The Ecology of the Ciliate Fauna of Hobson's Brook, a Cambridgeshire Chalk Stream

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SUMMARY: An investigation has been carried out into the association between ciliate Protozoa and bacteria in a chalk stream. The ciliates are discussed in the present paper. In general, the largest number of ciliate species and individual species were associated with a growth of green or blue-green filamentous algae. A zonation in the distribution of species along the length of the stream was observed, and particular species were found to undergo cyclical variations in the numbers of individuals. Though there was no correlation between total bacterial numbers and total numbers of ciliates present at any time, the highest bacterial numbers occurred when diatom-eating ciliates were dominant. Statistical analysis showed that the presence of Gram-negative rods in the bacterial flora was correlated with the presence of bacteria-eating ciliates. Gram-positive rods, on the other hand, were correlated with the presence of diatom-eating ciliates. The bacterial flora of the stream was in part determined from that of the surrounding soil. The periods of abundance of ciliates seemed to be related to weather conditions, since ciliates were found to increase in numbers after heavy rain or during prolonged droughts when the banks tended to crumble. Various observations suggest that the ciliate faunas of soil and brook are largely identical. The addition of a small quantity of a broth culture of a micrococcus isolated from the brook to cultures of soil ciliates in soil-extract hay-infusion medium led to a rapid multiplication of ciliates.

An investigation of the ecology of the ciliate fauna of a chalk stream (Hobson's Brook) was carried out over a period of 2 years, the immediate object being to ascertain whether Hassall (1855) was correct in his assumption that fresh-water ciliates are constantly associated with sewage pollution. This paper discusses the results obtained so far as they concern the ciliate fauna. The stream has been described in detail in a previous paper (Gray, 1951). It flows from chalk springs known as the Nine Wells, the term Nine denoting an indefinite number (Conybeare, 1897), and its fall is gradual except in a gravel reach 300 yards below the springs where the water crosses the 45 ft. contour. Nevertheless, the force of water is always considerable. Most of the springs are rheocene in character with neutral or slightly acid waters (pH 6.6-7.0) and like other chalk springs flow at a practically constant temperature throughout the year. The biota have been described in a previous paper (Gray, 1951).

Methods

Samples were taken from the current core at twelve fixed stations along the stream in four arbitrary zones of the upper reach of the Brook (Gray, 1951); from communities of filamentous algae (*Ulothrix*, *Spirogyra*, *Cladophora*) which occurred at the sides or on the bottom of the stream, though not generally in

mid-stream owing to the scouring action of the current; from the flowering plants in the centre of the Brook; from the bottom mud. When the region of maximum abundance had been determined, samples were taken only from the current core.

Samples were taken in the manner previously described (Gray, 1951). The temperature of the stream was determined at the time and place of sampling, and pH values were determined colorimetrically on return to the laboratory.

Water samples were either centrifuged or filtered through gauze and sand, and the deposit or residue examined. When filamentous algae or other macroscopic plants were present, these were agitated in the original water sample after it had been poured into a dish. The plants were then discarded and the contents allowed to settle in a conical funnel. Not less than six uniform drops of the sediment obtained by these methods was examined, and the ciliates were recorded as absent, present, or numerous according to whether 0-5, 5-10, or more than 10 individuals were present (cf. Hausman, 1917).

The numbers of individuals and of species of ciliates were generally small (Table 1). Sometimes a few individuals of several species were found, when all were recorded as present. Occasionally the drops contained large numbers of one or two species only, in which case the species present in greatest numbers was recorded as dominant and the less numerous form as subdominant. It was not uncommon to find that on a given day, six or more drops contained only a very few individuals of several species of ciliate, whereas a day or two later a sample from the same place might teem with individuals of one or two species to the exclusion of all others.

During the summer of 1947 a series of 100 bacteriological counts at 22° were made, and the total numbers and the food habits of the associated ciliates recorded. In this series of examinations a count was made of all ciliates in not less than six drops, both as regards total numbers and as regards the numbers of bacteria-eating or diatom-eating forms. At all other times only the relative abundance of ciliates was recorded (i.e. absent, present, numerous) as for example during the summer of 1948, when the morphology of the bacteria was considered in relation to the dominance of bacteria-eating or alga-eating ciliates, and the relative abundance of the dominant forms determined.

The spring waters themselves were not found to contain any ciliates, but these occurred in small pools formed in fissures of the chalk where dead leaves collected. Samples were taken from the springs throughout the investigation, but the ciliate population was negligible.

A considerable number of samplings were made at various points on cross-sections of the stream between Zone 2 and Zone 4 (Gray, 1951; Fig. 1), and it was found that on an average the greatest number and variety of ciliates occurred in the current core. To obtain a picture of the microfauna and bacterial flora in the current core, twelve fixed stations were established in relation to objects on the banks (Gray, 1951; Fig. 2). When an organism of particular interest appeared at a given point, the microfauna in this region was examined at least three times within as many days. As a routine, the microfauna at the twelve stations was examined at least once a week, samples

usually being taken between 9 and 10 a.m.; occasional samples were also taken late in the afternoon and at midnight. Sampling was done working upstream.

Table 1. *Ciliates isolated from the current core of Hobson's Brook from October 1946 to June 1949*

The numbers indicate the Zones where the organisms were found.

HOLOTRICHA	
Sub-order Gymnostomata	Sub-order Trichostomata
<i>Amphileptus gutta</i> 4	<i>Colpoda cucullus</i> 2, 3, 4
<i>Chaenia teres</i> 3, 4	<i>Colpidium colpoda</i> 3, 4
<i>Chilodon cucullus</i> 3, 4	<i>Dallasia</i> sp. 2, 3, 4
<i>Didinium nasutum</i> 3	<i>Paramoecium caudatum</i> 3
<i>Holophyra</i> sp. 2, 3, 4	Paramoecidae sp. 3, 4
<i>Lacrymaria olor</i> 4	<i>Pleuronema chrysalis</i> 4
<i>Lionotopsis anser</i> 3	<i>Saprophilus</i> sp. 3, 4
<i>Loxophyllum rostratum</i> 2, 3, 4	<i>Trichoda pura</i> 2, 3, 4
<i>Loxodes rostrum</i> 2, 3, 4	
<i>Nassula ornata</i> 2, 3, 4	
<i>Prorodon ovum</i> 3	
<i>Spathidium spathula</i> 3, 4	
HETEROTRICHA	
<i>Condylostoma</i> (?) sp. 3, 4	
<i>Halteria grandinella</i> 2, 3, 4	
<i>Stentor polyphemus</i> 3, 4	
<i>S. niger</i> 3	
<i>Spirostomum ambiguum</i> 3	
HYPOTRICHA	
	<i>Aspidisca costata</i> 2, 3, 4
	<i>Euplotes patella</i> 1, 2, 3
	<i>Oxytricha gibba</i> 2, 3, 4
	<i>Stylonichia mytilus</i> 3, 4
	<i>Urostyla</i> sp. 3, 4
	Unidentified sp. 2, 3, 4
PERITRICHA	
<i>Vorticella nebulifera</i> 1, 2, 3, 4	The peritrichous ciliate <i>Epistylis</i> was only encountered once, in Zone 3; <i>Vorticella microstoma</i> was only occasionally encountered in Zone 3, but was more common in the soil on the banks of Zone 3. <i>V. nebulifera</i> was quite common in all Zones and was also found in colonies of blue-green algae floating down the stream.
<i>Vorticella microstoma</i> 3	
<i>Epistylis flavicans</i> 3	

Unidentified hypotrichous ciliates were common in all Zones all the year round. Some of them were morphologically identical with unidentified hypotrichous ciliates found in the soil of the surrounding land. *Stylonichia putrina* was very common in the summer of 1947 in the soil surrounding the Brook. It was not, however, encountered in the stream, where *S. mytilus* colonized Zone 3 all the year round, but was found once in Zone 2 and only on three occasions in Zone 4.

Holotrichous ciliates resembling *Paramoecium caudatum* (i.e. species of Paramoecidae) were most common in Zone 3 during the summer of 1948.

RESULTS

Association of plants and ciliates

Colonization of the Brook by the filamentous diatom *Melosira varians* was accompanied in spring 1947 and spring 1948 by an abundance of diatom-eating ciliates. In 1947 the dominant form was a *Condylostoma* species (Fig. 1), while in 1948 species of *Loxodes*, *Loxophyllum* and *Stylonichia* were abundant.

Blooms of blue-green algae found floating down the current core, from the upper third of Zone 2 to the lower third of Zone 4 in October 1946, March 1947 and January 1948, were associated with a diverse and numerous microfauna: *Halteria grandinella*, *Actinophrys sol*, *Vorticellae* sp.; Paramoecidae sp., *Pleuronema chrysalis*, *Loxodes rostrum*, *Holophyra* sp., *Colpoda* sp.; together with *Amoeba proteus* and colourless and green flagellates. Generally, the greatest variety of species of ciliates, and very often the greatest numbers, were

Fig. 1. The abundance of diatoms and of diatom-eating ciliates in the stream during January to June 1947.

associated with green or blue-green algal communities (cf. Picken, 1937). The age of the growth of green algae appeared to be of some importance in relation to the abundance of ciliates. Samples taken from young actively growing masses of *Spirogyra*, *Vaucheria*, *Ulothrix* and *Cladophora* had high bacterial counts, but in general very few ciliates. Older growths of green algae had high bacterial counts and a numerous and diverse ciliate fauna. Throughout the investigation the pH value of the water entangled in young blooms of filamentous green algae was often at or just below neutral point (pH 6·6–7·0), while that of mature algae was alkaline (pH 7·4). Decaying green algae and decaying leaves of green plants (with the notable exception of *Sparganium erectum*; see later) had high bacterial counts but in general very few ciliates. In contrast to what was observed in growths of green algae, the age of blooms of blue-green algae did not appear to affect the variety or numbers of associated ciliates.

On the four occasions when blooms of *Oscillatoria* occurred the pH value of the water was *c.* 7.4 and did not vary appreciably with the age of the bloom.

The leaves of green flowering plants in general had a poor ciliate population, but the leaves of *Sparganium erectum* provided a marked exception. Perhaps because of their virtual confinement to the centre of the stream, and their vertical growth (which may prevent the accumulation of silt on the leaf surface) they were usually coated with algae and diatoms and at all ages bore a numerous and varied ciliate fauna.

Zonation of ciliate species

Only a few species of ciliates were recorded. The total numbers of any one species varied within wide limits but during the investigation the same species constantly recurred. The respective Zones each tended to have their characteristic microfauna, as if the topography and physical factors defined a distinct environment in each Zone. Thus when diatom-eating ciliates were dominant, *Loxodes rostrum* and *Loxophyllum rostratum* were found in Zone 2, *Stylonichia mytilus* in Zone 3, and only *Nassula ornata*, *Euplotes patella* and certain Oxytrichidae in Zone 4. Throughout the investigation such zonation was repeatedly encountered. The most striking example was provided by *Stylonichia mytilus*. This organism was virtually confined to Zone 3. It was only found once in Zone 2 and once or twice in Zone 4, and was usually the most common ciliate in Zone 3. Although this zonation was marked with the diatom-eaters, it also occurred, though to a lesser degree, among the bacteria-eating forms. A large diatom-eating form (a *Condylostoma*) dominated the whole rural reach in March 1947 to the virtual exclusion of all other ciliates, but disappeared 6 weeks later. It was not observed again until the autumn of 1948, when it was found at the junction of Zones 2 and 3, the point at which it was first recorded in January 1947.

Cycles of abundance of ciliates

The abundance of individual members of the ciliate fauna varied considerably at different times, and a sequence of species and series of maximum and minimum phases of individual abundance was observed. One ciliate tended to succeed another; bacteria-eaters might precede or succeed diatom-eaters. Whatever form was first observed to be dominant, it eventually ceased to be dominant or disappeared completely in periods from a few hours to 4 days, to be succeeded by another form not necessarily of related food habits. Once the new form had established itself, it might be dominant or share dominance with the preceding form, or both might disappear and be replaced by a third form (Fig. 2).

During a dominant phase, the given ciliate spread upstream and downstream, and in the minimum phase retreated to the upper levels. Thus in May 1947 a bacteria-eating ciliate, a *Colpoda* sp., spread from Zone 2 to the upper third of Zone 4. By the end of June it had retreated to the upper third of Zone 2 and it was not again recorded from Zone 3 until the middle of July. A species of

Holophyra present in Zone 3 in June 1947 was found throughout Zone 2 to Zone 3 and Zone 4 by the end of the month; subsequently it was only found in the upper third of Zone 2. *Halteria grandinella* spread in similar manner between Zone 2, Zone 3 and Zone 4. During the summer of 1947 *Stylonichia mytilus* colonized Zone 3; it was not found in Zone 2 and only on one or two occasions in Zone 4. In its maximum phase it was dominant in Zone 3 and occasionally spread to the upper third of Zone 4; during its minimum phase it retreated to the upper third of Zone 3. *Paramoecium caudatum*, a bacteria-eater, was only occasionally observed in 1947 but from Zone 3 it spread into Zone 4, retreated to Zone 2 and later spread through Zone 3 into Zone 4 again.

In 1947 and 1948 it was observed on several occasions that during the maximum phases of many of the ciliates (e.g. *Stylonichia mytilus* and other Hypotrichidae, *Loxodes rostrum*, *Chilodon cucullus* and species of *Colpoda* and *Holophyra*) forms in binary fission and conjugation were present. *S. mytilus*, for example, was observed in conjugation once, and in fission four times, in June 1947 during the weekly samplings at the twelve fixed stations, and in conjugation once, and in fission three times, in July 1948 during the twice a week samplings carried out at stations 6–8. In 1947 this reproductive phase was often followed by the appearance of small forms and a diminution in numbers; this was observed on twelve occasions in June in the weekly samplings from stations 1–12. In 1948, however, the maximum phase was often succeeded not only by the appearance of small forms of all species of ciliates, on twenty occasions in July in twice-weekly samplings at stations 6–8, but also by the appearance of monstrous forms, double monsters, and bent and swollen forms with irregular outlines. The latter were noted on sixteen occasions in July 1948 in the twice-weekly samplings from stations 6–8, when abnormal forms of *Paramoecia* were observed on six occasions and abnormal Hypotrichidae on five occasions. These abnormal forms tended to occur in the lower reaches, they were almost wholly limited to station 8 of Zone 3. It was often found that active healthy forms of a particular species were abundant at station 6, present in diminished numbers at station 7, and grossly distorted at station 8.

On the whole, the bacteria-eating *Paramoecium caudatum* and other *Paramoecia* were more common, especially in Zone 3, during 1948, than in 1947. They were never observed in conjugation but seen once in fission in July 1948 during an exceptionally abundant maximum phase.

Abundance in relation to numbers of bacteria

Although it is convenient to divide the ciliates into two groups according to their feeding habits (diatom-eaters and bacteria-eaters) the division is not absolute. Some diatom-eaters, e.g. *Stylonichia mytilus*, will ingest bacteria in the absence of diatoms, as Sandon (1932) found, or as epiphytic bacteria on diatoms. Harvey (1945) found the greatest numbers of bacteria in sea water in the samples richest in phytoplankton. It seems likely therefore that diatom-eating ciliates will also tend to diminish the numbers of bacteria present, either because they are ingested accidentally as epiphytes on diatoms, or because they are an alternative food for certain species. A study of the

relationship between the numbers of individuals and of genera of ciliates, and the number and kinds of bacteria present in the Brook, was made during the summers of 1947 and 1948. A series of 100 bacteriological counts of total numbers of bacteria at 22° were made in parallel with collections of ciliates at the twelve stations during June-July 1947. When the figures obtained were analysed statistically, no correlation was found between total bacterial numbers and total numbers of ciliates present at any time; nor were fluctua-

Fig. 2

Fig. 2. The relative abundance of seven ciliates in Zone 3 (the middle third of the Brook) from October 1947 to August 1948.

Fig. 3

Fig. 3. The prevailing microflora as shown by bimonthly examinations and the dominant ciliate fauna from October 1946 to October 1947.

tions in total bacterial numbers correlated with fluctuations in total ciliate numbers. It was found, however, that the highest bacterial numbers (up to 400,000/ml.) occurred when diatom-eaters were dominant.

A series of fifty samples taken in May-June 1948 from stations 6-8 in Zone 3 showed no correlation between the total ciliate numbers and the total bacterial numbers at 22° but, as in 1947, the highest bacterial numbers were associated with a dominance of diatom-eaters. When the number of bacterial colonies was small (six to twelve) these were picked off and stained by Gram's method. With counts of 50-100, twenty colonies were selected at random, picked off and stained. This examination of the bacteria suggested that dominance of diatom-eaters or of bacteria-eaters was associated with the presence or absence of certain types of bacteria. There seemed to be some correlation between the presence of diatom-eaters and Gram-positive rods, while bacteria-eaters were associated with Gram-negative rods, and abundance of both groups was associated with micrococci. Thus from fifty samples taken at five stations (ten samples per station) on twelve occasions bacteria-eating ciliates were dominant when Gram-negative rods were more abundant than Gram-positive rods, or when only Gram-negative rods and micrococci were present. Diatom-eating ciliates were dominant on four

occasions when Gram-positive rods were more abundant than Gram-negative, and on two occasions when Gram-positive rods and micrococci were equally abundant. Statistical analysis of the data by Mr D. V. Lindley (Mathematical Laboratory, Cambridge) showed that the correlation of Gram-negative rods with the presence of bacteria-eaters and of Gram-positive rods with diatom-eaters was statistically significant.

These findings were supported by other observations made in the field. When either Gram-positive or Gram-negative bacteria dominated the microflora of the stream, one or other of the two groups of ciliates tended to be dominant (Fig. 3). When the microflora was largely composed of Gram-negative rods, bacteria-eaters (e.g. *Colpoda* spp.) were common; when Gram-positive rods were more numerous than Gram-negative rods, diatom-eaters supplanted bacteria-eating forms. The bacterial flora of the stream came largely from the surrounding land, as shown by the appearance of soil bacteria after heavy rain or periods of drought (Gray, 1948, 1951) and the character of the soil bacteria tended to be modified by agricultural operations. In a tributary stream flowing into the Brook from a farmyard and Shelford village (Gray, 1951) the greatest numbers of *Paramoecia* were found where sewage pollution (i.e. by Gram-negative rods) was highest. Where the tributary entered the Brook and sewage pollution was least, *Paramoecia* disappeared and diatom-eating ciliates became prominent (Gray, 1951).

The influence of weather conditions

The cycles of abundance of ciliates could not be related to total numbers of bacteria growing at 22°, pH value or temperature of the water. *Stylonichia mytilus*, however, appeared to exhibit lunar periodicity (see later). With this exception, although the cycles could not be related to any one of the above factors, they were related to weather conditions. The ciliates increased in numbers and spread down the stream after floods or heavy rain, or during prolonged periods of hot dry weather when the banks tended to crumble and soil fell into the water (Fig. 4). The latter occurred particularly in 1947, when floods in the spring were succeeded by months of drought. By contrast, the summer of 1948 was dull and wet. In this year, in twenty-two examinations over the entire length of the reach of the Brook examined, thirteen were made within 24 hr. of storms or heavy rain. In the samples taken during eight of these latter examinations, bacteria-eating ciliates were dominant; on three occasions diatom-eaters were dominant, while on two occasions dominance was shared between bacteria-eating and diatom-eating ciliate protozoa. It did not seem probable that this phenomenon was due solely to mechanical transport by the increased volume of water, for the maximum peak of abundance of a particular species had often passed at a particular point while a spate was still passing downstream or while rain was still falling. It was noted that the actual numbers of individuals of particular species (rather than of all species) increased at the sampling points as they did when soil was crumbling into the water; that is to say, only one species out of perhaps half a dozen originally present would increase in number at a particular sampling station.

Lunar periodicity in Stylonichia mytilus

When the numbers of this organism were plotted against the date, it was found that during 1947 maximum phases occurred on an average 7–10 days after a new moon or a full moon, especially the latter. The two largest maxima, when the organism dominated the whole of the middle reach of the stretch under examination, occurred 9 days after a new moon in July and 4 days after a full moon in December. By contrast with the dry weather and clear nights of 1947, the year 1948 was wet and dull, and *S. mytilus* was only recorded from May to September. Nevertheless, its numbers rose to a maximum on five occasions averaging 8 days after a new or a full moon. A statistical analysis of the numbers of *Stylonichia* (as obtained by the methods described) considered in relation to lunar periodicity in days, was made by Mr D. V. Lindley, and suggested that *S. mytilus* has a maximum about 4–7 days before and after a full moon, with some slight evidence for a fall in numbers at the full moon. More data would be necessary to establish the possible periodicity (Gray 1951*a*).

Possible influence of the macrofauna

One of the features of the changing fauna of the Brook was a peak of numbers of dipterous insects in late summer (Gray, 1951). Although the relative abundance of individuals of various species of ciliate continued all the year round, their total numbers fell steadily throughout the summer. During this period there was an increase of dipterous larvae, especially chironomids and simuliids; these were particularly common in Zone 3. They are known to be omnivorous microphages. Since they occur in maximal numbers in the current core, where the largest ciliate population was found, it is possible that the fall in total numbers of ciliates from the current core during the summer is correlated with this enormous increase in the population of microphagous larvae (Fig. 5). Barker (1946) found a similar association in sewage filter beds, where seasonal peaks of fly output were accompanied by a depression of ciliate protozoa.

Identity of soil and stream ciliates

The numbers of ciliates at the sampling points increased greatly after heavy rain or during periods of drought. Especially after rain, the increase in numbers was too great, too sudden, and too general, wholly to be accounted for by multiplication of the existing ciliate population as a result of some change in natural conditions. Moreover, soil bacteria appeared in the microflora at such times (Gray, 1948). It was considered possible, therefore, that not only soil bacteria but also soil ciliates were entering the Brook. When the soil ciliate population of the banks of the stream and of the soil 200 yards away on the banks of ditches emptying into the Brook was examined, the same genera were found to be present as in the stream (i.e. *Colpoda*, *Oxytrichidae*, *Halteria*). Ciliates of the same genus as those in the stream were also obtained from the interior of clods of dried mud originally excavated from the stream-bed and cast on adjoining fields. These observations (Table 2)

suggested that the ciliate faunas of the soil and Brook are virtually identical, one habitat receiving recruits from the other (Gray, 1948).

Fig. 4

Fig. 5

Fig. 4. Rainfall, temperature, and abundance of ciliates at 2-day intervals in Zone 3 (the middle third of the Brook) during June and July 1948.

Fig. 5. The abundance of dipterous larvae and of ciliates in Zone 3 (the middle third of the Brook) during the summer of 1947.

Table 2. *A comparison of the infusoria obtained from the soil round Hobson's Brook in the early summer of 1947 as compared with forms found in the Brook during the investigation*

In the soil		In Hobson's brook
<i>Ciliata</i>		
<i>Colpidium colpoda</i>		<i>Colpidium colpoda</i>
<i>Colpoda cucullus</i>		<i>Colpoda cucullus</i>
<i>Halteria grandinella</i>		<i>Halteria grandinella</i>
<i>Holophyra</i> sp.		<i>Holophyra</i> sp.
<i>Holotrichous</i> sp.		<i>Holotrichous</i> sp.
Paramoecidae		Paramoecidae
<i>Oxytricha</i> sp.		<i>Oxytricha</i> sp.
Oxytrichidae		Oxytrichidae
<i>Spirostomum ambiguum</i>		<i>Spirostomum ambiguum</i>
<i>Vorticella microstoma</i>		<i>Vorticella microstoma</i>
<i>Rhizopoda</i>		
<i>Actinophrys sol</i>		<i>Actinophrys sol</i>
<i>Amoeba limax</i>		<i>Amoeba limax</i>
<i>A. proteus</i>		<i>A. proteus</i>
Testaceous rhizopods		
<i>Diffugia</i> sp.		<i>Diffugia</i> sp.
<i>Arcella</i>		Not found
<i>Rotifera</i>		
<i>R. vulgaris</i>		<i>R. vulgaris</i>
<i>R. elongatus</i>		Not found; but found in River Cam
<i>Philodina</i> sp.		<i>Philodina</i> sp.

Multiplication of soil ciliates in the presence of broth cultures of micrococci and pseudomonads

Cultures of soil ciliates were obtained by covering a few grams of soil in a conical flask with sterile tap water containing 0.2–0.5 % (v/v) hay infusion and 1 % (v/v) soil extract. The hay infusion was prepared from good quality hay as tea is prepared (Heath, n.d.), the soil extract was prepared according to the formula of Cunningham (1947). The numbers of ciliates in cultures made with this medium increased but slowly when the sole food supply was the natural soil microflora. Ciliates were not found in the cultures until these had been incubated at room temperature for several days or even as long as 2–3 weeks. Following the observation of Singh (1946) cultures were inoculated with micrococci from the stream. A massive multiplication of ciliates resulted in cultures so inoculated. A few drops of broth cultures, or of filtrates of broth cultures, of two micrococci 3/2 and 3/3 isolated from Zone 3 in the summer of 1947, caused a great multiplication of the soil bacteria, initially of spore-forming bacteria, followed in a few hours by a great increase in the numbers of soil ciliates. Strain 3/2 had not so powerful an action as 3/3 and died out within a short time, whereas 3/3 was subcultured for 3 years and provoked an identical action at the end of that time. It retained its morphology, showing that it was not a pleomorphic strain of *Bact. globiforme* Conn. A few drops of broth only caused a multiplication of bacteria in cultures of soil ciliates but no increase in the numbers of the ciliates. During the increase in numbers of ciliates after inoculation with broth cultures of micrococcus 3/2 or 3/3, ciliates were seen in fission. A few drops of hay-infusion cultures of micrococcus 3/2 or 3/3, or filtrates of such cultures, did not cause either a multiplication of bacteria or an increase in the numbers of ciliates. There was, however, some increase in the numbers of flagellate protozoa. After a few drops only of broth cultures of micrococcus 3/2 or 3/3 had been added, the bacterial flora of the culture at the time the numbers of soil ciliates were increasing, was composed largely of long and short Gram-negative rods, among which pseudomonads were most common, together with the strain of micrococcus that had been added, some chromogenic micrococci, and an occasional spore-former. Four to seven days after multiplication of the ciliates had ceased, the microflora was almost wholly composed of long and short Gram-negative rods (pseudomonads prominent) and the micrococcus.

These experiments showed that increase in the numbers of bacteria-eating soil ciliates followed inoculation of hay-infusion soil-extract cultures with a few drops of the micrococcus culture; no experiments were undertaken to establish whether micrococci alone among the stream microflora were capable of stimulating this increase.

Single cysts of *Colpoda* and *Colpidium* spp. were picked out from mixed cultures of soil ciliates, and pure cultures of these bacteria-eating ciliates were obtained by passing them through a series of sterile baths. It proved possible to remove gross bacterial contamination by such methods, but the cysts could not be freed from a Gram-negative rod. This was a species of *Pseudomonas*

identical with that isolated from mixed cultures of soil ciliates after addition of micrococcus 3/2 or 3/3.

Cultures of *Colpoda* could be established successfully in liquid media containing no bacteria except the pseudomonas, and in media containing only the pseudomonas and a brook micrococcus, by transferring single individuals or cysts to the media in question. It was observed, however, that although the ciliates multiplied in the presence of such bacterial foods, the multiplication was not unlimited. Pure cultures of *Colpoda* and *Colpidium* grew and multiplied during the first 24–48 hr. but multiplication then ceased and some ciliates encysted, although food bacteria were still abundant in the medium and would support vigorous multiplication of soil ciliates if reinoculated.

DISCUSSION

The conclusion reached by Hassall (1850, 1855), that ciliates (notably Paramoecidae) are constantly associated with sewage pollution because they find in pollution 'their means of subsistence and growth', probably marks the first tacit recognition of the dependence of certain ciliates on a supply of bacteria rather than green algae as a source of food. Two years later, in 1852, Pritchard published the third edition of his *Infusorial Animalcules* in which the nutrition of ciliates is only vaguely indicated: 'The digestive cells of Paramoecidae are demonstrated in one species by its usual green food.... In all Kolpoda digestive cells have been indicated by coloured foods....' Hassall's observations were largely made on the Thames; but in spite of the importance of sewage pollution of rivers, they are only paralleled in modern times by those of Lackey (1938) on ciliates as pollution indicators in the Scioto River.

Cycles of maximum and minimum abundance of different species of ciliates, noted in the Brook, have been recorded by many workers (Calkins & Summers, 1941); they also occur in the soil (Russell, 1923). The present study suggests that in many instances they are directly related to climatic conditions, since peaks of abundance occur after heavy rain, or after drought when soil is washed or crumbles into the Brook. At such times there was a peak of abundance of soil bacteria in the Brook and a maximum of bacteria-eating ciliates.

It was noted, however, that the peak of ciliate numbers (especially bacteria-eaters) which followed pollution of the Brook by soil formed so rapidly that it could hardly be accounted for by simple multiplication of the existing population induced by an increased food supply. Since ciliates occur in the soil, it was thought that they might have entered the stream with soil bacteria. The suggestion was therefore made that soil and fresh-water ciliates are in fact identical, one habitat being the source of the other's population (Gray, 1948). Soil (ciliate) protozoa were known to Ehrenberg (1838), and many later workers have commented on their similarity to fresh-water forms. Russell (1923) observed that at one time it was held that the soil was recruited at random by protozoan cysts carried by wind from dried-up ponds and streams, but added that to 'Martin & Lewin (1915) must be ascribed the distinction of first proving that the soil possesses an active population'. Again, it was suggested by Calkins & Summers (1941) that 'water-dwelling forms might occasionally be

found in soil and interpreted as casual soil-dwelling types'. Woodruff (1912), on the other hand, in a classical study of the ciliates of hay-infusions, gave air, water, and hay as their source, but did not mention soil; similar sources, exclusive of soil, were given by Kent (1881). An examination of the micro-fauna of the soil round the Brook revealed that, with the exception of species of *Stentor*, all the ciliates isolated from the Brook during the 2½ years of this inquiry were forms recorded by Sandon (1927) as common in the soil, to an extent suggesting that the soil may be the chief source of the ciliate population of inland waters.

Since it is known that individual crop-plants are associated with characteristic bacterial flora, it was of interest to examine the nature of the crops surrounding the Brook during the investigation. During this period, the crops sown on adjoining land which largely drained into the Brook, were almost wholly a cereal rotation (Gray, 1951). Wheat and barley were sown on each bank of Zone 3 during 1947; linseed and wheat, during 1948. The banks of Zone 4 were sown with oats and sugar-beet, and rape-cole and kale, in 1947; with oats and wheat, in 1948. One bank of Zone 2 is old permanent grassland; while the other bank was sown with barley in 1947, and with meadow hay in 1948. Lochhead, Timonin & West (1940) showed that the rhizospheres of plants, notably cereals, roots, and clover, are characterized by a distinct microflora, among which Gram-negative rods are very abundant. It seemed possible, therefore, that the crop-rotation on the banks was providing bacteria-eating ciliates with their chosen bacteria, viz. Gram-negative rods, since bacteria-eating ciliates were dominant when soil entered the Brook.

Gram-negative rods derived from the rhizosphere would be most plentiful when the crops were growing, that is, in the late spring and early summer. When the land was ploughed and dressed with farmyard manure other Gram-negative rods (e.g. *coliform* bacteria) would enter the stream, while many would be derived from the droppings of sheep then folded beside the Brook. Severin (1895; quoted by Waksman, 1927) found that bacterial rods predominated in freshly ploughed soil, but that within 2 weeks micrococci were abundant. I found that micrococci tended to dominate the microflora of the Brook in the autumn (Gray, 1951).

Singh (1941) showed that the bacterial food supply is the chief factor controlling the abundance of soil Protozoa. The restricted number of species of soil ciliates found during this investigation may be attributable to the same cause; for if soil and stream ciliates are identical, the same factor of bacterial food supply would tend to restrict the number of species found in the Brook.

Anscombe & Singh (1948), in an investigation on the limitation of bacteria by micro-predators in soil, showed that two species of Myxamoebae (*Dictyostelium giganteum*, *D. mucoroides*) and two species of Myxococcaceae (*Myxococcus fulvus*, *Chondrococcus exiguus*) prefer Gram-negative bacteria. The cycles of abundance of ciliates may therefore be attributed to variations in the bacterial food supply and to variations in the numbers of soil ciliates entering the Brook. Bacteria-eating ciliates seem to be particularly favoured at those times when Gram-negative rods are abundant in the soil microflora. There

appears to be a division among the bacteria-eaters themselves according to the character of the Gram-negative rods, for Paramoecidae were only abundant when *Bact. coli* was numerous, as in the tributary, whereas other forms (e.g. *Colpoda*) were associated with abundance of Gram-negative rods not strains of *Bact. coli*. In this respect the investigation supports the emphasis laid by Noland (1925) on the importance of the food supply in the ecology of fresh-water ciliates.

In connexion with the occasional presence of ciliate monsters in the Brook, and the apparent importance of a micrococcus in determining the abundance of the Brook ciliates it is interesting that Sonneborn (1932) induced the formation of double-monsters and chain-forms in *Colpidium campylum* by feeding this ciliate on a strain of *Micrococcus sensibilis*. The two strains, 3/2 and 3/3, of micrococcus isolated in the present work were not identified. It was noted that double-monsters and other abnormal forms of both diatom-eating and bacteria-eating ciliates (e.g. *Loxodes rostrum* and Paramoecidae) were present on more than one occasion when unidentified micrococci dominated the bacterial flora of the brook. They were particularly noticeable among the Paramoecidae, though in addition abnormal forms of *Loxodes*, *Loxophyllum rostratum* and Oxytrichidae were also noted. Certain of them closely resembled Sonneborn's figures of degenerate *Colpidium campylum* fed on a strain of *Micrococcus sensibilis*.

It is suggested, therefore, that the periods of ciliate abundance may be induced by the nature and quantity of the bacteria made available as food by climatic conditions, when these cause a massive and general contamination of the stream with soil bacteria and ciliates.

Noland (1925) emphasized the supreme importance of food supply in the ecology of fresh-water ciliates; the present inquiry, in stressing this conclusion, points to the soil surrounding the aqueous habitat of the ciliates as the major source of food. Diatoms also may perhaps come from the soil, but both diatom-eaters and bacteria-eaters ingest bacteria which may also be derived from the soil. Climatic conditions may be responsible for the quantity, and present and past agricultural operations for the quality, of the food bacteria provided. The quantity of suitable bacteria available in the soil at any one time will vary, for Taylor (1936) has recorded short-period fluctuations in the numbers of bacterial cells in soil. While fresh-water ciliates may justly be regarded as indicators of bacterial pollution, the present study has shown that pollution may derive from the soil in the absence of sewage as such. The ecology of the fresh-water ciliates suggests how close is the association between the stream and the soil through which it runs.

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