bacteria on their leaves; generally substantially less than 10^6 cm^{-2} (released by 5 min treatment in a stomacher and assessed by AODC's; 3.4). These densities were however very low when compared to natural

Plate 9.1 Minimally-colonized Apium nodiflorum plants grown on inorganic-nutient agar for (a) c.3 weeks and (b) c.12 weeks.

(a)

(b)



populations on submerged leaf surfaces of up to 5.3 x 10^7 cm^{-2} on A.nodiflorum in Church Beck, Millington Beck and Mill Beck (Table 6.1) and 13.1 x 10^7 cm^{-2} for all species at the source of Mill Beck (7.5).

9.2.ii Attachment rate in the laboratory.

A laboratory-grown plant of A.nodiflorum with a weight attached (and a replicate at a later date) was submerged in 5 litres of freshly collected Mill Beck water, with suspended cell concentration (by AODC) of 2.3 (or 1.8, replicate run) x 10^5 ml^{-1}, and maintained in subdued light at 10°C. At intervals over 10 h, five leaflets (one apical, two first and two second leaflets all from different petioles) were removed to 10 ml of 0.2 um membrane-filtered water for immediate stomaching. At 10 h, the plant was transfered to 5 litres of 0.2 µm membrane-filtered stream water and five more leaflets were removed after a further 10 (or 14) h.

9.2.iii Attachment rate in the field.

Weighted, individual laboratory-grown plants were submerged in Church Beck (Fig. 2.1.b and c), in October 1984 at c.75 m, 200 m and 400 m downstream from the source, where suspended cell concentrations were 2.9, 7.8 and 10.1 x 10^5 ml^{-1}, respectively. Water temperature was c.10°C. At 2 h intervals over 10 h, five leaflets were removed (as in 9.2.ii) from each plant and transfered to 10 ml of 0.2 µm membrane-filtered, 2% formaldyhyde solution for preservation prior to stomaching.

9.2.iv Attachment rate in the field over a range of concentrations of suspended bacteria.

A range of concentrations of suspended bacteria was obtained by utilizing the natural downstream increase in Church Beck. Batches of five

plants were submerged on two dates during September and October 1984 at c.75, 125, 200, 300 and 400 m downstream of the source, where concentration of suspended bacteria increased from 3.1×10^5 ml^{-1} to 11.4×10^5 ml^{-1} (day 1) or 2.9×10^5 ml^{-1} to 10.1×10^5 ml^{-1} (day 2). Water temperature was c.10°C. At 0 h and 9.5 (or 10) h, five leaflets were removed from each plant (as in 9.2.ii) and the 25 leaflets from each site were combined in 10 ml of 2% formaldyhyde.

9.2.v Assessment of attached epiphytic bacteria.

The batches of removed leaflets were treated by stomaching for 5 min (6.1.i-ii) then cell concentration was determined by AODC. The mean surface area of leaflets was estimated from photocopies on gridded paper, of five or 10 representative leaflets from each plant. Linear regression lines of density of epiphytic bacteria against attachment time or concentration of suspended bacteria, were fitted using SPSS New Regression (Hull & Nie, 1981). The mean proportion of epiphytic bacteria removed by stomaching from A.nodiflorum growing naturally was 0.65 (Table 6.1). To check that a similar proportion was removed from laboratory grown plants with short colonization periods, two further comparisons between PAB counts and AODC's on stomachates were carried out. This yielded values of proportion removed of 0.61 and 0.67. The mean value of 0.64 was used to correct numbers of epiphytic bacteria in the three investigations.

9.3 Results.

The laboratory investigation demonstrated a linear increase over 10 h in density of epiphytic bacteria on A.nodiflorum leaflets in stream water, which ceased on transfer to 0.2 µm-filtered stream water (Fig. 9.1). The regression coefficients (attachment rates) were 2.1 and 2.2×10^5

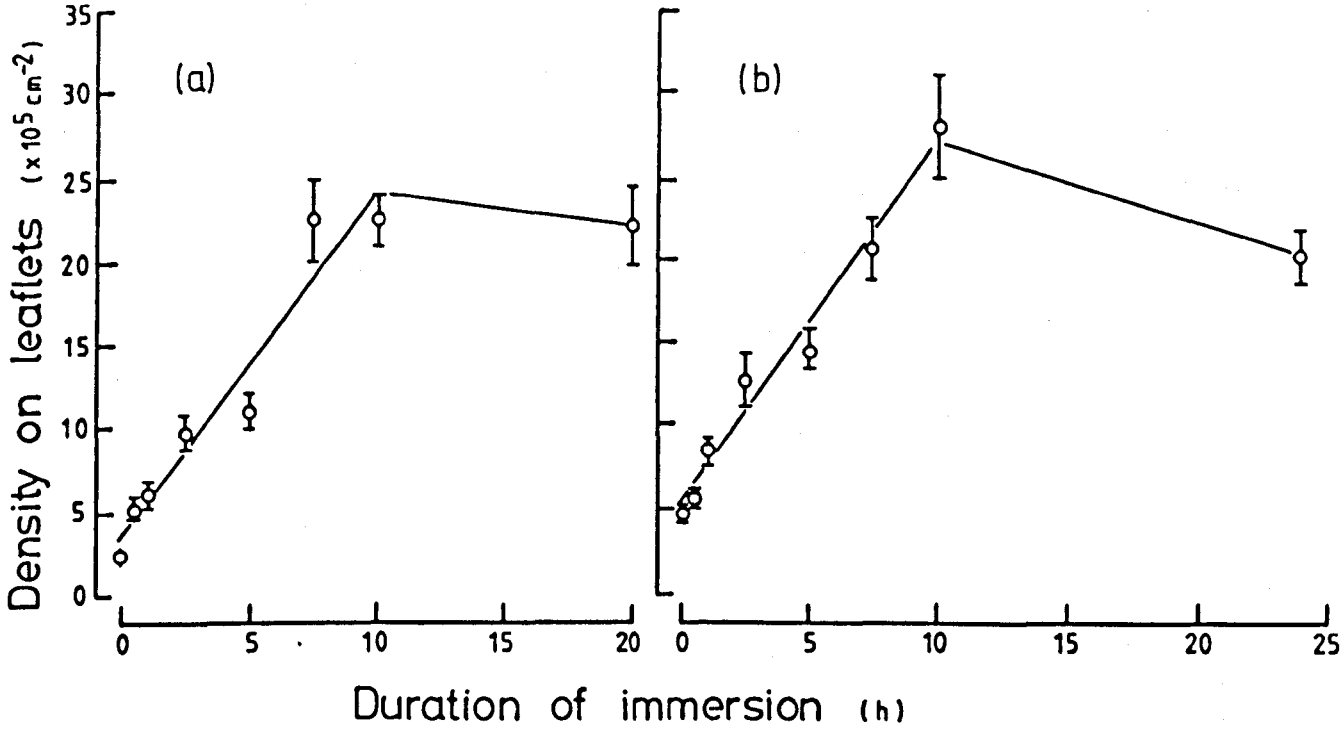


Figure 9.1 Apium nodiflorum immersed in stream water in the laboratory. The relationship between density of bacteria on leaflets and time (vertical bars indicate 95% confidence limits). Mean concentrations of suspended bacteria over 0–10 h were (a) 2.34 x 10^5 ml^{-1}; and (b) 1.81 x 10^5 ml^{-1}; plants were transfered to membrane-filtered (0.2 µm) stream water at 10 h. F values for regression over 0–10 h were (a) 69.4 ($P < 0.001$) and (b) 204.6 ($P < 0.001$); the regression coefficients (attachment rates) equalled (a) 2.1 x 10^5 $cm^{-2}h^{-1}$ and (b) 2.2 x 10^5 $cm^{-2}h^{-1}$.

bacteria $cm^{-2}h^{-1}$. A linear increase was also obtained in the field (Fig. 9.2), with attachment rates of 0.9, 2.0 and 1.7 x 10^5 bacteria $cm^{-2}h^{-1}$, at 75, 200 and 400 m. When plants were submerged along the length of the stream, over a range of concentrations of suspended bacteria, the attachment rate increased linearly with increase in concentration of bacteria in the surrounding water (Fig. 9.3). Attachment rate values from Fig 9.2 were also included in Fig. 9.3. The regression was highly significant ($P < 0.001$), and the regression coefficient (Fig. 9.3) gave an attachment rate relative to bacterial concentration of 1.7 x 10^4 bacteria $cm^{-2}h^{-1}$ for each 10^5 bacteria ml^{-1} in the surrounding water.

9.4 Discussion.

The increase in density of epiphytic bacteria on leaf surfaces in stream water in the laboratory, over 10 h (Fig. 9.1), was potentially due to both attachment and cell division, although it probably chiefly represented attachment because, (1) there was no increase in cell density after transfer to 0.2 um filtered water, (2) the relationship was linear rather than exponential and (3) the doubling time required to produce the observed 5-10 fold increase by cell division (1-2 h), is much shorter than most published values for bacteria in freshwater (1.1.iii and 7.5). The linear increase obtained in the field (Fig. 9.2), therefore can also probably be ascribed to attachment.

Since laboratory studies have shown that attachment to surfaces is dependent on suspended-cell concentration (Fletcher, 1977; Paul, 1984), it is reasonable to suggest that the greater attachment rates at the more downstream sites in Church Beck were a direct result of increased concentration of suspended bacteria. There may, however, have been a

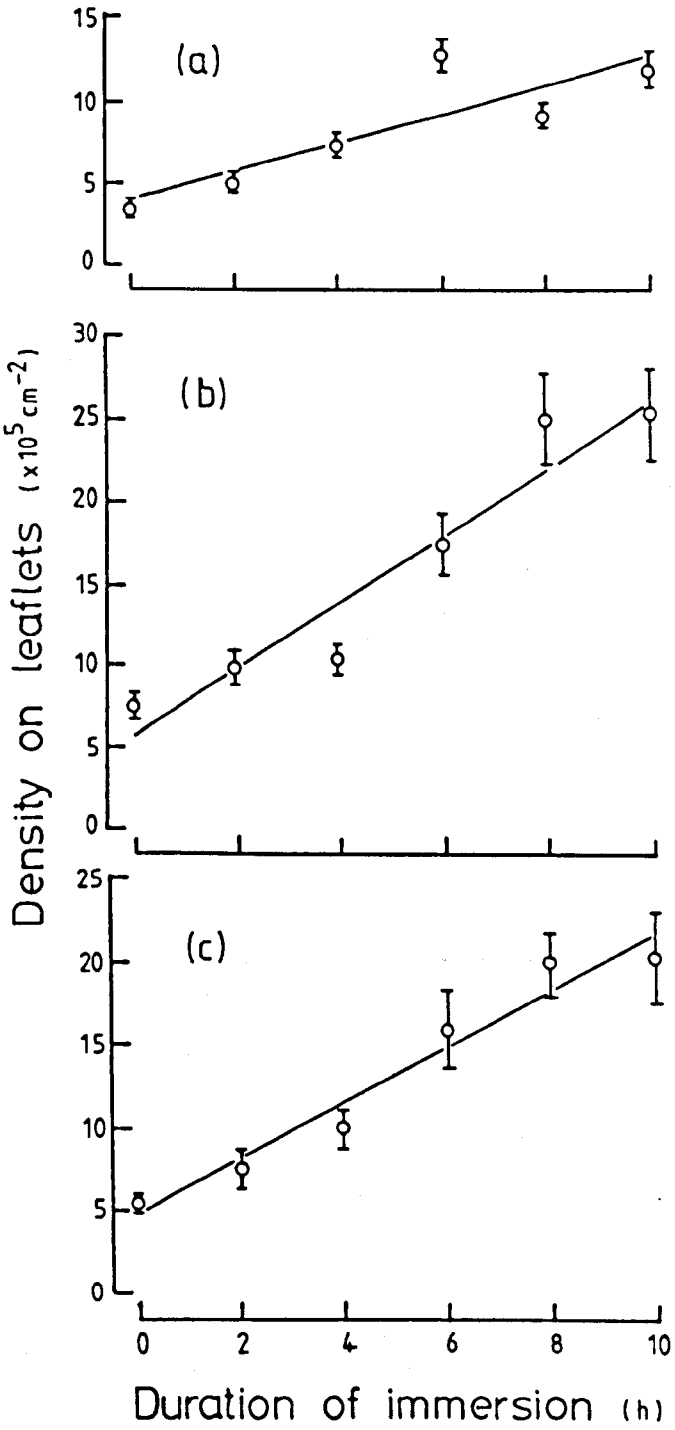


Figure 9.2 Apium nodiflorum immersed in Church Beck, 3 October 1984. The relationship between density of bacteria on leaflets and time (vertical bars indicate 95% confidence limits). Plants were submerged at about (a) 75 m, (b) 200 m and (c) 400 m below the source; concentrations of suspended bacteria at these stations were (a) 2.87 x 10^5 ml^{-1}, (b) 7.79 x 10^5 ml^{-1} and (c) 10.13 x 10^5 ml^{-1}. F values for regression were (a) 11.5 ($P < 0.05$), (b) 44.9 ($P < 0.01$) and (c) 87.6 ($P < 0.001$); the regression coefficients (attachment rates) equalled (a) 0.9 x 10^5 $cm^{-2}h^{-1}$, (b) 2.0 x 10^5 $cm^{-2}h^{-1}$ and (c) 1.7 x 10^5 $cm^{-2}h^{-1}$.

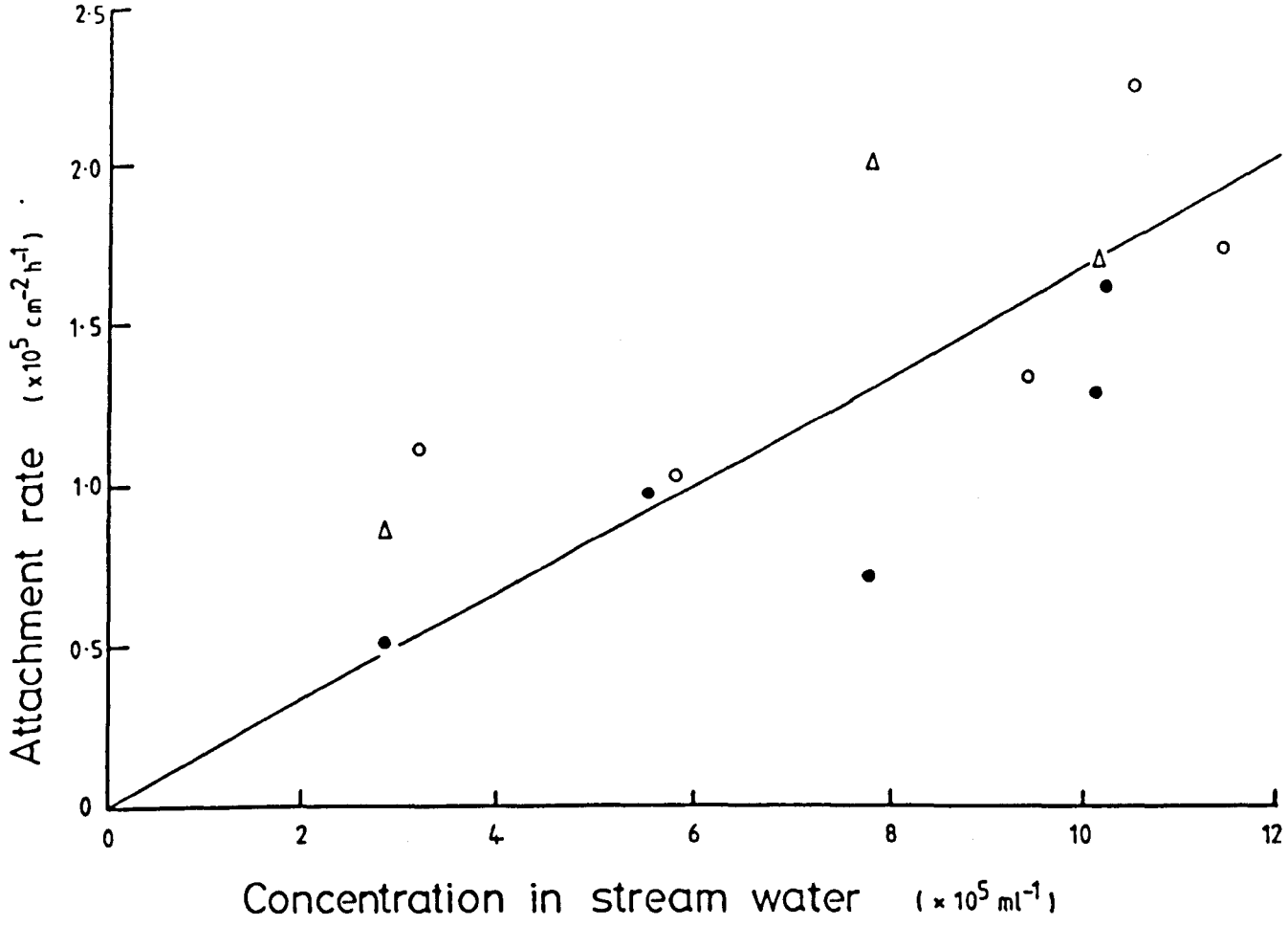


Figure 9.3 Apium nodiforum immersed in Church Beck. The relationship between rate of attachment of bacteria to leaflets and concentration of bacteria in the stream water. Plants were submerged, at distance intervals, for 9.5 or 10 h on 29 September (open circles) and 3 October 1984 (closed circles). Three further attachment-rate values, obtained from the data in Fig. 9.2, are also included (open triangles). The regression line was forced through the origin, $F = 154.8$ ($P < 0.001$). The regression coefficient (attachment rate relative to bacterial concentration) equalled 1.7×10^4 $cm^{-2}h^{-1}$ for each 10^5 ml^{-1}.

change in physiological state and taxonomic composition of bacteria along the stream and this might account for some of the increase in attachment rate. Also, velocity may have varied from site to site, although Baker (1984), found that attachment of bacteria to an artificial substratum in a chalk stream was similar at current speeds of both <0.1 m s^{-1} and >0.3 m s^{-1}.

The attachment rate relative to bacterial concentration of 1.7×10^4 $cm^{-2}h^{-1}$ for each 10^5 bacteria ml^{-1} in the surrounding water was somewhat higher than the approximate rate of $0.1\text{-}0.5 \times 10^4$ $cm^{-2}h^{-1}$ for each 10^5 bacteria ml^{-1} calculated from Fig. 1 in Fletcher (1977). However, Fletcher was assessing attachment to polystyrene by a marine pseudomonad in culture at concentrations of 10^9 ml^{-1}, moreover, she was also removing loosely attached cells before counting.

Short colonization periods were used to minimize cell losses due to detachment and predation. The laboratory investigation, however, showed a slight decrease in density of epiphytic bacteria after transfer to 0.2 µm membrane-filtered water (Fig. 9.1), which may have been due to detachment of loosely adsorbed cells. The attachment rates in the laboratory ($2.1\text{-}2.2 \times 10^5$ cells $cm^{-2}h^{-1}$) were somewhat higher than those obtained in the field ($0.9\text{-}2.0 \times 10^5$ cells $cm^{-2}h^{-1}$) despite lower suspended cell concentrations ($1.8\text{-}2.3 \times 10^5$ ml^{-1} compared with $2.9\text{-}10.1 \times 10^5$ ml^{-1}). One explanation of this is increased predation in the field experiment, where the plants were in contact with the stream bed fauna. If there was appreciable loss by detachment and predation, the attachment rates are underestimates of the true values. They are, however an order of magnitude higher than values of $0.4\text{-}1.6 \times 10^4$ $cm^{-2}h^{-1}$ for artificial substrata (Baker, 1984) and 2.4×10^4 $cm^{-2}h^{-1}$ for Lemna minor (Hossell & Baker, 1979c) in the calcareous River Frome, where colonization periods were from 11-20 days, and there was thus much more opportunity for

Table 9.1 Mill Beck, source to 48 m, May–June 1983. Estimation of attachment rate of suspended bacteria to submerged vegetation.

	10–13 May	24–27 May	7–10 June
Total dry weight of vegetation (kg) (a)	81	85	100
Total surface area of vegetation (m^2) (b)	3848	2755	4750
Mean concentration of suspended bacteria ($x10^5 ml^{-1}$) (c)	0.58	0.57	0.65
Attachment rate ($x10^4$bacteria $cm^{-2}h^{-1}$) (d)	0.99	0.97	1.11
Total attachment ($x10^{12}$bacteria d^{-1})	9.1	6.4	12.6
Total epiphytic bacteria ($x10^{15}$) (e)	3.6	4.1	6.2
Attachment as % of total epiphytic bacteria (d^{-1})	0.3	0.2	0.2

(a) From data in Table 7.3.
(b) Using surface area per unit dry weight conversion factor of 475 cm^2g^{-1}, mean for all species during May–June 1984 (n = 12), Table 10.1.
(c) From data in Table 7.1.
(d) Calculated using attachment rate of 1.7×10^4 bacteria $cm^{-2}h^{-1}$ per 10^5 bacteria ml^{-1}.
(e) From data in Table 7.2.

the development of a community of grazers.

The attachment rate relative to bacterial concentration of 1.7×10^4 bacteria $cm^{-2}h^{-1}$ for each 10^5 bacteria ml^{-1}, can be used to estimate total reattachment of suspended bacteria to plant surfaces within a section of stream. If it is assumed that attachment rate relative to concentration of suspended bacteria was similar in Mill Beck to that in Church Beck, an estimation can be made of the reattachment of suspended bacteria which took place during the study of the 48 m section below the source of Mill Beck described in Chapter 7 (Table 9.1). By applying the conversion factor for mean surface area of macrophyte per unit dry weight (Chapter 10) for all species categories in Mill Beck during May-June 1984 ($475\ cm^2g^{-1}$, n=12; Table 10.1) to the total dry weight of vegetation (81-100 kg; Table 7.3), the estimated total plant-surface area was 2755-4750 m^2. The mean concentration of suspended bacteria (derived from Table 7.1) gave attachment rates of $0.97\text{-}1.11 \times 10^4$ bacteria $cm^{-2}h^{-1}$, and when applied to the total area of vegetation, yielded overall attachment rates of $6.4\text{-}12.6 \times 10^{12}$ bacteria d^{-1}. This represented a very low proportion, at 0.2-0.3% d^{-1} of total epiphytic bacteria ($3.6\text{-}6.2 \times 10^{15}$; Table 7.2). Net daily drift loss ($4.3\text{-}6.7 \times 10^{14}\ d^{-1}$; Table 7.1) was thus not a serious underestimate of total drift loss.

CHAPTER 10

Relationships between suspended bacteria, epiphytic bacteria and submerged vegetation in Mill Beck over a spring growing period (January-June).

10.1 Introduction.

The investigation described in Chapter 7, showed that the population of epiphytic bacteria over 48 m below the source of Mill Beck, during May-June 1983 was probably adequate to sustain the observed downstream losses of suspended bacteria. The study did not exclude, however, the possibility of input of cells from groundwater or release from stream sediments. Moreover, the density of the epiphytic population and rate of release of bacteria to the water column may vary according to, for example, discharge, current velocity, water temperature and extent, species composition and age of submerged vegetation. Therefore, a further study was carried out to investigate the relationships between suspended bacteria, epiphytic bacteria and submerged vegetation, in a vegetated section of Mill Beck, over a five month period (January-June 1984), during which time there was a seasonal fall in discharge and increase in vegetation. In addition, in order to assess any downstream changes in the suspended bacterial population that might occur in the absence of submerged vegetation and its associated epiphytic population, a further section of stream was studied, which was physically similar, apart from being heavily shaded and thus devoid of vegetation.

10.2 Description of sites.

Mill Beck was described in 7.2.i. The sites studied were: (1) a richly vegetated 135 m section in open pasture (Plate 10.1), which extended from a point about 225 m below the source to a bridge carrying a farm track over the stream (section (b) in Fig. 7.1); (2) a heavily shaded, vegetation-free 150 m section (Plate 10.2), in woodland, which extended downstream from a point approximately 1100 m below the source (section (c) in Fig. 7.1).

In the vegetated section, the plants were rooted in silt or chalky gravel; in the vegetation-free section the stream bed was principally of silt with some accumulation of tree debris. Physical features such as width, depth and current velocity were superficially similar in the two sections.

10.3 Methods.

Field work was during eight replicate two-day blocks over January-June 1984, except in early March when a day intervened between sampling days.

On day one, discharge at the upstream and downstream limits of both sections of stream was estimated from measurements of depth and velocity at 0.6 of depth (Ott small current meter), at 0.10 or 0.15 m intervals across stream widths of 1.25-3.3 m. In the vegetated section these measurements were made at convenient points with regular cross-section, i.e. an open brickwork culvert adjacent to the upstream limit and beneath the bridge at the downstream limit. In the vegetation-free section points with regular cross-section were dug out at the upstream and downstream limits. Stream temperature (mercury-in-glass thermometer) was measured at the upstream limit of both sections; pH, conductivity (laboratory meters) and relative

Plate 10.1 Mill Beck, April 1986. Vegetated section, 225-360 m below the source. View from c.355 m, looking upstream. The emergent plants are mainly Glyceria fluitans with Rumex longifolius in the foreground. Submerged Nasturtium officinale and Apium nodiflorum can be seen on the left of the photograph.

Plate 10.2 Mill Beck, May 1986. Vegetation-free section, 1100-1250 m below the source. View from c.1225 m looking upstream.

Plate 10.3 Mill Beck, showing increase in submerged and emergent macrophytes from January to June 1984, in the vegetated section, 225-360 m below the source. View from c.360 m looking upstream. (a) 30 January, (b) 16 April and (c) 25 June.

turbidity (laboratory nephelometer) were measured at both upstream and downstream limits.

Water samples (10 ml) were collected on day one, at 15 m intervals along both sections. Samples were taken (downstream samples first) from mid-stream at c.10 cm depth using a displacement pipette, and transfered to sterile universal bottles which contained 0.5 ml of 0.2 µm membrane-filtered neutral formaldehyde; this gave a final concentration of 2% formalin. During the May and June samping blocks, a replicate series of samples was collected in the vegetated section on day 2. Total bacteria were counted in these samples within 10 days (AODC method, 3.4) and downstream increase in concentration of suspended bacteria was obtained by linear regression of concentration against distance (SPSS New Regression; Hull & Nie, 1981). Drift loss of bacteria per day from within the section equalled downstream increase in concentration of bacteria times the discharge. When replicate values of downstream increase were available from successive days, the mean was used.

On day two, submerged plant shoots were collected from the vegetated section for assessment of density of epiphytic bacteria. The procedure was slightly different from that used during the study at Mill Beck source (Chapter 7), this was in order to gain more information about possible between-species variation, and difference in colonization between leaves and stems.

The section of stream was divided into three 45 m segments, and two points were located in each segment using random co-ordinates. Two plant shoots representing each of the following categories: (1) Nasturtium officinale, (2) Apium nodiflorum, (3) Glyceria fluitans and (4) other species, were collected at, or as near as possible to each of six random points (after removing any emergent foliage). One set of shoots was used to determine the surface area to dry weight ratio for each plant category

(6.2.ii). The shoots in the second set were transfered to sterile stomacher bags (three shoots of the same category per bag); the six shoots in each category were separated into (i) leaves and (ii) stems plus petioles, using forceps and scissors, and each fraction was replaced in one of their original stomacher bags. Epiphytes were removed from the plant material by stomaching for 5 min (6.1.i-ii), using 150 ml of both stomaching and rinsing water. The total bacteria in the stomachates were determined by AODC; results were expressed per gram dry weight of plant material (6.1.v) and corrected for 62% stomaching efficiency (6.1.iii).

Eighteen 25 x 25 cm quadrats of submerged vegetation were harvested on day two, for the determination of dry weight of plant material per unit area of stream bed, as described in 6.2.i. In addition, six of the harvested quadrats were sorted into (i) leaves and (ii) stems plus petioles for each vegetation category, and each fraction was dried and weighed separately. The percentage cover of vegetation was obtained by recording the presence (submerged or emergent) or absence of vegetation at 150 randomly located points (method 2; 6.2.iii). The total stream bed area of the vegetated section was determined from a map drawn in the field at 1:100 (method 1; 6.2.iii) on 3 June 1984.

Stream depth was determined at 24 random locations within the vegetated section during each sampling block and the mean value was combined with stream bed area and discharge to estimate retention time of water within the section and mean current velocity.

The epiphyte and vegetation data were combined, as in Chapter 7, to yield (1) density of epiphytic bacteria per unit dry weight of each vegetation category, (2) total epiphytic bacteria, (3) drift loss per day of suspended bacteria from the vegetated section expressed as percentage of total population of epiphytic bacteria and (4) doubling time required by the epiphytic bacteria to compensate for drift loss.

The plant-surface area per unit dry weight for each vegetation category was combined with vegetation dry weight data to estimate (1) density of epiphytic bacteria per unit surface area of each vegetation category, (2) mean density on (i) leaves and (ii) stems plus petioles and (3) total submerged plant-surface area in the vegetated section. Estimation of plant-surface area per unit dry weight was not made during January, therefore the February results were used to convert the January dry weights to surface areas.

An estimate was made of the number of suspended bacteria which became attached per day to submerged vegetation within the vegetated section. The attachment rate relative to concentration of 1.7×10^4 bacteria $cm^{-2}h^{-1}$ for each 10^5 bacteria ml^{-1} (Chapter 9) was used, together with estimates of the total surface area of submerged vegetation and the mean concentration of suspended bacteria in the vegetated section, which was taken to be the value at mid-point along the section, from the regression equation of concentration against distance (day 1).

10.4 Results.

10.4.i Physico-chemical variables.

Discharge (Fig. 10.1.a), which increased from late January to a maximum in mid February and then declined steadily to late June, was on average 16% greater in the vegetation-free, downstream section (range $0.43\text{-}3.38 \times 10^4$ m^3d^{-1}) than in the vegetated, upstream section ($0.41\text{-}3.24 \times 10^4$ m^3d^{-1}). In neither section were there appreciable differences in discharge between the upstream and downstream limits. Mean current velocity over the vegetated section showed a similar pattern (Fig. 10.1.a) with a maximum value of 0.25 m s^{-1} in mid-February decreasing to 0.04 m s^{-1} in June. Temperature, pH, conductivity and relative turbidity

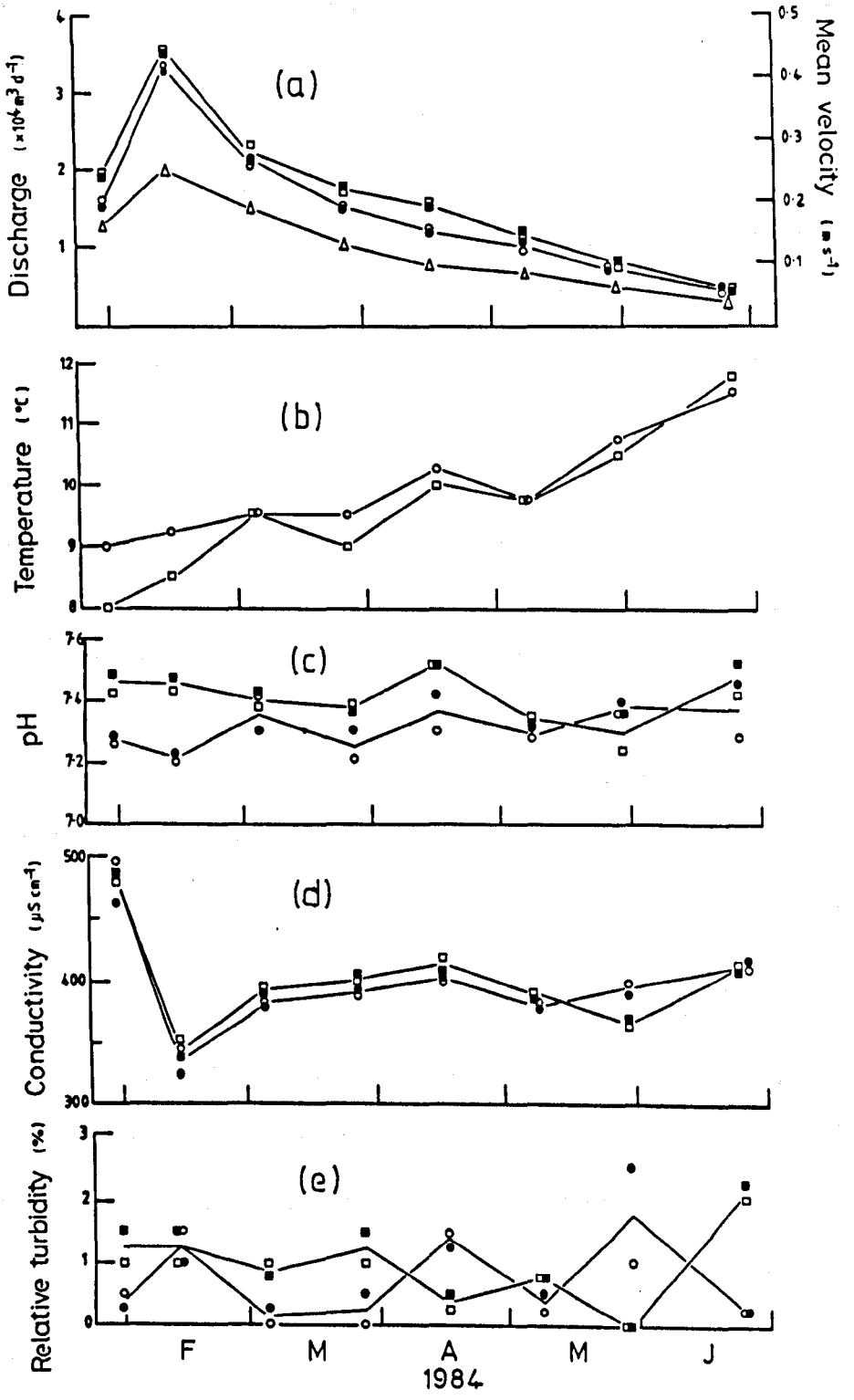


Figure 10.1 Mill Beck, January-June 1984. Physico-chemical variables; (a) discharge and current velocity, (b) water temperature, (c) pH, (d) conductivity and (e) turbidity. Circles indicate vegetated section (c.225-360 m below the source), squares indicate vegetation-free section (c.1100-1250 m below the source), open circles and squares indicate upstream measurements, solid circles and squares indicate downstream measurements and triangles indicate mean velocity over the vegetated section.

are shown in Figs. 10.1.b-e. Stream temperature increased slightly, over the sampling period. Spring water at the source is a constant c.10°C thus water temperature ranged only over 9-11.5°C in the vegetated section and 8-11.75°C in the vegetation-free section; the water cooled or warmed up along the stream depending upon air temperature. pH remained relatively constant from late January to June, ranging from 7.2-7.5. Conductivity was similar in both sections on each sampling occasion (range 324-495 $\mu S\ cm^{-1}$), and varied inversely with discharge. Relative turbidity was negligible (<3.0%) during the whole period.

10.4.ii Downstream drift loss of suspended bacteria as percentage of standing crop of epiphytic bacteria.

Downstream change in concentration of bacteria is shown in Fig. 10.2. In the vegetated section there was a significant ($P < 0.05$) downstream increase on all sampling occasions except for 30 January, whereas in the vegetation-free section there was usually no downstream change, although on two occasions there was a significant downstream decrease in concentration.

The downstream increase in concentration of suspended bacteria over the 135 m vegetated section (Fig. 10.3.a) ranged from $0.3 \times 10^5\ ml^{-1}$ in mid-February to $1.4 \times 10^5\ ml^{-1}$ in late May. Drift loss of bacteria per day from the vegetated section (Fig. 10.3.b) ranged from $0.4 \times 10^{15}\ d^{-1}$ in late June to $1.4 \times 10^{15}\ d^{-1}$ in early May. With the vegetation-free section, since there was never a significant downstream increase in bacterial concentration (Fig. 10.2), there was never a measurable drift loss of bacteria.

Mean density of epiphytic bacteria per unit dry weight of submerged vegetation (Fig.10.4.a) ranged from $1.0 \times 10^{10} g^{-1}$ to $4.0 \times 10^{10} g^{-1}$. The density decreased from late January to early March and then increased until June, with a high value in April. The dry weight of submerged

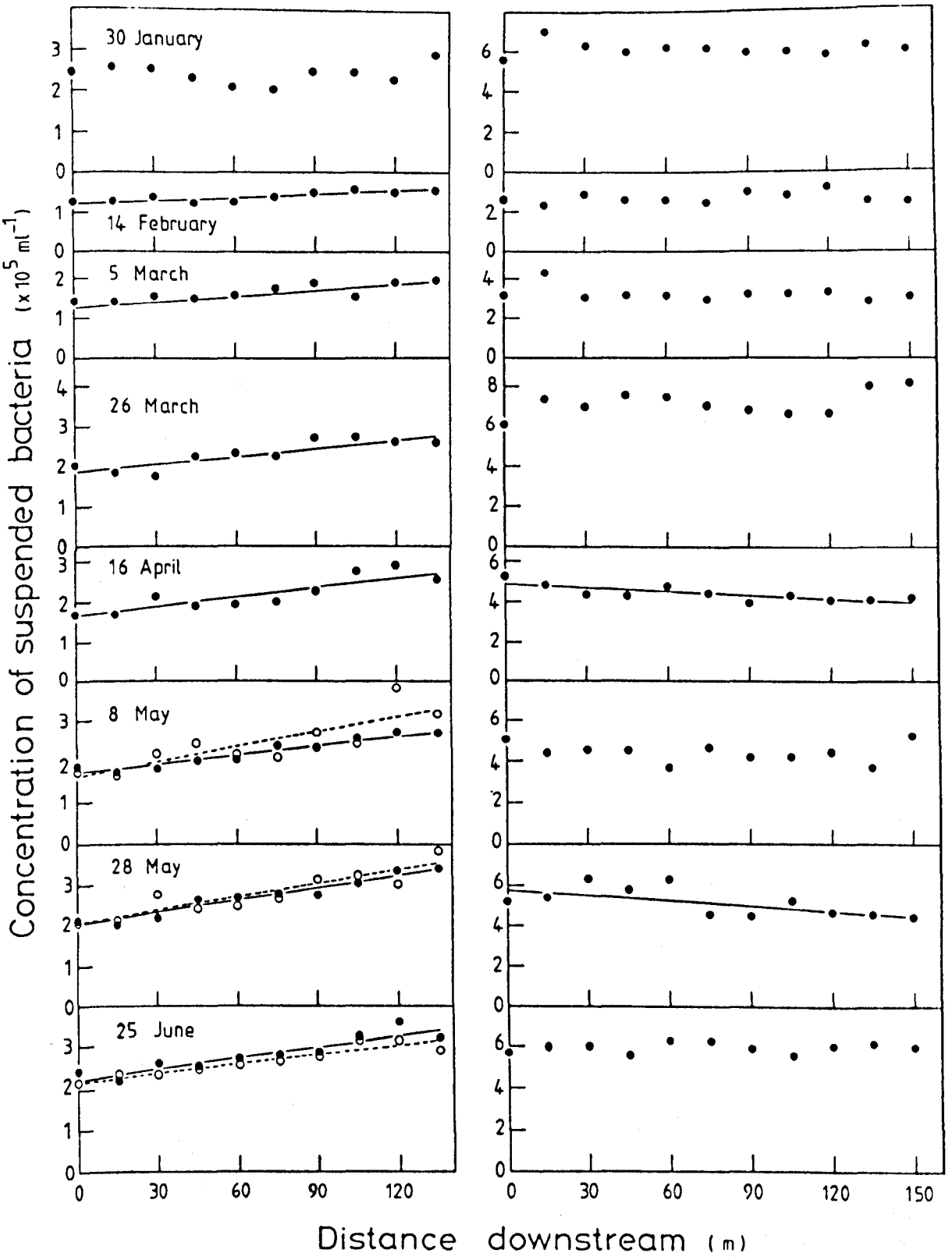


Figure 10.2 Mill Beck, January-June 1984. Relationship between concentration of suspended bacteria and distance; vegetated section c.225-360 m below the source (left), vegetation-free section c.1100-1250 m below the source (right). 95% confidence limits were plus or minus < 10% of the values shown. Straight lines indicate significant regression ($P < 0.05$); open symbols and broken lines refer to replicate samples taken on the day following the date shown.

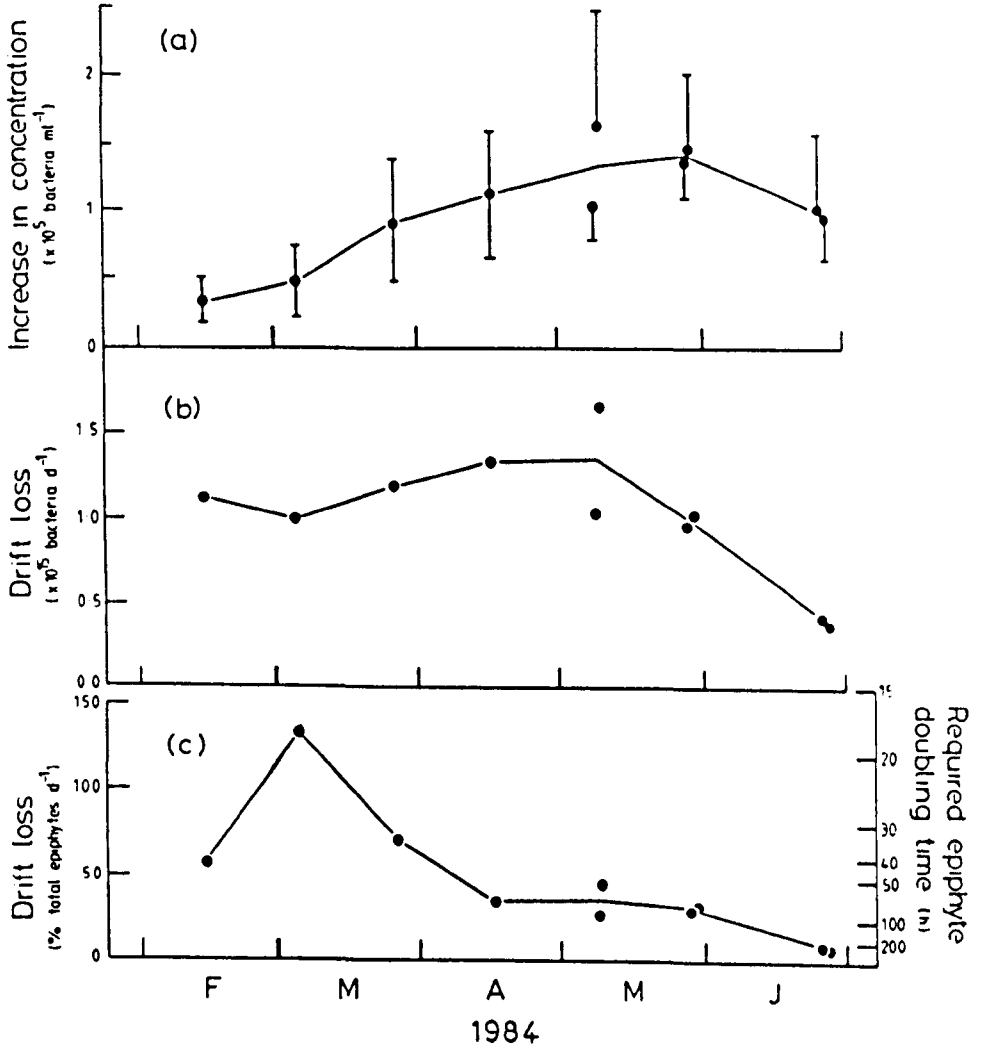


Figure 10.3 Mill Beck, January-June 1984. (a) Downstream increase in concentration of suspended bacteria over the vegetated section (c.225-360 m below the source); vertical lines are 95% confidence limits, derived from confidence limits around the regression coefficients obtained from regression of concentration against distance (Fig. 10.2). (b) Daily drift loss of suspended bacteria from within the vegetated section. (c) Daily drift loss of bacteria from within the vegetated section as percentage of total population of epiphytic bacteria, and doubling time required by epiphytic bacteria to maintain constant population.

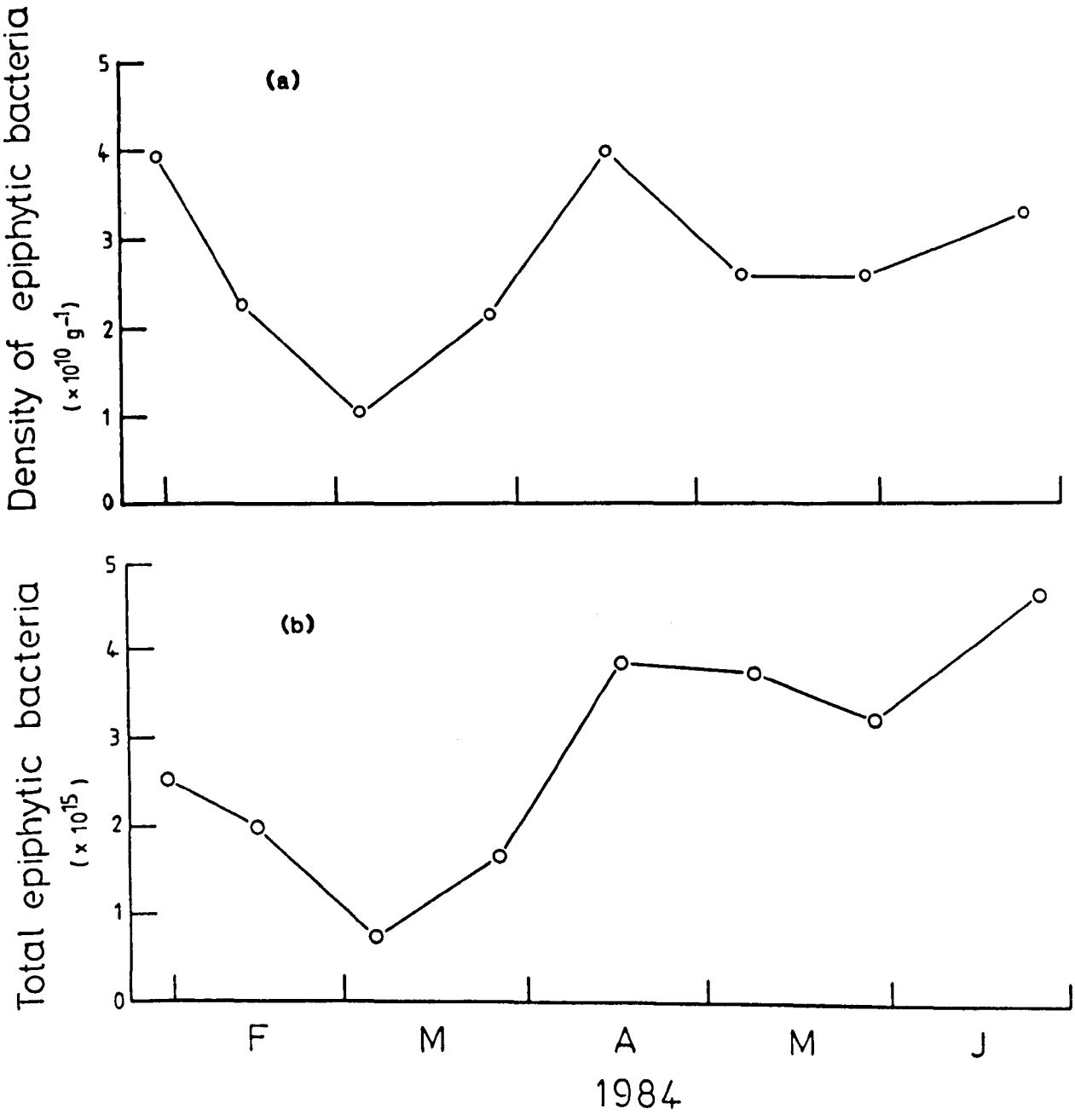


Figure 10.4 Mill Beck, January-June 1984. (a) Density of epiphytic bacteria per unit dry weight of submerged vegetation in the vegetated section (c.225-360 m below the source). Each value is derived from direct counts on eight suspensions of epiphytic bacteria (four vegetation categories; (i) leaves and (ii) stems plus petioles in each category). (b) Total epiphytic bacteria in the vegetated section.

vegetation per unit area of stream bed in the vegetated section (Fig. 10.5.a) increased from a minimum of 109 g m^{-2} in late January to a plateau by May-June (maximum 249 g m^{-2} in early May). The percentage cover of vegetation (Fig. 10.6.a) was fairly constant at 89-96%. The total area of stream bed, from the June survey (Fig. 10.7), was 640 m^2. Total dry weights of submerged vegetation in the vegetated section (Fig. 10.5.a) were thus found to increase from 67 kg in January to 131-150 kg during May-June. The number of total epiphytic bacteria (Fig. 10.4.b) decreased from 2.5 x 10^{15} in late January to a minimum of 0.8 x 10^{15} in early March and then increased to a maximum of 4.7 x 10^{15} in late June.

Drift loss per day of suspended bacteria, as percentage of total epiphytic bacteria (Fig. 10.3.c) increased from 57% d^{-1} in mid February to 132% d^{-1} in early March but then decreased rapidly to 35% d^{-1} by mid-April and to 9% d^{-1} by late June. Doubling times for maintenance of constant population of epiphytic bacteria, thus ranged from 18-255 h (Fig. 10.3.c) but were > 30 h on all but one occasion (early March) and > 65 h on five out of seven occasions.

10.4.iii Attachment of suspended bacteria to submerged plant surfaces.

Attachment rates of formerly-suspended bacteria, and the information used in their derivation, are given in Table 10.1. Submerged plant-surface area per unit dry weight ranged from 350-1021 cm^2g^{-1}, values for A.nodiflorum were usually higher than for the other vegetation categories, otherwise there was much irregular within- and between-category variation. The total plant-surface area of submerged vegetation ranged from 4143-7620 m^2, and the mean concentration of suspended bacteria in the vegetated section increased from 1.3 x 10^5 ml^{-1} in February to 2.8 x 10^5 ml^{-1} in June, thus attachment rate of bacteria per unit plant-surface area increased from 2.2 x 10^4 $cm^{-2}h^{-1}$ to 4.7 x

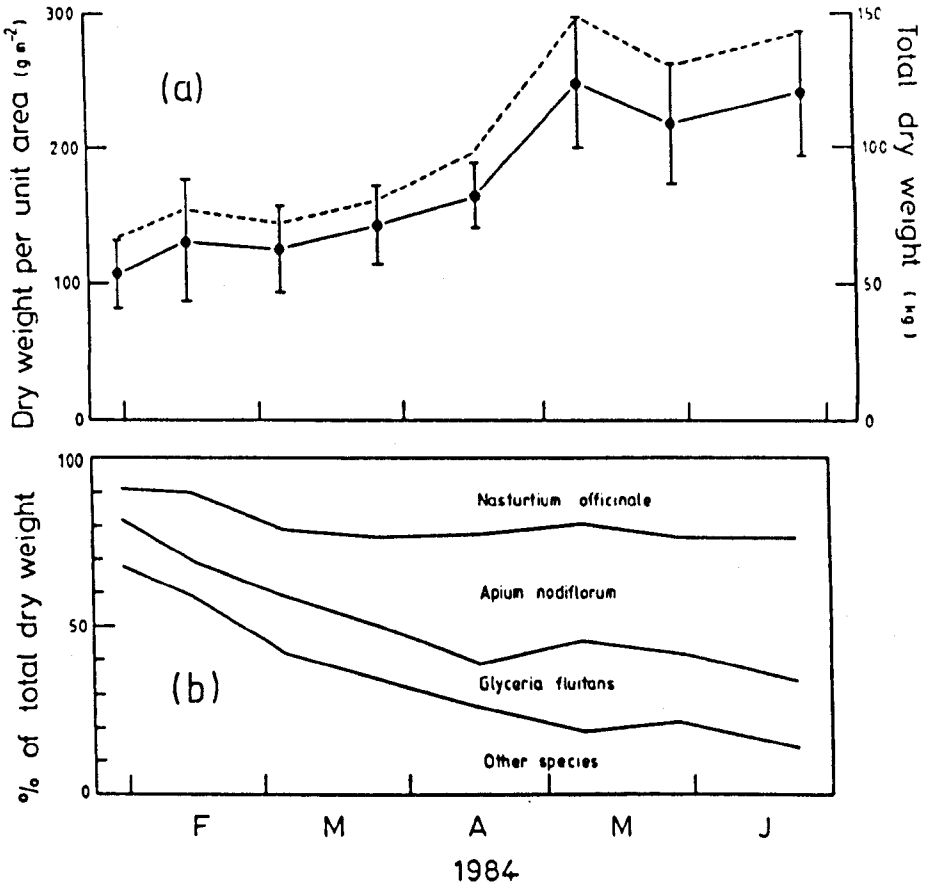


Figure 10.5 Mill Beck, January-June 1984. (a) Dry weight of submerged vegetation per unit area of stream bed (solid circles) with 95% confidence limits, and total dry wight of submerged vegetation (broken line) in the vegetated section (c.225-360 m below the source). (b) The percentage of total dry weight which was due to each vegetation category. The species composition of the other species category is given in the text.

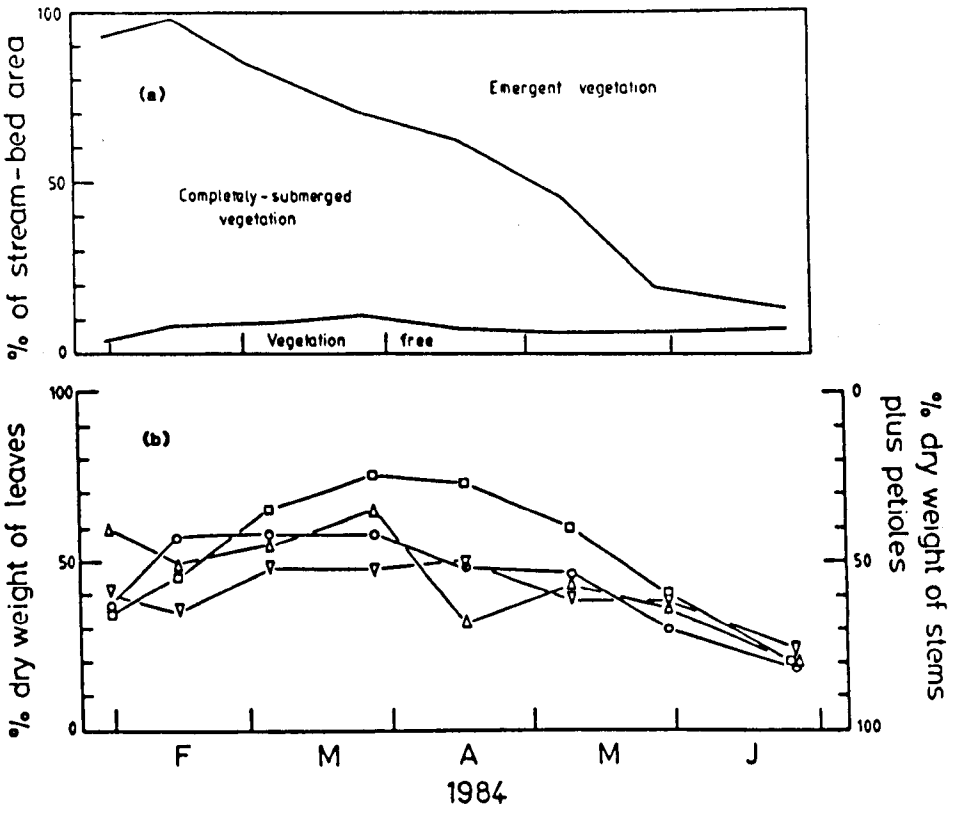


Figure 10.6 Mill Beck, January-June 1984. (a) The percentage of stream-bed area in the vegetated section (c.225-360 m below the source) which was free of vegetation, occupied by completely-submerged vegetation, or by emergent vegetation. (b) The percentage dry weight of submerged vegetation in the vegetated section, which comprised (i) leaves and (ii) stems plus petioles. Values are means for four vegetation categories; Nasturtium officinale (circles), Apium nodiflorum (squares), Glyceria fluitans (triangles) and other species (inverted triangles). The species composition of the other species category is given in the text.

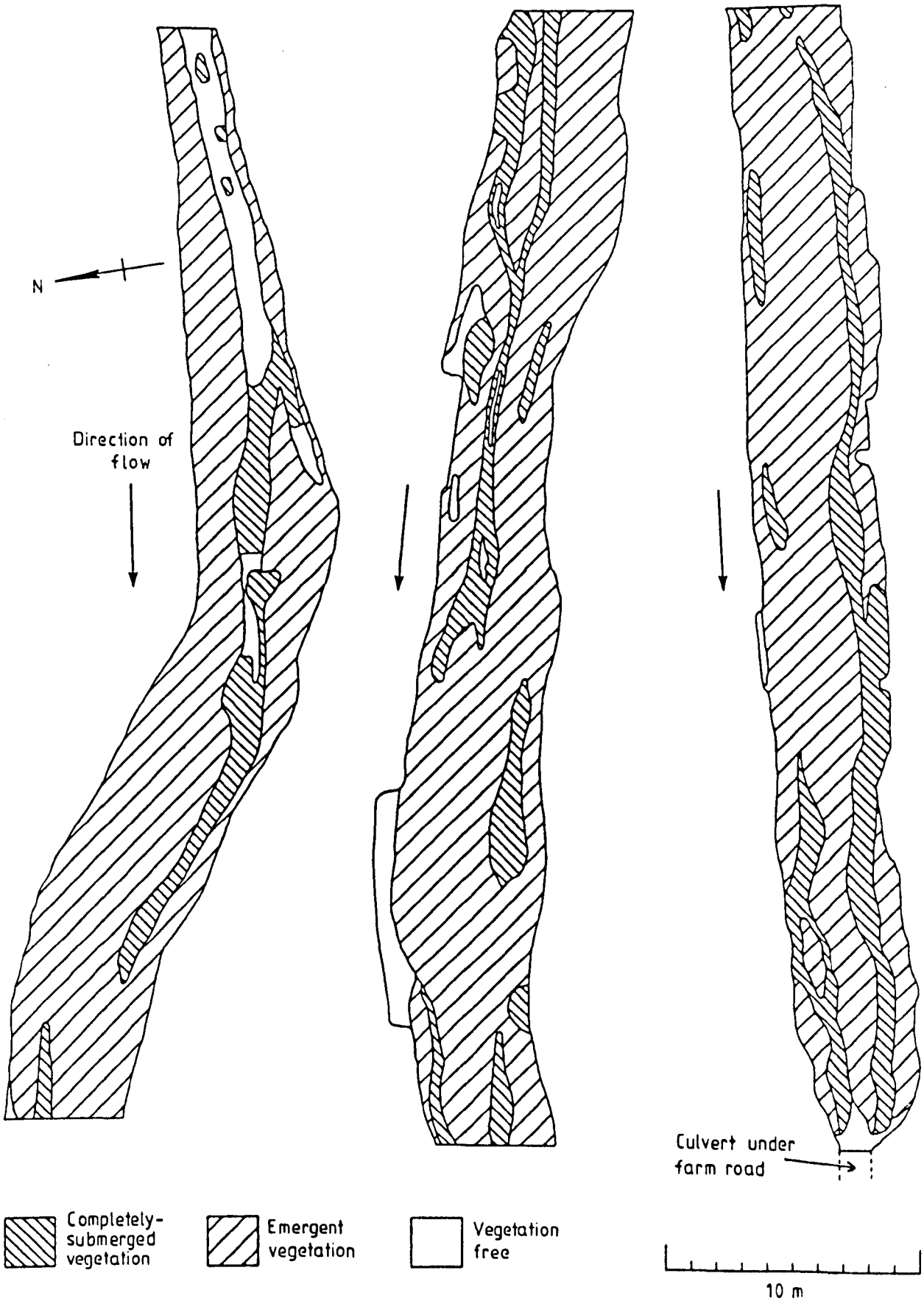


Figure 10.7 Mill Beck; map of the vegetated section (c.225-360 m below the source), from survey of 3 June 1984, divided into three 45 m segments.

Table 10.1 Mill Beck, c.225-360 m below the source, January-June 1984. Estimation of attachment rate of suspended bacteria to submerged vegetation.

Vegetation category	Sampling date							
	30-31 January	14-15 February	5-7 March	26-27 March	16-17 April	8-9 May	28-29 May	25-26 June
Surface area of submerged plants per unit dry weight ($cm^2 g^{-1}$) (a)								
Nasturtium officinale	-	593 (124)	546 (144)	496 (74)	566 (121)	388 (83)	350 (104)	376 (79)
Apium nodiflorum	-	921 (523)	770 (212)	710 (75)	1021 (563)	621 (98)	496 (69)	600 (313)
Glyceria fluitans	-	629 (257)	655 (388)	561 (80)	630 (152)	503 (158)	454 (242)	412 (279)
Other species (b)	-	584 (301)	440 (74)	360 (90)	665 (255)	372 (53)	471 (190)	655 (324)
Total surface area of submerged plants (m^2) and % due to each vegetation category (c)								
Total area	4191	4937	4143	4238	7620	7441	5885	7455
N.officinale	9	9	20	22	16	15	18	16
A.nodiflorum	13	26	27	37	51	44	39	50
G.fluitans	14	10	20	17	11	27	20	16
Other species	64	55	33	24	22	14	23	18
Mean concentration of suspended bacteria ($x10^5 ml^{-1}$) (d)	2.4	1.3	1.7	2.3	2.2	2.3	2.7	2.8
Attachment rate (x 10^4 bacteria $cm^{-2}h^{-1}$) (e)	4.1	2.2	2.8	3.9	3.7	4.0	4.7	4.7
Total attachment ($x10^{13}$ bacteria d^{-1})	4.1	2.6	2.8	4.0	6.8	7.1	6.6	8.4
Attachment as percentage of total epiphytic bacteria (d^{-1}) (f)	1.6	1.3	3.7	2.4	1.7	1.9	2.0	1.8

(a) Values are means from six shoots; standard deviations in brackets.
(b) Species composition given in text.
(c) Calculated using dry weight data in Fig. 10.5.
(d) From data in Fig. 10.2.
(e) Calculated using a relative attachment rate of 1.7 x 10^4 bacteria $cm^{-2}h^{-1}$ per 10^5 bacteria ml^{-1} (Chapter 9).
(f) Calculated using data in Fig. 10.4.b.
(-) Indicates no measurement.

10^4 $cm^{-2}h^{-1}$ over that period, with a high value of 4.1×10^4 $cm^{-2}h^{-1}$ in January. Total attachment of bacteria per day ranged from 2.6×10^{13} to 8.4×10^{13}, this represented a low and relatively constant proportion (1.3-3.7% d^{-1}) of total epiphytic bacteria.

10.4.iv Vegetation composition.

There was no increase in total dry weight of vegetation after early May (Fig. 10.5.a), however these weights represented only submerged shoots. There was a steady increase in the area occupied by emergent foliage (Plates 10.3.a-c), from 2% in February to 87% in June (Fig. 10.6.a), with a parallel increase from the end of March to June in the proportion of stems plus petioles in the six sorted, harvested vegetation quadrats (Fig. 10.6.b).

The proportion of submerged vegetation belonging to each vegetation category is shown in Fig.10.5.b. The importance of N.officinale, A.nodiflorum and G.fluitans, increased through the study period until by late June they together made up 86% of total dry weight of vegetation. A.nodiflorum, in particular, became more dominant, increasing in total dry weight from 6 kg in January to 62 kg in June. The other species category consisted mainly of Agrostis stolonifera, which accounted for 61% of total vegetation dry weight in late January, diminishing to 9% in June, with a reduction in total dry weight from 41 kg in February to 13 kg in June. The following species were also harvested: Veronica beccabunga, Rumex longifolius, Berula erecta, Veronica anagallis-aquatica, Ranunculus repens, Myosotis scorpioides, Epilobium hirsutum, Equisetum fluviatile, Ranunculus aquatilis and Scrophularia auriculata, but only contributed 5-10% of total dry weight.

Plate 10.3 Mill Beck, showing increase in submerged and emergent macrophytes from January to June 1984, in the vegetated section, 225-360 m below the source. View from c.360 m looking upstream. (a) 30 January, (b) 16 April and (c) 25 June.

(a)

(b)

(c)

10.4. v Density of epiphytic bacteria.

The density of epiphytic bacteria per unit plant-surface area for each vegetation category ranged from 0.90-10.61 x 10^7 ml^{-1} (Fig. 10.8). Other species apart, the categories followed a similar pattern, with a decrease to early March, then an overall increase to June with high values in April. Densities of epiphytic bacteria were generally highest for G.fluitans and lowest for A.nodiflorum. The trend may have been obscured in the other species category by variation in species composition. The 48 shoots randomly collected over the study period comprised Veronica beccabunga (22 shoots), Rumex longifolius (8), Agrostis stolonifera (7), Berula erecta (6), Veronica anagallis-aquatica (2), Ranunculus repens (2) and Myosotis scorpioides (1).

Fig. 10.9 shows the mean density of epiphytic bacteria per unit area of (i) leaves and (ii) stems plus petioles for the four vegetation categories. The range of values generally overlapped, however, stems plus petioles appeared to support a consistently higher density of epiphytic bacteria than leaves, particularly in January and April-June, when overall densities of epiphytic bacteria were higher.

Spearman's rank correlation coefficients between combined densities of epiphytic bacteria on the four vegetation categories (Fig. 10.8) and physico-chemical variables in the vegetated section (Fig. 10.1) are shown in Table 10.2. Values of discharge and mean velocity had the same rank order, and showed a significant (negative) correlation at the 1% level. Correlations (positive) of density of epiphytic bacteria with temperature and conductivity were less significant (5%); pH and turbidity showed no significant correlation.

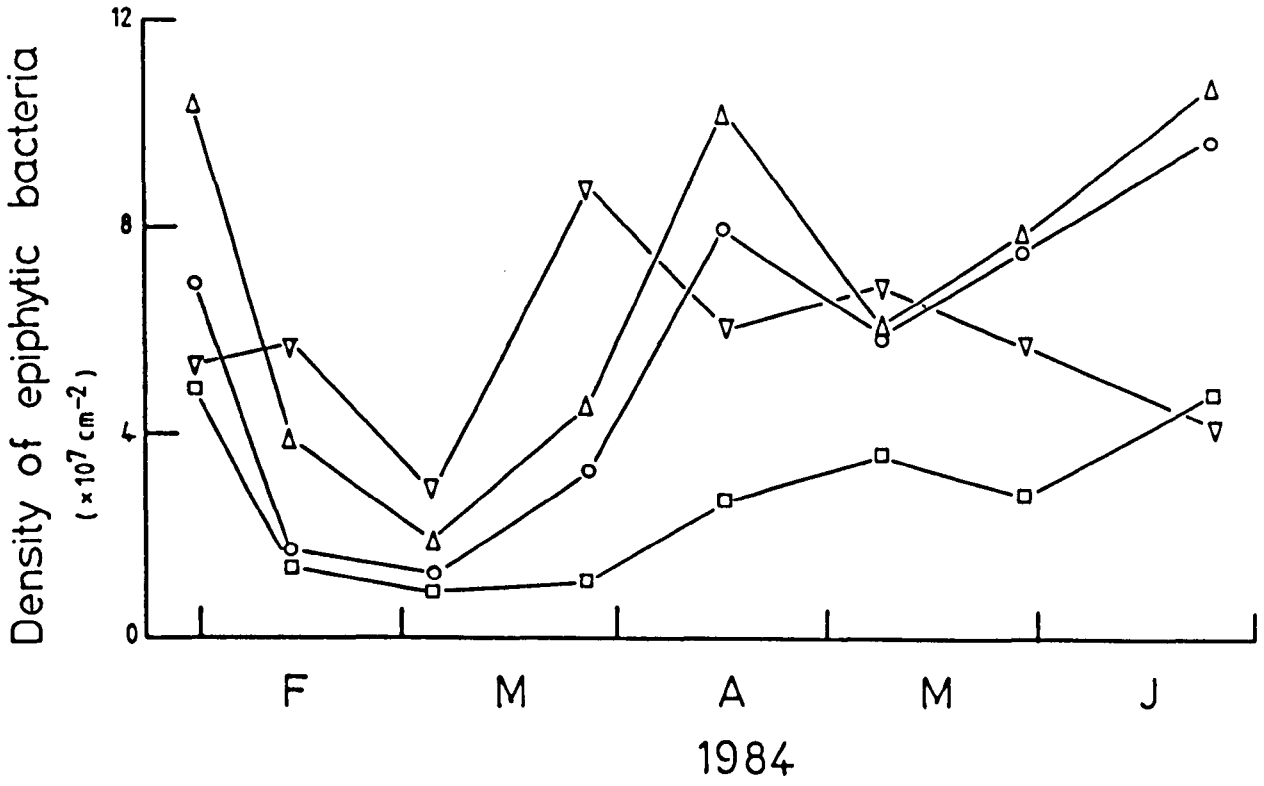


Figure 10.8 Mill Beck, January-June 1984. The density of epiphytic bacteria per unit plant-surface area of submerged vegetation in the vegetated section (c.225-360 m below the source), for four vegetation categories: Nasturtium officinale (circles), Apium nodiflorum (squares), Glyceria fluitans (triangles) and other species (inverted triangles). Each value is derived from direct counts on two suspensions of epiphytic bacteria ((i) leaves and (ii) stems plus petioles).

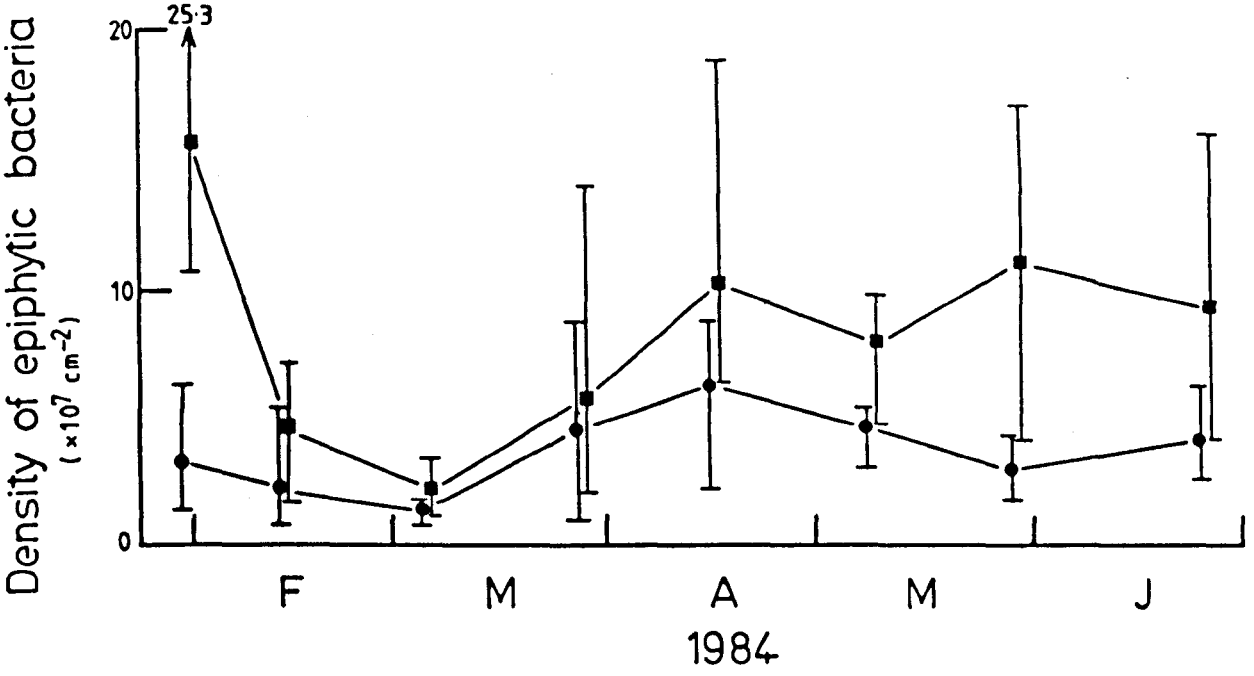


Figure 10.9 Mill Beck, January-June 1984. The density of epiphytic bacteria per unit plant-surface area of submerged vegetation in the vegetated section (c.225-360 m below the source), on leaves (circles) and stems plus petioles (squares). Values are means derived from direct counts on suspensions of epiphytic bacteria from four vegetation categories. Vertical bars indicate ranges.

Table 10.2 Mill Beck, c.225-360 m below the source, January-June 1984. Spearman's rank correlation between the density of epiphytic bacteria per unit surface area of submerged vegetation and environmental variables.

Environmental variable	Correlation coefficient r_s	P
Velocity / Discharge	-0.497	<0.01
Temperature	0.349	<0.05
Conductivity	0.392	<0.05
pH	0.216	N.S.
Turbidity	0.276	N.S.

10.5 Discussion.

Principal potential sources of the bacteria lost by downstream drift from within the vegetated section were: (1) release from submerged vegetation, (2) release from the stream bed and non-living submerged surfaces, and (3) input of soil bacteria with groundwater. In the vegetation-free section, only sources (2) and (3) were available. Input from groundwater and side springs may have been responsible for the 16% mean increase in discharge over the distance of c.750 m between the two sections (Fig. 10.1.a), however, there were no consistent differences between discharge at the upstream and downstream limits of either section, thus secondary input over both sections was too low to measure. In the vegetation-free section, there was never a significant downstream increase in concentration of suspended bacteria (Fig. 10.2), thus if release from the stream bed occured, it was balanced by reattachment. Therefore, sources (2) and (3) appeared to unimportant in Mill Beck, and bacteria lost from within the vegetated section were probably chiefly epiphytes dislodged from underwater vegetation.

From April to June, the daily drift loss of bacteria from the vegetated section equalled only a moderate proportion (9-35%) of the total population of epiphytic bacteria (Fig. 10.3.c). Hence doubling times required for maintenance of constant population (70-255 h) should have been readily achievable (1.1.iii and 7.5). During February-March, however, the daily drift loss was high at 57-132% of total epiphytic bacteria (Fig. 10.3.c), requiring doubling times of 18-42 h for maintenance of constant population, which may not have been achieved since total epiphytic bacteria decreased from late January to early March (Fig. 10.4.b). There was also heavy precipitation and snow melt at this time, with a high potential input of soil bacteria, perhaps from overland flow over the marshy margins or

from temporary springs which upwelled in the stream bed. The additional volume of water was not large, however, since there was no obvious increase in discharge over the section (10.1.a), but allochthonous bacteria may have contributed to the drift loss during this period, if the concentration of suspended bacteria in the water inputs was substantially higher than in the stream water.

Measurements of drift loss of suspended bacteria were net of attachment. Net daily drift loss at 9-132% of total epiphytic bacteria (Fig. 10.3.c) was substantially greater than attachment (1.3-3.7%), and thus not a serious underestimate of total (gross) drift loss.

The decrease in total epiphytic bacteria from January to March (Fig. 10.4.b) was due entirely to decrease in density of epiphytic bacteria (Fig. 10.4.a), since the total dry weight of vegetation (Fig. 10.5.a) was constant or increased slightly. The subsequent increase in total epiphytic bacteria, from March onwards (Fig. 10.4.b) was, however due to increase in both density of epiphytic bacteria (Fig. 10.4.a) and submerged plant biomass (Fig. 10.5.a).

The increase in dry weight of submerged vegetation in the vegetated section from January onwards (Fig. 10.5.a) was presumably initially encouraged by the relatively high water temperature of 9-10°C early in the year (Fig. 10.1.b). The recorded dry weights from February onwards represented a decreasing proportion of total shoot biomass because of the increase in extent of aerial foliage (Fig. 10.6.a, Plates 10.3.a-c). The plateau during May-June (Fig. 10.5.a), and the decreasing proportion of dry weight of leaf material (Fig. 10.6.b) suggests that most growth at that time was above water, not that biomass increase had ceased.

From September to December, there is usually little, or no water flow in the vegetated section of Mill Beck (Goulder, 1984), which may have encouraged the growth of Agrostis stolonifera, a plant of damp grassland

(Hubbard, 1984). The reduction in total dry weight of A.stolonifera from 41 kg in February to 13 kg in June suggested that growth was poor under submerged conditions. A.nodiflorum by contrast, increased in dry weight by over 10 fold and become increasingly dominant through the season. The proportion of (i) leaves to (ii) stems plus petioles was also higher for A.nodiflorum than the other vegetation categories from early March to late May (Fig. 10.6.b) suggesting that a flush of young growth may have been responsible for increase in biomass.

There was no obvious change in density of epiphytic bacteria per unit surface area on any one vegetation category relative to the other categories (Fig. 10.8) apart from the other species category, which had considerable variation in species composition. Density of epiphyic bacteria was generally highest on Glyceria fluitans, however, this may have been due to an underestimate of surface area, since the tubular leaf sheaths were included in the stem fraction and thus their adaxial surface area was ignored. A.nodiflorum supported consistently lower densities of epiphytic bacteria than G.fluitans or N.officinale (Fig. 10.8), particularly from early March onwards. This may partly reflect the age of the shoots, since higher densities of epiphytic bacteria on older plant surfaces have been reported for N.officinale and Lemna minor (Hossell & Baker, 1979a), Ranunculus penicillatus (Hossell & Baker, 1979b), Potamogeton crispus (Rogers & Breen, 1981) and Veronica beccabunga (Baker & Orr, 1986). The lower proportion of stem plus petiole to leaf material for A.nodiflorum (Fig. 10.6.b) may also have affected the density of epiphytic bacteria, since stems plus petioles appeared to support higher densities of epiphytic bacteria than leaves (Fig. 10.9). Kudryavtsev (1984) also found that densities of epiphytic bacteria on macrophytes in the littoral of a reservoir were from 1.3-3 times higher on stems than on leaves. Possible reasons for the differential distribution might include differences in

grazing pressure, surface morphology, physical conditions and length of time that the surfaces have been exposed for colonisation. Surface pH may possibly affect the distribution of epiphytic bacteria; Baker & Orr (1986) suggested that the lower density of epiphytic bacteria on the adaxial surfaces of leaves may be due to elevated pH consequent on hydroxyl ion release following photosynthetic bicarbonate utilization.

The pattern of temporal change in density of epiphytic bacteria on the four vegetation categories (Fig. 10.8) was perhaps influenced by several environmental variables. The most significant correlation was with discharge and current velocity, and although correlation does not imply causality, it nevertheless seems likely that the high current velocity associated with high discharge at the beginning of the season may have increased the shear stress exerted on the epiphytes (Silvester & Sleigh, 1985), resulting in greater percentage release and lower densities of epiphytic bacteria. The subsequent increase in density of epiphytic bacteria from early March to June coincided with a steady decrease in discharge and velocity. The current velocity, however, represents the mean value for the whole section and there was probably considerable spatial variation. Faster moving water, particularly at times of low discharge, was generally confined to one or more channels, indicated by the bands of completely submerged vegetation in Fig. 10.7. In the dense stands of emergent vegetation, current velocities may have been well below the mean values of 0.04-0.25 m s^{-1} (Fig. 10.1.a). Madsen & Warncke (1983), using a small-sized flowmeter, found that compared to open water, current velocities within beds of Callitriche stagnalis were reduced by 25-92%. Thus, in densely vegetated parts of Mill Beck, towards the end of the sampling period, current velocity may have been reduced to near zero. As discharge decreased from February to June, there would also have been less dilution of macrophyte organic exudates and this may have encouraged

development of epiphytic bacteria.

The density of epiphytic bacteria also showed significant correlations with temperature and conductivity (Table 10.2). Although the range of temperature was not great (9-11.5°C, Figure 10.1.b) the gradual temperature increase over the sampling period probably encouraged growth of both macrophytes and epiphytic bacteria. Conductivity varied inversely with discharge (Fig. 10.1.a and d) and may have been related to changes in groundwater, thus, the significant correlation with density of epiphytic bacteria was probably not a causal relationship.

Baker & Orr (1986) found no significant correlation of epiphyte density on Ranunculus penicillatus with discharge or temperature in the calcareous Bere Stream in Dorset, although the discharge was more than double the maximum value recorded at Mill Beck. They also found no significant seasonal change in density of epiphytic bacteria on R.penicillatus. Yakushin (1979), however, reported fluctuations in the density of epiphytic bacteria on the surface of Phragmites sp. in the North Crimean canal, with lowest densities in spring and highest densities in autumn, and Kudryavtsev (1984) monitored an increasing density of epiphytic bacteria on macrophytes in the littoral of a lake from early summer to autumn, which was ascribed chiefly to change in water temperature.

The results from Mill Beck during the early part of 1984 therefore support the conclusion reached in Chapter 7, that downstream increase in concentration of suspended bacteria can be accounted for by release of epiphytic bacteria. They also demonstrate a temporal change in density of epiphytic bacteria, which was correlated with, and may have been a response to environmental variables.

By refering to the diagramatic representation of sources and losses of suspended bacteria in running water (Fig. 1.1), the situation near the source of Mill Beck can be envisaged as follows: Upstream input of suspended bacteria is generally low, the stream being spring-fed from deep ground water. Development of a population of suspended bacteria is probably chiefly through autochthonous release of epiphytic bacteria from the surface of submerged macrophytes. Growth and cell division within the suspended population is probably negligible over short distances, and reattachment of suspended bacteria to plant surfaces within the stream is likely to be unimportant. Physico-chemical variables such as temperature may affect the development of the population of suspended bacteria by influencing the growth rate of the source population (standing crop of epiphytic bacteria), hence the supply of cells available for release to the stream water; discharge and current velocity appear to infuence the process of release of bacteria from the source population to the stream water.

The extent to which such relationships between epiphytic and suspended bacteria occur generally in headstreams, is unclear. The pattern observed in Mill Beck might possibly be peculiar to this seasonally intermittent chalk headstream. However, the frequent pattern of linear downstream increase in concentration and heterotrophic activity of suspended bacteria in Birdsall Beck, Church Beck, Eastburn Beck and Millington Beck during 1982-1983 suggests that bacteria sloughed from within-stream surfaces may also be an important source of suspended bacteria in other Wolds streams.

The situation may be rather different in upland streams. In Dungeon Burn, for example, there appeared to be little release of bacteria to the water column from within-stream surfaces despite a large standing crop of epiphytic bacteria. The majority of the suspended bacteria at least during non-spate conditions may therefore perhaps be allochthonous in origin. The Galloway streams are fed principally by shallow groundwater which may

already contain substantial bacterial populations. Physico-chemical variables such as low pH and the oligotrophic nature of the water may retard the activity and growth rate of epiphytic, epilithic and groundwater bacteria.

It could perhaps be postulated that in the Galloway streams, a higher proportion of autochthonous bacteria in the suspended population might be expected after an intense rainstorm event, producing a rapid increase in discharge and current velocity, with increased sloughing of bacteria from vegetation and rock surfaces. Conversely, a similar rainstorm event on the Wolds, would be unlikely to affect the discharge of spring water, but may increase the likelihood of inputs of groundwater through interflow and overland-flow, and thus, possibly increase the proportion of allochthonous bacteria in the suspended population. The extent to which such a reversal in origin of suspended bacteria in spring-fed and shallow-groundwater-fed streams occurs, under base-flow and storm-flow conditions, however, requires further investigation.

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APPENDICES

APPENDIX 1

Aquatic vegetation in Wolds and Galloway streams, 1982-1983. Wolds streams: Birdsall Beck (BB), Church Beck (CB), Eastburn Beck (EB) and Millington Beck (MB). Galloway streams: Dargall Lane (DL), Dungeon Burn (DB), Garrary Burn (GB), and Minnigall Lane (ML). Submerged and marginal species (those liable to submergence during high flow) are included, with an estimate of the maximum percentage of stream cross-section occupied by each species, at any point along the sampled length of stream; (X) indicates < 5% cover, (-) indicates no record.

Species	Wolds streams			
	BB	CB	EB	MB
Angiosperms				
Agrostis stolonifera	X	X	X	X
Angelica sylvestris	-	-	-	X
Apium nodiflorum	100	100	5	100
Berula erecta	-	-	60	100
Callitriche sp.	-	-	X	-
Caltha palustris	-	-	-	X
Cardamine pratensis	-	-	-	X
Carex acutiformis	-	-	-	50
C. riparia	-	-	-	100
Catabrosa aquatica	-	-	-	100
Cirsium palustre	X	X	-	-
Deschampsia cespitosa	X	-	X	X
Epilobium hirsutum	100	100	X	100
Filipendula ulmaria	-	-	-	X
Glyceria fluitans	100	50	50	100
Groenlandia densa	-	-	75	-
Hedera helix	-	X	-	-
Holcus lanatus	X	X	-	-
Hypericum tetrapterum	-	X	-	-
Iris pseudacorus	-	-	X	100
Juncus bufonius	-	-	X	-
J. effusus	X	-	-	X
J. inflexus	X	X	X	20
Lolium perenne	X	-	-	-
Lychnis flos-cuculi	-	-	-	X
Mentha aquatica	50	75	X	50
Mimulus guttatus	-	-	X	-
Myosotis scorpioides	-	-	5	50
Nasturtium officinale	-	100	20	100
Petasites hybridus	X	-	X	X
Poa annua	X	-	-	-
P. trivialis	-	-	-	X
Polygonum persicaria	-	-	X	-
Ranunculus fluitans	-	-	100	-
R. repens	X	-	-	-
R. sceleratus	-	-	-	X
Rubus sp.	X	-	-	-
Rumex longifolius	X	X	X	X
Scrophularia auriculata	-	10	X	X
Sparganium erectum	-	-	X	-
Stachys sylvatica	X	X	-	-
Urtica dioica	X	X	-	-
Veronica anagallis-aquatica	-	-	X	-
V. beccabunga	100	100	5	50
Pteridophytes				
Equisetum palustre	-	X	-	X
Bryophytes				
Calliergon cuspidatum	X	-	-	X
Cratoneuron commutatum	X	-	-	-
Eurhynchium praelongum	X	X	-	-
Lophocolea bidentata	X	-	-	-
Pellia endiviifolia	X	X	X	-
Plagiochila asplenoides	-	X	-	-
Plagiomnium undulatum	X	X	-	-
Rhynchostegium riparioides	X	X	50	X

Species	Galloway streams			
	DL	DB	GB	ML
Angiosperms				
Agrostis canina	-	-	X	X
A. capillaris	X	X	X	X
Anthoxanthum odoratum	-	-	X	X
Calluna vulgaris	X	X	X	X
Carex echinata	X	X	X	X
C. demissa	X	-	X	X
C. nigra	-	-	X	X
C. panicea	-	-	X	X
C. pauciflora	-	X	-	-
C. rostrata	100	-	-	100
Carum carvi	-	-	X	X
Cirsium palustre	-	-	X	X
Danthonia decumbens	-	-	X	X
Deschampsia cespitosa	X	-	-	-
Drosera rotundifolia	-	X	-	-
Erica tetralix	-	X	X	X
Eriophorum angustifolium	10	-	100	X
Festuca vivipara	X	-	-	-
Galium saxatile	-	-	X	X
Juncus acutiflorus	100	-	100	75
J. bulbosus	100	5	75	80
J. conglomeratus	-	-	X	X
J. squarrosus	X	-	X	X
Littorella uniflora	-	-	30	30
Lobelia dortmanna	X	-	-	-
Molinia caerulea	100	X	10	30
Myrica gale	X	X	X	X
Myriophyllum alterniflorum	10	-	-	-
Nardus stricta	X	X	10	10
Narthecium ossifragum	10	X	X	10
Pinguicula vulgaris	X	-	X	X
Potentilla erecta	-	-	X	X
Potomogeton polygonifolius	20	-	X	X
Ranunculus flammula	X	-	X	X
Stellaria alsine	-	-	X	X
Trichophorum cespitosum	X	X	50	X
Vaccinium myrtillus	X	-	-	-
Viola palustre	X	X	X	X
Pteridophytes				
Blechnum spicant	-	-	X	X
Equisetum fluviatile	-	-	30	30
E. palustre	100	-	-	-
Oreopteris limbosperma	X	-	X	X
Phegopteris connectilis	-	-	X	X
Bryophytes				
Amphidium mougeotii	-	-	X	-
Andreaea sp.	X	X	-	-
Anthelia julacea	X	50	-	-
Bryum alpinum	-	-	X	X
B. pallens	-	-	X	X
Campylopus atrovirens	-	X	X	X
Campylium stellatum	-	-	X	X
Diplophyllum albicans	X	50	-	-
Drepanocladus revolvens	X	-	-	-
Hyocomium armoricum	-	-	X	-
Marsupella aquatica	-	100	-	-
Nardia compressa	100	100	100	100
Pellia epiphylla	X	X	20	20
Pleurozia purpurea	X	-	-	-
Polytrichum commune	X	X	X	X
Pohlia nutans	-	-	X	X
Racomitrium aciculare	X	-	10	10
R. aquaticum	-	-	10	10
Scapania undulata	X	100	50	50
Sphagnum auriculatum	X	50	-	-
S. compactum	-	X	-	-
S. palustre	100	50	50	80
S. papillosum	-	50	-	-

APPENDIX 2

Wolds and Galloway streams 1982-1983. Physico-chemical variables. (-) indicates no measurement.

Wolds streams; Birdsall Beck

Sampling date	Distance below source (km)									
	0	0.09	0.18	0.27	0.36	0.45	0.54	0.63	0.72	0.81
	pH									
28 10 82	7.7	8.0	7.9	8.2	8.1	8.0	8.0	7.9	7.9	7.8
18 11 82	7.4	7.7	7.8	7.9	7.9	7.9	7.9	8.0	8.0	8.0
20 12 82	7.5	7.7	7.9	7.9	8.0	7.9	8.0	8.0	8.1	8.1
25 08 83	7.4	7.8	7.8	7.9	7.9	7.9	7.9	7.8	7.9	7.9
27 10 83	7.5	7.9	7.9	8.0	8.0	8.0	8.0	7.9	7.9	8.0
16 12 83	7.3	7.6	7.6	7.7	7.7	7.7	7.8	7.8	7.8	7.9
	Conductivity ($\mu S\ cm^{-1}$)									
28 10 82	340	343	363	369	369	372	400	400	403	406
18 11 82	349	369	389	398	409	409	432	443	440	418
20 12 82	323	329	352	355	358	355	372	372	378	372
25 08 83	389	400	406	412	412	403	426	426	429	420
27 10 83	352	360	386	389	395	389	420	432	432	420
16 12 83	372	389	426	432	426	429	463	475	463	469
	Temperature (°C)									
28 10 82	8.75	8.5	8.75	8.75	8.75	8.5	8.0	8.0	7.75	7.5
18 11 82	8.75	9.0	9.25	9.25	9.5	9.5	9.5	9.5	9.5	9.25
20 12 82	8.75	8.0	7.75	7.25	6.75	6.75	5.75	5.75	5.5	5.5
25 08 83	9.0	9.75	10.0	10.25	11.0	11.0	11.75	11.75	11.75	12.0
27 10 83	8.75	9.0	9.0	9.0	8.75	8.75	9.0	8.75	8.5	8.5
16 12 83	8.5	8.0	7.75	7.75	7.5	7.25	7.25	7.0	6.75	6.75
	Relative turbidity (%)									
28 10 82	0	2.25	4.25	7.75	8.0	5.75	10.5	9.75	8.25	7.25
18 11 82	0.75	2.5	2.75	4.0	4.0	3.75	6.25	6.25	5.75	5.75
20 12 82	0.25	1.5	1.75	2.5	3.25	4.5	4.75	4.75	5.0	5.0
25 08 83	0	0.5	4.0	4.0	7.0	6.5	15.0	14.75	9.0	7.5
27 10 83	0	1.75	3.5	3.25	3.75	5.0	41.5	59.5	53.0	17.75
16 12 83	0.5	2.0	2.75	3.25	3.5	3.5	6.0	6.0	5.0	5.25
	Discharge ($x10^{-3}m^3s^{-1}$) *									
28 10 83	4.2									
18 11 82	9.0									
20 12 82	9.6									
25 08 83	5.2									
27 10 83	2.7									
16 12 83	27.0									

(*) Discharge at c.0.8 km below the source.

Wolds streams; Church Beck

Sampling date	Distance below source (km)									
	0	0.025	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4
	pH									
10 09 82	6.6	-	7.0	7.1	7.1	7.1	7.1	7.1	7.2	7.3
25 10 82	7.4	7.5	7.5	7.7	7.7	7.7	7.7	7.8	7.8	7.8
16 11 82	7.5	7.6	7.6	7.6	7.7	7.6	7.6	7.7	7.7	7.9
16 12 82	7.3	7.5	7.5	7.6	7.6	7.7	7.7	7.7	7.7	7.7
19 08 83	7.0	7.3	7.4	7.5	7.5	7.6	-	7.6	7.5	7.5
28 10 83	7.5	7.8	7.8	7.8	7.8	7.8	7.7	7.8	7.8	7.9
14 12 83	7.2	7.5	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
	Conductivity ($\mu S\ cm^{-1}$)									
10 09 82	523	-	486	512	481	515	521	526	529	535
25 10 82	426	415	406	415	412	418	426	420	423	426
16 11 82	418	426	418	420	446	438	438	440	455	466
16 12 82	415	395	523	400	415	423	429	423	423	426
19 08 83	498	472	481	478	483	481	-	486	486	483
28 10 83	463	412	403	418	429	426	429	435	412	420
14 12 83	461	446	455	463	466	486	475	483	486	501
	Temperature (°C)									
10 09 82	9.75	10.75	11.25	11.5	11.75	11.75	11.75	12.0	12.25	12.0
25 10 82	9.5	9.5	9.5	9.5	9.25	9.0	9.25	9.25	9.0	9.0
16 11 82	9.5	9.25	9.0	9.0	8.75	8.5	8.5	8.25	8.0	7.75
16 12 82	9.25	8.75	8.5	8.0	7.75	7.25	6.75	6.25	6.0	6.0
19 08 83	9.75	10.0	10.25	10.75	10.5	11.5	11.75	11.75	11.75	12.0
28 10 83	9.25	8.75	8.25	8.25	7.75	7.25	6.75	6.5	6.25	6.25
14 12 83	8.75	8.0	8.0	7.75	7.5	7.25	7.0	6.5	6.25	6.25
	Relative turbidity (%)									
10 09 82	0	0.25	1.0	0.5	0.75	0.25	0.25	0.75	1.25	1.5
25 10 82	0	0	0.25	0.25	1.5	1.5	1.5	1.75	1.5	1.75
16 11 82	0.5	0.5	0.75	0.75	0.25	0.75	0.75	1.5	1.75	1.75
16 12 82	0	0	0	0	0	0.25	0.5	0.5	0.5	0.75
19 08 83	0.5	0.5	0.5	0.5	0.5	1.0	-	0.75	0.75	0.5
28 10 83	0.25	1.0	0.5	0.5	0.75	0.5	1.25	1.5	1.5	2.0
14 12 83	0.75	0.75	0.75	1.0	1.25	1.0	1.25	1.25	2.25	2.0
	Discharge ($x10^{-3} m^3 s^{-1}$) *									
10 09 82	6.5									
25 10 82	3.6									
16 11 82	3.6									
16 12 82	5.7									
19 08 83	9.8									
28 10 83	3.8									
14 12 83	5.9									

(*) Discharge at c.0.4 km below source.

Wolds streams: Eastburn Beck

Sampling date	Distance below source (km)							
	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4
	pH							
09 09 82	7.1	7.2	7.4	7.3	7.7	7.8	7.8	7.8
21 10 82	7.4	7.5	7.5	7.7	7.6	7.7	7.7	7.5
12 11 82	7.7	7.8	7.7	7.7	7.8	7.8	7.8	7.8
19 12 82	7.4	7.4	7.6	7.5	7.5	7.6	-	7.6
24 08 83	7.4	7.4	7.5	7.5	7.5	7.5	7.5	7.5
25 10 83	7.4	7.6	7.5	7.6	7.5	7.6	7.6	7.6
13 12 83	7.3	7.4	7.4	7.4	7.4	7.5	7.6	7.6
	Conductivity ($\mu S\ cm^{-1}$)							
09 09 82	423	423	403	415	415	409	423	423
21 10 82	343	340	349	346	346	346	349	346
12 11 82	346	340	346	352	352	318	332	346
19 12 82	318	330	335	323	329	332	-	343
24 08 83	363	360	366	369	363	363	369	372
25 10 83	335	340	336	340	336	340	340	340
13 12 83	383	378	398	389	389	403	406	398
	Temperature (°C)							
09 09 82	10.75	11.75	11.0	11.0	11.75	11.5	10.5	10.25
21 10 82	9.75	9.5	9.5	9.5	9.75	9.5	9.5	9.0
12 11 82	9.75	9.75	9.75	9.75	9.75	9.75	10.25	10.25
19 12 82	9.25	9.25	9.0	9.0	9.0	8.75	8.5	8.25
24 08 83	10.0	10.25	10.5	10.5	10.75	11.0	10.75	12.5
25 10 83	9.25	9.0	8.5	8.5	8.75	8.25	7.5	7.0
	Relative turbidity (%)							
09 09 82	0.75	0.25	1.0	0.5	1.75	1.5	1.25	1.25
21 10 82	0.5	0.5	0.75	0.75	1.25	2.0	3.25	3.75
12 11 82	0.5	2.0	1.0	1.5	8.0	22.75	44.0	12.25
19 12 82	1.75	2.0	3.0	2.75	2.0	3.75	3.0	3.5
24 08 83	0.25	0.5	0	0.5	0.5	0.25	1.0	0.5
25 10 83	0.75	1.0	1.0	0.75	0.75	1.0	1.0	2.0
13 12 83	1.5	1.75	2.25	2.25	2.0	2.75	4.0	7.0
	Discharge ($x10^{-3}\ m^3 s^{-1}$) *							
09 09 82	140							
21 10 82	59							
12 11 82	63							
19 12 82	690							
24 08 83	271							
25 10 83	91							
13 12 83	432							

(*) Discharge at c.1.3 km below source.

Wolds streams; Millington Beck

Sampling date	Distance below source (km)									
	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.7
	pH									
08 09 82	7.5	7.4	7.5	7.7	7.8	7.9	7.9	7.9	7.9	7.9
19 10 82	7.5	7.4	7.3	7.7	7.8	7.6	7.8	7.8	7.8	7.7
11 11 82	6.1	6.0	6.4	6.8	7.4	7.4	7.6	7.6	7.6	7.6
15 12 82	7.7	7.7	7.8	7.9	7.9	8.0	8.0	8.0	8.0	8.0
	Conductivity (μS cm^{-1})									
08 09 82	335	330	340	340	340	358	360	372	369	378
19 10 82	275	275	286	286	300	320	332	358	358	360
11 11 82	286	283	292	303	320	326	343	338	332	323
15 12 82	237	260	269	269	280	286	289	289	295	295
	Temperature (°C)									
08 09 82	9.75	12.0	12.0	11.75	12.0	12.25	12.0	12.0	12.0	12.0
19 10 82	9.0	8.5	8.0	8.0	8.0	8.0	7.75	7.5	7.5	7.25
11 11 82	9.25	8.0	8.0	7.75	7.75	7.75	7.5	7.5	7.5	7.5
15 12 82	9.25	9.5	9.5	9.5	9.5	9.25	9.0	9.0	8.75	8.75
	Relative turbidity (%)									
08 09 82	0	0	3.75	1.5	2.5	1.75	1.5	1.0	1.25	1.25
19 10 82	0	0.5	2.0	1.25	1.5	1.25	1.25	3.5	2.25	3.0
11 11 82	0.75	0.75	0.75	0.75	1.0	0.75	0.5	0.5	1.0	1.0
15 12 82	0	0	0.5	1.0	0.75	1.0	1.0	1.0	1.25	1.0
	Discharge (x10^{-3}m^{3}s^{-1}) *									
08 09 82	8.8									
19 10 82	6.2									
11 11 82	5.8									
15 12 82	34.0									

(*) Discharge at c.1.7 km below source.

Galloway streams; Dargall Lane

Sampling date	Distance below source (km)									
	0	0.42	0.77	1.12	1.47	1.82	2.17	2.52	2.87	3.22
	pH									
05 09 82	4.6	4.4	4.6	5.3	5.2	5.0	5.3	5.3	5.3	5.5
04 10 82	4.8	4.7	4.8	4.7	4.7	4.6	4.8	4.7	4.8	4.7
26 11 82	4.4	4.5	4.6	4.5	4.5	4.6	4.6	4.6	4.5	4.5
23 03 83	5.7	5.5	5.3	5.5	5.4	5.3	5.3	5.3	5.3	5.1
03 07 83	5.4	6.1	6.4	6.3	6.3	6.3	6.3	6.3	6.3	6.2
08 10 83	5.3	5.1	5.2	5.3	5.3	5.4	5.3	5.3	5.3	5.3
22 11 83	5.1	5.3	5.4	5.5	5.2	5.1	5.2	5.1	5.2	5.1
	Conductivity ($\mu S\ cm^{-1}$)									
05 09 82	40	37	39	38	36	36	36	36	37	38
04 10 82	25	25	22	22	22	22	23	23	23	23
26 11 82	32	28	28	28	28	28	28	29	29	29
23 03 83	50	61	58	58	59	60	62	61	61	61
03 07 83	33	29	29	27	27	27	27	27	27	27
08 10 83	29	32	28	30	28	28	30	30	30	30
22 11 83	33	30	30	30	31	32	32	33	33	33
	Temperature (°C)									
05 09 82	11.0	10.25	10.5	10.75	10.75	10.75	10.75	10.75	10.75	11.0
04 10 82	9.25	8.5	8.75	9.0	9.0	9.0	9.25	9.5	9.0	9.5
26 11 82	4.25	4.0	3.0	3.0	3.0	2.5	2.5	2.5	2.75	3.0
23 03 83	3.25	2.5	2.75	2.75	2.75	3.0	2.75	2.75	2.75	2.5
03 07 83	10.75	10.75	10.75	10.75	11.0	11.0	11.0	11.0	11.0	10.75
08 10 83	9.25	8.5	8.75	8.5	8.5	8.5	8.75	8.75	8.75	9.0
22 11 83	4.0	2.25	0.75	0.5	0.25	0.25	0.25	0.25	0.5	0.5
	Relative turbidity (%)									
05 09 82	0	0	0	0	0	0	0	0	0	0
04 10 82	0	0	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
26 11 82	0	0	0	0	0	0	0.25	0	0.25	0.5
23 03 83	0	0	0	0	0	0	0.25	0.25	0	0.25
03 07 83	0	0	0	0	0	0	0	0	0	0
08 10 83	1.25	0.5	0.5	0.75	0.5	0.75	0.75	0.5	0.25	0.25
22 11 83	0.25	0	0	0	0	0	0	0	0	0
	Discharge ($x10^{-3} m^3 s^{-1}$) *									
05 09 82	50									
04 10 82	1104									
26 11 82	104									
23 03 83	802									
03 07 83	85									
08 10 83	697									
11 11 83	11									

(*) Discharge at c.1.6 km below source.

Galloway streams; Dungeon Burn

Sampling date	Distance below source (km)									
	0	0.15	0.3	0.45	0.6	0.75	0.9	1.05	1.2	1.35
	pH									
23 07 82	4.3	-	-	-	4.2	-	-	-	-	4.7
03 09 82	4.2	4.1	4.1	4.2	4.2	4.3	4.4	4.3	4.3	4.4
03 10 82	4.4	4.3	4.3	4.4	4.4	4.3	4.4	4.3	4.3	4.3
25 11 82	4.2	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
24 03 83	4.7	4.9	4.9	4.9	4.8	4.9	5.0	5.0	4.9	5.1
04 07 83	4.8	4.8	4.7	4.7	4.7	4.8	5.1	5.0	5.0	5.0
07 10 83	4.8	4.8	5.0	5.0	5.1	5.1	5.0	5.3	5.2	5.2
23 11 83	5.2	4.8	4.8	4.9	4.9	4.8	4.9	4.9	4.8	5.2
	Conductivity (μS cm^{-1})									
23 07 82	32	35	36	34	34	34	29	32	30	30
03 09 82	40	42	41	40	39	39	34	34	35	31
03 10 82	23	24	23	23	23	23	22	22	23	23
25 11 82	34	34	36	35	34	36	35	35	33	34
24 03 83	53	52	51	52	49	53	52	51	51	51
04 07 83	30	30	30	30	29	30	29	29	29	27
07 10 83	32	33	33	33	32	33	33	33	34	34
23 11 83	35	40	40	40	38	35	35	36	36	34
	Temperature (°C)									
23 07 82	17.0	19.25	18.0	18.0	24.0	22.5	17.25	17.5	17.0	15.75
03 09 82	9.75	10.0	10.25	10.25	10.5	11.0	10.5	10.5	10.75	10.75
03 10 82	8.5	8.5	8.75	9.25	9.75	11.0	10.25	9.75	10.0	9.25
25 11 82	2.25	2.75	2.5	2.5	2.75	3.0	3.5	3.5	4.0	4.0
24 03 83	1.25	1.25	1.25	1.5	2.0	3.25	3.25	3.25	3.25	3.25
04 07 83	9.5	11.75	12.25	12.0	13.25	11.75	13.0	12.25	12.0	11.5
07 10 83	8.0	8.5	8.5	8.75	8.75	9.0	9.5	9.5	9.75	9.5
23 11 83	4.25	2.25	2.25	2.25	2.0	1.0	2.5	2.75	3.5	2.75
	Relative turbidity (%)									
23 07 82	0.5	0.5	1.0	0.5	0.25	0.25	0.25	0.75	0.5	0.5
03 09 82	0	0	0	0	0	0	0	0	0	0
03 10 82	0	0	0	0	0	0	0	0	0	0
25 11 82	0.5	0.25	0	0	0	0	0	0	0	0
24 03 83	0.25	0.25	0	0.25	0.25	0	0.25	0.25	0.25	0.25
04 07 83	0.75	0.5	0	0	0	0	0	0	0	0
07 10 83	0.25	0.5	0	0	0	0	0	0	0	0
23 11 83	0	0	0	0	0	0	0	0	0	0
	Discharge ($\times 10^{-3} m^3 s^{-1}$) *									
23 07 82	4									
03 09 82	22									
03 10 82	39									
25 11 82	74									
24 03 83	91									
04 07 83	19									
07 10 83	200									
23 11 83	8									

(*) Discharge at c.1.3 km below source.

Galloway streams; Garrary Burn

Sampling date	Distance below source (km)									
	0	0.52	1.04	1.56	2.08	2.60	3.12	3.64	4.16	4.68
	pH									
20 07 82	4.1	-	-	-	5.3	-	-	-	-	5.8
02 09 82	4.3	4.1	4.2	4.7	4.7	5.0	5.2	5.2	5.3	5.5
30 09 82	4.2	4.1	4.1	4.3	4.4	4.5	4.7	4.7	4.9	4.9
27 11 82	4.5	4.5	4.6	4.5	4.9	5.0	5.2	5.1	5.1	5.1
01 07 83	6.7	7.3	7.1	7.5	7.3	7.3	7.0	7.0	7.0	6.8
05 10 83	4.9	4.9	5.0	5.2	5.3	5.3	5.3	5.3	5.5	5.5
17 11 83	4.5	5.4	5.5	5.6	6.0	5.8	5.8	5.7	5.6	5.6
	Conductivity ($\mu S\ cm^{-1}$)									
03 07 82	28	25	27	22	23	22	22	22	22	22
20 07 82	29	26	27	27	26	26	27	27	28	29
02 09 82	38	35	34	34	36	34	34	34	38	39
30 09 82	27	28	26	25	25	24	24	24	25	25
27 11 82	32	31	30	30	29	29	29	29	30	30
01 07 83	30	27	28	30	29	30	31	32	31	32
05 10 83	33	34	35	34	33	34	38	36	38	36
17 11 83	38	39	39	38	37	37	38	38	40	39
	Temperature (°C)									
03 07 82	11.0	10.5	11.0	11.75	12.25	12.5	11.75	12.0	12.0	10.5
20 07 82	18.5	14.5	15.0	16.5	16.0	15.75	15.25	14.75	15.0	14.0
02 09 82	13.0	11.5	11.75	12.5	12.25	11.75	11.75	11.25	11.0	10.75
30 09 82	9.5	9.25	9.5	9.75	9.75	9.75	9.5	9.5	9.25	8.75
27 11 82	3.5	2.5	3.0	3.0	2.75	2.5	2.5	2.5	2.25	2.25
01 07 83	11.25	10.25	11.0	11.75	12.0	12.5	12.5	12.75	12.75	12.5
05 10 83	9.5	9.75	10.25	10.75	10.75	11.0	11.0	10.75	10.5	10.5
17 11 83	5.25	5.0	5.0	5.5	5.0	5.0	4.75	4.75	4.75	4.0
	Relative turbidity (%)									
03 07 82	0.5	0.5	1.0	0.25	0.25	0.5	1.5	1.0	0.5	0.75
20 07 82	0.25	0	0.75	0.5	0.5	1.0	1.0	0.5	0.75	0.5
02 09 82	0	0	0	0	0	0	0	0	0.25	0.25
30 09 82	0	0	0	0	0	0	0	0	0	0
27 11 82	0	0	0	0	0	0	0	0	0	0
01 07 83	1.0	1.0	1.0	0.5	0.25	0	0	0	0	0
05 10 83	0	0	0.25	0.5	0.5	0.75	1.0	1.0	1.0	0.75
17 11 83	0	0	0	0	0	0	0	0	0.25	0.25
	Discharge ($x10^{-3} m^3 s^{-1}$) *									
03 07 82	210									
20 07 82	25									
02 09 82	105									
30 09 82	317									
27 11 82	180									
01 07 83	18									
05 10 83	1024									
17 11 83	63									

(*) Discharge at c.4.5 km below the source.

Galloway streams; Minnigall Lane

Sampling date	Distance below source (km)									
	0	0.67	1.33	2.00	2.66	3.32	3.85	4.37	4.89	5.41
	pH									
06 07 82	4.5	-	-	-	5.0	-	-	-	-	5.5
21 07 82	4.7	-	-	-	5.8	-	-	-	-	6.2
01 09 82	4.3	4.2	4.4	4.5	4.5	4.6	4.8	4.7	5.0	5.2
02 10 82	4.6	4.4	4.3	4.3	4.3	4.2	4.3	4.4	4.5	4.7
24 11 82	4.5	4.4	4.4	4.5	4.6	4.5	4.6	4.6	4.6	4.5
22 03 83	4.9	4.8	4.9	4.9	4.9	4.7	5.6	5.5	5.5	5.6
02 07 83	5.3	5.5	7.0	7.1	7.1	7.1	7.1	7.0	7.0	6.7
06 10 83	4.8	4.8	4.8	4.8	4.8	4.7	4.7	4.7	4.7	4.6
21 11 83	4.9	5.1	5.0	5.0	5.2	4.9	4.8	4.6	4.5	4.6
	Conductivity ($\mu S\ cm^{-1}$)									
06 07 82	30	26	24	23	22	22	22	22	22	22
21 07 82	30	27	26	28	29	27	29	28	29	30
01 09 82	37	32	31	31	33	33	34	34	37	37
02 10 82	24	23	22	22	22	22	23	23	23	23
24 11 82	34	32	32	31	32	32	31	31	31	32
22 03 83	48	49	51	56	60	53	50	59	64	64
02 07 83	32	28	27	26	26	25	26	26	26	24
06 10 83	34	34	34	36	35	35	37	36	37	37
21 11 83	38	35	34	35	37	37	37	37	38	39
	Temperature (°C)									
06 07 82	10.5	12.0	13.5	13.5	13.25	12.75	12.5	12.25	12.25	11.75
21 07 82	15.0	13.75	18.0	17.25	17.0	16.5	16.5	16.5	16.5	16.25
01 09 82	12.25	12.25	14.25	14.5	14.0	12.75	12.75	12.5	12.25	12.0
02 10 82	9.75	9.75	10.0	10.0	10.0	10.0	9.75	9.75	9.5	9.0
24 11 82	4.75	4.0	3.75	3.5	3.25	3.0	3.0	3.0	2.75	2.75
22 03 83	3.5	3.0	3.0	3.0	0.5	0.75	1.0	1.0	0.5	0.5
02 07 83	10.75	12.0	13.25	13.75	13.25	12.25	12.0	11.5	11.25	11.0
06 10 83	10.25	10.5	10.75	11.0	11.0	10.75	10.75	10.75	10.75	10.75
21 11 83	6.0	3.75	2.25	1.75	1.25	1.5	1.0	0.75	0.5	0.5
	Relative turbidity (%)									
06 07 82	1.5	1.0	1.25	0.5	1.0	0.75	1.5	1.5	0.5	0.5
21 07 82	0.25	0.5	0	0	1.5	1.75	2.5	1.0	0.75	1.0
01 09 82	0	0	0	0	0	0	0	0	0.25	0.5
02 10 82	0	0	0	0	0	0	0.25	0.25	0.25	0.25
24 11 82	0	0	0	0.25	0.25	0.25	0.5	0.5	0.25	0.25
22 03 83	0.5	0.5	0.25	0.5	1.0	0.5	1.0	1.0	1.25	1.0
02 07 83	0	0	0	0	0	0	0	0	0	0
06 10 83	0	0	0.25	0	0	0.25	0.75	0.25	0.25	0.5
21 11 83	0	0	0	0	0	0	0	0	0	0

	Discharge ($x10^{-3}m^3s^{-1}$) *
06 07 82	270
21 07 82	22
01 09 82	211
02 10 82	1274
24 11 82	830
22 03 83	641
02 07 83	643
06 10 83	687
21 11 83	29

(*) Discharge at c.5.3 km below source.

APPENDIX 3

Wolds and Galloway streams, April-May 1985. Concentration of total bacteria, turnover rate, turnover rate per bacterium, Vmax, Vmax per bacterium and K+S, for glucose mineralization (by the kinetic approach).

Stream	Sampling date	Distance below source (km)	Total bacteria[a] ($x10^5 ml^{-1}$)	Turnover rate ($x10^{-3} h^{-1}$)	Turnover rate per bacterium ($x10^{-8} ml\ h^{-1}$)	Vmax[b] ($x10^{-3} \mu g\ l^{-1} h^{-1}$)	Vmax per bacterium ($x10^{-11} \mu g\ h^{-1}$)	K+S
	Wolds streams							
Birdsall Beck	15 05 85	0.18	7.8 (7.0-8.5)	13.3	1.7	84.5 (76.4-94.6)	10.9	6.4
		0.45	25.9 (24.2-27.6)	60.2	2.3	516.3 (246.3-571.1)	19.9	8.6
		0.81	22.6 (20.9-24.3)	57.1	2.5	440.5 (369.0-546.5)	19.5	7.7
Church Beck	13 05 85	0.05	1.9 (1.7-2.0)	4.6	2.5	34.5 (27.6-45.8)	18.6	7.6
		0.20	2.7 (2.4-2.9)	8.1	3.0	122.0 (101.7-152.2)	45.5	15.1
		0.40	3.4 (3.0-3.7)	7.4	2.2	116.1 (95.3-148.6)	34.7	15.7
Eastburn Beck	10 05 85	0	1.9 (1.8-2.1)	6.1	3.2	10.6 (9.4-12.1)	5.5	1.7
		0.60	1.2 (1.1-1.3)	2.5	2.0	15.7 (12.0-22.6)	12.9	6.4
		1.40	1.5 (1.4-1.6)	3.8	2.6	28.5 (24.7-33.6)	19.7	7.5
	Galloway streams							
Dargall Lane	28 04 85	1.47	1.9 (1.7-2.1)	0.1	0.1	1.5 (0.9-5.5)	0.8	12.6
Dungeon Burn	29 04 85	0	2.7 (2.4-2.9)	1.1	0.4	5.0 (3.4-9.7)	1.9	4.4
		0.60	2.8 (2.6-3.1)	0.4	0.1	2.7 (2.2-3.4)	1.0	6.8
		1.35	3.2 (2.9-3.5)	0.5	0.1	3.4 (2.4-6.0)	1.1	7.6
Garrary Burn	26 04 85	0	2.6 (2.3-2.8)	0.3	0.1	3.5 (2.1-10.2)	1.4	13.8
		2.08	2.5 (2.3-2.8)	0.8	0.3	7.8 (6.0-11.1)	3.1	9.5
		4.68	4.2 (3.8-4.6)	2.5	0.6	19.7 (13.7-34.9)	4.7	7.8
Minnigall Lane	27 04 85	0	2.0 (1.8-2.2)	0.3	0.2	1.5 (0.8-11.4)	0.8	4.4
		2.66	2.7 (2.5-3.0)	0.4	0.1	1.6 (1.1-2.9)	0.6	4.1
		5.41	5.5 (5.2-5.9)	0.8	0.2	20.4 (13.3-43.2)	3.7	24.3

(a) Mean and 95% confidence limits (in brackets) calculated using central-limit theorem (3.4).
(b) Mean and 95% confidence limits (in brackets) calculated using equation for small samples (6.1.i).

APPENDIX 4

Wolds and Galloway streams, November 1982 - October 1983. Bacterial cell volumes determined by transmission electron microscopy. Values are means, ranges and coefficients of variation (%). 100 cells were measured in each sample.

Stream	Sampling date	Distance below source (km)	Cell volume (μm^3) Mean	Range	CV
	Wolds streams				
Birdsall Beck	19 02 83	0.09	0.07	0.003-0.79	147
		0.81	0.07	0.002-0.65	144
	27 10 83	0.09	0.14	0.002-0.95	141
		0.36	1.15	0.002-0.89	107
		0.81	0.09	0.003-0.76	159
Church Beck	10 02 83	0.03	0.11	0.001-0.54	112
		0.40	0.08	0.001-1.01	202
	28 10 83	0.03	0.09	0.002-0.45	106
		0.15	0.11	0.002-0.73	120
		0.40	0.10	0.002-0.59	105
Eastburn Beck	25 10 83	0	0.12	0.002-0.64	100
		0.20	0.09	0.002-0.80	109
		1.40	0.11	0.001-0.75	118
Millington Beck	10 02 83	0.05	0.07	0.001-0.37	106
		1.70	0.06	0.003-0.65	140
	Galloway streams				
Dargall Lane	26 11 82	1.47	0.03	0.001-0.55	244
		3.20	0.02	0.001-0.22	241
	23 03 83	0	0.06	0.001-0.40	134
		1.47	0.04	0.001-0.50	205
		3.20	0.05	0.001-0.94	233
	03 07 83	0	0.08	0.001-0.45	131
		3.20	0.03	0.001-0.35	190
Dungeon Burn	24 03 83	0.61	0.05	0.001-0.40	150
		1.37	0.05	0.001-0.64	204
	04 07 83	0	0.06	0.001-1.10	255
		0.61	0.05	0.001-0.45	173
Garrary Burn	27 11 82	2.08	0.01	0.001-0.14	179
		4.68	0.04	0.001-0.21	105
	01 07 83	0	0.05	0.001-0.70	227
		2.08	0.06	0.001-1.99	336
Minnigall Lane	24 11 82	2.67	0.01	0.001-0.02	80
	22 03 83	0	0.05	0.001-0.40	144
		2.67	0.03	0.001-0.27	139
		5.42	0.04	0.001-0.57	174
	02 07 83	0	0.05	0.001-0.62	202
		2.67	0.05	0.001-0.79	171
		5.42	0.04	0.001-0.57	195

APPENDIX 5

Wolds and Galloway streams, 1982-1983. Rate of downstream increase in concentration of total bacteria and turnover rate for glucose assimilation per unit length of stream. Results are only from those sampling days when there was a significant ($P < 0.05$) linear regression with distance. (*) indicates non-significant linear regression. 95% confidence limits around the regression coefficient are given in brackets (calculated using the equation for small samples, 6.1.iv).

Wolds streams

Stream	Sampling date	Increase in total bacteria ($x10^5 ml^{-1} km^{-1}$)	Increase in turnover rate ($x10^{-3} h^{-1} km^{-1}$)
Birdsall Beck	28 10 82	13.8 (9.2-18.4)	28.1 (13.1-43.1)
	18 11 82	*	26.5 (16.3-36.7)
	20 12 82	15.7 (11.0-20.4)	19.1 (10.5-27.7)
	25 08 83	*	35.2 (27.9-42.6)
	16 12 83	*	17.9 (9.5-26.3)
Church Beck	10 09 82	13.4 (9.1-17.7)	10.2 (-1.1-2.2)
	25 10 82	14.5 (9.5-19.5)	16.1 (1.9-30.30
	16 11 82	21.4 (16.9-25.9)	18.9 (11.3-26.6)
	16 12 82	*	13.2 (7.5-18.9)
	19 08 83	12.7 (10.2-15.2)	18.2 (4.1-32.3)
	28 10 83	23.2 (14.7-31.7)	22.1 (5.8-38.7)
	14 12 83	29.6 (22.2-37.0)	31.4 (21.1-41.7)
Eastburn Beck	09 09 82	3.5 (2.0-5.0)	1.2 (0.2-2.1)
	21 10 82	4.3 (3.5-5.2)	*
	19 12 82	1.4 (1.3-2.6)	2.5 (0.8-4.2)
	24 08 83	5.5 (3.9-7.0)	2.8 (1.9-3.8)
	25 10 83	8.4 (6.1-10.8)	*
	13 12 83	3.8 (1.8-5.8)	5.1 (0.1-10.2)
Millington Beck	19 10 82	*	8.6 (5.9-11.3)
	11 11 82	4.1 (2.7-5.6)	*

Galloway streams

Stream	Sampling date	Increase in total bacteria ($x10^5 ml^{-1} km^{-1}$)	Increase in turnover rate ($x10^{-3} h^{-1} km^{-1}$)
Dargall Lane	23 03 83	0.7 (0.02-1.3)	0.1 (0.05-0.2)
	03 07 83	*	0.06 (0.01-0.1)
	22 11 83	0.5 (0.1-0.9)	*
Dungeon Burn	03 09 82	*	-0.07 (-0.1-0.01)
	03 10 82	-1.0 (-1.6- -0.4)	-0.2 (-0.3- -0.07)
	24 03 83	*	-0.2 (-0.2- -0.1)
	07 10 83	1.7 (1.2-2.3)	*
	23 11 83	*	-0.2 (-0.4- -0.05)
Garrary Burn	03 07 82	0.3 (0.2-0.4)	*
	02 09 82	0.6 (0.2-0.9)	*
	30 09 82	0.8 (0.4-1.2)	0.09 (0.05-0.1)
	27 11 82	0.3 (0.2-0.4)	0.09 (0.04-0.1)
	01 07 83	0.3 (-0.04-0.7)	*
	05 10 83	1.4 (1.0-1.8)	0.2 (0.04-0.3)
	17 11 83	0.7 (0.3-1.0)	0.08 (0.04-0.1)
Minnigall Lane	06 07 82	*	0.4 (0.2-0.5)
	01 09 82	0.3 (0.07-0.6)	*
	02 10 82	1.3 (0.9-1.7)	0.2 (0.08-0.2)
	24 11 82	0.2 (0.01-0.4)	*
	22 03 83	1.4 (0.9-2.0)	0.1 (0.07-0.2)
	02 07 83	3.5 (1.8-3.3)	*
	06 10 83	*	0.1 (0.02-0.2)
	21 11 83	0.4 (0.2-0.5)	*