STUDIES ON THE BIOLOGY OF SOIL CILIATES

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A B S T R A C T

The soil ciliate fauna consists of at least three major groups:

- (a) Species found wholly or chiefly in granular soils and litters and which may also be found in moss or sphagnum. These species are often larger than other soil species and structurally more specialized.
- (b) Species also commonly found in sewage, faeces or polluted water. These are chiefly small holotrichous ciliates which feed on bacteria. They tolerate a wide range of pH, salinity, temperature, oxygen and carbon dioxide tensions. They generally have a well developed cyst physiology.
- (c) Species of common fresh water genera which differ from the fresh water species chiefly in size. They are generally small holotrichous ciliates fairly widespread in soil although they have not yet been recorded from other habitats.

Parasitic species such as Balantidium and anaerobic species such as Trimyema occur very rarely. Suctorians occur occasionally.

In the laboratory most species grow best in a medium nearest their natural environment, viz. soil or peat extract. This is particularly true of the first and third groups. The second group grows well in enriched media which are not tolerated by the other species. In a laboratory culture most species are found associated with the bottom detritus but one species, Blepharisma steini, common in litters and occasionally pigmented, avoids the bottom of the culture and is found only at the surface. Soil ciliates are not affected by pH changes over a wide range.

In a soil laboratory culture there is less variation than in ordinary laboratory infusions, pH and Eh are fairly stable, and after the first week the bacterial flora consists principally of Pseudomonas. The ciliate fauna is present for about six weeks, the first species appearing within a few days, the majority being present after the first week, and finally the carnivorous species Dileptus alone being present after twenty weeks. The rhizopod fauna does not appear until after the majority of ciliate species have disappeared.

The effect of a heavy inoculation of bacteria into a mixed culture of protozoa causes the death of certain species, such as Euplotes aediculatus and the rapid growth of other species such as Paramecium caudatum, Cyclidium glaucoma and Vorticella microstoma which encyst or die when the bacteria are wholly consumed. Other species such as Vorticella striata neither die nor divide rapidly. Continued heavy inoculations of bacteria cause Vorticella microstoma to form unstable cysts due it is suggested to the accumulation of bacterial metabolites and the strongly reducing conditions. These cysts excyst when aerated. They are larger than the normal resting cyst which is formed on the exhaustion of food.

Only a few species such as Colpoda steinii, Colpoda inflata and Colpoda cucullus survive anoxia for any length of time at room temperatures. These facultative anaerobes continue to feed and move although they cannot divide or excyst. Rarely they form unstable cysts. Other ciliates which also survive for some time are Stentor roeseli and Vorticella microstoma both normally sessile

forms. The former becomes detached and swims freely in the medium with the aid of its peristomal cilia. Vorticella also becomes detached and forms a telotroch or swarmer which does not settle unless oxygen is present. The telotroch will survive in the absence of oxygen a little over two days. It cannot feed and it is suggested that the limited survival of these two ciliates is due partly to the exhaustion of their food reserves. Other species such as Stylonychia mytilus a common fresh water species and Halteria grandinella are extremely sensitive to anoxia.

Colpoda steinii, Colpoda inflata and Paramecium caudatum are very resistant to high carbon dioxide tensions and C. steinii will continue to move though not feed or divide at very high carbon dioxide tensions. Halteria grandinella is moderately resistant but Coleps hirtus and Stylonychia mytilus are extremely sensitive. Vorticella microstoma in response to high carbon dioxide tensions forms a telotroch which survives a comparatively long time. The trophic ciliate is much more sensitive. Stentor also becomes detached in high carbon dioxide tensions.

Colpoda steinii will grow in salinities up to 3% NaCl but division is progressively inhibited and unstable cysts are formed. Excystment takes place only if the environment is hypotonic to the ciliate. Growth of Colpoda steinii is most rapid at about 27°C. At high and low temperatures division is inhibited and unstable cysts are formed. This unstable cyst is similar to the reproductive cyst but unlike that cyst remains inactive until the inhibiting factor, e.g. temperature or salinity, is removed. The factors

which inhibit division also inhibit excystment and for this reason it is presumed that a common morphogenetic mechanism, identified with the 'activity' system found in other organisms, underlies them both. An interpretation of the physiology of Colpoda upon this assumption is used to explain the common effect of diverse environmental factors. Encystment and excystment are considered two distinct processes, contrary to the suggestion of Bridgeman (1948) who considered them complementary.

Excystment of Vorticella microstoma may be stimulated by very low oxygen tensions but is normally dependent upon the presence of bacteria. Activation of the encysted Vorticella leads to two processes: the differentiation of the telotroch and the escape of the ciliate from the cyst membrane. Following imbibition of water the ciliate bursts through the cyst pore the aboral end foremost. The posterior ciliary wreath is normally differentiated after the ciliate escapes from the cyst membrane and assumes the elongated body form of the telotroch. Sometimes the ciliate fails to escape and a fully differentiated telotroch is formed within the cyst membrane. Following excystment the telotroch becomes free swimming and finally settles. It swims with the aboral end and the posterior ciliary wreath directed anteriorly. Telotrochs are normally formed during excystment or by division but anoxia or high carbon dioxide tensions will also cause the trophic ciliate to form a telotroch. It does not encyst under these conditions contrary to prevailing opinion (Brand, 1923).

A consideration of the bionomics of ciliates shows that their

ecology is determined in their behaviour, life history and physiology. Soil species, such as Colpoda, are distinguished by their small size, their tolerance of a wide range of soil conditions and the efficiency of their cyst physiology. Fresh water species are excluded from soil either because they are not tolerant of such environmental conditions as high carbon dioxide tensions, e.g. Coleps hirtus, or because they have a poorly developed cyst physiology, e.g. Paramecium.

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Most ciliates are of cosmopolitan distribution. Some species are found in both marine and fresh water but the majority are limited to one or other of these habitats. Certain species and groups of species are commonly found associated with characteristic ecological conditions. A number of species are commonly found in polluted waters and these include Paramecium caudatum, which, since it is rarely found elsewhere in any great numbers, may be taken as an indication of pollution when found in natural waters. Other species, such as Frontonia leucas and Oxytricha chlorelligera, are rarely if ever found in polluted waters (Lackey, 1938b). Because of this specialization the free living ciliates can be classified into several groups which are the polysaprobies, living in an organically rich environment; the mesosaprobies, living in a more diluted organic environment; the oligosaprobies, living in an environment with little organic matter; and the katharobies, living in an environment devoid of organic matter. Under most natural conditions the environment is relatively unstable, with changing chemical, physical and organic properties. Correlated with these changes are changes in the ciliate fauna: Paramecium giving way to Stentor, and Stentor to diatom feeders (Gray, 1951, 1952). Such conditions, which may be observed in nature, are simulated in the laboratory by what is known as the culture sequence. The history of a laboratory infusion displays the same succession of species as those found in a stream, and this succession can be closely correlated with the other changing conditions of the infusion (Hetherington, 1933).

This close connection between field and laboratory conditions and the ciliate fauna has been known for a long time and there has been some attempt to account for it. In an extensive review of the whole problem in 1938, J.B. Lackey showed that none of the factors advanced by previous workers were by themselves satisfactory in explaining the ecological specialization of different species. He emphasised that species should not be classified ecologically until their range of tolerance of environmental conditions is known. He believed that many factors, such as food and temperature, have a quantitative but not a qualitative effect on the protozoan fauna.

The effect of environmental factors on protozoa has been widely studied. The effect of reduced oxygen tensions was first studied by Loeb and Hardesty (1895) and Putter (1905). A considerable literature of uneven value has accumulated on this subject and is reviewed by Brand (1946). The effect of carbon dioxide has been less studied although there is a classic paper by Jacobs (1912). One of the best papers on the effect of salinity is that of Hoare (1927) who experimented with the euryhaline species, Cyclidium glaucoma, Uronema nigricans, and Cohnilembus pusillus. A recent paper by Loefer (1939) deals with the tolerance of a species, Tetrahymena pyriformis, grown in pure, bacteria-free culture. There are many papers which show that growth takes place only within a limited pH range and that within this range there are often optima and pessima (Loefer, 1938). The growth of ciliates on different strains of bacteria has been studied by Burbank (1946), E. and M. Chatton (1927a, 1927b) and many others and the selective

effect of bacteria on the ciliate fauna was shown by Hetherington (1933).

The principal conclusion of these studies is that different species show very different tolerance of environmental factors. Some species, such as Cyclidium glaucoma, are euryhaline; some, such as Tetrahymena pyriformis, tolerate considerable changes of salinity; some, such as Tillina magna are tolerant of only slight changes. Similarly some species, such as Tetrahymena pyriformis are facultative anaerobes whereas others are obligate aerobes or obligate anaerobes. If the separate findings are correlated it is found that certain species are characterised by tolerance of a wide range of environmental conditions. Tetrahymena pyriformis is tolerant of anoxia, of high carbon dioxide tensions, and of a wide range of salinity, temperature and pH. Other species, such as Spirostomum ambiguum are much more sensitive to changing conditions. The tolerant species are those classified as polysaprobies whereas the sensitive species are mesosaprobies or oligosaprobies. There is a connection between the ecology and the physiology of the species, although the two are not absolutely correlated.

For any species there are two factors to consider: first, the range of culture or environmental conditions which the species will tolerate, and secondly, the behaviour of the species when conditions change. Mendelssohn (1902a,b,c,) showed that Paramecium placed in culture fluid of unequal temperature concentrates where the temperature is about 25°C. Saunders (1924) showed that Spirostomum ambiguum placed in culture fluid of varying pH concentrates where the pH is about 7.4. Rylov (1923) showed that Loxodes rostrum

avoids water of high or very low oxygen tension and is found chiefly in water from 9% to 45% saturation. Jennings (1904) showed that Stentor became detached when subjected to strong light and settles in less strongly lit regions. Jennings and Moore (1901) found that certain species were attracted by carbon dioxide whereas other species were not. These studies illustrate the selective behaviour of ciliates under diverse cultural or environmental conditions.

Half a century of careful research has revealed a characteristic ciliate fauna in soils (Sandon, 1928). A study of the fauna of New Zealand soils shows that it is systematically distinct from the fresh water fauna and that it may be divided into three major groups: one related to the moss and sphagnum fauna, one to the pollution fauna, and a third which is autochthonous. Since the soil fauna is ecologically distinct from the fresh water fauna the question arises as to whether there are significant differences in their physiology, behaviour or life history.

One most interesting distinction between the soil and fresh water fauna is the absence from soil of the common fresh water species of Paramecium and of the widespread Coleps hirtus. On the other hand the most common soil ciliates include species of Colpoda, Vorticella microstoma and Halteria grandinella. Of these ciliates Paramecium, Vorticella and Colpoda are polysaprophes while Coleps and Halteria are mesosaprophes. There is thus some factor other than the degree of organic decomposition which enables certain species to live in soil while it excludes others. Both Vorticella and Colpoda are distinguished by a highly developed cyst physiology and the formation of cysts is a characteristic and normal feature

of their life cycle. On the other hand cysts of Paramecium have only very rarely been recorded although it is one of the most commonly cultured ciliates and Paramecium has never been observed to excyst (Kudo, 1947). It seems probable that the regular formation of cysts greatly favours the life of ciliates in soil and that failure to form cysts may exclude some species. The soil fauna is not however homogenous and the factors that limit or favour each of the three ciliate groups may vary greatly. Some species are found only in litters or soils of good granular structure. These species are generally larger than other soil species and it may be that their size prevents them living in the smaller soil spaces, a factor which would also exclude many of the common fresh water species.

The chief purpose of the present thesis is to examine the response of different species, both soil and fresh water, to environmental conditions in order to establish a physiological distinction between the two groups and to test the effect of environmental conditions on the behaviour and life history of the ciliates. It is directed towards the general problem of interpreting the ecology of the ciliates in terms of their physiology, their behaviour and their life history.

The general conclusions drawn from earlier work have been confirmed and extended. Polysaprobic soil species such as Colpoda steinii and Colpoda inflata have been found to possess a greater tolerance of environmental conditions than fresh water species such as Stylonychia mytilus. In particular polysaprobic species are easily differentiated by their resistance to carbon

dioxide toxaemia and it is therefore suggested that the carbon dioxide concentration is an important factor affecting the composition of the fauna. In particular the extreme sensitivity of Coleps to carbon dioxide and the relative tolerance of Halteria is considered to account for the exclusion of Coleps from soil and the presence of Halteria.

The life history of Colpoda and Vorticella is shown to be affected by different environmental conditions and in Vorticella there is the ability to metamorphose from the sessile trophic form to a free swimming telotroch in response to changing environmental conditions, such as oxygen tension. The effect of environmental factors on division in Colpoda is identical with their effect on excystment and for this reason it is suggested that these two processes are governed by a single mechanism, the 'activity' system, which in other organisms, such as sea urchin eggs, is known to be dependent upon oxidative phosphorylation and aerobic glycolysis.

II

A DISCUSSION OF THE SYSTEMATICS AND ECOLOGY OF
SOIL CILIATES

- A. A DISCUSSION OF THE SYSTEMATICS OF NEW ZEALAND SOIL CILIATES.
 - i. Introduction.
 - ii. The Systematics of New Zealand Soil Ciliates and a Comparison of soil and Fresh Water Species.
 - iii. A comparison of the New Zealand and other Soil Faunas.

- B. THE ECOLOGY AND RELATIONSHIPS OF THE NEW ZEALAND FAUNA.
 - i. Introduction.
 - ii. The Ecology of New Zealand Soil Ciliates.
 - iii. The Relationships of the Fauna.

A. A DISCUSSION OF THE SYSTEMATICS OF NEW
ZEALAND SOIL CILIATES AND THEIR RELATION
TO THE FRESH WATER FAUNA.

1. Introduction.

A large number of ciliate species have been previously from soil (Table 1). Species so far recorded from New Zealand and its adjacent islands are given in Table 2.

Of the many investigators who have worked on soil ciliates few have described new species (Shibuya, 1931), although some species, such as Balantiophorus minutus, have been closely studied and redescribed (Watson, 1940; 1944; 1946a). Generally workers were content to identify the genera present (Sandon, 1927) or else identified the soil species with fresh water species (Fantham et al. 1922-1931). Despite this practice the workers almost invariably commented on the smaller size of the soil material. Fantham referred to 'smaller races' and Fellers and Allison (1922) referred to the smaller size of the soil fauna. This was also emphasised by Koffman (1934) and Kubiena (1938). Some, such as Fellers and Allison and Sandon, supposed that the soil harboured many new species and even new genera but none were described by these authors.

Most soil species are holotrichous ciliates and the systematics of this group have only recently been placed on a sound basis. The importance of the ciliary meridians and the morphology of the cytostome have been stressed by such authors as Hoare (1927), Taylor and Furgason (1938), Furgason (1940) and Watson (1940) and consequently many of the earlier species have been re-examined and re-defined. In the light of this new systematics a critical study of the ciliate fauna of New Zealand soils has revealed that,

contrary to prevailing opinion, the soil species are distinct from the fresh water species. The 'smaller races' are different species and are distinguished not only by their smaller size but also by such morphological characters as the number of ciliary meridians and the form of the macronucleus.

Of the 103 ciliate species so far recorded from soil and fresh water in New Zealand by Bary (1950a,b) and myself only seven species have been recorded from both habitats. These seven species are Cinetochilum margaritaceum, Cyrtolophosis mucicola, Cyclidium glaucoma, Halteria grandinella, Aspidisca lynceus, Vorticella microstoma and Vorticella striata. These are the only species whose distribution is not obviously restricted by ecological factors.

Table 3 gives a comparison of fresh water and soil species of the same genera. It shows that almost invariably the soil species are smaller than the fresh water species. Other differences are stressed in the following account of the New Zealand fauna. The New Zealand material was obtained from samples of soil, litter and peat collected throughout New Zealand and from Raoul Island, Niue Island and Samoa in the North and Macquarrie and Campbell Islands in the South. Methods of culture are described in the next chapter. Although further work requires to be done on the morphology and systematics of this material there is sufficient evidence to enable comparison of the soil fauna with the fresh water fauna.

TABLE 1

Ciliates Previously Recorded from Soil: after Fantham et al., (1922-1931) Yakimoff and Zeren (1924), Nowikbff (1922), Wolff (1912), Varga (1936), Perey (1922), Koch (1915), Waksman (1916), Allison (1924a), Sandon (1927, 1928), Bodenheimer and Reich (1934), Cunningham and Lohnis (1914), Martin and Lewin (1914), Dixon (1939) and Fellers and Allison (1920).

Didinium sp.
Mesodinium sp.
Coleps hirtus
Holophrya sp.
H. (simplex?)
H. ovum
Urotricha sp.
U. farcimen
U. lagenula
U. globosa
U. agile
Plagiocampa mutabile
Prorodon sp.
P. ovum
P. teres
P. armatus
Lacrymaria sp.
L. olor
L. apiculatum
Enchelys sp.
E. farcimen
E. pupa
Chaenea sp.
Amphileptus sp.
A. cygnus
Lionotus fasciola
Loxophyllum rostratum
L. flexilis
Dileptus sp.
D. gigas
Nassula sp.
N. elegans
Trochilia sp.
T. palustris
Chilodon sp.
C. cucullus
C. uncinatus
C. megalotrochae
Paramecium sp.
P. aurelia
P. caudatum
P. trichium
P. putrinum
P. bursaria

Colpoda sp.
C. cucullus
C. steinii
C. maupasii
C. saprophila
C. flavicans
Tetrahymena pyriformis
Leucophrys patula
Glaucoma sp.
G. scintillans
Colpidium campylum
C. colpoda
C. striatum
Trichoda pura
Uronema marina
U. sp.
Pleuronema sp.
P. chrysalis
P. glaucoma
Cyclidium sp.
Cyclidium glaucoma
Balantiophorus minutus
B. elongatus
Cohnilembus pusillus
Metopus es
Spirostomum ambiguum
S. teres
Blepharisma sp.
B. lateritia
B. ovata
Condylostoma patens
Halteria sp.
H. grandinella
Strombidium sp.
Strombilidium gyrans
Oxytricha fallax
O. pellionella
O. bifaria
O. gibba
O. ferruginea
O. affine?

TABLE 1 (Cont'd.)

Ciliates Previously Recorded from Soil.

O. sp.
Gonostomum affine
Uroleptus sp.
U. violaceus
U. mobilis
U. musculus
U. piscis
U. dispar
Stichotricha secunda
S. costata
Urostyla grandis
Pleurotricha lanceolata
Pleurotricha grandis
Pleurotricha sp.
Gastrostyla sp.
Stylonychia sp.
S. mytilus
S. pustulata
Onychodromus sp.
O. grandis

Euplotes sp.
E. patella
E. charon
E. harpa
E. carinata
Aspidisca lyncaster
A. costata

Vorticella sp.
V. microstoma
V. striata
V. microscopica
V. putrina
V. globularia
V. nebulifera
V. convallaria
Urceolaria sp.

Sphaerophrya sp.

Marine species
Anophrys sarcophaga
Anophrys sp.
Protocrucia pigerrima?

Parasitic Species
Protoopalina sp.
Balantidium entozoon

Table 2

Ciliate Fauna of New Zealand Soils

<u>Spathidium sp.1</u>	<u>Cyrtolophosis mucicola</u>
<u>Spathidium sp.2</u>	<u>Homalogastra setosa</u>
<u>Spathidium sp.3</u>	<u>Cyclidium glaucoma</u>
<u>Spathidium sp.4</u>	<u>Cohnilembus pusillus</u>
<u>Didinium balbiani?</u>	<u>Cohnilembus fusiformis</u>
<u>Urotricha agilis</u>	<u>Bryometopus pseudochilodon</u>
<u>Prorodon sp.</u>	<u>Bryometopus sp.</u>
<u>Lacrymaria sp.</u>	<u>Balantidium sp.</u>
<u>Enchelys sp.</u>	<u>Blepharisma steini</u>
<u>Chaenea sp.</u>	<u>Blepharisma sp.</u>
<u>Pithothorax ovatus</u>	<u>Phacodinium metschnikoffi</u>
<u>Trachelophyllum apiculatum?</u>	<u>Halteria grandinella</u>
<u>Litonotus sp.</u>	<u>Oxytricha fallax</u>
<u>Loxophyllum utriculariae</u>	<u>Oxytricha pellionella</u>
<u>Bryophyllum spathidioides</u>	<u>Oxytricha setigera?</u>
<u>Dileptus sp.</u>	<u>Oxytricha sp.</u>
<u>Dileptus americanus</u>	<u>Gonostomum affine</u>
<u>Dileptus gigas</u>	<u>Hemicycliostyla</u>
<u>Chilodonella sp.1</u>	<u>Psilotricha sp.</u>
<u>Chilodonella sp.2</u>	<u>Uroleptus musculus</u>
<u>Chilodonella sp.3</u>	<u>Urolentus piscis?</u>
<u>Trichopelma sphagnetorium</u>	<u>Uroleptus mobilis</u>
<u>Drepanomonas revoluta</u>	<u>Holosticha sp.</u>
<u>Drepanomonas sphagni</u>	<u>Onychodromus grandis?</u>
<u>Trimyema compressum</u>	<u>Euplotes sp.</u>
<u>Plagiopyla sp.?</u>	<u>Aspidisca lynceus</u>
<u>Paramecium sp.</u>	<u>Aspidisca costata</u>
<u>Colpoda cucullus</u>	<u>Rhabdostyla sp.</u>
<u>Colpoda inflata</u>	<u>Vorticella microstoma</u>
<u>Colpoda saprophila</u>	<u>Vorticella striata</u>
<u>Colpoda steinii</u>	<u>Vorticella sp.</u>
<u>Tetrahymena pyriformis</u>	<u>Sphaerophrya magna</u>
<u>Colpidium sp.</u>	<u>Sphaerophrya sp.1</u>
<u>Pseudoglaucoma muscorum?</u>	<u>Sphaerophrya sp.2</u>
<u>Saprophilus agitatus</u>	<u>Thecacinetia sp.</u>
<u>Saprophilus muscorum?</u>	
<u>Cinetochilum margaritaceum</u>	

Table 3

Ciliate Genera with Fresh Water and Soil Species Compared

Genus	Soil Species	Length mu	Fresh Water Species	Length mu
<u>Spathidium</u>	<u>S.sp.1</u>	40 - 100	<u>S.spathula</u>	100
	<u>S.sp.2</u>	90 - 180		
	<u>S.sp.3</u>	80 - 130		
	<u>S.sp.4</u>	180 - 200		
<u>Urotricha</u>	<u>U.agilis</u>	20	<u>U.farcta</u>	20 - 30
<u>Prorodon</u>	<u>P.sp.</u>	90	<u>P. discolor</u>	100-130
<u>Lacrymaria</u>	<u>L.sp.</u>	70 - 100	<u>L. olor</u>	400-1200
<u>Enchelys</u>	<u>E.farcimen?</u>	40 - 60	<u>E.curvilata</u>	150
<u>Chaenea</u>	<u>C.sp.</u>	130 - 150		
<u>Pithothorax</u>	<u>P.ovatus</u>	25		
<u>Trachelophyllum</u>	<u>T.apiculatum?</u>	160	<u>T.clavatum</u>	200
<u>Lionotus</u>	<u>L.sp.</u>	60 - 70	<u>L.fasciola</u>	100
<u>Loxophyllum</u>	<u>L.utriculariae</u>	70 - 100	<u>L.meleagris</u>	300-400
<u>Bryophyllum</u>	<u>B.spathidioides</u>	140 - 170		
<u>Dileptus</u>	<u>D.gigas?</u>	300 - 400	<u>D.anser</u>	250-600
	<u>D.americanus</u>	250 - 300		
	<u>D.sp.</u>	150		
<u>Chilodonella</u>	<u>C.sp.1</u>	60 - 90	<u>C.cucullus</u>	100-300
	<u>C.sp.2</u>	45		
	<u>C.sp.3</u>	100 - 130		
<u>Trichopelma</u>	<u>T.sphagnetorium</u>	25 - 40		
<u>Drepanomonas</u>	<u>D.revoluta</u>	30		
	<u>D.sphagni</u>	35		
<u>Trimyema</u>	<u>T.compressum</u>	65		
<u>Plagiopyla</u>	<u>P.sp.</u>	50	<u>P.nasuta</u>	100
<u>Paramecium</u>	<u>P.sp.</u>	80 - 90	<u>P.bursaria</u>	100-150
			<u>P.aurelia</u>	120-180
			<u>P.caudatum</u>	180-300
<u>Colpoda</u>	<u>C.cucullus</u>	80		
	<u>C.inflata</u>	60		
	<u>C.saprophila</u>	40		
	<u>C.steini</u>	40		
<u>Tetrahymena</u>	<u>T.pyriformis</u>	40 - 60	<u>T.vorax</u>	70- 80
<u>Colpidium</u>	<u>C.sp.</u>	30	<u>C.colpoda</u>	90-150
			<u>C.campylum</u>	50
			<u>C.striatum</u>	50
<u>Pseudoglaucoma</u>	<u>P.muscorum?</u>	12		
<u>Saprophilus</u>	<u>S.agitatus</u>	40		
	<u>S.muscorum?</u>	35		
* <u>Cinetochilum</u>	<u>C.margaritaceum</u>	35	<u>C.margaritaceum</u>	35
* <u>Cyrtolophosis</u>	<u>C.mucicola</u>	28	<u>C.mucicola</u>	28
* <u>Homalogastra</u>	<u>H.setosa</u>	30		
* <u>Cyclidium</u>	<u>C.glaucoma</u>	15 - 30	<u>C.glaucoma</u>	15-30
<u>Cohnilembus</u>	<u>C.pusillus</u>	30 - 50		
	<u>C.fusiformis</u>	60		

Table 3 (Continued)

Ciliate Genera with Fresh Water and Soil Species Compared

Genus	Soil Species	Length mu	Fresh Water Species	Length mu
<u>Bryometopus</u>	<u>B.pseudochilodon</u>	65		
	<u>B.sp.</u>	160		
<u>Balantidium</u>	<u>B.sp.</u>	50		
<u>Blepharisma</u>	<u>B.steini</u>	65-130	<u>B.lateritia</u>	130-200
	<u>B.sp.</u>	100-130		
<u>Phacodinium</u>	<u>P.metschnicoffi</u>	100-160		
+ <u>Halteria</u>	<u>H.grandinella</u>	30	<u>H.grandinella</u>	30
<u>Oxytricha</u>	<u>O.fallax</u>	150	<u>O.fallax</u>	150
	<u>O.pellionella</u>	110-120		
	<u>O.setigera</u>	50- 60		
<u>Gonostomum</u>	<u>C.affine</u>	100	<u>G.strenuum</u>	150
<u>Hemicycliostyla</u>	<u>H.sp.</u>	200-300		
<u>Psilotricha</u>	<u>P.sp.</u>	60-80	<u>P.acuminata</u>	80-100
<u>Uroleptus</u>	<u>U.musculus</u>	120-130	<u>U.dispar</u>	150-170
	<u>U.mobilis</u>	60-120		
	<u>U.piscis?</u>	170		
<u>Holosticha</u>	<u>H.sp.</u>	200	<u>H.vernalis</u>	180
<u>Onychodromus</u>	<u>O.grandis?</u>	200		
<u>Euplotes</u>	<u>E.sp.</u>	50-65	<u>E.patella</u>	90
			<u>E.aediculatus</u>	132
+ <u>Aspidisca</u>	<u>A.costata</u>	30-50	<u>A.costata</u>	30-50
+ <u>Rhabdostyla</u>	<u>A.lynceus</u>	30-50	<u>A.lynceus</u>	30-50
	<u>R.sp.</u>	80		
+ <u>Vorticella</u>	<u>V.striata</u>	25-35	<u>V.striata</u>	25-35
	<u>V.microstoma</u>	40-60	<u>V.campanula</u>	50-157
	<u>V.sp.</u>	60-65	<u>V.convallaria</u>	50-95
<u>Sphaerophrya</u>	<u>S.magna</u>	50		
	<u>S.sp.</u>	40		
	<u>S.sp.</u>	30		
<u>Thecacinetia</u>	<u>T.sp.</u>	52		

11. The Systematics of New Zealand Soil Ciliates and a Comparison of Soil and Fresh Water Species.

Holotricha

Gymnostomata

There are 22 gymnostomatous species. Of these some are very rare having been encountered only once or twice and then only a few individuals, e.g. Didinium balbiani? (Plate 1, fig.5) and Pithothorax ovatus (Plate 1, fig.7). Some have been found only in certain soils. These include the largest Spathidium sp.4 (Plate 1, fig.3) and Bryophyllum spathidioides (Plate 2, fig.4), both found only in Niue and Raoul Island samples. The larger species of Dileptus (D.gigas?) and D.americanus, Plate 2, figs. 6-8) occur both in New Zealand and Niue and Raoul Island samples but only those of better structure or where there is litter accumulation. Many of the species however are common and occur regularly in most soils. Enchelys is very widespread and the species encountered (Plate 1, fig.8) appears to be identical with that recorded by Sandon (1927) who does not give his material a specific name. It is distinct from E.pupa a commonly recorded species and also very probably from E.farcimen. Spathidium is represented by several species. The nucleus of the largest has not yet been determined but the others have massive type macronuclei which are either sausage shaped or C-shaped (Plate 1, figs. 1-4). There is a distinction of body form one type being more elongate (S.sp.1,2,4) and the other shorter and broader (S.sp.3). The species are also distinguished by the number of ciliary meridians, viz.8,12,24 and 36.

The common fresh water species which has been recorded in New Zealand by Bary (1950a) is S.spathula. This species has a ribbon shaped macronucleus and 16 ciliary meridians and is therefore quite distinct from the soil material (Woodruff and Spencer, 1922). Lionotus is represented in the soil by a single species with 4 ventral ciliary rows and two oval macronuclei (Plate 2, figs. 1, 2, 5). The common fresh water species is L.fasciola which has 14 ciliary rows and two spherical macronuclei (Kahl, 1926) and is therefore quite distinct from the soil species. Dileptus is represented by three species in the soil, all of which have massive sausage shaped macronuclei although the nucleus is liable to be fragmented in less healthy individuals. Two of these species have been identified with D.gigas and D.americanus, the latter a sphagnum species. Both these species occur only in soils of good structure or in litters. The common soil species is smaller with fewer ciliary meridians and five contractile vacuoles in the body (Plate 2, fig. 7). The common fresh water species is D.anser which has a beaded macronucleus (Hayes, 1938), which distinguishes it from all the soil material. Chilodonella is represented in soil by a number of species which may be compared with three fresh water species. The largest soil species which occurs only in soils or litters of good structure is similar to C.cucullus. It differs in the number of contractile vacuoles and also in size (Plate 3, fig. 9). The common soil material in which three varieties may be distinguished is similar to C.uncinatus (Plate 3, figs. 1-5). All have an oral basket composed of straight rods as in C.uncinatus (Kahl, 1931). Two varieties have three contractile vacuoles and

the other only two. Of the first two the larger has larger pharyngeal rods and a row of dorsal bristles. This genus is however subject to considerable intra-specific variation (Krascheninnikow, 1951; MacDougall, 1929, 1931). The third group of soil species consist of two varieties (Plate 3, figs. 6-8, 10, 11). One has dorsal ridges posteriorly, in the other these ridges are lacking. They are otherwise very similar. They resemble C.chauduri in some respects although the ridges are never as well developed as in that species. Bryophyllum, a moss genus, is represented by the uncommon species B.spathidioides so far found only in a Raoul Island sample. Loxophyllum is represented by L.utriculariae which is comparatively common in soils (Plate 2, fig.3). Prorodon is represented by a small species with about 20 ciliary meridians and an oval macronucleus. The common fresh water species of these genera have not yet been recorded in New Zealand soils.

Trichostomata

These include some of the most common soil ciliates. A single individual was identified as Trimyema compressum. Paramecium is represented by a single species more common in litters than in other soils. It is small and with a single contractile vacuole (Plate 2, fig.10). There are three types of body form in this genus: the cigar or torpedo shaped form of B.caudatum, P.aurelia and P.multimicronucleatum; the foot shaped form of P.bursaria; and the regular capsule shaped form of P.putrinum. The soil species is similar to the last but is distinguished by its single simple vacuole and its size.

Trichopelma sphagnetorium and Drepanomonas revoluta, both small ciliates, are comparatively common in soil. Drepanomonas sphagni has also been recorded. None of these species are generally recorded from fresh water and conversely the common fresh water species of Paramecium have not yet been identified in New Zealand soils.

Colpoda is probably the most common of all soil ciliates. It is represented in New Zealand soils by four species, C. cucullus, C. inflata, C. steinii and C. saprophila. Because it is studied more extensively in later chapters, the taxonomy of this genus is discussed here in detail.

Colpoda

1. History

Kudo (1947) mentions a record by Bonanni in 1691 but the genus was established by O.F. Muller (1773) who included 5 species. Later (1786) he included 16 species. An attempt to clarify the taxonomy of the genus was made by Ehrenberg (1838) who chose C. cucullus as the type species. Maupas (1883) established the identity of C. cucullus O.F.M. and described a new species which previous workers (Ehrenberg, 1838; Dujardin, 1841; Perty, 1852; Stein, 1854; Kent, 1881) had interpreted as C. cucullus. This new species was called C. steinii. Stokes (1888) described, under the genus Tillina, three species of Colpoda, C. inflata, C. flavicans, and C. saprophila. More definite diagnostic details were supplied by Enriques (1908) who described C. cucullus, C. steinii, and a new species C. maupasi. Photomicrographs of a ciliate identified as

C. steinii were given by Bresslau (1921). This material was described as a new species, C. aspera, by Kahl (1931) who recognised 16 and later 17 species of Colpoda. Taylor and Furgason (1938) gave a structural analysis of a ciliate they called C. duodenaria. This was identified as C. steinii by Burt (1940) who fully described this species, a variety (C. steinii var. piekarski) with a very small micronucleus, and four other species, viz. C. cucullus, C. inflata, C. maupasi and C. aspera. The earlier literature is reviewed in both these latter papers.

ii. Morphology

The morphology of the genus is fully analysed in the papers of Taylor and Furgason (1938) and Burt (1940). Earlier Enriques (1908) had studied the nucleus, Klein (1926, 1930, 1931) the silver line system, and Reichenow (1928) the Feulgen reaction. Piekarski (1939) had first described the small micronucleate variety of C. steinii. Taylor and Garnjobst (1939) studied the history of the silver line system during division.

Basically the animal is reniform in outline and depressed. The cytostome forms a depression of the ventral surface about mid-way of the body length. The cytostome is bordered by cilia which in one species, C. steinii, jut out to form a 'beard'. The ciliary meridians are arranged more or less regularly about the cytostome and their number is specific. The cilia are generally double. The nucleus is of two types one with and one without a complex karyosome figure. For convenience the genus may be divided into large species, which include C. cucullus, C. inflata and C. maupasi and small species which include C. steinii and C. aspera.

iii. Life History

The life history of Colpoda is illustrated diagrammatically overpage. It consists of three stages: a trophic or feeding stage, a reproductive or division stage, and a resting stage. Both the latter stages involve encystment and excystment (Brown and Taylor, 1938; Kidder and Claflf, 1938).

Under favourable conditions the ciliate feeds, grows and then after a time encysts to form a reproductive cyst. The encysted ciliate divides one, two or three times and then excysts and begins feeding again. If the food supply is exhausted resting cysts are formed. Depending upon the initial concentration of the ciliates these will either be temporary cysts or true resting cysts known as resistant or permanent cysts. In the resistant cysts nuclear reorganization takes place and is completed within about nine hours (Taylor and Garnjobst, 1939). Temporary cysts may excyst spontaneously but permanent cysts require a definite stimulus. The factors affecting these cysts will be described later.

Although this is the typical life cycle of the ciliate division may take place without prior encystment (Penn, 1937) and this condition may be experimentally induced (Stuart, Kidder and Griffin, 1940). Reynolds (1940) found that when C. steinii is parasitic in snails it divides without forming a cyst. If division is inhibited unstable cysts are formed. These are generally similar to reproductive cysts but neither division nor excystment take place until the inhibiting factor is removed. Both division and excystment are inhibited by various environmental

factors.

The life cycle of C.maupasi, which differs slightly from that of other species, has recently been described by Padnos et al. (1951).

COLPODA LIFE CYCLE

Colpoda Life Cycle:

There are three stages; the trophic ciliate, the resting cyst and the reproductive cyst. There are two types of resting cyst, permanent and temporary. If division is inhibited the monogenic cyst becomes an unstable cyst which resumes the normal life cycle when the inhibitory factor is removed:

Both Enriques (1906) and Wenyon (1926) record conjugation or syngamy in C. steinii. This species has been maintained in culture and subjected to a vast range of experimental conditions by workers in America and England. From these innumerable observations conjugation has never been reported. In the present studies on this ciliate only once has a process resembling syngamy been observed. In one culture a number of the ciliates were joined in pairs their ventral surfaces being apposed and partly united. Syngamy however in this case was not confirmed. Wenyon simply recorded a single instance of two ciliates 'becoming attached by their cytostomes and forming a common cyst'. This observation also does not appear sufficient evidence of syngamy which cannot yet be said to have been established in Colpoda. Taylor and Garnjobst (1939) maintain that the nuclear reorganization of the resistant cyst is a suppressed mitosis.

iv. Cyst Physiology

Cysts of Colpoda have been known and studied for more than a century from Stein (1954), Meunier (1865), Maupas (1883), Fabre-Domergue (1883), Stokes (1888), Rhumbler (1888), Butschli (1887-1889) in the XIXth century to the host of workers in the XXth century. The most important work of the present century has been done at Rothamsted, at Stanford University and at Amehurst College but papers by Adolph (1929) and Bodine (1923) are also important.

Most important is the distinction between the reproductive and resting cysts. The cyst of Colpoda is formed by the rounding and dedifferentiation of the ciliate and the secretion of a cyst membrane, held to be carbohydrate (Goodey, 1913). Three membranes are distinguished: an ecto-, meso- and endo-cyst. In reproductive cysts the membrane is generally thinner than in the resistant cyst.

The formation of reproductive cysts is a function of two factors, time and size (Adolph, 1929). The ciliates must attain a certain size before they can divide but after a certain time lapse the ciliates encyst although they may vary in size. The smaller ones divide only once but the larger ones divide twice. Where division is inhibited an unstable cyst is formed in which division or excystment will take place when the inhibiting factor is removed. When there is no food and the ciliates are not concentrated they encyst to form a temporary cyst and then excyst without division. This may take place several times the ciliate growing smaller until finally it dies. (Taylor and Strickland, 1939).

Normally starved ciliates when sufficiently concentrated encyst to form permanent resting cysts. The rate at which they encyst and the proportion of resting to temporary cysts depends upon their concentration. The former also depends upon the volume of the drop while the latter is independent of the volume (Taylor and Strickland, 1938, 1939, 1940). For this reason Taylor and Strickland believe that there are two factors one causing cyst formation and the other resting cyst formation.

Starvation is a necessary condition of resting cyst formation but Garnjobst (1947) has shown that this may be caused simply by a single nutritional deficiency such as a vitamin or growth factor.

The resting cysts decrease in size and weight with age and particularly there is marked water loss (Allison, 1924b). The respiration is depressed (Thimann and Commoner, 1940). These cysts survive indefinitely and are very resistant to desiccation, high and low temperatures, extreme atmospheric pressures and other adverse conditions. The cyst membrane is not permeable to absolute alcohol and is strongly resistant to acids (Bodine, 1923).

Excystment can be achieved simply by washing the resistant cysts in a hypotonic solution (Goodey, 1913; Kidder and Stuart, 1939). After an hour or so excystment takes place. There are three stages: first, the imbibition of water; secondly, the differentiation of the ciliate; thirdly the escape from the cyst membranes. The first is a simple diffusion process and will take place under suitable osmotic conditions; the second is marked by a sharp increase in respiration (Thimann and Commoner, 1940) and is dependent upon

respiration (Brown, 1939). The third is partly hydrostatic and partly chemical. By imbibition of water apparently aided to some extent by the activity of the contractile vacuole the volume of the cyst is greatly increased and consequently the two outer membranes burst and the ciliate still in its inner membrane escapes. The inner membrane is then dissolved by an enzyme (Goodey, 1913) and the animal escapes.

Although distilled water is a sufficient stimulus to excystment other media are more effective judged by the rate of excystment. Generally it is sufficient to initiate the process which once started continues until it is completed. Thus initiation of the osmotic reaction is all that is required. Amongst stimuli which are well known to be effective are yeast extract and bacteria and it was therefore thought (Bridgeman, 1948) that their efficacy was dependent upon a food factor just as encystment was dependent upon the absence of a food factor. Garnjobst (1947) however showed that a medium adequate for growth was quite ineffective as an excystment stimulus. Thimann and Haagen-Smit (1937), Haagen-Smit and Thimann (1938), Strickland and Haagen-Smit (1947, 1948) after considerable research found that the K ions were particularly effective and they finally devised a medium of dilute alcohol and potassium which was as effective as yeast extract. It is apparent that these factors act primarily through affecting the osmotic reaction. The rate of excystment increases with increasing temperature to an optimum about 30°C. Above this temperature and below 8°C excystment is inhibited, as is division (Thimann and Barker, 1934).

v. Distribution and Parasitism.

It can be seen from the history of the genus and the numerous studies that have been made on the different species that Colpoda is a common and widespread ciliate. It is common in infusions occurring generally in the first few days and then disappearing (Woodruff, 1912; Unger, 1939). It occurs in fresh water but is not as conspicuous as Paramecium as so great a part of its life history is spent in the encysted stage. It has been found in the pitcher plant (Hegner, 1926). In the soil it is perhaps the most characteristic ciliate, occurring in the majority of soils (Sandon, 1927). It may also occur in sewage (Lackey, 1938b), in filters and in faeces (Watson, 1946; Woodcock, 1916; Yakimoff and Kolpakowa, 1922).

Yakimoff and Kolpakoff (1921) gave a figure of Colpoda steinii showing its distinctive beard which had been recovered from human faeces in a case of colitis. This identification is apparently accepted by Burt (1940). Wenyon (1926) did not accept either this record or an earlier record, by Schulz (1899), of C. cucullus in human faeces as evidence of parasitism. A further record of Colpoda in human faeces was given by Demianov (1943). These authors suggested that Colpoda was associated with human colitis. Watson (1946) follows Wenyon in asserting that they are only coprophilic species. The only other record of Colpoda in faeces given by Watson is Alexieff (1929). Watson considered the presence of Colpoda in Demianov's case to have been due to cysts passing through the alimentary canal. There does not appear to be any

conclusive demonstration of Colpoda as a human parasite.

In 1936 Reynolds found that Agriolimax agrestis was infected with a ciliate which he identified as C.steinii. He found that the lungs were particularly affected and that infection ultimately led to the death of the host. His methods were unexceptionable. He isolated the ciliate from the slug and kept it in pure culture. He then subjected an uninfected slug to infection by feeding it lettuce leaves covered with Colpoda cysts. He found that these slugs were parasitized and he found that in nature there was a considerable percentage of parasitized slugs.

Reynolds was aware of Enriques' paper and his identification was based on Enriques' criteria. He also isolated Colpoda from the soil and compared the two strains. Watson (1946) was apparently unaware of this paper when he stated that no species of Colpoda were known to be parasitic. Kirby (1941) comments on Reynolds' paper and questions his identification observing that no illustration or description is given and that the parasites are said to resemble Balantidium more closely than the free living form. Kirby however accepts Mercier and Poisson's (1923) identification of a Colpoda parasitic in an insect. This doubtless because the authors had figured the ciliate which quite plainly is not a Colpoda. The nucleus is distinctive and it has not a ciliated cytostome.

Although Reynolds gives no figure he gives the size range of his ciliates, had obviously studied the nucleus, and pointedly noted the difference to Balantidium. He was aware that C.steinii

was a common free-living form and actually isolated a strain from the soil. He cultured his ciliate for two years in wheat and Liebig meat media. He compared the soil and parasitic forms and found them morphologically identical. He was aware of both Maupas' and Enriques' work and there seems no reasonable doubt that his identification was correct. Further he observed the typical form of division within a cyst, so characteristic of Colpoda. He observed that fresh faeces contained mostly trophic ciliates and his sections also showed trophic and dividing ciliates in the tissues and especially the lungs. The absence of a cyst membrane during division is not critical having been experimentally induced in this species by Stuart, Kidder and Griffin, (1940). The doubt raised by Kirby may therefore be dismissed and it thus seems that there is clear evidence of parasitism in Colpoda steinii and natural infection of the slug, Agriolimax agrestis.

In the three records of Colpoda from human faeces each associated with colitis there is no such clear cut evidence of parasitism although the authors all considered that the ciliate was responsible for the colitis. Both Wenyon and Watson consider that the ciliates were coprophilic contaminants although Watson also suggested that in Demianov's case contamination was due to cysts having passed unaffected through the alimentary canal of the patient. Both Wenyon and Watson assumed that there was no case of a parasitic Colpoda. Although Reynolds' paper does supply such a case it does not really support the possibility of Colpoda being a human coliform parasite for the conditions in

the lung of the slug and in the alimentary canal of man are very different. Moreover as will be shown later Colpoda divides only within a certain temperature range and at body temperature division would be inhibited. Generally there is no evidence that Colpoda is physiologically capable of living in the intestine or even of being able to pass through the alimentary canal of man as a cyst. For this reason the conclusion reached by Wenyon and Watson must be endorsed.

vi Systematics of the New Zealand Material

So far four species of Colpoda have been identified in New Zealand soil cultures. These are C.cucullus, C.inflata, C.steinii, and C.saprophila. The first three have been fully analysed by Burt (1940) and C.steinii has also been fully described by Taylor and Furgason (1938) as C.duodenaria. Only Stokes' original description of C. (Tillina) saprophila is still available and further information is therefore added to this description. The synonymy of these species is also discussed. Sandon (1927) and Varga (1936) have recorded C.maupasi from the soil but this species has not yet been identified in New Zealand cultures.

The characteristic form of each ciliate is described but like all ciliates this form is very plastic and will depend largely upon cultural conditions. Well fed ciliates tend to be gross and their proportions distorted. Burt cultured his strains on Aerobacter and remarked on the changes brought about in well fed organisms. C.inflata and C.cucullus when well fed are very similar, both with their cytoplasm stuffed with food vacuoles making the organisms dense and dark. At other times they can be easily distinguished by their body form. The morphological details given are taken largely from Burt's study, particularly the number of ciliary meridians. No such study has so far been made of the present soil strains although the number of ciliary meridians of C.saprophila has been recorded for the first time and on this information C.aspera is treated as a probable synonym.

Colpoda cucullus O.F. Muller 1773 (Plate 10, fig.3)

This is a large ciliate from 40 to 110mu and averaging 70-75mu. Generally it has a very marked thick pellicle and the shape of the body posterior to the cytostome gives the appearance of a protruding 'lower lip'. It is less plastic than other species. In dorsal or ventral view the body appears more nearly sub-circular than C.inflata. The cytostome is slightly anterior rather than lateral and is bordered by short cilia. The body ciliation consists of 29 - 34 meridians, eight of which reach the posterior border. The macronucleus is spherical with chromatic plates.

Although this is the ciliate which has been most commonly recorded by previous workers there seems little doubt that in many cases the material was C.inflata. This is apparently the case with Goodey and Sandon. It is however a fairly common soil species.

Colpoda inflata (Stokes, 1884) (Plate 10, fig.4)

Very similar in body proportions to C.cucullus but generally smaller. Average length about 60mu and range 35 to 90mu. Normally the most striking feature of the ciliate is the posterior body-half which is much more clearly distinguishable than with C.cucullus. It is as Stokes (1888) wrote: 'rounded, inflated, conspicuously widened and somewhat oblique'. Just as C.cucullus gives the appearance of a protruding lip, C.inflata gives the appearance of a protruding chin. In general outline this species appears bean-shaped. The ciliation consists of 21-24 ciliary meridians, seven of which reach the posterior border. The cytostome is similar in position to that of C.cucullus and is also bordered by short cilia. The macronucleus is of the C.cucullus type.

Stokes found this species in the water in which bulbs of a Chinese Narcissus were growing and he thought that it was confined to this habitat. It has since been recovered from a variety of habitats (Kahl, 1931; Burt, 1940). It has never been recorded previously from the soil although it has proved the most common of N.Z. species. Fellers and Allison (1920) recorded C. (Tillina) flavicans (Stokes). In size and general structure the two species are identical and Stokes remarks that the movements of C.inflata are very similar to those of C.flavicans but he distinguishes them principally because the 'rotation of the endoplasm so noticeable in C.flavicans is seldom visible in C.inflata'. Plainly this is no adequate distinction and C.flavicans which Stokes recovered from standing water with dead

leaves must be considered a synonym of C.inflata and the record of Fellers and Allison further evidence of the occurrence of this ciliate in soil.

It is unfortunate that generally European workers have failed to study the American literature and vice versa. This is particularly true of Stokes whose work does not appear to have been well known in Europe. But even Burt (1940) working in America had not read Stokes and was unaware that Stokes' was the original description of C.inflata and not Kahl's (1931). Much of the confusion in the literature is due to this ignorance with the result that European workers in soil protozoology (Goodey, Sandon) called their large Colpoda, C.cucullus and their small Colpoda, C.steinii, the two species best known in Europe, whereas the American workers (e.g. Fellers and Allison) called their large species C.flavicans and their small species C.saprophila, after Stokes. The confusion was further established by one of the few early European papers which mentioned Stokes, viz. Enriques (1906). This author considered C.inflata to be a synonym of C.cucullus a not unreasonable but incorrect assumption which was followed by Kahl (1931). This error influenced both Goodey and Sandon and largely accounts for their failure to record C.inflata.

Goodey (1911, 1913) recorded C.cucullus and gives a figure of his ciliate escaping from a cyst (Goodey, 1911, fig.5; 1913). He records that there was a larger strain of Colpoda cucullus but he found the smaller strain more satisfactory for his experiments on excystment. Fig.4 in his 1911 paper doubtless

illustrates this larger strain. The size of the smaller strain was about 45 μ . Its form is identical with that of C.inflata with the characteristically swollen posterior half and the rather pointed anterior end. From this it seems most likely that the smaller strain was C.inflata and the larger C.cucullus. Goodey was not aware of Stokes' paper.

Sandon (1927) records C.cucullus as the most common of soil ciliates. He gives the following description: 'C.cucullus is a large form, 40 to 80 μ in length with the part behind the mouth very swollen and almost globular, while C.steinii is smaller (length 25 to 60 μ) and not inflated behind, so that the ventral surface is quite flat except for the notch which leads to the mouth; the anterior end is also more pointed than in C.cucullus.' Sandon adds that both species are facultative anaerobes but cites no evidence. The only illustration of this C.cucullus is one taken from Enriques which cannot be distinguished as either C.cucullus or C.inflata. It is obvious however that the ciliate with the 'inflated' posterior half recorded by Sandon was Stokes' species which it resembles both in size and shape. It is possible however that Sandon also encountered C.cucullus but the common species is obviously C.inflata. Sandon states that C.cucullus and C.steinii are the two most common soil ciliates occurring in 58% and 49% of the soils respectively. The New Zealand figures for C.inflata, C.steinii and C.cucullus are 62%, 47% and 25% respectively. If it is correct to identify Sandon's species as C.inflata the disparity between the records of Colpoda in the soil disappears and Sandon's

figures are strictly comparable with the New Zealand figures. The difference is the absence of C.maupasi from New Zealand soils and the absence of C.saprophila from Sandon's list. C.inflata and C.steinii are in both cases the most common species.

Burt in describing this species refers to it as C.inflata Kahl, apparently not being aware of Stokes' work. Kahl had assigned Roux' C.steinii (1901) to a new species C.rouxi in 1926. In 1931 he identified this species with Stokes' Tilling inflata and consequently called it Colpoda (Tillina) inflata (Stokes, 1885). This is of course the correct designation.

was accepted
subgenus?

Colpoda steinii Maupas (Plate 11).

This is a small species 15 to 42mu in length and averaging about 30mu. Although small it is easily distinguished by a number of distinctive morphological characters. Generally reniform like the other members of the genus it is variable in shape. The cytostome is marked by distinctive posterior pseudomembranelles longer than the body cilia which protrude from the cytostomal cleft to give the appearance of a "beard". This is one of the most distinctive features of the ciliate and the "beard" has been recognized and recorded by many workers. The body ciliation is in 12 ciliary meridians, five of which reach the posterior border. There is further a pair of longer caudal cilia which can be observed with some difficulty. The nucleus is distinct from that of C.cucullus and C.inflata being ellipsoidal with a central spherical plasmosome. The micronucleus is typically similar to that of the other species but a variety, distinguished as C.steinii var. piekarskii by Burt has a minute micronucleus. It is otherwise identical. In the present studies the typical micronucleate form was used.

This species has been recorded by most soil investigators since it was first described by Maupas (1883) and again distinguished by Enriques (1908). It is very widespread and was the only ciliate recorded by Bodenheimer and Reich from a poor Palestinian soil. Its biology will be very fully discussed throughout this thesis.

Colpoda (Tillina) saprophila (Stokes, 1884) (Plate 10, figs. 1 & 2)

This is also a small ciliate about 25 μ to 35 μ . In general appearance it differs from C. steinii by being more pointed anteriorly with a straighter ventral face. The cytostome is typical and the ciliary meridians in the New Zealand material number 15 to 16 with seven reaching the posterior border (Plate 10, fig. 2). The cilia of the anterior part of the body appear longer than those posteriorly but they have not yet been extensively studied. Nor has the nucleus, which Stokes recorded as spherical.

This species has occurred only occasionally and at the time it was identified with Stokes' material it was not realized how important the nuclear apparatus and the exact arrangement of the cilia were. These facts still need to be determined. The species was considered by Kahl (1931) to be synonymous with C. steinii but Stokes particularly remarked on the short cytostomal cilia comparing them with the long and very fine body cilia. He could hardly have overlooked the characteristic "beard" in so detailed an examination. It is however very similar to Colpoda aspera Kahl as fully described by Burt. They are both similar in general size and shape and the preliminary information on the ciliary meridians here presented shows that the two are similar, for Burt records 14 to 16 meridians in his material, although only five reach the posterior border. One difference is that in the present material the cilia were recorded as single whereas they are generally double. Closer study may remove this difference. C. aspera has a macronucleus

of the spherical C.cucullus type as distinct from that of C.steinii.

Although further work needs to be done on the material identified as C.saprophila it seems very probable that C.aspera is a synonym of Stokes' species. C.aspera is apparently a common saprobic species and euryhaline (Lackey, 1938b; Finley, 1930). C.saprophila has been recorded by a number of workers from soil, including Fantham and Fellers and Allison. It is possible as Sandon (1927) suggests that C.steinii has been recorded as C.saprophila by these authors but the occurrence of the latter species in New Zealand soils indicates that both are true soil species.

c. Hymenostomata

These include the very widespread species Cyclidium glaucoma and Cinetochilum margaritaceum. Other species are Homalogastra setosa, Cohnilembus fusiformis, Cyrtolophosis mucicola, and Saprophilus agitated which are recorded rarely from fresh water although more commonly either in sphagnum or from polluted water. The species of Saprophilus may prove on further study to be identical, as Hoare (1927) suggests, with Uronema nigricans. The common fresh water species of Colpidium are C.colpoda and C.campylum. The dimensions of the former are 90 to 150mu (Furgason, 1940) and of the latter about 50mu. The Colpidium species recorded from the soil is only about 30mu long. Although Sandon (1927) records Colpidium colpoda from soil the dimensions he gives, viz. 30 to 45mu do not agree with this identification. His species may have been either C.campylum or C.striatum but it is even more probable that it is the New Zealand species which is quite distinct from the fresh water species not only in size but also in the number of ciliary meridians (Plate 4, fig.5). A species very similar to, but slightly smaller than Kahl's Pseudoglaucoma muscorum also occurs occasionally (Plate 4, fig.10). The common fresh water species of Colpidium and Glaucoma, like the fresh water Paramecium have not so far been recorded from New Zealand soils although they have been recorded by Bary (1950a) from fresh water. Tetrahymena pyriformis has twice been identified in soil cultures. The history and identity of this species is discussed by Corliss (1952) and his nomenclature is accepted. Common synonyms for this much studied species include

Glaucoma pyriformis (or piriformis) in France and Tetrahymena geleii, Colpidium striatum and Colpidium campylum (partim) in America.

d. Spirotricha

These are generally more common in soils or litters of good structure and are often larger than other soil species.

A single individual of Balantidium has been recorded (Plate 5, fig.4), but not yet identified. Blepharisma is represented by two species, one B. steini more common in litters (Plate 5, fig.2) and the second more common in ordinary soil. Phacodinium metschnikoffi (Plate 5, figs. 5,6) was recorded by Framé (1921) from forest soils. It has been recorded twice in New Zealand both times from beech litter. This is a large and elaborate ciliate with the peristome exceptionally well developed. Ciliation consists of large cilia and there are more ventral than dorsal meridians. Bryometopus pseudochilodon occurs both in soil and litter and a large species of Bryometopus has been recorded only from litter. Halteria grandinella is widespread in all waters and is common in soils.

Although the hypotrichous ciliates are structurally more complex than other ciliates their form is variable with changing cultural conditions. Both the nucleus and ventral cirrhi are liable to vary (Kay, 1945a). For this reason it is not possible to separate ciliates solely on this basis if only a limited amount of material is examined. One of the most variable and also one of the most common of soil ciliates is Gonostomum affine. The number and arrangement of the ventral cirrhi is shown for

two individuals (Plate 6, figs.7, 8). A similar difference is illustrated by Kahl (1931). For this reason the Hemicyliostyla illustrated in Plate 7, fig.5 found in Raoul Island soil (under Pohutakawa) and that illustrated in Plate 8, fig.1 found in beech litter at Taupo are not distinguished although there are obvious differences in the ciliation. Both differ from Stokes' species (1888) in having a collecting canal associated with their contractile vacuole and also in size and ciliation. The genus is however essentially a sphagnum-litter group. A ciliate identified as Onychodromus grandis? was also recorded from beech litter (Plate 8, fig.2) and a Niue Island sample (Plate 7, fig.7). Again this material is variable and differs slightly from the original description.

Oxytricha is represented by the common O.pellionella and O.setigera. O.fallax typically a fresh water species is rare. Uroleptus musculus, Uroleptus mobilis and Holosticha sp. are all quite common. Psilotricha is represented by variable material distinct from P.acuminata the fresh water species.

Euplotes is represented by a species of the 'patella' group (Plate 6, figs.9-12). It is smaller than the other species of this group and the peristomal organelles are relatively indistinct (Pierson, 1943). It has a simple C-shaped macronucleus and except in size most closely resembles E.patella. Sandon describes species as E.charon although its size (35 to 80 μ) is much smaller than the typical E.charon. It seems very likely that his species is identical with the New Zealand material.

Aspidisca is represented by two species identified as

A.costata and A.lynceus. A species of Rhabdostyla is occasionally recorded.

Of the three species of Vorticella so far recorded from New Zealand soils one is comparatively rare and has not so far been identified. The other two are V.microstoma and V.striata. These two species are very similar and are not always easily distinguishable. Because of the confusion of their taxonomy and because some experimental work has been done with these two species their systematics is discussed at greater length.

Vorticella

1 History

This ciliate was first described by van Leewenhoek in a letter to the Royal Society in 1675. It was figured by Rosel in 1755 and Linneaus (1758) called Rosel's species Hydra convallaria and later (1767) Vorticella convallaria. O.F. Muller (1773, 1786) listed 127 species of Vorticella. The genus was redefined by Ehrenberg (1838) and has since been extensively studied, Lachmann, Greeff, Kent, Stokes, Butschli, Schroder, Faure-Fremiet and Penard being listed by Noland and Finley as of especial importance. Noland and Finley (1931) give a critical review of the taxonomy of the genus. Over 220 names and descriptions were examined and of these only 40 appeared as probable species of Vorticella, many being rotifers, other ciliates or synonyms. These authors give a careful and detailed description of the following species:

- V.convallaria (Linneaus)
- V.campanula Ehrenberg
- V.nebulifera O.F.M. var. similis (Stokes)
- V.microstoma Ehrenberg

V. striata Dujardin var. octava (Stokes)
V. picta Ehrenberg
V. monilata (Tatem)

11 Morphology

The morphology of this interesting ciliate has been examined and described by numerous authors. This work is critically reviewed by Taylor (1941).

Vorticella is a bell-shaped animalcule with a triple row of cilia forming the peristome. There is a vestibule and pharynx, the former ciliated. There are well developed contractile fibres both in the body and in the stalk. There are often granules in the thecoplast of the stalk and these are useful diagnostic characters. However in both the present species they are absent.

111 Life History

Finley (1936, 1939) has studied the life history of V. microstoma and a diagrammatic figure is given overpage. There are two distinct cycles, trophic and sexual. In the trophic cycle the stalked ciliate with its peristome everted feeds actively and increases in size. Division takes place with the division between the two daughter cells slightly asymmetric so that one remains attached to the parent stalk. The other forms a posterior ciliary wreath and with its peristome retracted becomes free-swimming telotroch. The polarity of the telotroch is the exact reverse of the stalked individual, the posterior ciliary wreath being anterior. Ultimately the telotroch settles, secretes a stalk, resorbs the posterior ciliary wreath, everts the peristome and begins feeding as a normal trophic individual.

VORTICELLA MICROSTOMA
LIFE CYCLE

Vorticella microstome Life Cycle:

This consists of an encystment-excystment cycle, a division cycle and a sexual cycle which has been worked out by Finley (1936, 1939). In the first two cycles there are three distinct stages: the trophic form, the cyst and the telotroch. The telotroch is formed by the excysting vorticellid, by one of the daughter vorticellids formed during division, and under certain circumstances by the trophic vorticellid. The telotroch normally settles to form a trophic vorticellid. It cannot feed, divide or encyst. The sexual cycle involves a pre-conjugation division giving rise to a macrogamete and a free-swimming microgamete. Conjugation involves the fusing of a microgamete and a macrogamete. If the macrogamete fails to conjugate it becomes a normal trophic vorticellid. If the microgamete fails to conjugate it dies. It cannot feed, encyst, divide or settle.

On the exhaustion of food and perhaps under the influence of other environmental factors the peristome is retracted and the ciliate secretes a cyst membrane. When stimulated to excyst the ciliate escapes from the membrane as a telotroch and reverts to the normal trophic cycle. Under certain circumstances conjugation takes place. Pre-conjugation division leads to the formation of a macrogamete and a microgamete, the latter very much smaller but otherwise morphologically identical with the telotroch. The free swimming microgamete becomes fused with a macrogamete and conjugation takes place, the microgamete being completely absorbed. The microgamete can conjugate with its sister macrogamete but not with an ordinary trophic vorticellid. Unlike the telotroch it dies if it fails to conjugate. It cannot metamorphose into a normal trophic individual, although the macrogamete can. After conjugation the vorticellid reverts to the normal trophic cycle (Finley, 1936, 1939).

The life cycle of Vorticella is therefore more complex than that of Colpoda and the sexual cycle is interesting because of the development of sexual dimorphism, also illustrated by Metopus es (Noland, 1927). One change not mentioned in the above account is the habit of telotroch formation by the trophic individual. This is in response to a number of environmental factors of which oxygen lack and high carbon dioxide tensions are two demonstrated in the present thesis. The experimental modification of the life history will be discussed more fully in a later section.

Although the normal vorticellid, such as V.microstoma, has these two distinct stages: one sessile and trophic, and the other non-trophic and free-swimming, occasionally the stalked individual becomes detached from its substrate. When this happens the individual will swim freely in the medium propelled by the beat of its peristomial cilia which normally cause a current of food particles to be swept towards the vestibule. While this behaviour is exceptional for V.microstoma, it is the normal behaviour of V.mayeri Faure-Fremiet which lives in open waters using its unattached stalk as a large flagellum as well as the powerful current caused by the peristomial ciliary wreaths. Conversely V.crateriformis O.F.M. exists permanently as a telotroch and upon these grounds was removed by Kent from the genus Vorticella and called Telotrochidium crateriformis. This is also the case for V.vaga Romer which divides in the telotroch stage and does not form a stalk. These species are interesting in that they can both feed and divide in the telotroch stage, thereby being markedly dissimilar to V.microstoma. Further cysts of both V.vaga and Telotrochidium (Opisthonecta) henneguys have been described by Romer and Faure-Fremiet so that encystment of the telotroch stage must take place (Brand, 1923).

iv Cyst Physiology

Brand (1923) studied the cyst physiology of Vorticella and gives an extensive review of the earlier work.

Brand gives a full description of the cyst and its history including that of the nucleus. The peristome is retracted and a cyst membrane of several layers secreted. The contractile vacuole pulsations gradually slow down and the vacuole ultimately disappears. There is complete dedifferentiation of the protoplasm but no nuclear reorganization. Excystment is marked by the formation of the contractile vacuole which pulsates and becomes very large. The ciliate escapes from the cyst membrane through a cyst pore which is forced open by hydrostatic pressure due to the activity of the contractile vacuole. The ciliate differentiates as a telotroch, becomes free-swimming and finally settles as a normal trophic vorticellid (Plate 13).

Brand does not distinguish this excystment pore although he described two pores during encystment, one anterior and one posterior, which are not immediately covered by the cyst membrane. In the present material the excystment pore can be clearly seen and the cyst membrane is not ruptured as shown by Brand (Plate 13). Plate 13 also shows that vestibule is early differentiated although feeding does not take place. Excystment, that is the escape from the cyst membrane, is independent of differentiation. The latter may take place without the former and vice versa. Usually an apparently undifferentiated mass with an excessively large contractile vacuole forces its way through the narrow cyst pore and this mass subsequently differentiates a posterior ciliary

wreath and adopts the form of a telotroch. It then swims away. Sometimes a fully differentiated telotroch may be found activated within an intact cyst membrane from which it has failed to escape.

The mechanics of excystment are generally similar to those of Tillina (Beers, 1945a) and Colpoda in that the build up of hydrostatic pressure accompanying the activity of the contractile vacuole finally ruptures the cyst membrane. The generally subsequent differentiation of the telotroch is comparable to Bursaria (Beers, 1948) in which the immediately excysted ciliate is dissimilar to the fully differentiated Bursaria.

Of the factors causing encystment absence of food is generally accepted as essential. Enriques (1907) regarded chemical influences as also affecting the encystment of V. microstoma and putrefaction and dessication have been suggested as possible factors (Brand, 1923). Brand himself inferred from his experiments that starvation and lack of oxygen were the principal causes of encystment and that dessication could cause encystment. The validity of these conclusions will be discussed in connection with the present experimental work. Brand did not find that either the pH or the accumulation of Paramecium metabolites affected encystment.

Brand found that excystment was affected by a number of factors including bacteria, oxygen and pH. Moreover he found that cysts differed in their response some excysting under one set of circumstances and others under different conditions. An alkaline pH he thought was inhibitory and neutralization in removing this inhibitory factor permitted excystment to take place. ~~Lack of~~ Oxygen and neutralization ^{were} ~~was~~ sufficient to cause excystment of over 30% of the cysts. The addition of bacteria caused the excystment of about 70% of the cysts. These three factors then accounted for the excystment of almost all the cysts and Brand therefore believed that they were the most significant. These results will be discussed in relation to the present experiments.

Generally whereas the causes of encystment in V. microstoma are somewhat similar to the conditions in Colpoda the causes of excystment differ in that the presence of bacteria is necessary before the great majority of cysts are activated. In this V. microstoma resembles Didinium (Beers, 1946b) for which bacteria or bacterial metabolites are a necessary excystment factor. However in the case of Didinium, which is a carnivore, the bacteria cannot be thought of as nutritional factor and although Vorticella is bacteriophagous it does not necessarily imply that the effect of the bacteria is nutritional.

What, then, is it

v. Distribution

Vorticella is a common and widespread genus containing very many species (Noland and Finley, 1931; Kahl, 1935). Most are fresh water or marine species. The distribution of V.microstoma is more specialized and it has been recorded from faeces, sewage, soil, and laboratory infusions.

(a) Alexieff (1923, 1929) is the sole reference given by Watson (1945) recording the occurrence of this ciliate in dung, faeces and other coprophilic habitats. Apparently it is not common in these conditions.

(b) It is one of the common sewage protozoa (Pillai, personal communication; Lackey, 1938b). The strain used in the present experiments was obtained from an anaerobic meat digester and was found with such ciliates as Metopus es and Cohnilembus pusillus. Lackey (1938b) gives the following distribution:-

<u>Habitat</u>	<u>Number of Records</u>
Ponds, pools or still areas along stream margins	-
Open waters of lakes	-
Tree or stump holes	-
Atlantic Ocean, Harbour at Woods Hole, Mass.	-
Polluted streams	20
Imhoff tank	6
Trickling filter	8
Activated sludge chamber	17

(c) It is the Vorticella commonly found in the soil. Sandon (1927) records it in 12% of soils. Fellers and Allison (1920) record V.striata as one of the six most common soil protozoa and Sandon suggests that the same species, not mentioned by other authors has been recorded as V.microstoma. It seems

probable however that Fellers and Allison were dealing for the most part with this latter species although both species are common in New Zealand soils.

(d) Although the species is recorded from fresh water by Wang (1927) it is not recorded by either Noland (1925a) or Lackey, nor have I noticed it in any of the fresh water samples I have examined. It seems likely that it is found only in polluted waters, certainly in the trophic state.

(e) It is commonly recorded from long standing infusions (Muller, 1776).

(f) It was recorded from muds "shown to be oxygen-free" by Kolkwitz and Marsson (1909).

Like Paramecium caudatum and Colpoda steinii this ciliate is found in organically rich habitats and is rare elsewhere.

vi. Prey-Predator Relation of Vorticella microstoma and Prorodon.

In the study of an anaerobic digester a species of Prorodon was encountered which has not yet been identified with any of described species. Generally in its life history it resembles P. griseus (Tannreuther, 1926). It forms thick walled or permanent cysts and thin walled or temporary cysts. These two cysts and the free-swimming form are so far the only stages studied.

(a) Morphology (Plate 14)

The ciliate is highly variable in shape and size. While in a starved culture it may be as small as 60 μ in a well fed culture it may be as large as 160 μ . Further whereas the starved or smaller forms show a typically oval outline, gross individuals

are found in well fed cultures which are broader than their length.

The structure of the ciliate is similar to other species with a well developed oral basket and about 50 ciliary meridians composed of short cilia. Because the cytoplasm is normally so stuffed with food masses it is impossible to study the nucleus in the free-swimming form (Tannreuther, 1926). The only record of the oval macronucleus is from encysted material (Plate 14, fig. 2).

(b) Systematics

The number of ciliary meridians is generally specific. P. discolor has a similar number of meridians but the nucleus of this species is elongate ellipsoid. P. ovum is very similar though I can find no record of the number of ciliary meridians. The nucleus of this species is described by Roux (1901) as spherical and is shown as oval. Kahl (1930) does not describe the nucleus but also shows an oval nucleus. Tannreuther (1926) states that the macronucleus of P. griseus varies considerably in shape.

The systematic position is therefore uncertain but the behaviour of this ciliate is so characteristic that had it been previously studied it must have been recorded.

(c) Behaviour

The ciliate swims in a most erratic fashion. It darts first one way then another. Suddenly it will strike a trophic vorticellid. Generally the Prorodon becomes attached to the vorticellid and the rotary swimming ceases. The predator then

swallows its prey. The whole process of capture takes about 30 seconds and the ciliate again darts about until it strikes another Vorticella when the process is repeated. A flourishing culture of trophic Vorticella may be reduced very rapidly to a few isolated individuals. I have never seen the Prorodon attack cysts or swallow other protozoa nor have I seen it feed on anything other than Vorticella. When the Vorticella are almost exterminated the Prorodon are consequently starved and become much smaller. In a well fed culture with many Vorticella many thin walled Prorodon cysts can be seen, the ciliate still revolving within the membrane or at rest. These encysted ciliates are absolutely stuffed with food and are very dense (Plate 14, fig. 1). They escape from their cysts however often before there is any noticeable diminution in the food mass. I have never observed division of either the free-swimming ciliates or of the thin walled cysts. Tannreuther states that P.griseus divides both in the free-swimming and the encysted state. The only evidence of division is the cyst containing two ciliates shown in Plate 14, (fig.2). These may be exconjugants. As the numbers of Prorodon increase in a well fed culture it is obvious that they divide readily although this has not been observed.

Although this Prorodon sp. has never been recorded from soil its intimate connection with the biology of Vorticella microstoma as well as the intrinsic interest of its habits make its record well worth while. As can be judged from the above account it is remarkably similar to Didinium nasutum in its behaviour and particularly in its method of capturing its prey. Just as

Didinium feeds chiefly on Paramecium, so this Prorodon feeds exclusively on Vorticella. The carnivorous habit of this Prorodon is apparently unique in the genus which is otherwise wholly herbivorous (Sandon, 1932).

Structurally Prorodon is very similar to Didinium the latter characterised by the loss of the normal ciliary meridians in the fully developed ciliate although they are present in the recently excysted ciliate (Beers, 1945b). The cyst physiology of the present Prorodon sp. has not yet been experimentally studied.

Vorticella microstoma Ehrenberg (Plate 12, figs.6-12.)

This species is highly variable in shape. It is typically pyriform with a narrow peristome and symmetrically swollen so that it is broadest centrally and tapers evenly to the peristome anteriorly and the stalk attachment posteriorly. The average size is 55mu by 35mu and the peristome width 16mu. The ratio of length to breadth is therefore 1.6:1. The striations are fairly clearly marked and so too is the line of the posterior ciliary wreath. This form is illustrated in fig.8 and is well described in Noland and Finley's paper.

When starved the ciliate is thinner and therefore appears more elongate. The form shown in Fig.9 is 40mu by 20mu and the peristome width is still 15mu. The ratio of length to breadth is therefore 2:1.

When fed on a rich bacterial infusion the form changes dramatically. The ciliate becomes markedly swollen and pyriform

and greatly increased in body volume. The striations are no longer visible. These forms are over 70 μ long and up to 55 μ in breadth. The ratio of length to breadth is then 1.3:1. The peristome width is 23 μ . The difference between individuals before and after bacteria have been supplied is shown in figs.9 and 11.

Fig.6 is a typical soil form with the proportions little distorted. Fig.7 shows a remarkable individual with two short stalks. These were not observed to contract and indeed it is difficult to imagine how they could be synchronised. This form is interesting from a theoretical point of view since the morphogenesis of vorticellids has been subjected to considerable study (Summers, 1941). The stalk connection is held to inhibit the formation of a posterior ciliary wreath, and, one would imagine, a second stalk. In this case the morphogenetic mechanism has been disturbed, dominance has not been established and two stalks instead of one are the result.

The cysts of V.microstoma also vary in size depending upon the previous history of the culture. Fig.10 shows a small cyst from a starved culture and Plate 13, drawn to the same scale the excystment of a large cyst. Cysts formed in a hanging drop and therefore well aerated ranged from 28.8 to 40.0 μ in diameter and averaged 35.2 μ whereas cysts in an anaerobic digester ranged from 33.6 to 46.4 μ and averaged 38.4 μ . This indicates that factors other than starvation affect cyst formation.

Vorticella striata Dujardin

This vorticellid is also variable in shape and size. It is

typically much smaller than V.microstoma (figs. 1, 2, and 3). This form may be described as 'vase shaped, about twice as long as broad, widest centrally, tapering posteriorly and slightly constricted beneath the everted and revolute border of the peristome whose width is less than the greatest width of the body; cuticular surface strongly and conspicuously striate transversely'. The dimensions are length 23mu, breadth 11.5mu and peristome width 10.6mu.

When fed the ciliate changes in shape, becomes swollen and very like V.microstoma. Fig. 5 shows a ciliate that has been fed on bacteria. Drawn to the same scale as the V.microstoma next to it (fig.6) it is conspicuously larger. Its dimensions are 50mu by 32mu and the peristome width 22mu. Most noticeable however is the pyriform shape of the body and loss of the graceful vase-like figure of the normal form. The scopula which is very distinct in the small form is hardly distinguishable in the large. This applies also to the striations.

Discussion

The systematics of these small vorticellids is more difficult than that of other ciliates because the absence of body cilia deprives the systematist of one of his most valuable diagnostic characters. V.microstoma and V.striata are distinguished by Noland and Finley in the following terms: 'Specimens occur which are almost intergrades between this species (V.microstoma) and V.striata, but in general V.microstoma differs from the latter species in having a longer nucleus, less conspicuous pellicular

striae, a larger size and a greater tolerance to bacterial action in infusions'. The present account agrees with that of Noland and Finley but shows that the definiteness of the pellicular striae and the size vary with cultural conditions. With the possible exception of the nucleus, itself a very variable character in ciliates, there is no definite specific character to distinguish the two species, and it is very difficult when faced with only one or two individuals to determine to which species they properly belong.

Noland and Finley appear to recognize these two species as the principal small vorticellids and consequently consider small vorticellids, previously described, as synonyms of these two species. Following is their synonymy of V. microstoma.

Vorticella hians O.F. Muller 1773
Vorticella microstoma Ehrenberg 1830
Vorticella infusionum Dugardin 1841
Vorticella sphaerica d'Udekem 1864 (?)
Vorticella constricta Fromental 1874 (?)
Vorticella fluvialis Fromental 1874 (?)
Vorticella mammillata Fromental 1874

Muller (1773) described Vorticella hians as 'Vorticella simplex citriformis pedunculo retortili'. This was an infusion form. Subsequently Kent (1880-1882) figured the species and described it as 'body elongate, pyriform or lemon shaped, widest and inflated posteriorly; about twice as long as broad; pedicle very short, scarcely as long as the body'. But Kent adds that this description fits only one type of V. hians and that the stalk may be of normal length.

Fauré-Fremiet (1905) was the first to connect this infusion species with V. microstoma Ehrenberg. He considered it an anaerobic

variety of the latter species and described his material as 'ovoid and more often pyriform, swollen at the base and very narrow at the apex'. The size of his material was 90 μ by 53-60 μ and the peristome width 22 μ compared with 27 μ in Faure-Fremiet's normal form. He also recorded abnormalities of colour, peristome, and nucleus in V. hians.

There is little doubt then that it is V. hians that is illustrated in figs. 11 & 12 which show the lemon-shaped body first described by Muller. It is also clear that it is an abnormal variety of V. microstoma due however not to anaerobic conditions but to a rich bacterial diet. Strictly, this synonymy established, Ehrenberg's name should be discontinued and the species called V. hians. However none of the modern authors - Faure-Fremiet, Noland and Finley, or Kahl - have suggested this radical step.

In identifying their fresh water material with the marine species of Dujardin, Noland and Finley established an extensive synonymy of Vorticella striata.

<u>Vorticella striata</u> Dujardin 1841	Marine
<u>Vorticella sphaerica</u> d'Udekem 1864	
<u>Vorticella pyrum</u> Mereschowsky 1879	Marine
<u>Vorticella octava</u> Stokes 1885	Fresh water
<u>Vorticella utriculus</u> Stokes 1885	Fresh water
<u>Vorticella rhabdostyloides</u> Kellicott 1885	Fresh water
<u>Vorticella lemnae</u> Stokes 1886	Fresh water
<u>Vorticella aqua-dulcis</u> Stokes 1887	Fresh water
<u>Vorticella pusilla</u> Stokes 1887	Fresh water
<u>Vorticella striata</u> Fromentel 1874	Fresh water
<u>Vorticella platysoma</u> Stokes 1887	Fresh water
<u>Vorticella conochili</u> Stokes 1889	Fresh water
<u>Vorticella oceanica</u> Zacharias 1906 (?)	Marine
<u>Vorticella striatula</u> Dons 1915	Marine
<u>Vorticella microstoma</u> var. <u>abbreviata</u> Keiser 1921	

Kahl (1935) adopts a different course. Of the different species involved he separates V. striata Dujardin absolutely and gives no synonyms not even V. pyrum or V. striatula. He does not mention Fromentel's species. He also separates V. microstoma but he allows V. infusionum Dujardin, V. striata Kent and V. hians to be synonyms of this species. Of the other species mentioned by Noland and Finley he groups Stokes' species (V. octava, V. aquae-dulcis, V. pusilla, V. conochili, and V. lemnae) with V. submicrostoma Ghosh and V. subprocumbens Ghosh. This group he separates from V. utriculus Stokes on the one hand and V. microstoma on the other, the former because of its more slender elongate form and the latter because of its longer nucleus and saprobic habit. Of the fresh water group which Noland and Finley identified with V. striata Dujardin, Kahl remarks that "it is a pity that we cannot tell from Noland's paper whether the variability of his form 'octava' fell within a single population. Otherwise he could have combined other species also. This requires to be tested!" In other words Kahl is unwilling to admit the identity of the fresh water group until their ecological range has been experimentally verified. This has so far not been done but it is known from Hoare's work that this is a difficult task.

Of the other species mentioned by Noland and Finley, viz. those of Fromentel, Kellicott and d'Udekem, Kahl associates V. sphaerica V. rhabdostyloides, V. margaritifera Fromentel 1874 V. globularia O.F. Muller 1776, V. constricta, V. mammilata, V. communis Fromentel and V. fluvialis in one group; V. platysoma and V. abbreviata

with other species in another group; and the remaining species separately.

It seems impossible to reach any definitive position on the previously described species. The present material appears to be identical with V. utriculus Stokes 1885 and as this was one of the first species described by Stokes this would be a satisfactory identification. V. parasita Stokes 1887 could be considered a synonym. It seems most likely that the V. octava group are the same species. The relation between the fresh water and marine species is less certain. Fromentel's V. striata was a fresh water species which Noland and Finley consider to be identical with the marine. Provided the strain of ciliate is described there seems no objection to calling them all V. striata as they are morphologically indistinguishable. I can find less justification however for designating the fresh water strain with a varietal name as is done by Noland and Finley, who term their material V. striata var. octava. This practice is without precedent. Either the fresh water and marine strains are identical or they are not. There is no question of a varietal difference when they are morphologically indistinguishable. The correct practice is to identify their material with V. octava Stokes and then to determine whether or not this species is a synonym of V. striata Dujardin. Either it is or it is not. If it is then the correct name becomes V. striata, if it is not then the correct name is V. octava. The same argument holds for the present material. It is identified with V. utriculus Stokes. If this species is identical with V. striata Dujardin then Stokes' name must be discontinued.

If it is identical with V.octava then either or other of the names must be suppressed. Both descriptions are of the same date. For convenience I will refer to the species as V.striata assuming the identify of the fresh water and marine species.

f. Suctoria

Suctoria have been previously recorded from the soil only by Fantham who found Sphaerophrya. They have been recorded only occasionally in New Zealand soils. One a Thecacinetia species (Plate 8 fig. 6) has been found only once in beech litter associated with a rich and specialized ciliate fauna. Two of the other species, probably Sphaerophrya, have also occurred only once and their life history is unknown. The fourth species (Plate 9 figs. 2 & 3) probably Sphaerophrya magna has occurred several times and once a culture containing dividing ciliates was observed which enabled the systematics to be positively determined. I have not observed the predatory habits of any of these species.

iii. A comparison of the New Zealand and other Soil Faunas.

This review of the systematics of the soil ciliates shows that although many of the species have been previously described and named a considerable number, about a third of the total list, cannot be identified with the common species previously described from fresh water. Occasionally the soil species has been identified with a rarely recorded species such as Loxophyllum utriculariae but the common fresh water species such as L. meleagris, or Dileptus anser have not been recorded. This finding is contrary to previous work on the soil fauna which has generally identified the species encountered with well known fresh water species. It is therefore necessary to consider this discrepancy.

Few of these workers give an intimate systematic account of the soil fauna. Fantham, for example, does not give any descriptive information and simply lists the specific names of his material. Varga also gives no systematic detail. Fellers and Allison give a number of figures, some original and some reproduced from earlier standard texts. They give brief descriptions of the figured species including the size. However their work is not critical as can be judged from the conflicting opinions they offer. They state that identification was difficult because of the confusion of earlier literature and because the same species was listed under several names. They state that nearly all the soil species were found in adjoining fresh water but in different abundance. But they also state that many of the common soil ciliates are 'undoubtedly new species and some

may even have to be given new generic names'. They remark on differences of size, colour and other characteristics between the same organism taken from soil and fresh water. They conclude by stating that the characteristic soil protozoa are "those small hardy forms which are capable, by means of rapid encystation or otherwise, to withstand successfully extremes of heat and cold, dessication and aeration and such other inhibiting factors as are natural to their home in the soil". It is very difficult to estimate the value of their systematics but it is evident that they were not aware of the finer details of morphology, such as the number of ciliary meridians. A number of their identifications however may be queried.

It has already been mentioned that Sandon queried their identification of Colpoda. They record C.saprophila as 'abundant' and C.cucullus as 'extremely abundant'. They record C.flavicans (?) as 'rare'. Only the first two species are described; 'C.saprophila 25 to 40 μ (30); ventral border anteriorly notched, recurved pharynx very conspicuous, wide ventral groove; C.cucullus Stein 30 to 35 μ (35), nucleus central, contractile vacuole posteriorly located, distinct groove ventrally'. This latter species is of course C.steinii. Sandon who suggested that their C.saprophila may have been C.steinii was apparently unaware of the identity of C.cucullus Stein. I have already suggested that C.flavicans is a synonym for C.inflata. They describe Prorodon teres (?) Ehrbg. as '60 to 150 μ (75), oral opening terminal, nucleus central' and they comment that the species is usually described as larger than this. They describe D.gigas with a moniliform nucleus. This

species is D.anser (Hayes, 1938). They describe Blepharisma ovata (?) Stokes as '80 to 150mu (100), body much flattened, anterior end acute, membrane covering lower part of the peristome sometimes visible'. They describe Vorticella striata as '35 to 45mu (40), striae prominent'. Generally their identifications are unexceptional but it seems probable that when they were uncertain as e.g. with Prorodon and Blepharisma the species they saw were not the fresh water species but the species mentioned in the present chapter. Their Vorticella may have often been small V.microstoma judging by the dimensions given.

The principal source of systematic detail on soil species is the Rothamsted work done by Goodey and Sandon in particular. Goodey (1911, 1913) recorded only a few ciliate species but one unnamed Chilodonella species of the C.uncinatus group is very similar to present material. In the preface to the 1927 work Sandon states that 'the soil, undoubtedly harbours some protozoa at present unidentified and possibly quite new to science. Many of the genera and species here recorded are very imperfectly known and more complete knowledge of their morphologies and life histories may necessitate considerable modifications in the nomenclature and classification that has been adopted'. Sandon then gives an extensive systematic treatment of the fauna. It is difficult to detect any obvious errors. Lionotus fasciola is described 'neck not so distinct as in Amphileptus and body more flattened, cilia in longitudinal rows on one side only, length 80 to 100mu, macronucleus consisting of two ellipsoidal masses'.

L. fasciola has two spherical macronuclei. He gives the length of V. microstoma as 60 to 100µ which is excessive. The confusion with Colpoda and the possible error with Euplores have already been mentioned. The most interesting fact is that in the list of ciliates given in his survey he identifies specifically only 17 species out of a total of 35, that is less than half. The species listed by name are those common species for the most part present also in New Zealand soils. All other holotrichous ciliates are left simply in their genera.

The unexpected number of unidentified species found in the New Zealand survey does not contradict the earlier work as much as might at first appear. It seems very probable that the earlier workers, who were conscious (as the present author) of deficiencies in their systematics, came across the species so widespread in New Zealand soils. These they either assigned to described species, e.g. Fantham et al., Fellers and Allison, or else left unidentified, e.g. Sendon. The constant reference to the smaller soil races or strains or species is borne out with the New Zealand material but these 'races' are really distinct species. Certainly I have not yet encountered in the soil any of the common large fresh water species such as Dileptus anser or Lionotus fasciola, which are recorded by both Fantham et al. and Fellers and Allison. The species of these genera which I have encountered are not only smaller but are quite distinct both with regard to the number of ciliary meridians and the form of the macronucleus. The 'large' species I have encountered quite commonly in fresh water samples. They are not therefore excluded by any geographical barrier. The

conclusion seems inevitable that earlier workers were mistaken in recording these fresh water species, certainly as common, and that they mistook for these species the smaller soil species dealt with in this chapter.

Although it is not easy to correlate the work of Sandon with the New Zealand survey there are certain important agreements concerning the absence rather than the presence of species. Thus Sandon remarks particularly on the absence of Paramecium, Coleps hirtus, Colpidium and Stylonychia. He himself recorded Stylonychia once with two queries, and Colpidium twice with two queries out of a total of 145 samples. The other two ciliates he did not record at all. Both Fantham et al. and Fellers and Allison record Paramecium, Colpidium, and Stylonychia and Fantham et al. records Coleps hirtus. The discrepancy between Sandon's and the New Zealand survey on the one hand and Fantham et al. and Fellers and Allison on the other, is therefore present not only in the species recorded but also in the species not recorded.

B. THE ECOLOGY AND RELATIONSHIPS OF THE SOIL FAUNA

A comparison of the ciliate fauna of New Zealand soils with the fresh water fauna shows that they are systematically distinct and that they are composed often of different genera of different species. A number of surveys have been made of the incidence of free-living ciliates in different habitats and under varying ecological conditions. These include those of Noland (1925a), Wang (1928), Picken (1937) and Lackey (1938b). Early work had been done by Lauterborn (1916) on the fauna of mud and there is a

considerable literature on sewage biology.

The work of Lackey (1938b) is the most comprehensive. He examined the protozoan fauna of eight characteristic ciliate habitats. The following table summarises his results:

Habitat	No.Samples	No.Species	No.Spp; in 25% Samples
1. Ponds, pools and still areas along stream margins	70	102	5
2. Open waters of lakes	20	19	3
3. Tree or stump holes	25	27	1
4. Atlantic Ocean, Harbour at Woods Hole Mass.	40	36	2
5. Polluted stream	40	52	11
6. Imhoff tanks	50	29	3
7. Trickling filter	50	65	8
8. Activated sludge chamber	40	44	11
<u>Total</u>		<u>118</u>	

Species	Habitat							
	1	2	3	4	5	6	7	8
<u>Aspidisca turrita</u>				2			1	1
<u>Carchesium lachmanni</u>					24	1	1	
<u>Colpoda aspera</u>			2		10	5	2	
<u>Dactylochlamys sp.</u>			2		2			
<u>Drepanomonas revoluta</u>			3		3	1		
<u>Dysteriopsis minuta</u>					1	2		
<u>Epistylis plicatilis</u>					5		13	27
<u>Hexotrichia caudatum</u>					1	4		
<u>Holophrya sp.</u>					12	13		
<u>Lionotus lamella</u>					6	2	7	9
<u>Loxophyllum meleagris</u>					1		2	6
<u>Opercularia spp.</u>					2	6	42	36
<u>Trimyema compressa</u>			1		2	27	1	
<u>Trochiliopsis opaca</u>					1		2	5
<u>Vorticella nebulifera</u>								2
<u>V. microstoma</u>					20	6	8	17

There are also a number of species which occurred rarely in the first habitat but frequently in one or more of the polluted habitats or else as with Paramecium caudatum the numbers were sparse in the first habitat while very great in polluted waters.

From these studies Lackey was able to conclude that although protozoa were cosmopolitan in their distribution a given habitat may be prohibitive to many species because of local environmental conditions. Further he was able to conclude that the various types of sewage disposal plants have definite protozoan populations characterized by a large number of species in trickling filters, a much smaller number in activated sludge chambers and the smallest number in Imhoff and septic tanks. Polluted streams also have highly characteristic protozoan populations. Lackey also pointed out that although we correlate definite habitat types with

particular protozoa or groups of protozoa we fail to recognize the specific determinative factors. These can be known only when the tolerance of each species has been investigated.

Kolkwitz and Marsson (1909) introduced a classification of the different types of habitat which is still valuable. They distinguished the following faunas:

Katharobic: living in an environment devoid of organic matter; highly oxygenated with very few bacteria; e.g. fresh water springs.

Oligosaprobic: living in an environment of well oxygenated, relatively clean water in which mineralization of organic matter is largely completed; chlorophyll bearing forms are particularly abundant in such a habitat and may occur in very great numbers so as to seriously affect the water;

Mesosaprobic: living in an environment with both organic matter and bacterial activity and with adequate dissolved oxygen; this is generally the richest collecting place for fresh-water protozoa as can be seen from Lackey's Table.

Two types of mesosaprobe are distinguished, the alpha and beta mesosaprobe the former being associated with higher organic content and grading into the latter type.

Polysaprobic: living in an environment rich in high molecular decomposable organic food material in which decomposition due to the enormous number of bacteria present is going on actively and consequently there is very little oxygen, but high sulphide and carbon dioxide concentrations. These include the anaerobes of sewage disposal plants and the 'sapropelic' mud fauna of Lauterborn (1901, 1916).

Under natural conditions a particular habitat may comprise simply one faunal type but in many cases such as in polluted streams or sewage disposal works there are constantly changing conditions and a constantly changing fauna.

In most fresh water habitats in which ciliates are found they are not freely suspended in an aqueous medium but are supported by a plant substrate which under normal conditions is not part of the diet of other organisms in the community. In nature it is in general only at surfaces of discontinuity between gas and liquid, or liquid and solid, that an accumulation of essential food substances sufficient to support a considerable population can take place. Thus one finds a dense population in surface films of bacteria or Cyanophyceae over the surface of submerged plants whether continuous leaf surfaces or the surface of a mass of filamentous algae (Picken, 1937). As Picken and Noland point out the protozoan population is greatest, in number of species, in these plant or fungal associations. This is confirmed by Lackey. The history of a typical community is discussed by Faure-Fremiet (1950).

In polluted waters and in laboratory infusions there is sufficient concentration of the food supply in suspension and solution to support a free-swimming population. This is because the enriched solution can support a dense bacterial growth which first concentrates the dissolved food source and is then consumed by the bacteriophagous ciliates, such as Paramecium and Colpoda (Butterfield, 1929). There is still a tendency for these ciliates, particularly Paramecium, to clump in thick bacterial suspensions

and this is largely due, as Picken suggests, to the presence of carbon dioxide (Jennings, 1915). It may also be partly due, in the case of Colpoda, to a separation of assimilation and movement, the latter being largely inhibited when the former is functioning normally. In nature this bacteriophagous association may also be confined by the fine fungal mesh.

There is then this distinction between a ciliate fauna bound to plant surfaces and largely associated with diatoms and algae and a free-swimming fauna, largely associated with bacteria. In the soil there are two different conditions. Where there are large pore spaces, worm and root holes and soil fissures water may be held for a short time as in puddles and fungi and algae may develop. In such spaces there is better aeration and the soil solution is more dilute. Such conditions are very similar to those of the laboratory culture as Picken points out. Where there are capillary pore spaces of microscopic dimensions there is poor aeration and chiefly bacterial activity. In this case, as Kubiens (1938) found, the ciliates are closely bound to the soil particles and retreat into the smallest inter- and intra-granular spaces as the soil dries out. These conditions are very similar to those of the coprophilic fauna. Finally in litters there is good aeration and water retention but as with Picken's community the ciliates are closely associated with their plant substrate, in this case dead, and the fungi and algae which grow on it.

11 The Ecology of New Zealand Soil Ciliates

In Table 4 is summarised the distribution of the ciliate fauna in soil and litter samples, whether or not they have been recorded from fresh water in New Zealand, and any distributional peculiarities.

This table shows that a number of species have been recorded chiefly or solely from granular litters and soils. In particular beech litters have harboured Phacodinium metschnikoffi a species not recorded elsewhere in New Zealand and previously recorded from soil only by Francé who worked on forest soils (Sandon, 1927). Other species such as Blepharisma steini and Paramecium are found more frequently in litters than in mineral soil and the only occasion when a pigmented B. steini was observed was in a litter sample. Some of the characteristically litter species, found particularly in beech litter, have also been recorded from the granular soils of Niue and Raoul Islands. Hemicyliostyla recorded in New Zealand only from beech litter was recorded three times in these Islands in soils under cocoanut or scrub. Onychodromus grandis? also recorded only from beech litter in New Zealand was found in a Raoul Island sample under Pohutakawa and grass. On the other hand the largest Spathidium sp.4 has so far been recorded only from Raoul and Niue Islands. Although this appears to indicate that the ecology of each species differs slightly from that of other species, those species which are found wholly or largely in granular soils and litters are considered as one ecological group which for convenience may be termed the litter fauna. They are generally larger and structurally more elaborate

than other soil ciliates and this is particularly true of Phacodinium metschinicoffi. Systematically they are generally spirotrichous ciliates, these being morphologically the most specialized.

Of the remaining species the majority are fairly common in both soils and litters although a number of species such as Cyrtolophosis mucicola are rare in litter and relatively common in soil. These species fall into two groups: those which have been identified with previously described species and those which have not. The former consist of species of Trichopelma, Drepanomonas, Colpoda, Tetrahymena, Saprophilus, Cyclidium, Cohnilembus, Oxytricha, Gonostomum, Uroleptus, Aspidisca and Vorticella. In all there are about thirty species. Some of these species such as Oxytricha pellionella, Cyclidium glaucoma and Vorticella microstoma are commonly found in polluted waters, sewage and faeces. Others are often associated with decaying vegetation such as in sphagnum or moss, e.g. Trichopelma sphagnetorium, Drepanomonas sphagni and Cohnilembus fusiformis. Although the whole group may be considered saprobic there is a difference between the polysaprobic pollution species and mesosaprobic sphagnum species. All these species are characteristically small in size and simple in structure. Often as with Vorticella microstoma their cyst physiology is highly developed.

The species which have not been identified with previously described material number about twenty species, some of which are confined to litter. Of those fairly widespread throughout the soil types, the majority are small and simple ciliates similar in this respect to the better known saprobic species. This group may

be called the autochthonous soil fauna although further research may reveal the presence of these species in other habitats. Besides these three major groups there are a number of records of considerable interest. These are the occurrence of a single individual of Trimyema compressum and Balantidium sp. The former is found chiefly in Imhoff tanks and is really an anaerobe (Lackey, 1938b). The latter is a parasitic species and it is interesting to find that Fantham (1931) also recorded Balantidium from soil which he attributed to contamination by bull frogs. Fantham, in the same paper, also recorded a suctorian which he thought was Sphaerophrya which is an interesting confirmation of the New Zealand records.

Perhaps the most interesting point however is the few species found in soil which are also commonly recorded from fresh water. The majority of such species are polysaprophes such as Cyclidium glaucoma or such widespread species as Halteria grandinella. In other words these species can hardly be considered ecologically as fresh water species since they occur throughout the whole range of ciliate habitats, fresh water and marine. Typical fresh water species such as Coleps hirtus and Stylonychia mytilus are not recorded at all. Although a notable distinction between most soil species and many fresh water species is the smaller size of the former there is no such distinction between the 'litter group' and the fresh water species and many of the 'litter' species are as morphologically specialized as fresh water species. It is obvious then that size is not the sole factor distinguishing the faunas for soils of good structure harbour quite large species.

Table 4

Distribution of the Ciliate Fauna of New Zealand Soils

c - common; o - occasional; r - rare; - no record;
x - recorded from fresh water.

Species	Soil	Litter	Fresh Water	Comment
<u>Spathidium sp.1</u>	o	o	-	
<u>Spathidium sp.2</u>	o	o	-	
<u>Spathidium sp.3</u>	r	o	-	
<u>Spathidium sp.4</u>	r	-	-	Raoul and Niue Is. only
<u>Didinium balbiani?</u>	r	-	-	Single record, few individuals
<u>Urotricha agilis</u>	o	o	-	
<u>Prorodon sp.</u>	o	o	-	
<u>Lacrymaria sp.</u>	o	o	-	
<u>Enchelys sp.</u>	c	c	-	
<u>Chaenea sp.</u>	o	o	-	
<u>Pithothorax ovatus</u>	r	-	-	Single record, few individuals
<u>Trachelophyllum apiculatum?</u>	r	r	-	Raoul Is., beech litter only
<u>Litonotus sp.</u>	o	o	-	
<u>Loxophyllum utriculariae</u>	o	-	-	
<u>Bryophyllum spathidioides</u>	r	r	-	Raoul Is., beech litter only
<u>Dileptus sp.</u>	o	o	-	
<u>Dileptus americanus</u>	r	r	-	Raoul Is., Samoa, litter only
<u>Dileptus gigas</u>	r	r	-	Niue Is.
<u>Chilodonella sp.1</u>	r	r	-	Granular soils only
<u>Chilodonella sp.2</u>	o	r	-	
<u>Chilodonella sp.3</u>	r	-	-	
<u>Trichopelma sphagnetorium</u>	c	c	-	
<u>Drepanomonas revoluta</u>	o	r	-	
<u>Drepanomonas sphagni</u>	r	-	-	
<u>Trimyema compressum</u>	-	r	-	One individual
<u>Plagiopyla sp.?</u>	c	c	-	
<u>Paramecium sp.</u>	r	o	-	
<u>Colpoda cucullus</u>	o	o	-	
<u>Colpoda inflata</u>	c	o	-	
<u>Colpoda steinii</u>	c	o	-	
<u>Colpoda saprophila</u>	r	r	-	
<u>Tetrahymena pyriformis</u>	r	r	-	
<u>Colpidium sp.</u>	c	o	-	
<u>Pseudoglaucoma muscorum?</u>	r	-	-	
<u>Saprophilus agitatedus</u>	o	r	-	
<u>Cinetochilum margaritaceum</u>	r	-	x	

Table 4 (Continued)

Species	Soil	Litter	Fresh Water	Comment
<u>Cyrtolophosis mucicola</u>	o	r	x	
<u>Homalogastra setosa</u>	r	-	-	
<u>Cyclidium glaucoma</u>	o	o	x	
<u>Cohnilembus pusillus</u>	r	-	-	
<u>Cohnilembus fusiformis</u>	r	-	-	
<u>Bryometopus pseudochilodon</u>	r	r	-	
<u>Bryometopus sp.</u>	-	r	-	Beech litter only
<u>Balantidium sp.</u>	r	-	-	One individual only
<u>Blepharisma steini</u>	r	o	-	
<u>Blepharisma sp.</u>	o	r	-	
<u>Phacodinium metschnikoffi</u>	-	r	-	Beech litter only
<u>Halteria grandinella</u>	c	c	x	
<u>Oxytricha fallax</u>	r	r	-	
<u>Oxytricha pellionella</u>	c	o	-	
<u>Oxytricha setigera?</u>	c	o	-	
<u>Gonostomum affine</u>	c	o	-	
<u>Hemicycliostyla sp.</u>	r	r	-	Beech litter, Raoul and Niue Is. only
<u>Psilotricha sp.</u>	r	r	-	
<u>Uroleptus musculus</u>	o	r	-	
<u>Uroleptus piscis?</u>	r	-	-	
<u>Uroleptus mobilis</u>	c	o	-	
<u>Holosticha sp.</u>	o	c	-	
<u>Onychodromus grandis?</u>	r	r	-	Beech litter, Raoul Island only
<u>Euplotes sp.</u>	o	-	-	
<u>Aspidisca lynceus</u>	r	r	-	
<u>Aspidisca costata</u>	r	-	x	Campbell Island only
<u>Rhabdostyla sp.</u>	r	r	-	
<u>Vorticella microstoma</u>	o	o	-	
<u>Vorticella striata</u>	o	o	-	
<u>Sphaerophrya magna</u>	r	-	-	
<u>Thecacinetia sp.</u>	-	r	-	Beech litter only

iii The Relationships of the Fauna

Relation to Litter and Sphagnum Faunas

It has been stressed that certain species are generally restricted to soils and particularly litters of good structure. Many of these species have been previously described from litter and sphagnum, such as Bryometopus pseudochilodon and Blepharisma steini, and almost always they belong to typical moss or sphagnum genera, e.g. Bryophyllum and Hemicycliostyla. This element in the soil fauna indicates an overlap between the two habitats characterised chiefly by typically large species which occur only soils or litters of good structure. This appears to indicate that good aeration and good water holding capacity are of greatest importance for this fauna and this is confirmed by the fact that such species are found in granular podocarp litters but not in non-granular podocarp litters and that they are generally found in beech litters which have a good structure. (Good) in what sense

The saprobic species which are common both in soil and sphagnum are not restricted to soils of good structure but are widespread throughout all soil types. In this case the association appears to be with the organic content of soil and sphagnum which favours their growth.

Relation to the Sewage Fauna and Fauna of Polluted Waters

Four of the seven species common to both soil and fresh water are most frequently found in polluted streams, sewage filters, activated sludge or enriched infusions. These are Cinetochilum margaritaceum, Cyclidium glaucoma, Aspidisca costata

and Vorticella microstoma. This fauna is well represented in soil particularly widespread and common of all soil ciliates and their rapid growth and powers of encystment and excystment make them particularly well adapted to soil conditions. Further they are generally capable of withstanding a wide range of environmental conditions.

Relation to the Coprophilic Fauna

According to Watson (1946c) all coprophilic species have been recorded from the soil and the coprophilic ciliates may therefore be understood as a special section of the soil fauna very closely related to the sewage and pollutional species. They are particularly adapted to resist dessication and to grow in organically rich media.

III

AN EXPERIMENTAL ANALYSIS AND DISCUSSION OF THE RESPONSE OF CILIATES TO CULTURE FACTORS AND OF THE CULTURE SEQUENCE

A. AN EXAMINATION OF CULTURE METHODS

- i. Growth in Enriched and Unenriched Media
- ii. Growth at Different Depths
- iii. Growth in Media of Different pH

B. AN EXAMINATION OF THE RELATION BETWEEN BACTERIA AND CILIATES

- i. Growth of Mixed Fauna in Bacterial Suspensions
- ii. Growth and Encystment of Vorticella microstoma in Bacterial Suspensions

C. AN EXAMINATION OF THE CULTURE SEQUENCE AND OF THE EFFECT OF STEAM TREATMENT

- i. Culture Sequence of Greywacke Cultures
- ii. Culture Sequence of Steam Treated Soil

D. GENERAL DISCUSSION

Soil Conditions and Their Relation to Protozoan Activity

A. AN EXAMINATION OF CULTURE METHODS

1. Growth in Enriched and Unenriched Media

Introduction

The simplest method of establishing a soil culture is to add water to the soil keeping the culture in a closed dish or test tube to prevent evaporation and contamination. This method was employed by some of the earlier workers, including Goodey (1911), and also by Fantham et al. (1922-1931) in their survey of South African soils. It was also used for the first few samples of the New Zealand survey.

The second method is to use an enriched culture. Much of the earlier work of Cunningham and Lohnis (1914), Martin and Lewin (1915), Koch (1915a) and others was concerned with devising satisfactory culture media. Fellers and Allison (1920) used soil extract, horse dung and hay infusion in their survey of New Jersey soils and Sandon (1927) used nutrient agar, dilute hay infusion and sterile tap water.

Sandon and other of the early workers found that different culture media gave rise to different faunas and this was confirmed in a study by Dixon (1937). She found that with different media (peptone agar, soil extract and hay infusion) the peptone favoured specialized species of bacteria and protozoa whereas soil extract permitted growth of many species in small numbers. For this reason she found the soil extract agar medium the most satisfactory for estimating the soil protozoan population. It was a modification of this medium which was used for nearly all the New Zealand soil samples.

Material and Methods

To test the selective effect of different culture media on the protozoan fauna a number of different media were prepared.

- i. Soil extract: this was prepared by steaming soil in a steam sterilizer for an hour or more in about a litre of water. The water was subsequently poured off and diluted before use.
- ii. Peat extract: this was similarly prepared from initially dried peat.
- iii. Yeast extract: this was similarly prepared from commercial bakers' yeast.
- iv. Peptone: de Witte's peptone in concentrations of 2% to 5% was used.
- v. Yeast extract-peptone-glucose: this medium was prepared with 1% yeast extract, 2% peptone and $\frac{1}{2}$ % glucose.

The various media were placed in Syracuse watch glasses and inoculated with protozoa from a flourishing beech litter culture. The species occurring in the various culture media were recorded and their approximate abundance.

*Temp.
Davis - 506*

Results

Table 5 gives a list of the different species and their occurrence in the different culture media. The relative abundance of the different species is shown by the number of asterisks. No protozoa grew or lived in the peptone cultures. 15 species were recorded from both the soil and the peat extract media; 7 species from the yeast extract medium with two species of Colpoda very abundant; and only three species of ciliate lived

in the yeast extract-peptone-glucose medium and only one species, Colpoda steinii, multiplied, the other two species dying out. C.steinii however in this last medium attained very great numbers.

Discussion and Conclusions

Fuller discussion of the relation between culture conditions and the protozoan fauna will be left for a later section. Here it is sufficient to note that the results are in agreement with those of Dixon. Soil extract and peat extract which are nearest the natural habitat of the fauna supported the greatest number of species although not always the greatest number of individuals. Yeast extract and yeast extract-peptone-glucose, typical enriched media, permitted the growth of only a limited fauna but particular species showed greatly increased growth rate in these media and this is particularly noticeable for Colpoda inflata and C.steinii in the first and C.steinii in the second. It should be noted that C.cucullus did not show any marked increase of growth in the first enriched medium and did not survive at all in the second. This species, in contradistinction to the other two species, has proved much more difficult to maintain in serial sub-culture using yeast extract although it grows very well in hay extract, soil extract or peat extract. In yeast extract if kept in permanent culture not only is the division rate depressed but abnormality of shape and size often occur. It will be remembered that this species is common in hay infusions and occurs occasionally in fresh water (Wang, 1928) but is less common in soil than the other two species. The effect of the enriched media, in distinction to the 'natural media'

is to select certain species of the original fauna and favour their growth and multiplication. This laboratory demonstration parallels the selective effect of different habitats in nature and moreover the species which flourish in laboratory infusions and enriched cultures are the same species which are to be found in selective habitats, such as sewage filters, activated sludge, or polluted streams in nature (Noland, 1925a; Lackey, 1938b).

It may therefore be concluded not only that the non-selective or natural media are most satisfactory as an indication of the soil fauna but that the effect of the enriched media is similar to the effect in nature when enriched conditions arise in the soil.

Table 5

Growth of Protozoa in Different Media

Protozoan Species	Peat	Soil	Yeast G1.Pep.Y.	
<u>Biomyxa vagans</u>	X**	X		
<u>Mayorella vespertilio</u>	X	X	X	
<u>Trichamoeba sp.</u>	X	X		
<u>Spathidium sp.</u>		X		
<u>Enchelys sp.</u>	X			
<u>Chilodonella sp.</u>		X		
<u>Trichopelma sphagnetorium</u>	X***	X***	X**	X
<u>Colpoda steinii</u>	X**	X**	X***	X****
<u>Colpoda cucullus</u>	X	X	X	
<u>Colpoda inflata</u>	X	X	X***	X
<u>Colpidium sp.</u>	X	X		
<u>Cyrtolophosis mucicola</u>		X		
<u>Cyclidium glaucema</u>	X	X		
<u>Halteria grandinella</u>	X	X		
<u>Gonostomum affine</u>	X		X	
<u>Oxytricha setigera</u>	X			
<u>Oxytricha pellionella</u>	X	X		
<u>Holosticha sp.</u>	X***	X	X	

?
Sewage
etc

11. Growth at Different Depths

Introduction

It has been found with laboratory cultures that certain species are found only, or predominately, at the surface of the culture while others are found only, or predominately, at the bottom. Some from both top and bottom are found in the intervening space (Woodruff, 1942). It is of interest whether soil species show the same segregation as fresh water species.

Material and Methods

Peat extract medium was placed in three 4" x 1" tubes to a depth of three inches. The tubes were inoculated with protozoa from the beech litter culture previously used. Surface and bottom samples were pipetted off at regular intervals and the protozoan fauna recorded.

Results and Discussion

The results are given in Table 6. The chief distinction between the two faunas is the presence of the rhizopods in the bottom samples and their absence, except for one species, from the surface. This may be attributed largely to their amoeboid habit and their concentration on a solid substrate. Of the ciliates, eleven species are common to both faunas, one species was found only in the surface fauna and seven only in the bottom. The single species peculiar to the surface, viz. Blepharisma steini, has been recorded chiefly from litters and may prefer the better aeration such conditions offer. This species is sometimes pigmented and Giese (1938) has shown that the pigment is bleached by white light. The segregation of seven species to the bottom

of the cultures may be attributed partly to their habit of creeping over surfaces (Picken, 1937) or in the case of Vorticella to their attachment to the substrate. They may also be associated with the debris at the bottom of the tube which afforded a source of food.

Table 6

Vertical Distribution of Protozoan Species in Test Tube Cultures

<u>Protozoan Species</u>	<u>Top</u>	<u>Bottom</u>
<u>Rhizopoda</u>		
<u>Biomyxa vagans</u>		X
<u>Mayorella vespertilio</u>		X
<u>Trichamoeba sp.</u>	X	X
<u>Raphidiophrys elegans</u>		X
<u>Trinema lineare</u>		X
<u>Euglypha denticulata</u>		X
<u>Pontigulasia compressa</u>		X
<u>Diffugia arcuata</u>		X
<u>Ciliata</u>		
<u>Enchelys sp.</u>		X
<u>Plagiopyla sp.</u>		X
<u>Trichopelma sphagnetorium</u>	X	X
<u>Paramecium sp.</u>	X	X
<u>Colpoda cucullus</u>	X	X
<u>Colpoda inflata</u>	X	X
<u>Colpoda steinii</u>		X
<u>Cyrtolophosis mucicola</u>	X	X
<u>Cyclidium glaucoma</u>	X	X
<u>Bryometopus pseudochilodon</u>	X	X
<u>Blepharisma steini</u>	X	
<u>Phacodinium metchnikoffi</u>	X	X
<u>Halteria grandinella</u>	X	X
<u>Oxytricha setigera</u>		X
<u>Oxytricha pellionella</u>	X	X
<u>Onychodromus grandis?</u>		X
<u>Holosticha sp.</u>	X	X
<u>Gonostomum affine</u>		X
<u>Vorticella striata</u>		X

111. Growth in Media of Different pH.

Introduction

Ecological surveys such as those of Noland (1925a) and Wang (1928) of fresh water and Sandon (1927) and Fantham et al. (1922-1931) of soil have suggested that pH limits the distribution of certain species. Darby (1929) has attributed the greatest significance to changes in pH in explaining the protozoan sequence of ponds. Noland pointed out that calcium and magnesium carbonates were natural buffers preventing the development of too acid pH in many waters. High alkalinity was the result of photosynthetic activity which reduces the carbon dioxide content of the water, the chief determinant of pH, and in particular removes the bicarbonates of calcium and magnesium leaving the more alkaline carbonates. Noland did not consider pH to be a limiting factor within these conditions, the pH of his samples varying from 6.0 to 9.8. Wang suggested that certain species, including Coleps hirtus, Lionotus fasciola, Cinetochilum margaritaceum, Halteria grandinella and Aspidisca costata, tolerated the complete range of pH in his pond (viz. 6.2 to 7.05), while certain species were restricted to a narrow range and other species did not occur in the more acid water.

Cutler and Crump (1935) using glucose filters found that certain species, viz. Cinetochilum margaritaceum, did not occur below pH 5.7 and others, viz. Pleuronema chrysalis, increased in numbers above pH 6.0. The other ciliates, Colpidium colpoda and Paramecium putrinum also reached their maxima between 6.0 and 7.0 Arcella vulgaris on the other hand preferred a strongly acid reaction below pH 6.0.

Sandon concluded that pH was not a factor limiting the distribution of flagellates, amoebae and ciliates in soil but that testaceans were limited to acid soils. He considered that Pantham's data supported this conclusion. Sandon's conclusions are generally supported by the New Zealand survey and in particular the restriction of most testacean species to an acidity greater than pH 5.7 is confirmed.

Other evidence on the effect of pH comes from pure cultures. These generally show that most common fresh water species will grow over a relatively wide range of pH but that they have an optimum range and often distinct maxima separated by considerable pH differences (Loefer, 1938; Darby, 1929). Some species have a very restricted range (Jenkin, 1927; Saunders, 1924). Watson (1944) working with Balantiophorus minutus, a coprophilic species sometimes recorded from soil, found that it had a range from 4.65 to 9.25 with maxima at 7.0 and 8.7 and minima at 5.0 and 8.0 which Watson attributed to increased viscosity of mucus at these values. Hoare (1927) found the range of three coprophilic ciliates to be very similar. Nasir, quoted by Sandon and Cutler and Crump, found the pH range of C. cucullus to be 3.5 to 9.75 and Mores (quoted by Kudo, 1947) found that the same species had a range 5.5 to 9.5 with maxima at 6.5 and 7.5.

Collett (1919, 1921) showed that it was not only the pH but also the type of ion, particularly the type of anion, which is important in determining the toxicity of an acid solution to ciliates. This may often explain conflicting reports of the pH tolerance of the same species.

Soil Conditions

Records of the pH of soils are fairly common and Sandon and Fantham et al. both give the pH of the soils they examined.

In New Zealand, of the soils sampled, there was a marked distinction between organic and mineral soils. Litters were generally very acid with pH about 4.0 or less. However a puriri litter had a pH of 6.7 and while some beech litters were very acid, others were less acid (pH 5.7 and 6.0). It was only the very acid litters (5.2 or less) that had large testacean populations. It is not only the litter but the pH which limits the distribution of this fauna.

Most New Zealand soils under humid or sub-humid conditions are markedly acid (pH 5.0 to 6.0). Soils under semi-arid conditions are weakly acid or alkaline (pH 6.5 to 7.5) as are recent soils including the soils of the Pacific Islands (Raoul, Niue and Samoa). Exdeptional soils include those of White Island where very great acidity is due to the presence of sulphuric acid.

Although pH is a limiting factor for testaceans it does not appear to affect the total ciliate population for the highest ciliate populations have been recorded from Pacific Island soils which are generally weakly alkaline and beech litters which have a pH below 4.0. The effect on individual species however may prove to be different. Cinetochilum margaritaceum has not so far been recorded from acid soils, which is to be expected if the findings of Cutler and Crump are considered. Fantham and Robertson (1928) record Lacrymaria olor from soils of pH 4.8 and

5.2 which is surprising as both Darby (1929) and Noland (1925) record this species from alkaline water (pH 7.2 to 7.5).

The direct effect of soil pH is probably less than its indirect effects. pH has an important effect on the solubility of various ions and the pH of the soil will therefore determine whether or not these ions will pass into the soil solution or remain precipitated or bound in the soil. Ions involved include iron and manganese, and phosphate release is also affected by pH. Such ions may not always be of direct importance to the protozoa but they are important for plant and bacterial growth and therefore largely affect the tempo of biological activity in the soil. However extreme values of acidity and alkalinity will directly affect protozoan protoplasm.

It is important to realize that the pH measurements given are an indication only of the general pH level of the soil and that microscopically there is considerable local variation. So Kubiena (1938) found the pH range of a soil recorded by the conventional macromethod as 7.5 to vary between 6.3 and 8.4. The pH of microscopic dimensions is particularly influenced by accumulation of salts or by lime or fertilizer particles. Great changes may also be produced by the activity of filamentous fungi, of bacteria or at plant roots. So Sekera, quoted by Kubiena, found that in a soil of pH 6.0, the pH near root hairs ranged from 4.9 to 5.8 due to the evolution of carbon dioxide. Naturally the variation in any particular soil will depend upon its buffering capacity but it is obvious that in the microscopic habitats in which microorganisms, including protozoa, are active,

the pH may vary considerably, and generally on the acid side from the general soil pH.

Material and Methods

Culture fluid of known salt concentration was prepared. The salts used were: NaCl, 0.4g; KCl, 0.016g; CaCl₂, 0.024g; distilled water 4,000 ml. Buffer was added (0.2% KH₂PO₄) and the pH adjusted with KOH to pH 4.4, 5.2, 6.4 and 7.4. Non-nutrient agar in Syracuse watch glasses was used as a base for these sub-cultures and inoculations of bacteria from glucose-peptone slopes were used as food.

The sub-cultures were inoculated with ciliates from a thriving beech litter culture which was strongly acid (pH 4.0). From these sub-cultures further sub-cultures were prepared using the above salt and phosphate medium adjusted to pH 4.4 in equal proportion with peat extract.

Results

Table 7 shows the growth of the different species in the initial sub-cultures. This indicates that although more species grew in the sub-culture nearest in pH to the original beech culture, most of the species also grew at pH 6.4. At pH 7.4 the five species present included four of the most common soil species, viz. Trichopelma and the three Colpoda species, and the fifth a fairly common species was especially numerous in the inoculum. These results indicate that more species were encountered in the acid than in the alkaline cultures and that most species were found in the sub-culture nearest in pH to that

of the original culture. The most common soil species however appear unaffected by pH changes.

Table 7

Growth of Protozoa in Sub-Cultures of Different pH

<u>Species</u>	<u>4.4</u>	<u>5.2</u>	<u>6.4</u>	<u>7.4</u>
<u>Biomyxa vagans</u>	x	x	x	
<u>Mayorella vespertilio</u>	x	x	x	
<u>Trichopelma sphagnetorium</u>	x	x	x	x
<u>Colpoda cucullus</u>	x	x	x	x
<u>C. inflata</u>	x			x
<u>C. steinii</u>	x	x		x
<u>Colpidium sp.</u>	x	x	x	
<u>Gyrtolophosis mucicola</u>	x			
<u>Oxytricha setigera</u>			x	
<u>O. pellionella</u>	x			
<u>Holosticha sp.</u>	x		x	x
<u>Blepharisma steini</u>	x			
<u>Phacodinium metschnikoffi</u>	x		x	

In the further sub-cultures at pH 4.4, Phacodinium metschnikoffi, Bryometopus pseudoehilon, Blepharisma steini and Holosticha sp. all grew well. In the case of Bryometopus this was particularly interesting as active ciliates were not recorded in the original sub-culture and the ciliate must therefore have excysted in response to some excystment factor.

Discussion

The survey of the New Zealand soil protozoa demonstrated quite clearly that pH was not a limiting factor for the ciliate fauna within the range sampled. The only exceptions to this may have been the extremely acid soils of White Island but even there protozoa were recovered from samples taken from the bare surface adjacent to a gannetry and to a sulphur fumarole. The

protozoa in these cultures may have been cysts distributed from other parts of the island but 14 ciliates found in one sample under Pohutakawa where the acidity of the soil culture was 4.2 and of the initial sample less shows the extreme range tolerated by the soil fauna.

Certainly there are no comparable records of the common fresh water species occurring under such acid conditions either in nature or in laboratory culture. The range given by Lackey (1938b) for Coleps birtus (5.5 to 7.4) is typical of many fresh water species and even bacteria-feeders such as Paramecium and Colpidium do not tolerate extreme acidity. It is noticeable that two of the species with the most acid range quoted by Lackey, viz. Cyclidium glaucoma (2.9 to 8.0) and Halteria grandinella (4.2 to 7.5) are both common soil species.

The litter ciliates used in the present experiments grew better at pH 4.4 than in less acid media. It may be that their optima lie within the strongly acid range and the optima of typical fresh water species (e.g. Paramecium bursaria or Coleps birtus) within the mildly acid (pH 5.0 to 7.0) or mildly alkaline range. Darby has shown that this is the case both for Paramecium and Stylonychia pustulata.

However there are two points to be remembered. First, it is not so much the pH but the specific ion concentration which is important. This explains the sometimes conflicting results e.g. with Paramecium (Darby (1929) and Wichterman (1948)) in which factors other than pH affect the growth and activity of the ciliates. Secondly, pH differences will not explain the

distinction between the soil and fresh water fauna since the soil fauna of weak acid or weakly alkaline soils resembles the fauna of the more acid soils and not the fauna of fresh water.

It is not only the tolerance of acid or alkaline conditions which is important in the distribution of protozoa. The reaction of protozoa to adverse conditions is also important. Darby (1929) has shown that if the pH falls below or rises above a certain critical level Stylonychia pustulata encysts, provided there are sufficient numbers and that it excyst again when favourable pH conditions arise. Cyst physiology is not however so well developed in other ciliates. From laboratory and field evidence it seems that Paramecium and Spirostomum migrate to the surface or the bottom to find the most favourable pH conditions. For these ciliates encystment is not commonly recorded or is not recorded at all. Their survival then is dependent upon a habitat sufficiently diverse to preserve in some section favourable conditions. This is probably the case for most typical fresh water species in which encystment has not been recorded or is very uncommon. In the present experiments Paramecium died out in the shallow culture dishes as the cultural conditions changed. There was no encystment as there is with Colpoda.

B. AN EXAMINATION OF THE RELATION BETWEEN
BACTERIA AND CILIATES

Introduction

Many free-living ciliates and most soil ciliates are bacteria-feeders. The nature of the bacterial flora is therefore of great importance in their ecology. Two important factors are the edibility of the bacteria and the ciliates' power of selection. Work on these problems up to 1932 is reviewed by Sandon (1932). There is also much information in Kahl (1930-1935). Only the more recent work will therefore be reviewed here.

Leslie (1940) working with Paramecium and using carefully standardised conditions found that not only the strain of bacterium but also the age of the bacterial inoculum was important. He found Pseudomonas fluorescens suitable between the ages of 4 and 30 days. If taken from a twenty four hour old slant this bacterium is unsuitable. Judged by the division rate of the ciliate (Paramecium multimicronucleata) and by its survival in different suspensions there are some bacteria which maintain a high fission rate over a long period, others a low fission rate, and others cause the premature death of the ciliate. Leslie found, as did Hetherington (1933), that different strains varied as much as different species in suitability. He also found the initial concentration of the bacteria to be important, and that too little or too great a concentration were unfavourable to ciliate growth. Bragg (1936) examined the selection of food by Paramecium trichium.

Burbanck (1942, 1946) worked with Colpidium colpoda. He obtained growth on 27 out of 31 strains of bacteria tested. He found the Gram-negative Enterobacteriaceae to support the highest division rates and the Bacillaceae the lowest. He also found however that different species of the same genus gave different results. In a later paper (1946) he suggested that because different bacterial strains supported different division rates this fact could be used to check the identification of bacterial species. An important point noted by Burbanck was that the division rate of the ciliate grown on a particular bacterial strain was determined partly by the culture medium in which it had been previously kept. Colpidium taken from Pseudomonas fluorescens and Aerobacter aerogenes cultures gave significantly different division and survival rates when grown on the same bacterial strain. Hetherington (1933), whose findings are similar to those of Leslie and Burbanck, found that the fission rate differed with the same bacterial strain grown in broth and suspended in salt solution.

Kidder and Stuart (1939a,b) found that with Colpoda steinii different bacterial strains gave significantly different fission rates. Their results were similar to those of Burbanck except that they did not obtain good growth with Pseudomonas. This bacterium gave good growth with Taylor and Strickland (1938) and it seems possible that Kidder and Stuart were not using an aged culture which Leslie has shown to be important. The results of Singh (1941) generally confirm those of Kidder and Stuart and Singh made the interesting observation that the ciliates differed

from the amoebae in their bacterial preference.

Chromagenic bacteria had been found by Chatton and Chatton (1927a,b) to be unsuitable as food for ciliates and caused cytolysis in Glaucoma scintillans, Colpidium colpoda, C. steini, Colpoda cucullus, Paramecium caudatum and Glaucoma (syn. Tetrahymena) pyriformis. They thought that the cytolytic effect was not due to the pigment itself but to a combination of a glucoside and a lipoid. Kidder and Stuart confirmed that pigmented strains of both Pseudomonas and Serratia were toxic both to active and encysted Colpoda. They showed that this toxicity was entirely due to the pigment which diffused through the cyst membrane. They also found that the ciliate could become adapted to Serratia but not Pseudomonas. Earlier Philpott (1928) had shown that Paramecium could become adapted to one strain of toxic bacteria, B. pyocyaneus, but not to another, B. enteritidis.

Hetherington found that Glaucoma scintillans and Colpidium campylum grew well on E. coli but poorly on B. subtilis whereas E. coli was toxic to C. colpoda which grew well on B. subtilis. Both E. coli and Ps. fluorescens and their metabolites were toxic to Stentor coeruleus. Chatton and Chatton (1925a, b, c) found that a certain strain of E. coli caused abnormalities in Colpidium campylum. These included chain formation. Sonneborn (1932) recorded the same effects with Micrococcus and Colpidium campylum. Chatton and Chatton also found that the different bacterial strains affected conjugation in Glaucoma and Colpidium and that the latter was particularly affected by E. coli.

In a culture of mixed bacteria the ciliates will select only

the edible strains and leave the inedible strains which will consequently increase in numbers. This was found to be the case by Kidder and Stuart (1939a). It also explains why Phillips (1922) found that mixed bacterial strains gave better results than single strains for there is a better chance of edible strains being present in a mixed culture. If an edible strain is supplied however best growth is obtained with just a single strain (Leslie, 1940). Singh (1941) found that in the presence of an inedible strain Colpoda encysted as it would have done in a non-nutrient medium.

Protozoa may effect a marked and rapid reduction of bacterial numbers. Hoffman (1905) and Huntermuller (1905) reported protozoa feeding on typhoid bacteria. In activated sludge and other places where bacterial digestion is taking place they assist by constantly feeding on the bacteria and so maintaining a young and vigorous bacterial culture, by aerating and mixing the suspension by the constant beat of their cilia, and by flocculating the particles, partly by the secretion of mucus (Barker, 1946; Watson, 1946c). An important role attributed to bacteria by Purdy and Butterfield (1918) and Butterfield (1929) is the concentration into bulk of dissolved substances. The same function can be attributed to the bacteria-feeding protozoa and this intermediate role in the food chain is stressed by Bond (1933) and Lucas (1947). The larger zooplankton feed on this microplankton and thereby indirectly derive their energy from dissolved and bacterial substances. In the soil the protozoa must play a similar role to that of the sewage and planktonic faunas and many sewage species

also occur in the soil. The soil protozoa are confined to capillary moisture where usually bacterial rather than fungal degradation takes place. They are primarily bacteria-feeders with few carnivores. It may be for this reason that Coleps hirtus which is not bacteriophagous is not found in the soil whereas bacteriophagous species such as Halteria grandinella are found quite often.

Not only do the protozoa select the bacteria, the bacterial flora also has a selective effect on the protozoan fauna (Crozier, 1923). Since ciliate species differ not only in the tolerance of different bacterial strains but also in tolerance of different stages of bacterial decomposition the fauna will change with the flora. This has been demonstrated by Hetherington (1933) in the laboratory and observed in nature by Gray (1951, 1952).

Soil Conditions

One of the earliest findings at Rothamsted was the fluctuation of bacterial numbers and the correlation of protozoan numbers with this change in bacterial numbers. For this reason Russell thought that the protozoa were an inhibitory factor in soil microbiology. Later it became apparent that bacterial numbers and the composition of the bacterial flora were dependent more upon other soil conditions than the depredations of the protozoan fauna.

The bacterial flora of the soil is determined by the substrate, by the moisture content and by the aeration of the soil. In the presence of oxidizable sulphur under aerobic conditions sulphur oxidising organisms, Thiobacillus, will develop. Under reducing

conditions sulphate-reducing bacteria will be active (Desulfovibrio). In the presence of cellulose, cellulose decomposing bacteria will be present while sugars and proteins will stimulate other sections of the soil flora. Under aerobic conditions Azotobacter will fix nitrogen and under anaerobic conditions Clostridium. Also under anaerobic conditions nitrates are reduced to nitrogen or ammonia while normally the reverse process occurs under aerobic conditions.

Of the different groups of bacteria in soil which are connected with direct decomposition B.megatherium, B.mycoides and B.subtilis form one group and Ps.fluorescens another. The former according to Greig-Smith (1912) is associated with sick soils and the latter with healthy soils. It is interesting to note in this respect that of the strains tested Pseudomonas is generally more edible than the subtilis group which agrees with the greater protozoan activity in healthy soils.

i. Growth of Mixed Fauna in Bacterial Suspensions

Material and Methods

A sample taken from a drain overhung by willows and with little or no photosynthetic flora was found to contain principally two ciliates, viz. Paramecium caudatum and Euplates aediculatus. Vorticella striata and Lembadion bullinum occurred in small numbers but no other ciliates species were observed. In order to secure a mass culture of Paramecium some of the material was pipetted into Syracuse watch glasses and several loops of bacteria added. The bacterial strain used was a Proteus sp.

Results

After twenty four hours there was little change in the fauna

EXPERIMENTAL CULTURE SEQUENCE

NUMBERS

DAYS

although the Euplotes were toxic and fewer in number. After forty-eight hours the Paramecium were present in very great numbers, massed together and dividing rapidly. Chilodonella dentata, Cyclidium glaucoma, and Oxytricha pellionella were also present in large numbers. Euplotes had died out completely and V.striata, though still present, showed no change in numbers although the individuals had increased in size. After ninety-six hours the culture, previously turbid with bacteria, had cleared. There were many dead but no living Paramecium. Chilodonella and Cyclidium had disappeared. Oxytricha was still present in large numbers and a few V.striata were dividing. Algae, both green and yellow brown, were increasing in numbers. Subsequently the ciliates disappeared entirely from the culture which was filled with algae. The diagram overpage shows the change in the fauna following the addition of heavy suspensions of bacteria. Three stages may be distinguished. An initial stage when the fauna is unchanged and which ends with the death of the sensitive species, Lembadion bullinum and Euplotes aediculatus. A period of rapid growth of Paramecium caudatum, Cyclidium glaucoma, Chilodonella dentata and Oxytricha pellionella. This period ends with the clearing of the culture as the bacteria are wholly consumed. Finally the ciliates disappear, after the protracted growth of Vorticella striata, and algae dominate the culture.

Discussion and Conclusions

Although Paramecium caudatum is often stated to be widespread in nature (Kudo, 1947), this assertion is misleading. Bary (1950a) in his survey of typical fresh water ponds and pools recorded the species only once and then in small numbers. The present sample and the sample from an oxidation pond (in which E. aediculatus was also present) are the only two occasions on which it has been found by the present author. In this latter case it occurred in very great numbers, comparable to the conditions of the present experiment. It has also been recorded in very great numbers by Green (1925) in an open sewer and by Hassall (1850, 1855) in the Thames near sewer mouths. There is little doubt then that the species is an indicator of pollution and that it is found in considerable numbers only where there is great bacterial activity (Lackey, 1938b).

The most interesting record of Paramecium under natural conditions is that of Gray (1951, 1952) who found P. caudatum and other species present in very large numbers in a polluted stream and occurring whenever the stream became turbid after rains and organic and faecal matter were washed into it. When these conditions disappeared Paramecium disappeared also. This is the condition simulated by the present culture: the death of the non-pollution species (Euplotes and Lembadion), the growth of pollution species (Cyclidium, Oxytricha, Chilodonella and Paramecium), and the disappearance of the ciliates when the culture clears. Under natural conditions it is very probable that Paramecium would survive in small numbers at the

bottom of the stream where conditions are closer to its favoured habitat and not die as in the present experiments. Normally the end of a hay infusion culture is marked by the presence of the ciliates at the bottom of the culture (Jones, 1930), and Green found them in greatest numbers at the bottom of the sewer. Lindemann (1942) and Lauterborn (1916) amongst others have recorded P. caudatum from bottom, though not mud, samples and Lindemann has shown that they survive for a long period even under anaerobic conditions if the temperature is low.

This experiment is of interest to soil protozoology since two of the pollution species, viz. Cyclidium glaucoma and Oxytricha pellionella are common soil species. V. striata is also common in soils and the experiment shows, that the reaction of different soil species to bacterial suspensions is not the same, for V. striata, unlike the other two species, and unlike V. microstoma, does not thrive under such conditions although it does not succumb to them. The experiment shows: first, the selective effect of concentrated bacterial suspensions on the protozoan fauna, and secondly, the dependence of certain species upon such bacterial concentrations.

ii. Growth and Encystment of Vorticella microstoma in Bacterial Suspensions.

Material and Methods

Vorticella microstoma was obtained both from an anaerobic digester and an oxidation pond set up by the Auckland Metropolitan Drainage Board as an experimental plant to handle trade meat wastes.

The digester which was maintained at 80°F. was fed regularly with meat waste. The appearance of the raw meat waste was greenish-brown, opalescent, with much settleable solids and fat in small lumps. It had a typically meaty odour, like stale soup, and the pH was 7.5 to 12. After two days digestion at 80°F. the waste was light to deep amber in colour, clear and bright; initially becoming turbid on standing and depositing solids with colloidal sulphur. It had a peculiar amine smell and some sulphides were present. The pH was 7.1 to 7.7. After ten days retention in the oxidation pond the liquor was deep green somewhat opalescent with few stringy solids but some colloids. There was free ammonia at times but never any sulphides. The pH was 8.0 to 9.5. The Biochemical Oxygen Demand fell during digestion from 1553 in the raw waste, to 134 in the digester, to 53.2 in the oxidation pond. (B.O.D. in 5 days at 65°F.). During this digestion gas was evolved at a rate of 219,000 cubic feet per million gallons treated and at 37.17 cubic feet per pound of dry matter destroyed. The most important chemical transformations are the conversion of organic sulphur bodies to sulphides and the oxidation of sulphides in the oxidation pond (complete removal in less than half an hour); and the conversion of protein nitrogen to ammoniacal nitrogen with the release of ammonia in the oxidation pond following pH rise.

Biologically the raw wastes are virtually inactive, a few flagellates occasionally appearing. In the digester there are many active ciliates and flagellates and chains of Cyanophyceae are formed on the surface of the liquor after shaking. Some

Vibrios and Chlamydomonas have been identified. In the oxidation pond there is a dense population of Chlorella and many Chlamydomonas. In sunny weather particularly, Paramecium occurs in very great numbers. The full ciliate fauna recorded from the digester and the oxidation pond is given in Table 8.

Material from the digester was sub-cultured onto agar plates and into Syracuse watch glasses. The principal ciliate species, and often the only species to develop was V.microstoma. It was observed however that trophic ciliates were never recovered on direct examination of the digester fluid. Large cysts were present which readily excysted on the slide or in the sub-culture and the excysted ciliates were of the large or 'hians' form. Normally encystment of Vorticella microstoma takes place on the exhaustion of food. The occurrence of many large cysts in the digester appeared contradictory to the usual experience with this ciliate, and an attempt was therefore made to induce experimentally the formation of these large cysts.

When V.microstoma was present by itself or with some of the smaller ciliates (Enchelys, Colpoda) the growth of the ciliates was normal but in some cultures there was also present the Prorodon species described earlier. When this ciliate was present the vorticellids did not increase and occasionally were reduced to quite small numbers. This was due to the predatory behaviour of Prorodon, a genus previously recorded as principally vegetarian (Sandon, 1932). The behaviour and life history of this ciliate have been previously described. This ciliate was not found on direct examination of the digester liquor but only

in the sub-cultures. The only ciliate which definitely appeared to be trophic in the digester liquor and which was not obtained in sub-culture was Metopus es.

Table 8

Ciliate Fauna of Anaerobic Digester and Oxidation Pond

<u>Species</u>	<u>Digester</u>	<u>Oxidation Pond</u>
<u>Prorodon sp.</u>	x	
<u>Enchelys sp.</u>	x	
<u>Lionotus sp.</u>		x
<u>Chilodonella sp.</u>		x
<u>Paramecium caudatum</u>		x
<u>Cyclidium glaucoma</u>		x
<u>Lemmus pusillus</u>	x	
<u>Metopus es.</u>	x	
<u>Oxytricha pellionella</u>	x	x
<u>Kahlia acrobates</u>	x	
<u>Euplotes aediculatus</u>		x
<u>Aspidisca lynceus</u>		x
<u>Vorticella microstoma</u>	x	
<u>Podophrya fixa</u>	x	x

Experiments and Results

Loops of bacteria (Proteus sp.) were added to sub-cultures of Vorticella. The addition of one or two loops of bacteria to a Syracuse watch glass containing the ciliates resulted in their excystment within an hour or two if they were encysted, and the development of intense trophic activity. At room temperature the ciliates divided 6 times in twenty four hours the total population thereby increasing very rapidly. This rapid division continued until the bacteria were wholly consumed. The ciliates would then mostly encyst. The addition of fresh bacteria would bring about repetition of this cycle. In these cases when only a loop or two of bacteria were added and the culture left the cycle would

take about 48 hours and the ciliates would first increase in size in high bacterial concentrations to the 'hians' type and then with decreasing bacterial concentrations decrease in size to the 'microstoma' form. Encystment was therefore of the small form and small cysts were produced. This is similar to the history of the large ciliates which excysted from the digester liquor and which without the addition of bacterial divided and decreased in size to the 'microstoma' form. In all these cases then the vorticellid passed through a 'hians' stage in high bacterial concentrations but on the disappearance of the bacteria became a 'microstoma' before encysting. The conditions of the digester liquor were not reproduced, i.e. encystment of the 'hians' form.

Instead of adding a few loops of bacteria and waiting for the sub-culture to clear in the next forty eight hours many loops of bacteria were added and before these were wholly consumed and while the sub-culture was still turbid more loops were added until the sub-culture became very turbid with unconsumed bacteria. In this case it was observed that large cysts were formed and despite the heavy concentration of edible bacteria no trophic forms were found. If however a drop or two of this thick sub-culture containing the 'hians' cysts were placed on a slide or in a Syracuse watch glass, these large cysts excysted spontaneously in exactly the same manner as the cysts from the digester and the excysted ciliates began feeding and dividing. The conditions of the digester had therefore been experimentally reproduced.

Discussion and Conclusions

These experiments show that Vorticella microstoma is an

avid feeder on bacteria and that under certain conditions it will clarify bacterial suspensions in a very short time in the same manner as Paramecium caudatum or Colpoda steinii. They also show that the swollen 'hians' form which Faure-Fremiet (1905) thought was due to anaerobic conditions is really due to the rich bacterial diet. They also indicate that concentrated vorticellids normally encyst on depletion of the food supply.

The most interesting problem however is the apparent inactivity of the vorticellids in the digester liquor. The reactions of V. microstoma to carbon dioxide and to lack of oxygen are described in a later section. It is shown that the complete absence of oxygen leads to telotroch formation in the trophic ciliate and that the telotroch will not settle and feed unless oxygen is present. It is obvious then that under anaerobic conditions the ciliate will not be trophic in the digester. But the digester fluid is strictly anaerobic and is maintained under strictly anaerobic conditions. The only possible explanation of the presence in large numbers of Vorticella is that for a short time after the introduction of the raw waste there is sufficient oxygen in the digester to permit the growth of the ciliates. As the ciliates can divide very rapidly a short interval of such conditions would be sufficient to produce the large numbers found in the liquor.

However there still remains the problem that large cysts are found in the digester and no telotrochs. Normally encystment takes place only on the exhaustion of the food supply, but these large and unstable cysts must be formed under different conditions.

Now the experiment shows that massed bacteria will induce the formation of these large cysts and aeration will induce excystment. It is for this reason that Brand (1923) believes that oxygen lack causes encystment. This is incorrect as is shown later, yet adequate aeration will prevent formation of the large cysts. Encystment in this case must be due either to the accumulation of bacterial toxins or to a low redox potential. The two may be identical and the low redox potential may be due to the production of reduced bacterial metabolites. Aeration achieved by increasing the surface area of the liquor will permit oxidation of these metabolites and consequently a rise in the potential. This is what happens in the oxidation pond where the process is greatly helped by the green Chlorella. The potential of the digester was -290mv. whereas the potential of the oxidation ponds was c.200mv, i.e. there was an intensely reducing potential in the digester and of course a high B.O.D. compared with the oxidation pond or the sub-cultures. It does not seem unreasonable then to attribute cyst formation to the accumulation of bacterial metabolites associated with a low redox potential and excystment with oxidation of the metabolites and raised redox potential. This hypothesis would explain why the vorticellids were found encysted in the digester, for admitting the initial presence of a trace of oxygen the ciliates would first grow and divide rapidly but a bacterial activity became intense or a different flora develops and the Eh falls to a negative potential (Hewitt, 1936) the vorticellids would encyst before the oxygen was exhausted and would not therefore form telotrochs. Then as long

as these reducing anaerobic conditions obtained the ciliate will remain encysted, but on aeration perhaps by placing in an aerobic sub-culture excystment and division will take place and the normal, 'aerobic' cycle completed. The marked difference of Eh between aerated and unaerated bacterial cultures shown by Hewitt (1936) is sufficient to explain why without any other change the transference of a drop of encysted vorticellids from a thick bacterial suspension to a slide or dish will bring about sufficient change to induce encystment.

C. AN EXAMINATION OF THE CULTURE SEQUENCE
AND OF THE EFFECT OF STEAM TREATMENT

Historical

Although the successive changes in laboratory infusions have been known since the time of van Leewenhoek, detailed study of these changes was not made until the present century. Peters, Woodruff, Fine, Bodine, Prathi, Jones and Darby have since made extensive study of various cultural factors. Their work has been done principally on the typical laboratory hay infusion and their results therefore can be easily correlated. This is important since these authors generally recorded only one or two of the changing factors of the laboratory culture. More recently Hetherington has studied the change in bacterial flora and protozoan fauna, a change studied under natural conditions by Gray (1951, 1952).

Laboratory infusions, like natural ponds, fall into two classes: buffered and unbuffered, and these two classes are characterised both by different culture histories and by

different protozoan faunas. Ordinarily the hay infusion is of the unbuffered kind and its history falls into three periods. The first is characterised by a sudden drop in pH, Eh, and alkaline reserve as the culture becomes turbid with bacterial activity. The pH of the culture which is initially near neutrality may fall as low as 5.0 or even lower (Bodine, 1921; Pruthi, 1927, Fine 1912; Jones, 1930; Darby, 1929; Hetherington, 1933). The Eh falls and a negative potential, as in bacterial cultures, may develop (Efimoff, Nekrassow, and Efimoff, 1928; Hewitt, 1936). Concomitant with a marked increase in the carbon dioxide concentration the alkaline reserve falls (Pruthi, 1927). The bacterial flora consists of strains breaking down the readily available carbon sources and multiplying throughout the culture very rapidly producing concentrated toxic metabolites and making the culture cloudy (Hetherington, 1933). The protozoan fauna of this period consists only of those forms capable of tolerating the acid, reducing conditions and the bacterial flora associated with it. Glaucoma scintillans and Colpidium colpoda are two such species recorded by Hetherington and we must include Colpoda steinii. Jones found that while Paramecium would survive the acid conditions of this early period provided it was not too prolonged and provide the acidity did not fall much below pH 5.0, it did not multiply until the pH was more favourable (i.e. above 6.0). When the most readily available substances have been utilized the first bacterial flora is succeeded by a second flora using less readily available substances and the decomposition products of the first flora. Unlike the initial

decomposition this period is fairly stable and may persist for some time. The metabolites are less toxic for protozoa and the culture less acid. The culture is still cloudy with bacteria however, still acid, and still with fairly high carbon dioxide concentration although the reduction potential has risen. The ciliate associated with this period in Hetherington's cultures is Colpidium colpoda. It is at this pH range that Paramecium also becomes active multiplying rapidly in the rich bacterial suspension. Oxytricha pellionella and Cyclidium glaucoma are other typical species (Section B).

The rapid growth of the protozoa clears the culture, consequently the bacteria in the second phase, which is associated with this clarification, are aggregated in clumps around less easily metabolised substances, such as cellulose, and form a scum at the surface which eventually sinks to the bottom. The pH in an unbuffered culture is still rising and the carbon dioxide tension falling. Hetherington found Stentor coeruleus in his cultures at this stage at a pH of 6.8 and above. There are naturally less toxic byproducts and the bacterial flora again changes. During this period of slowly decreasing acidity and increasing alkalinity the most common protozoa occur as the pH reaches a favourable range. As the alkalinity increases further however the species die or encyst (Darby) and finally at about pH 9.0 few if any ciliates are left.

The last stage is marked by great algal and diatom activity and consequently strongly oxidising and alkaline conditions. Provided the pH is not too high a ciliate fauna feeding on the

algae will develop (Gray 1951; Picken, 1937).

These conditions may be easily followed in the laboratory and occur also in nature. Gray has distinguished the three different stages in a stream subject to pollution. With the first bacterial flora (essentially coliform bacteria and Pseudomonas, typical soil forms) are associated the bacteria feeding ciliates, Colpoda, Colpidium and Paramecium; with the disappearance of this flora Stentor occurs in great numbers for a short time, to be succeeded by the diatom eating ciliates as the algae develop. This distinction of fauna has also been observed by Noland (1925) and Picken (1937).

Although the correlation between the physical, chemical and biological changes of the culture and the protozoan sequence have now been firmly established, the explanation of this association is still in some doubt. Of the factors first favoured as the primary determinant pH has had the greatest support. Pruthi found that Paramecium did not occur until the pH was alkaline, irrespective of the age of the culture. Jones found that the inoculum of Paramecium would survive but not grow in the early acid phase of the culture. The ciliates aggregated on the surface which Fine (1911) had shown to be less acid. In the second phase when the Paramecium grew and divided they were distributed throughout the culture and in the third phase when the culture becomes alkaline they aggregate at the bottom where it is least alkaline. Similarly Spirostomum has been shown to have a narrow pH range in which it grows and in water of different pH it is found to aggregate within its

favoured range, 7.3 to 7.5 (Saunders, 1924; Jenkin, 1927). Darby (1929) showed that the division rate of Paramecium was greatest in the range 6.0 to 8.0, although Wichterman (1948) obtained satisfactory growth at more acid pH with P.bursaria and P.polycarum. Generally however fresh water species have a relatively restricted pH range whereas polysaprobic species have a very wide range. Species with a narrow range, e.g. Spirostomum, will therefore occur in a protozoan sequence only when the pH is favourable. Other species, e.g. Stylonychia pustulata, which encyst when the pH falls or rises markedly and which only excyst within a narrow pH range, will also occur only within this range (Darby, 1929). Although pH may be important in some cases Darby admits that other factors may also be involved.

Of other factors the bacterial flora has already been discussed in section B. Carbon dioxide and oxygen tensions are also important but as these factors are more fully investigated in the next chapter they will be discussed then.

Oxidation-reduction potentials, while comparatively easy to measure, are difficult to evaluate in biological terms although their theoretical significance is obvious (Hewitt, 1936; Jahn, 1941; Needham, 1930; Thomas, 1942; Michaelis, 1949). Jahn (1933) studied the growth of Chilomonas in bacteria-free cultures in which definite potentials had been established by the addition of peroxide and sulfhydryl compounds. Although the results suggested that the growth rate of the protozoa was influenced by the potential the evidence was not conclusive and the results may have been due to the poisoning systems themselves. Comparable

experiments were carried out during the present researches using cysteine hydrochloride but the same inconclusive results were obtained and it was impossible to attribute the results to potential differences when differences in the chemical composition of the media necessarily varied so greatly. The other method of varying the potential is to vary the oxygen tension but as Jahn pointed out in 1941 this makes an experiment containing only one variable seemingly impossible to execute and the theory is not therefore amenable to experimental approach. A discussion of the significance of Eh in anaerobiosis will be given later.

1. Culture Sequence of Greywacke Samples

Material and Methods

Eighteen samples of Greywacke soils all from the same area but under different conditions of vegetation were cultured. A full description of these samples is given in Table 9.

Agar plates were poured in 5" petri dishes. A trace of sodium acid phosphate and sodium nitrate together with soil extract was added to the agar. A few grammes of soil was added and then about 20 ml. of distilled water. Two controls lacking the soil but otherwise similarly treated were also prepared. The cultures were kept at room temperatures.

The plates were examined regularly and the protozoan fauna listed. Using glucose ($\frac{1}{2}\%$)-peptone (2%)-agar (1%), a number of bacterial plates were prepared from the soil cultures and controls. The pH was read electrometrically using a Macbeth Continuous pH Meter and glass and calomel electrodes. The instrument was standardised before use with borate and tartrate buffers (pH 3.7

and 9.2 respectively). Eh was read with the same instrument using a bright platinum electrode and the calomel electrode. These were standardised with quinhydrone buffer. The electrodes were rinsed with distilled water between each reading.

Table 9

Description of Soils used in Culture Experiment

Area: Orari hills, Geraldine

Parent Material: Greywacke

Classification: Weakly weathered Yellow Brown Earth

Soil Type: Kaikoura

Sample	Horizon	Depth	Description	Vegetation
1.	C	0-3"	Pale brown fine sandy slit loam, crumbly and rooty.	Bare ground between burnt snow grass.
2.	B&C	0.3"	Fine granular grey brown silt loam, heavy silt loam	"
3.	A	0.3"	Soft granular blackish loam	<u>Danthonia, Festuca</u>
	B	8-11"	Crumbly pinkish brown silt loam	"
4.	A0	0-1"	Fine granular moist dark brown mor	<u>Celestia viscosa</u>
	A1	1-5"	Fluffy granular dark grey brown peaty loam	"
	A2	7-12"	Fine granular dark brown heavy loam	"
	B.C.	14-18"	On pale grey brown heavy silt loam	"

Soil Type: Tengawai

5.	C	0-3"	Powdery, light grey brown gritty sandy silt loam	Bare ground between <u>Festuca</u>
6.	A	0-4"	Soft granular dark brown grey gritty silt loam	Fescue, tussock & matagouri

Table 9 (Continued)

6.				
Sample	Horizon	Depth	Description	Vegetation
	AB	6-10"	Slightly lighter in colour and more rocky	Fescue, tussock & matagouri
	B.C.	11-13"	On pale brownish rocky gritty sandy silt loam	"
7.	A	0-3"	Fluffy dark brown grey fine sandy silt loam	<u>Danthonia</u> , grasses
	A.B.	4-10"	Darker brownish grey	"
	B.C.	12-15"	On pale yellow brown, rocky, gritty heavy silt loam	"

The Protozoan Fauna and the Faunal Patterns of the Greywacke Samples

The fauna of these samples is given in Table 10. Much the same species are found in all the samples and these are common soil species. Specialized species are absent. The only other sample examined from the weakly weathered yellow brown earth was a sample taken from the head of the Shag Valley at 1800 feet.

This was under tussock (Poa caespitosa) and the fauna was:

Mavorella vespertilio
Euglypha rotunda
Nuclearia sp.
Chaenea sp.
Colpoda steinii
Trichopelma sphagnetorum
Spathidium sp.
Oxytricha setigera
Gonostomum affine
Verticella microstoma

The pH of this sample, a dark greyish brown heavy silt loam, depth 0 to 2 inches, was 6.7. The fauna of this soil is obviously very similar to the fauna of the Geraldine samples.

1.C	2:7
2.B&C	-:6
3.A	3:5
B	1:4
4.AO	3:8
A1	5:9
A2	2:8
BC	1:1
5.C	-:8
6.A	5:9
AB	3:6
BC	1:6
7.A	2:6
AB	2:5
BC	-:5

Protozoan Fauna of Geraldine Soil Samples

[illegible]

These ratios illustrate a number of points. First, the effect of depth. The profiles sampled from different horizons show that the depth of 1 to 4 inches has the greatest number of protozoan species. It is interesting that the A₁ horizon of Sample 4 is richer in species than the A₀ horizon. With increasing depth the number of species diminishes but the rhizopods disappear more rapidly than the ciliates, e.g. Sample 6. Secondly, these samples show the close relation between rhizopod numbers and litter accumulation. Samples 1, 2, and 5 taken from bare ground with no plant cover have few or no rhizopods; Samples 4 (under a dense mass of Celestia) and Sample 6 (with fescue, tussock, Voaulia australis, Primelia sp., sweet vernal, catsear, and matagouri) each have a total of five species; Samples 3 and 7 are each under Danthonia and other herbs and grasses. These have an intermediate number. Thirdly, the ratio for these samples is very similar to that of the Shag Valley sample, i.e. 3:7, and is also very similar to the ratios of other Zonal soils. It is interesting to note that Soil Type is of no demonstrable significance, the Tengawai and Kaikoura samples being indistinguishable.

Results

The Protozoan Sequence

Table 11 gives the protozoa identified in the eighteen Greywacke samples on different days after culture. The Table shows:-

First, that there is a time lag in the development of the fauna as only 4 species were recorded in the first week.

Secondly, that the ciliate fauna develop more rapidly than the Rhizopod fauna which was not fully present until three months after the culture was established, whereas by this time the ciliate fauna was reduced to one species.

Thirdly, that the ciliate fauna, which was active chiefly from the eighth to the thirty second day of culture, changed slightly in composition and could be classified into three groups; viz.

- (a) An early group which includes Colpoda inflata and C. cucullus;
- (b) A later group which included Spathidium, Holosticha and Vorticella;
- (c) A group present throughout the whole 'ciliate period', which includes Enchelys, Chilodonella, Plagiopyla, Trichopelma, Colpidium, Homalogastra, Cyclidium, Pseudoglaucoma, Bryometopus, ^{Uroleptus} ~~Epielintes~~ and Gonostomum.

Table 11

Protozoan Sequence: List of Species and Their Occurrence

Days after culture	2	4	8	10	12	15	18	22	32	52	143
<u>Mayorella vespertilio</u>	x	x	x	x	x	x	x	x	x		x
<u>Trichamoeba sp.</u>							x	x		x	
<u>Thecamoeba sp.</u>											x
<u>Cryptodifflugia oviformis</u>									x		x
<u>Difflugia arcula</u>					x						
<u>Difflugia sp.</u>											x
<u>Trinema enchelys</u>					x			x	x		
<u>Trinema lineare</u>	?		x		x	x	x		x	x	x
<u>Euglypha rotunda</u>			x								
<u>E. ciliate v. glabra</u>							x				x
<u>E. dentata</u>											x
<u>Nuclearia simplex</u>						x	x	x	x	x	
<u>Spathidium sp.</u>							x		x		
<u>Enchelys sp.</u>	x	x	x	x	x	x	x	x	x		
<u>Prorodon sp.</u>						?					
<u>Chilodonella sp.</u>			x	x	x	x	x	x	x		
<u>Plagiopyla sp.</u>			x	x	x	x	x	x	x	x	
<u>Trichopelma sphagnetorium</u>	x			x	x	x	x	x	x	x	
<u>Drepanomonas sp.</u>						?					
<u>Colpoda inflata</u>			x								
<u>C. cucullus</u>				x							
<u>Chaenea sp.</u>			x			x		x			
<u>Litonotus sp.</u>			x								
<u>Dileptus sp.</u>			x			x			x	x	x
<u>Colpidium sp.</u>			x	x	x	x	x	x	x		
<u>Pseudoglaucoma sp.</u>				x	x	x	x	x	x		
<u>Saprophilus sp.</u>						x		x			
<u>Homalogastra setosa</u>			x	xx	x	x	x	x			
<u>Cyclidium glaucoma</u>			x		x	x	x	x	x	x	
<u>Cohnilembus fusiformis</u>				x					x		
<u>Bryometopus pseudochilodon</u>				x	x	x	x	x			
<u>Uroleptus mobilis</u>			x	x	x	x	x	x	x		
<u>Gonostomum affine</u>	x	x	x	x	x	x	x	x	x	x	
<u>Oxytricha setigera</u>			x	x							
<u>Oxytricha pellionella</u>			?	x	x	x	x				
<u>Holosticha sp.</u>			?			x	x	x	x		
<u>Vorticella microstoma</u>						x	x	x	x		

The Bacterial Flora

The following strains of bacteria were isolated on the plates which had been cultured at room temperature.

1. Motile bacillus, 6 μ or more in length; Gram-negative; non-sporing; Colonies on glucose-peptone agar, small, white, smooth and discrete.
2. Minute motile bacillus, 2-3 μ ; Gram-negative; non-sporing; Colonies on glucose-peptone agar, smooth and coalescing, white or yellow or green.
3. Large motile bacilli, 6 μ ; Gram-negative; non-sporing; Colonies on glucose-peptone agar, discrete, jelly-like and colourless.

The first strain was the sole bacterial strain which grew on plates inoculated from the control cultures, i.e. agar plates with distilled water but without soil. The strain was not isolated from the soil cultures.

The third strain was isolated from soil cultures on the first plates but was not present later. The early plates inoculated from the soil cultures showed a variety of unidentified strains.

After ten days the second strain was alone recovered from the soil cultures. This strain appears to be closely related to Pseudomonas fluorescens, a common soil and water species, but the group is imperfectly known and there are a large number of Gram-negative motile rods so far unidentified (Gray, 1951).

Oxidation-Reduction Potential Eh

Graph 1 gives the range and fluctuation of the Eh readings of the different soil cultures (unbroken line) and controls (broken line).

This graph indicates that the readings throughout the period both for the soil cultures and controls fluctuated between 350mv. and 430mv., with the soil cultures generally reading below 400mv. For most soil cultures there was initially a slight fall in potential.

Hydrogen Ion Concentration pH

Graph 2 gives the range and fluctuation in pH readings of the different soil cultures (unbroken line) and controls (broken line).

This graph indicates that the control cultures are less acid than the soil cultures. Secondly, that during the first week to ten days there is some fluctuation in the readings but that after this period the pH both of the soil cultures and the controls is fairly constant. Readings taken after three months showed a slight rise in pH. Thirdly, after the initial period of fluctuation the pH of the soil cultures lies between 5.0 and 6.0, and of the controls between 6.2 and 6.7. The highest pH reading for the soils was 6.8 and for the controls 7.1; the lowest 4.55 and 5.75 respectively.

Graph 1. Fluctuations of E_h of soil and control cultures over the first month of culture.

Graph 2. Fluctuations of pH of soil and control cultures over the first month of culture.

Discussion

These results confirm the protozoan succession of cultures. The history of the present cultures can be divided into three periods: an initial period of about a week to ten days of relatively slight protozoan activity; a period of some three weeks, 'the ciliate period', when the cultures are dominated by ciliate species; and a third period after the disappearance of most of the ciliate species when the cultures are dominated by the rhizopods.

The first period is marked by a mixed bacterial flora, generally by an initial drop of about 40 mv. in the Eh (Graph 1) and overall a drop in pH of about 1.0. The second period is marked by the dominance both of the ciliates and Pseudomonas spp.?, by a stable pH (5.0 - 6.0) and an Eh fluctuating between for the most part between 350mv. and 400mv. The third period was not so closely studied but pH and Eh readings after three months were not significantly different from those of the second period.

It is apparent that in soil cultures fluctuations of Eh and pH cannot account for the biological succession, for the total pH range lies between 4.5 and 7.0 and during the period of greatest protozoan activity, i.e. the second period, the pH is virtually stable. The Eh fluctuation of 90mv. is not significant and continued throughout the first and second periods when the readings were taken. The fluctuations may have been due simply to the inaccuracy of the readings. A bright platinum electrode was used and merely rinsed in distilled water between readings.

It is evident, however, on surveying the literature that this technique is not as reliable as the platinized-platinum, or black electrode. The bright electrode is liable to surface reaction (oxidation) and what is known as 'adaptation lag' - the electrode readings being affected by previous readings. The unexpectedly great fluctuations of the control cultures may have been due to this adaptation lag as these cultures were poorly poised compared with soil. It is sometimes held that the Eh is simply another measurement, and less accurate, of the pH. It is apparent from the history of these cultures that in the present case this was not so, for the Eh continued to fluctuate after the pH had stabilised.

The restricted analysis of the bacterial flora does not permit any conclusive statement on this important factor. Only the one culture medium (glucose-peptone agar) was employed and only the bacterial strains growing on this medium were recorded. Moreover no quantitative analysis was undertaken. The absence, in the second period, of strains which occurred in the first period may be taken to indicate partly the absence of suitable metabolites and partly the reaction to the waterlogged, semi-anaerobic conditions which were established. The ciliate activity appeared to be associated with the dominance of the Pseudomonas spp. It has been shown (Burbanck, 1942; Singh, 1941; Taylor and Furgason, 1938) that this type of bacterium is a very suitable bacterial food for Paramecium and Colpoda and Gray (1951) has shown that in natural conditions in a stream Colpoda and

Colpidium are associated with Pseudomonas (derived from soil) and that with the disappearance of the flora, the ciliates disappear. It is not unreasonable to presume that the presence of this flora in the cultures supported the ciliate population during the second period. Kidder and Stuart (1939a) have shown that if Colpoda is maintained with a mixed bacterial culture with initially a high Aerobacter population (another edible bacterium) the Aerobacter are eaten but the remaining flora multiplies and the non-edible species are more numerous at the end than at the beginning of the culture. Singh (1941) has shown that Colpoda favours certain strains rather than others and that if given an inedible strain it will encyst, in the same manner as if it were starved. Kidder and Stuart (1939b) have also shown that certain pigmented bacteria (Achromobacter) are toxic to this ciliate. Singh (1941, 1945) has shown that amoebae and ciliates may prefer different bacterial strains. Although the bacteriology of the third period of the present cultures was not determined it is reasonable to conclude that it was characterised by a different bacterial flora suitable as food for the rhizopods, but not for the ciliates, which consequently encysted.

Although the majority of the ciliate species in the culture were primarily bacteria-feeders, e.g. Colpoda, Cyclidium, Enchelys, Oxytricha pellionella, Vorticella, other species, e.g. Dileptus, are carnivores and may even feed on the rhizopods. This may account for the persistence of Dileptus in the cultures.

11. Culture Sequence of Steam Treated Soil

Introduction

At the beginning of the century a phenomenon was observed known as sick soil or sewage soil. It was found that growth after a time became poor in such a soil and there was an increased incidence of disease. Soil reactions such as nitrogen fixation slowed down and bacterial activity was depressed. Such soils did not respond to normal manuring practice. This phenomenon is still common especially in greenhouse soils.

At about the same time great interest was being taken in bacterial activity in the soil following the pioneer work of Pasteur and Winogradsky and it was thought that the key to the problem lay in bacterial activity. One method which was found to be satisfactory in curing such sick soils was partial sterilization either with such chemicals as carbon disulphide or toluene or by steaming.

In 1909 Russell and Hutchinson put forward the view that sick soils owed their infertility to low bacterial action; that bacterial action was in such soils inhibited by some factor; that this factor was removed by partial sterilization which permitted vigorous bacterial growth; and finally, as they had come to notice that the inhibiting factor was the protozoan fauna.

This celebrated thesis did more for the study of soil protozoology than all the treatises of the XIXth century. In the next decade innumerable studies were made on partial sterilization, on soil bacteria and on soil protozoa. By 1917 Kopeloff and Coleman could review an extensive literature filled

with many conflicting opinions and statements. Certain facts were however established: the presence of an active protozoan fauna in the soil largely feeding on bacteria; the alternation of bacterial and protozoan populations; and the undoubted stimulatory effect of partial sterilization on bacterial activity.

Finally by 1948 (Anon., Soils and Fertilizers) it was realised that partial sterilization, particularly steam sterilization, had widespread effects on the soil equilibrium and that no one factor was sufficient to explain its beneficial effects. It is now recognised that the effect of steaming the soil is more extensive than its biological effect and that physical and chemical characteristics of the soil are also affected. These include water-holding capacity, capillary properties, destruction of the colloidal film surrounding soil particles, increased concentration of the soil solution and increased solubility of soil constituents (e.g. Ca, Mg, Mn, and phosphate and also to a lesser extent K, Si and Al). The biological effects are an initial inhibition of bacterial activity and seed germination followed by greatly stimulated bacterial and plant growth.

Sterilization, which may be effected by steam or chemicals, can also take place in fallow soils under very hot and dry conditions. This is the case with the soils of Egypt which are left fallow during the Sheraqi period of summer fallow. The soil dries out and then for a period of a few months very high soil temperatures are experienced (Taylor and Burns, 1922, Prescott, 1920). Temperatures reach 55°C at the surface, 37°C at

10cm. depth and 33°C at 20cm. depth. The soil becomes caked and the surface cracks. After this period and following irrigation, it shows all the characteristics of a sterilized soil including increased nitrate production. The high temperatures and dry conditions do not affect the viability of the protozoan fauna. The protozoa which encysted when the soil dries up show little or no depletion of numbers during the Sheraqi period. Following irrigation the numbers of amoebae and flagellates increase slightly as the protozoa excyst and multiply. Laboratory tests showed that the protozoa could survive temperatures up to 73°C for ciliates and 80°C for amoebae and flagellates. It is clear that the most extreme temperature conditions obtaining in soil under natural conditions are not sufficient to kill the protozoa provided they are encysted. Moreover such extreme temperature conditions are rare. Kraus (1911) observed a soil bare of vegetation which had a temperature 28°C above the maximum air temperature but normally in temperate climates the soil temperature shows less fluctuation than air temperature although it is generally slightly higher than the mean air temperature (Cutler and Crump, 1935).

Egyptian soils are heavy clay and when irrigated harbour a large number of active protozoa, especially ciliates (Taylor and Burns, 1922). The extreme conditions of the Sheraqi period are compensated then by favourable conditions during the growing season. Indeed, as the results of steam sterilization show, protozoan activity is greater after partial sterilization than in the unsterilized soil.

In other soils subject to extreme temperatures, e.g. the sandy soils of the Mackenzie country or the Palestine soil sampled by Reich and Bodenheimer (1934), the protozoan population is very low. But in these cases the soil is quite different, being a poor sandy soil which does not benefit either from irrigation or crop growth. Further with the Mackenzie country soil the high summer temperatures and dry conditions are succeeded by very low winter temperatures which also inhibit protozoan activity. There is not then an adverse period alternating with favourable conditions but almost persistently unfavourable conditions.

Experimental Methods

It appears that since the time of Russell and Hutchinson no one has actually examined the effect of partial steam sterilization on the protozoan fauna of the soil. For this reason an opportunity was taken to examine the fauna of a greenhouse soil (from the Cawthron Institute) which had been steam treated.

The soil was sterilized by steam injection to a depth of 18" by pipes inserted from above. 100 days later samples were taken from the 0 to 4 inches and 20 to 24 inches horizons. Samples were also taken from unsterilized soil. These were cultured 24 days later and the development of the protozoan fauna followed. The culture methods employed were the same as employed for the Greywacke samples.

Protozoan History of the Cawthron Samples

The cultures were set down on the 11th October. On the 12th there was little protozoan activity, Saprophilus sp. appearing in the unsterilized topsoil and Vorticella microstoma in the unsterilized subsoil.

On the 13th, flagellates were omnipresent, but whereas the sterilized subsoil showed no ciliates and the sterilized topsoil only a few Colpoda inflata, the unsterilized samples showed a number of species; the subsoil having C.inflata, C.steinii, Saprophilus sp. and a rotifer; and the topsoil had these three ciliates together with Oxytricha pellionella, Chilodonella sp., and nematodes.

On the 14th, the sterilized subsoil had C.inflata, and the sterilized topsoil added Oxytricha pellionella to its fauna as did the unsterilized subsoil. At this date the unsterilized topsoil was readily distinguished by the greater variety of species present and still more by the greater number of individuals, especially C.inflata and C.steinii, which far exceeded the numbers present in other cultures.

On the 22nd, Chilodonella sp., Oxytricha, Vorticella, Euplotes and Trichopelma were noted in the unsterilized topsoil, the two Colpoda having disappeared. In the unsterilized subsoil Oxytricha, Vorticella, Enchelys, Mayorella, and Sphenoderia were present. In the sterilized subsoil C. inflata, Oxytricha and Vorticella were present, while in the sterilized topsoil only two species were present, C. inflata and Oxytricha but these were present in phenomenal numbers. (With a lens combination L.P. x 10, up to 100 being observable in one field anywhere on

the plate).

Except for the occurrence of Spathidium and Lionotus in the sterilized subsoil, the subsequent history of the culture was of no particular interest.

A full list of the fauna occurring in the four samples is given in Table 12.

Table 12

Protozoan Species in Cawthron Soils

	UNSTERILIZED		STERILIZED	
	0-4"	20-24"	0-4"	20-24"
<u>MASTIGOPHORA</u>				
<u>Bodo sp.</u>	x	x		x
<u>Bodo sp.</u>	x	x		x
<u>Monas sp.</u>	x	x		
<u>Euglena sp.?</u>		x		x
<u>Cercomonas sp.</u>	x	x		x
<u>Astasia sp.</u>	x	x	x	x
<u>Oikomonas sp.</u>	x	x		
<u>Mastigamoeba sp.</u>	x	x		
<u>RHIZOPODA</u>				
<u>Mayorella vespertiliox</u>		x	x	x
<u>Trichamoeba sp.</u>	x		x	x
<u>Cryptodifflugia</u>				
<u>oviformis</u>	x	x		
<u>Trinema lineare</u>	x			x
<u>Sphenoderia dentata</u>	x	x		x
<u>Euglypha rotunda</u>	x			
<u>Nuclearia simplex</u>				x
<u>CILIATA</u>				
<u>Spathidium sp.</u>	x	x		
<u>Enchelys sp.</u>	x	x	?	
<u>Chilodonella sp.</u>	x	x		
<u>Lionotus sp.</u>				x
<u>Trichopelma</u>				
<u>sphagnetorium</u>	x			x
<u>Colpoda steinii</u>	x	x		
<u>C. inflata</u>	x	x	x	x
<u>Colpidium sp.</u>		x	x	x
<u>Pseudoglaucoma sp.</u>		x		x
<u>Saprophilus sp.</u>	x	?		?
<u>Cinetochilum</u>				
<u>margaritaceum</u>				
<u>Cyclidium glaucoma</u>	x	?		
<u>Cohnilembus</u>				
<u>fusiformis</u>	x	x		x
<u>Oxytricha pellionella</u>	x	x	x	x
<u>Euplotes sp.</u>	x	x		
<u>Vorticella</u>				
<u>microstoma</u>	x	x		x

DISCUSSION

These observations demonstrate several important facts. First, as shown in Table 12, the number of species in the sterilized topsoil is greatly reduced. There were only 6 species compared with 25 in the unsterilized topsoil, i.e. a reduction by steam treatment of 76%. The sterilized subsoil however was little affected, 20 species compared with 23 in the unsterilized subsoil.

Secondly, the protozoan fauna of the steam treated soils appeared some days after that of the untreated soils. That is, there was a time lag in the development of the fauna.

Thirdly, protozoan activity in the treated soils was far greater than in the untreated soils after the fauna had developed. This was particularly true of the treated topsoil. The two chief ciliate species occurring in this culture were Colpoda inflata and Oxytricha pellationella and after eleven days of culture these ciliates were present in very great numbers which have never been paralleled by any other soil culture in my experience.

From these results it is obvious that while Russell and Hutchinson were correct in surmising that the protozoan population would be drastically reduced, they were in error when they attributed the heightened activity of the bacteria to the absence of protozoa. For not only are the protozoa present in the treated soil but they are present in very great numbers and their activity is stimulated by sterilization just as much as

the activity of the bacteria. Both show an initial inhibition followed by greatly enhanced activity of that part of the flora and fauna which is not killed by steaming.

Work on the life cycle of ciliates which involved serial culture in pure, but not bacteria-free, media showed the presence of 'rhythm' - periods of good growth succeeded by periods of poor growth (Calkins, 1933). The early workers believed that these rhythms were innate in the life cycle of ciliates but later work under standardised conditions, particularly of the bacterial flora, has shown that these rhythms can be suppressed (Taylor and Strickland, 1938). There is no doubt that they are due to cultural conditions and in particular the bacterial flora. Generally this effect is ascribed to the accumulation of toxic metabolites and indeed there is no more specific knowledge of the factors involved. The effect of these toxic metabolites is twofold: first, growth itself is depressed, and secondly, excystment is often inhibited.

Kidder and Stuart (1939a,b) found that the growth of Colpoda steinii was depressed in a mixed bacterial culture and that ultimately the ciliates died out. They also found that cyst formation was inhibited and the ciliates which normally divide within a cyst membrane failed to do so. Singh (1941) confirmed the depressing effect which old culture filtrate had on the growth of Colpoda steinii even when adequate food was available. He showed however that the toxic effect of the filtrate could be removed by heating for one hour at 70 to 80, °C. Kidder (1939) found that old culture medium had both an inhibitory and

acceleratory effect on bacteria-free cultures of Tetrahymena pyriformis. The acceleratory factor was absorbed on a Seitz filter (Richards, 1941).

Penn (1935) found that old culture filtrate increased the rate of encystation of Pleurotricha lanceolata which he attributed to the presence of salts. McIntyre (1949) showed that excystment of Tillina magna in an excystment medium could be interrupted and blocked by returning the cysts to their encystment medium. The same is true of Colpoda (Brown and Taylor (1938)). Brand (1923) found that the encystment medium of Vorticella microstoma also inhibited excystment, a fact which has been further investigated in the present thesis.

It is apparent that there is a toxic factor in old culture fluid due to the activity of bacteria which is thermolabile. The effect of steam sterilization is to remove this factor which will have the double effect of removing an excystment inhibitor and an inhibitor of ciliate growth. It will account therefore for the greatly stimulated growth of Colpoda in the present cultures.

Hall and Loefer (1938) working with Colpidium campylum (syn. Tetrahymena pyriformis) found that autolysed filtrate of old culture medium accelerated growth when added in the proportion of 1:9 to fresh medium. In this case the possibility of trace elements and perhaps growth factors cannot be ruled out as the medium employed was simply 1% Bactotryptone. Nevertheless it is further evidence of the beneficial effects following autolysis of old culture medium.

The present view of steam sterilization then with which this

work agrees is that the destruction of organisms destroys possible disease-causing or antibiotic organisms and also releases plant nutrients both by autolysis of microbial protoplasm and by reducing microbial competition for nutrients. Deficiencies of trace elements can also be removed by partial sterilization. The whole phenomenon can be viewed as a gross reorganization of the soil equilibrium and the stimulated bacterial, protozoan, and plant activity as resultants of the new equilibrium reached.

D. GENERAL DISCUSSION

SOIL CONDITIONS AND THEIR RELATION TO PROTOZOAN ACTIVITY.

The experiments described in this chapter have dealt with
the culture of the soil protozoa in different media. The results

clearing turbid and polluted water (Barker, 1946). They are chiefly found only when bacterial decomposition is actively taking place. In the absence of food they encyst. No ciliate demonstrates this cycle more perfectly than Colpoda. Excystment, growth and encystment are directly related to the bacterial activity (Taylor & Strickland, 1938).

Whereas the 'natural' culture media (soil and peat extract) supported growth of the whole range of ciliate species, only a limited fauna grew in yeast extract. These species, however, of Mayorella, Trichopelma, Colpoda, Gonostomum and Holosticha are among those most commonly encountered in soil. They are all found for example in the Greywacke samples which did not have a large protozoan population. Their distribution then is correlated with a greater tolerance of cultural conditions.

Noland (1925a) observed that the species occurring in laboratory infusions were different from the ordinary fresh water forms and he attributed this to their greater tolerance of bacterial infusions and of the accompanying conditions of semi-anaerobiosis, viz. high carbon dioxide and low oxygen tensions. Noland observed that under laboratory conditions with higher temperatures and higher concentration due to evaporation the bacteria replaced the algae and consequently the bacteriophagous ciliates replaced the diatom and algae feeding forms. The laboratory species particularly mentioned by Noland were Colpoda steinii, C. cucullus, Glaucoma (syn. Tetrahymena) pyriformis, Glaucoma scintillans and Paramecium caudatum, together with the predatory species Dileptus gigas (anser?) and Spathidium spathula.

These he observed are rare in the field.

The investigation of the soil culture and the protozoan sequence indicated that conditions were very stable apart from bacterial and protozoan activity. The conditions of the laboratory culture are comparable to conditions in the field. Most soils are acid and this is certainly true for the Zonal soils of New Zealand. The pH as with the soil culture lies between 5.0 and 6.0. Kubiena (1938) has shown however that there are considerable variations within any one soil and that in particular the secretion of carbon dioxide by root tips causes a marked increase in acidity. If anything then soil conditions will be more acid than culture conditions for the soil culture is prepared by a gross dilution of the soil solution and consequently a dilution of soil acidity. This acid condition is important for it differs very markedly from conditions in most ponds and streams where generally neutral to alkaline conditions obtain. Further the acidity of the one and the alkalinity of the other are enhanced by the activity of their respective floras. Bacterial activity generally increases the acidity of a solution by the production of carbon dioxide and other acid end products; algae on the other hand by their fixation of carbon dioxide reduce the acidity and increase the alkalinity. This difference is shown very well in the digestion (bacterial) and oxidation (Chlorella) ponds described in Section B. The pH limits of most fresh water ciliates generally lie close to neutrality. The acidity of soil conditions is one barrier to this fresh water fauna. However they are not found in the alkaline soils

(Intrazonal and Azonal). There must then be a further barrier other than pH.

The oxidation-reduction potential of soils was first measured by Gillespie (1920). Since then a large number of workers have made readings of different soil conditions and of different soil types. Readings have also been made of lakes. The latest and best work on soils was done by Quispel (1947a) who used a platinized-platinum electrode. He found that the Eh of the soil was a good measure of the oxygen tension and that it fell with waterlogging and consequently poor aeration. On draining a soil the Eh would rise. When flooded or waterlogged the potential fell as low as -200mv. When well drained it rose above 600mv. Burrows and Cordon (1936) found that the type of decomposition taking place in the soil had a marked effect on the potential. Decomposition of casein was found to result in strongly positive potentials and of carbohydrates of more negative but not extremely negative potentials. The effect of clay poisoning systems was examined by Puri and Sarap (1938) and Wight and Starkey (1945) showed that iron was an important poisoning system (i.e. ferrous-ferric). Pearsall (1938) examined different types of soil and found that woodland soils had strongly oxidising potentials. Other soils had more reducing though not negative potentials and waterlogged soils had low potentials. Lafond (1950) found that mulls had oxidising and more reducing potentials and that duff mulls were intermediate between the two. Other Wisconsin workers (Wide, Trach and Petersen, 1950) found that peats had strongly reducing potentials particularly moss

peats with a potential of -255mv. Culliford (unpublished) has confirmed these findings working with New Zealand litters.

Pearsall and Mortimer (1939) found that the potential of lakes falls with increasing depth and that in bottom muds there are reducing conditions and ammonia and sulphide production. This was confirmed by Hutchinson et al., (1939) and Allgeier et al. (1941). Strongly oxidising conditions obtain at the surface and strongly reducing conditions at the bottom with a gradual fall of potential with increasing depth. Hutchinson et al. stress the importance of poisoning systems, particularly ferrous-ferric, and pointed out that the Chironomus fauna was closely correlated with the redox potential.

The fall in potential accompanying bacterial growth has been fully studied by Hewitt (1936). He showed that this fall to a strongly reducing potential can be offset by aerating the culture, when the potential after an initial drop rises again to its original level. Bhaumik (1948) found that bacterial activity in moistened soil was marked by two maxima, the first he attributed to aerobic bacteria and the second to anaerobic. He also showed that bacterial activity is greater in dry soils than in waterlogged soils. Boynton and Compton (1944) found that bacterial activity judged by carbon dioxide production was greatest in midsummer with hot, dry conditions. They found anaerobic conditions in soils waterlogged in winter. Quispel recognized the persistence of anaerobic pockets where aeration was impeded. Such conditions are particularly liable to occur where there is accumulation of organic matter, intense bacterial activity and a compact clay soil.

It is apparent then that the conditions of the soil culture are not very different from soil conditions but that if anything the field conditions would be more reducing when saturated and more oxidising when drained. Generally soil offers conditions where moisture and bacterial activity are associated with reducing and anaerobic conditions particularly in clay soils. Consequently the fauna will be reduced to those species capable of withstanding such conditions. Soils with better structure, such as the Intrazonal soils sampled, and with a different clay composition will offer better conditions of aeration and less fluctuating conditions and will have therefore a greater ciliate population.

Litters on the other hand, as shown by Culliford and Lafond, have strongly oxidising aerobic conditions associated partly with the absence of bacterial decomposition and the presence of actinomyces and fungi. Although conditions are very acid there is good water retention and good aeration. Consequently more ciliates are present, and this is particularly so of granular litters. Peats on the other hand tend to be waterlogged and less well aerated. They approach soil conditions and have a reduced ciliate fauna. Fresh water (surface waters) differs from these three habitats first, by its alkalinity, secondly, by its strongly oxidising conditions, and thirdly by the predominance of an algal and diatom flora.

Besides the differences of acidity and aeration, soil is also characterised by marked changes in the soil solution. The moisture content of the soil fluctuates greatly varying with the type of soil and the climate. This fluctuation of soil moisture

results, as Kubiena has shown, in the precipitation of salts in the minute soil chambers formed by adjoining soil particles. From the type of efflorescence in the chamber it is possible to judge whether they were formed under aerobic or anaerobic conditions. It is obvious however that, besides variation of oxygen and carbon dioxide tension, the salt concentration of the soil solution will vary greatly.

In a well drained soil the changes in moisture content are as follows. After rain the soil is saturated with water which fills both the capillary and non-capillary pore spaces. The water then drains from the non-capillary pore spaces and in this drained condition the soil is said to be at field capacity. Further evaporation causes the soil moisture to be restricted to the capillary pores which may be about 100mm in diameter. Naturally different soils will vary greatly both to the degree to which they retain moisture and in the structure of the capillary and non-capillary spaces. The non-capillary spaces when drained of water permit aeration so that aeration and water content are inversely proportional. Sands which permit excellent drainage and good aeration have poor water retention; clays have poor drainage, poor aeration but good water retention. However different types of clay vary in structure and in water holding capacity and the amount of organic matter and the plant cover are also very important.

It is then in the small capillary spaces that water is best retained and it is for this reason that bacterial and protozoan activity is concentrated in these spaces. But because of their

small size and poor aeration these spaces tend to be anaerobic (Boynton and Compton, (1944); Russell and Appleyard (1913); Cutler and Grump (1935).

To counterbalance these disadvantages the soil solution has various advantages. First, soil is generally well buffered, the hydrogen ion concentration varies, but not very greatly whereas in fresh water or hay infusion very great variation is common. The stability of the pH is shown in graph 2. Secondly, soil soil possesses a number of poisoning systems including clays and metals (e.g. ferrous-ferric). Thirdly, partly due to microbial activity the soil possesses commonly a number of chelate compounds which poise or buffer the concentration of metallic ions. Organic acids such as citric and oxalic are active chelating compounds and these are typical microbial metabolites. Finally the activity of clays in binding bases and in holding proteins and enzymes in their lattice structure is important. The bases may be released by organic acids and then utilized in microbial metabolism. The clays also largely determine the texture and structure - what Kubiena calls the fabric - of the soil.

Soil conditions will however vary. Increasing acidity will release metals such as iron into solution. Sodium will flocculate while calcium will aggregate soil colloids or clays. Persistent tillage ruins the tilth of a soil which loses its crumb structure. Litter accumulation and the whole organic cycle greatly modify soil conditions.

The effect of these differences and changes in the soil on

the protozoan fauna have been discussed earlier. It is necessary however to be more precise about the specific factors which are important. It is in comparing soil conditions with conditions obtaining in fresh water and litter that the effect of specific factors can best be judged.

The most obvious distinction between soil and fresh water is the greatly restricted water capacity of the former and to this may be attributed the conspicuously smaller size of soil ciliates. Where there is better water retention, as in litters, larger and more complex species are found comparable with fresh water species. Many small fresh water species however are not found in soil, e.g. Coleps hirtus, so that size is not the only limiting factor.

Two factors that remain to be considered are oxygen and carbon dioxide tensions. In these factors soil differs markedly from fresh water and their effect on ciliate species will be considered in the next chapter.

IV

AN EXPERIMENTAL STUDY AND DISCUSSION OF THE RESPONSE OF
CILIATES TO ANAEROBIC CONDITIONS

A. THE EFFECTS OF ANOXIA

Anaerobiosis and Soil Conditions

- i. Reaction of Fresh Water Ciliates
- ii. Reaction of Soil Ciliates

B. THE EFFECTS OF HIGH CARBON DIOXIDE CONCENTRATIONS

- i. Reaction of Fresh Water Ciliates
- ii. Reaction of Soil Ciliates

C. THE EFFECT OF AMMONIA

- i. Reaction of Colpoda

D. GENERAL DISCUSSION

The Causes of Death in Anaerobiosis, Ecological
Importance, and Physiological Significance.

A. THE EFFECTS OF ANOXIA

Anaerobiosis and Soil Conditions

Anaerobiosis

'Anaerobic' is a term loosely employed and 'anaerobic' conditions cover a number of factors. Basically anaerobic conditions imply low oxygen tensions or the complete absence of free oxygen. Generally associated with such conditions in nature are low oxidation-reduction potentials, and high carbon dioxide tensions. Ammonia, hydrogen sulphide and occasionally methane and free hydrogen may also be associated with low oxygen tensions.

Anaerobic habitats may be divided into two types, parasitic and non-parasitic. The parasitic habitats include the gut of various hosts. The non-parasitic include the benthic muds of lakes and oceans, Imhoff tanks, and sewers, where intense organic breakdown is taking place, e.g. the anaerobic digester mentioned in the last chapter. Soil may sometimes be classed with these habitats.

Biologically there is associated with such anaerobic conditions a characteristic fauna and flora. Included amongst the parasitic and endocommensals are rumen ciliates, termite flagellates and endamoebae. Non-parasitic faunas are the sapropelic species associated with the benthic muds and the sewage species associated with the benthic muds and the sewage species associated particularly with Imhoff tanks (Lackey, 1924, 1925, 1926, 1932; Lauterborn, 1894, 1901, 1916). Many free-living species however have been recorded 'in the complete absence of oxygen' (Noland, 1925a; Brand, 1946). The anaerobic fauna may also be classified physiologically into obligate anaerobes (those which require

constantly anaerobic conditions), and facultative anaerobes (those which survive under anaerobic conditions but which normally live and grow under aerobic conditions). The rumen ciliates and termite flagellates are both obligate anaerobic faunas, whereas Balantidium and Nyctotherus are both facultative anaerobes. The sapropelic and sewage ciliates are obligate anaerobes but the ordinary free-living ciliates occasionally found in such habitats are facultative anaerobes.

With soil ciliates we are concerned chiefly with facultative anaerobes although the occasional record of Trimyxa and Metopus indicate that obligate anaerobes may also be found. The chief point of interest is the period which facultative anaerobes will survive under anaerobic conditions and the factors which favour or hinder survival.

There are two sources of evidence on anaerobic survival: first ecological and the second experimental. The ecological evidence consists of records of ciliates from habitats, particularly benthic muds and Imhoff tanks, which are under anaerobic conditions. Lauterborn (1894, 1901, 1916) was the first to record a characteristic protozoan fauna associated with the 'anaerobic' muds. This fauna, which was later studied by Noland (1927), Wetzel (1928) and others, was termed the sapropelic fauna and for the most part consists of ciliates systematically distinct from other free-living species. Such species are found only where strongly reducing or strictly anoxic conditions obtain. They are characterised by an extremely low rate of metabolism and Noland (1927) found that Metopus es., a typical sapropelic ciliate,

took at least a week to complete conjugation. These ciliates are slow moving and have a slow ciliary beat. Besides this sapropelic fauna there are a few species which occur regularly in Imhoff tanks. These are Saprodinium putrinum, Metopus es and Trimyema compressa. All these species appear to be truly anaerobic in that their life cycle takes place in the entire absence of oxygen (Lackey, 1938b).

Lauterborn also recorded a number of typically free-living ciliates which he found not in the mud but just above it where there was also no oxygen. The same ciliates have been recorded by other workers including Lindeman (1942). This worker tested the survival of the ciliates at different temperatures. He found that they survived for a month at 0°C and 5°C but that only a few species, including Coleps birtus survived at 10°C. Fauré-Fremiet et al. (1929) working with Paramecium caudatum also found that this species survived better at lower temperatures.

The experimental evidence, with which Lindemann's may be classed falls into two groups: that which involves the bubbling of gases through the culture (Nikitinsky and Mudrezowa-Wyss, 1930) and that which involves the removal of oxygen either by use of a bell jar or by a small chamber in which a hanging drop is suspended.

It is the last method - the suspension of a hanging drop in a small chamber which is then flushed with the required gas - that was used in the present tests. Previous work with this apparatus or with a manometer under much the same conditions have given very diverse results even when the same species is being tested. There are three different reactions described: first, the immediate

blistering of the ciliate (Gersch, 1937; Specht, 1934); secondly, the survival of the ciliate for a short period followed by blistering (Kitching, 1939a, b; Johnston and Evans, 1941a; Adolph, 1929); thirdly, the survival of the ciliate indefinitely (Thomas, 1942; Watson, 1944).

A comprehensive review of anaerobiosis in ciliates is given by Thomas (1942) and Brand (1946) who list both the ecological and experimental evidence given in the literature. Not only is there some conflict between the experimental results obtained with the same species by different workers but there is also a marked discrepancy between the experimental and the ecological evidence.

Kitching (1939b) points out that for genuine anoxic test the oxygen content of the medium must be so small that the ciliate is unable to utilize it, and further there must be no generation of oxygen by accompanying organisms, e.g. algae or plant cells. It is doubtful whether in the experiments of Putter (1905) and Nikitinsky and Mudrezowa-Wyss (1930) these conditions obtained and for this reason their results are not true tests of anoxia.

Typical of the conflicting results are the tests with Paramecium caudatum. Putter (1905) recorded survival of this ciliate at room temperatures for 10 days, Nikitinsky and Mudrezowa-Wyss (1930) also at room temperatures recorded survival for 18 days, Gersch (1937) recorded death at room temperatures in ten seconds, Barbarine (1938) recorded survival of starved ciliates for an hour and fed ciliates for more than two days, Kitching (1939b) recorded maximum survival of 12 hours at 24° to 28°C.,

Liebmann (1936) recorded survival for 3 days at room temperatures and Lindeman (1942) recorded survival for less than 30 days at 10°C. but more than 30 days at 0°C. and 5°C. Watson (1944) recorded that P. caudatum survived less than two hours at room temperatures although Balantiophorus minutus survived indefinitely under the same conditions.

These results with Paramecium caudatum are so conflicting that they are difficult to evaluate. However the experiments of Kitching and Watson both assured the complete absence of oxygen and their results may be taken as a general guide. These indicate the survival of the ciliate for several hours and under the most favourable conditions although at a relatively high temperature for 12 hours. The results of Gersch must therefore be dismissed and death in these experiments ascribed to some factor other than anoxia. On the other hand the survival of the ciliate at room temperatures for several days strongly indicates the presence of traces of oxygen and this is supported by the fact that Noland (1925a) did not record this ciliate in the complete absence of oxygen although it was present both in the field and in laboratory cultures when the oxygen tension was only 0.4cc./l. It may be concluded then P. caudatum is a facultative anaerobe but that it can survive only a limited period in the complete absence of oxygen. Of the factors which favour survival two are apparent in the evidence cited, viz. food and low temperatures. Well fed ciliates survive better than starved ciliates and the ciliates survive longer at lower temperatures than high, a point also made by Fauré-Fremiet et al. (1929a,b). Kitching found that the

ciliates survived much longer in their own culture fluid than in a buffer and if kept in the buffer the ciliates died almost instantaneously (within ten minutes), and comparatively quickly in the culture fluid without hay (within two hours). The ciliates even blistered in air when transferred to phosphate buffer from the culture fluid without hay. Kitching also found that the ciliates survived longer when subjected to hydrogen or nitrogen plus carbon dioxide than without carbon dioxide. The effect of the carbon dioxide was to stabilize the pH at approximately 7.1. Kitching stressed the need to avoid any impurities in the gas which may be harmful also the importance of watching such secondary effects as pH shift.

Another ciliate for which there is a marked discrepancy between Lindeman's findings and the experimental evidence is Spirostomum ambiguum. Lindeman found that in his experiments this ciliate survived for over a month at 0°C. and 5°C. although not at 10°C., i.e. similar to P. caudatum. Specht (1934) who measured the oxygen consumption of this ciliate at different oxygen tensions recorded immediate blistering of the ciliate in the complete absence of oxygen. It seems probable however that the death of the ciliates in Specht's experiments was due to factors other than anoxia and that this ciliate is similar to P. caudatum, a facultative anaerobe of limited period of survival.

Nearly all the experiments that have been recorded have been done with impure cultures. The only exception is the work done on pure sterile cultures of Tetrahymena pyriformis by Thomas (1942) and Pace and Ireland (1945). Thomas, who studied the anaerobic

carbohydrate metabolism of the ciliate, reported that the ciliates under anaerobic conditions were sluggish, moved slowly, and did not divide. Some of his experiments lasted 52 hours without the death of any of the ciliates. Pace and Ireland, using an apparatus which involved initial partial evacuation of the experimental chamber and subsequent replacement with gas of known constitution, recorded death of the ciliates in one experiment and growth in another. Obviously their technique was unsatisfactory and in this case there is no reason to doubt that Tetrahymena pyriformis is a true facultative anaerobe. This conforms with the ecological evidence. Noland (1925a) recorded Glaucoma pyriformis in his laboratory cultures in the complete absence of oxygen.

The only other indisputable case of facultative anaerobiosis in a free-living ciliate is that of Balantiophorus minutus reported by Watson (1944) which like T. pyriformis moves sluggishly but does not divide although it survived in Watson's experiments for over 20 days.

Metopus es, although it may occur in water with a trace of oxygen, is a typical obligate anaerobe (Noland, 1927) and differs from the facultative anaerobes in that its physiology is not affected by the absence of oxygen and it can both divide (Lackey, 1938b) and conjugate (Noland, 1927) in the complete absence of oxygen.

Generally then three groups of ciliates can be distinguished. First, the obligate aerobes which die immediately on the exhaustion of oxygen. Second, the facultative anaerobes which consist of

those species able to survive more or less indefinitely, e.g. Balantiophorus minutus and T. pyriformis and of these species which can survive for only a limited period, e.g. Paramecium caudatum and Spirostomum ambiguum. These species cannot divide in the absence of oxygen. Third, the obligate anaerobes which cannot be successfully cultured at high oxygen tensions but which grow well in the complete absence of oxygen, e.g. Metopus es . A careful review of the literature shows that there is not such a great discrepancy between the experimental and ecological evidence as at first appears. In many cases faulty technique invalidates the experimental work and in other cases, e.g. Lindeman's results, the very low temperature and perhaps the presence of traces of oxygen, which at such temperatures would be only slowly exhausted, account for the longer survival period than that recorded by other workers. There remains the possibility mentioned by Krogh (1941) and endorsed by Brand (1946) that the 'continued provision of quite small and in themselves absolutely insufficient amounts of oxygen is essential for the successful resistance against asphyxiation'. This possibility should be borne in mind when experimental and ecological evidence is compared.

There are a number of other factors which may also affect anaerobic survival.

1. Small size: this may be a benefit in conditions of very low oxygen tension. The smaller the ciliate the more rapidly the oxygen may diffuse into the protoplasm.
2. Acidity and alkalinity: increasing acidity or alkalinity

may be more toxic to some species than others.

3. Toxic byproducts: the accumulation of toxic byproducts may be important. Putter (1905) found that ciliates survived longer in larger drops than small. This may be the explanation.
4. Food: well fed ciliates survive longer than starved ciliates and as Kitching suggests survival may be prolonged by providing food for the ciliates.
5. Temperature: ciliates survive longer at low temperatures than at high. This may account for discrepancies between different experiments.
6. Perhaps the most important factor in anoxic survival is the ultimate fate of the ciliate. In many cases the ciliate blisters, e.g. Paramecium and Spirostomum. In Colpoda however the ciliate encysts and can thereby survive indefinitely. There is also the possibility that whereas sudden changes of oxygen tension may prove fatal the ciliate may become adapted to gradual changes. This possibility is discussed by Juday (1908) and von Brand (1934; 1946) with regard to the discrepancy between experimental and ecological findings.

Soil Conditions

It might be thought that in well drained soil anaerobic conditions would not occur or would occur very rarely (Brandt, 1946). Such a view is erroneous. With adequate pore space a great part of the soil will be under aerobic conditions with the oxygen tension only slightly below and the carbon dioxide tension only slightly above those of the atmosphere (Russell and Appleyard, 1909; Boynton and Reutner, 1939; Boynton and Compton, 1944;

Cutler and Crump, 1935). However microclimatic conditions are quite different (Kubiena, 1938).

The relation of soil air to soil conditions and its effect upon soil life is reviewed by Peterson (1950) and Chang and Loomis (1945). There are in any soil two variables to a large degree interdependent, viz. oxygen tension and carbon dioxide concentration. Soil conditions which effect one often affect the other. It is necessary to distinguish between oxygen tension and oxygen diffusion. The latter is biologically the more important for most organisms can utilize oxygen at a very low tension. The problem therefore is one of minimum oxygen levels, maximum carbon dioxide tolerance and expected gas concentrations in various environments.

Different soils and different habitats offer varying conditions of oxygen supply and carbon dioxide tension. These conditions are met in two ways. Either the organism is sufficiently tolerant of a wide range of conditions or a special fauna and flora is found.

One of the principal factors affecting the soil air is soil moisture. Peterson cites Wollny who found that the carbon dioxide content of soil air increases ten times as the moisture content changes from 6.8% to 26.8%. Waterlogged soils and swamps offer especially poor conditions of soil aeration. It is therefore not remarkable that Fantham et al. who have made the most extensive survey of the protozoan fauna of waterlogged soils should find only half the species which they found in the non water-logged soils.

Another important soil condition affecting aeration is soil structure. Wollny (1881, 1886) found that granular soils have less than half as much carbon dioxide as powdery soils. The relative amount of carbon dioxide increases with an increase of depth and with density and compaction of soil. Any soil property resulting in large air capacity, such as granular structure, will result in lower carbon dioxide and higher oxygen tensions. Increasing the non-capillary pore space will both allow more rapid diffusion of oxygen and carbon dioxide and it is the rate of diffusion which is important.

It is almost a century since the first researches on soil air were carried out by Boussingault and Lewy. Recent reviews are given by Clements (1921) and Peterson (1950) while techniques are described by Hutchins (1926) and Taylor (1950). Soil air is generally sampled from a reservoir which is exhausted by a vacuum. The air so obtained is calculated to be from all the non-capillary pore spaces down to a diameter of 20 μ . This air has been analysed by Russell and Appleyard (1914) and Boynton and Compton (1944). They found that it was little different from atmospheric air but with slightly increased carbon dioxide and slightly decreased ^{oxygen}~~carbon dioxide~~. It showed however considerable seasonal fluctuation and the oxygen was sometimes as low as 2% and the carbon dioxide as high as 10%. Oxygen tension was at a minimum during winter and carbon dioxide tension at a maximum during mid-summer when there was the greatest microbial activity.

The lowest oxygen concentrations are obtained in waterlogged soils and the highest carbon dioxide tensions, sometimes up to

20% (Clements, 1924) are near plant roots. The effect of water-logging is both to fill up the pore space and so diminish the air space of the soil and initially to stimulate organic breakdown. Soils with heavy plant cover or rich in organic matter have the highest carbon dioxide tensions and pastoral land has higher tensions than arable.

Hutchins and Livingston (1923) devised an ingenious apparatus for testing the oxygen supplying capacity of soil. Hutchins (1926) showed that the oxygen supplying capacity of a saturated soil was only half that of the unsaturated soil and that if the surface was sealed the oxygen supplying capacity became negligible. Generally Hutchins' results support the conclusions of the other workers with respect to the effect of saturation, compaction and drainage.

Study of the oxidation-reduction potential of soils according to Quispel (1947a) also indicates the oxygen tension of soil. He showed that an inundated soil the potential fell from a strongly oxidising to strongly reducing but that on drainage there was a gradual rise in potential. In Quispel's view the potential was a direct reflection of the oxygen tension and he showed that replacing the soil air with nitrogen gave the same results.

Besides the soil air found mainly in the non-capillary pore space there is the gas dissolved in the finer capillaries and intragranular spaces, and in the films of moisture which clothe the soil particles. Russell and Appleyard found that when they had removed the initial soil air they continued to get from their vacuum a soil air consisting principally of carbon dioxide and nitrogen and with very little or no oxygen. In some cases the

carbon dioxide percentage was as much as 99%. This they believe to be the 'bound air' dissolved in the soil moisture films. Jones (1950) showed that the nitrogen is due to the anaerobic decomposition of nitrate and the carbon dioxide is also the produce of microbial metabolism. Boynton and Compton agree that the air of the smaller capillaries is principally carbon dioxide and nitrogen and has very little oxygen. This view is endorsed by Clements and indeed all the authors agree that in the finer capillaries anaerobic conditions, similar to those obtaining in waterlogged soils, are liable to occur, particularly in soils of poor structure such as clays and those liable to waterlogging or rich in organic matter.

Evidence that this is the case and that anaerobic conditions do obtain in the minute soil spaces is given by Kubiena (1938). He showed that a soil with spongy fabric possessed spaces of various orders. These were the large pore spaces, worm holes and root holes; the smaller or second order spaces of capillary pores and intergranular spaces; and finally the first order or intra-granular spaces.

In a well drained soil at field capacity the non-capillary pore space is filled with soil air. Soil in equilibrium with this soil air is then under aerobic conditions and as the air is replaced by atmospheric air of lower moisture tension efflorescences of salts are formed on the surrounding soil surfaces. Moreover due to this evaporation both salts and peptizable colloids 'creep' to the aerated surface. Aerated soil can therefore be recognized by the presence of such efflorescences. Anaerobic conditions on the other hand can be recognized by the formation of crystal chambers

and the absence of such efflorescences.

Biologically the change between aerobic and anaerobic conditions is marked. Fungi and actinomyces are confined to aerobic conditions where they are primarily responsible for the breakdown of plant remains and humic substances. With increasing moisture bacteria replace the fungi and actinomyces and first an aerobic and then an anaerobic flora develops. The presence of moisture and the development of this flora is an important factor in establishing anaerobic conditions. Moreover the increasing carbon dioxide tension increases the acidity of the soil solution while with gradual evaporation the soil solution retreats to the smaller capillary and intragranular spaces.

The microfauna is divided into the mites and collembola which live in the larger non-capillary pore spaces and which feed on the fungi and organic debris; and the protozoa and nematodes which are confined within the moisture film and develop, particularly the ciliates, in association with the bacterial flora. As the soil solution retreats into the smaller capillary spaces and intragranular spaces the ciliates either encyst or retreat with the solution and restrict themselves to moisture films and crevices in and amongst the soil particles. The site of such bacterial and ciliate activity can therefore be later recognized by the cysts lining the walls of such soil spaces.

Soils offering good water retention, rich organic sources and which are frequently watered offer excellent conditions for soil ciliates. Intermittent drying of the soil is not a

disadvantage (Bodine, 1923; or the Sheraqi soils) since the ciliates are adversely affected by the accumulation of metabolites in the soil solution and the development of an inedible or toxic bacterial flora. In a well drained and well watered soil frequent aeration and leaching prevent these conditions. For this reason the ciliate fauna of permanently waterlogged soils is not as great as of non-waterlogged soils although certain species may occur under such conditions which do not occur in well aerated soil.

These conditions under which the ciliate fauna is most active account for two important characteristics of the fauna: first, the dwarfed growth or smaller 'races' which are the common soil species and which, as has been shown, are really distinct species, and secondly, the life history and physiology of these species which it has been the purpose of this thesis to examine. Foremost amongst the physiological characteristics which might be expected of such a fauna are tolerance of anaerobic conditions. The present chapter investigates therefore anaerobiosis in soil ciliates, and, for purposes of comparison, fresh water ciliates.

Apparatus and Methods

A modification of Kitching's apparatus was used. A glass ring about 2 cm. in diameter with an inlet and outlet tube was sealed onto a 3" x 1" slide. A hanging drop preparation was made and the cover slip sealed to the top of the glass ring. The atmosphere of the glass ring was then changed by passing gas through the chamber. The gases employed were oxygen, nitrogen, and carbon dioxide or mixtures of these gases. They were obtained commercially in cylinders and the gas flow was adjusted by fine adjustment valves. Before being passed through the chamber the oxygen and carbon dioxide were bubbled through distilled water. The nitrogen was bubbled through dilute sulphuric acid (to remove any ammonia present), alkaline pyrogallol (to remove any traces of oxygen) and distilled water. The gas escaping from the chamber was bubbled through distilled water and in the case of nitrogen through alkaline pyrogallol in order to prevent any oxygen diffusing back into the chamber.

Erlenmeyer flasks; glass tubing; and rubber tubing and stoppers were used throughout to connect up the apparatus. The chamber was generally kept on the movable stage of the microscope where the reactions of the ciliates could be closely observed.

Overpage is a diagrammatic representation of the apparatus.

ANAEROBIC APPARATUS

Anaerobic Apparatus: Diagrammatic representation showing the purification of the nitrogen obtained from a commercial cylinder. The hanging drop chamber was normally mounted on the stage of the microscope. Much the same apparatus was used to test the effect of oxygen and carbon dioxide. These gases were also obtained from commercial cylinders but were simply bubbled through distilled water before and after being passed through the hanging drop chamber.

Material

1. The fresh water ciliates were taken from fresh samples collected from reservoirs, ponds and streams.
2. The soil ciliates tested were taken from laboratory cultures, generally yeast extract.
3. Vorticella microstoma was obtained directly or indirectly from the anaerobic meat digester.
4. Metopus sp. came from an old laboratory infusion of soil and vegetable matter.

Results

Nitrogen was used to replace the oxygen in the chamber and maintain the ciliates in an oxygen-free atmosphere. Some difficulty was experienced both in maintaining the chamber in an oxygen-free state and in securing reproducible results. It was found that if the ciliates were transferred to the chamber immediately after the preparation of the hanging drop and the oxygen removed death would occur whereas if the drop was left for a little while (say over night) better survival could be obtained. Also slow replacement of the oxygen secured better survival than rapid replacement.

Nevertheless the results clearly indicated that facultative anaerobiosis under the conditions of the experiment was limited to only some ciliates, and these included the soil ciliates, whereas many typical fresh water species survived only a very short time after the removal of oxygen although recovery could be obtained if oxygen were reintroduced. The results are summarised in Table 13.

One difference between the tests with fresh water and soil ciliates was that the latter were free of any photosynthetic organisms whereas these were occasionally present in the hanging drop preparations of the fresh water ciliates and Paramecium bursaria has of course a photosynthetic symbiont. A trace of oxygen may therefore have been evolved by photosynthetic activity in some of these experiments.

Table 13

Survival of Ciliates under Anoxic Conditions

Ciliate (Fresh Water)	Survival Time (Hours)	Comment
<u>Coleps hirtus</u>	124	No marked adverse effects
<u>Lionotus sp.</u>	48	"
<u>Lionotus fasciola</u>	24	"
<u>Chilodonella sp.</u>	48	"
<u>Paramecium bursaria</u>	124	"
<u>P. aurelia</u>	76	Variable results
<u>P. caudatum</u>	72	
	113½	With <u>Chlorella</u>
<u>Glaucoma scintillans</u>	20	
<u>Cinetochilum margaritaceum</u>	76	Variable results
<u>Cyclidium glaucoma</u>	124	
<u>Stentor roeseli</u>	24	Detached and free-swimming
<u>Halteria grandinella</u>	24	Usually toxic within 1 hour
<u>Oxytricha pellionella</u>	113½	With <u>Chlorella</u>
<u>Stylonychia mytilus</u>	1	Becomes toxic very rapidly
<u>Euplotes patella</u>	48	
<u>E. aediculatus</u>	22	Few ciliates tested
<u>Vorticella campanula</u>	2	Forms telotroch, becomes detached from stalk and soon toxic

Ciliate (Soil and Sewage)	Survival Time (Days)	Comment
<u>Trichopelma sphagnetorium</u>	24	
<u>Colpoda steinii</u>	7	Encysts on exhaustion of food
<u>C. inflata</u>	3	"
<u>C. cucullus</u>	3	"
<u>Cyclidium glaucoma</u>	1	Did not survive with <u>Trichopelma</u>
<u>Metopus sp.</u>	7	No adverse effects
<u>Vorticella microstoma</u>	2	Forms telotroch

1. Reactions of Fresh Water Ciliates

The maximum survival time of the fresh water ciliates tested is given in Table 13. The results of some typical experiments are given below.

A hanging drop containing Paramecium bursaria, Coleps hirtus, Cyclidium glaucoma and Cinetochilum margaritaceum was placed in the chamber for one hundred and twenty four hours. Cinetochilum, of which there was only one individual, died. No Paramecium died but a few Coleps and Cyclidium.

A hanging drop containing Stylonychia mytilus, Glaucoma scintillans, Chilodonella sp., Vorticella campanula and V. convallaria, was placed in the chamber. Stylonychia was dead in little over an hour and the vorticellids were very toxic and survived little longer. Glaucoma died in twenty hours and only one Chilodonella out of thirty five survived forty four hours. A Lagynophrya sp. and Peranema trichophorum survived forty eight hours.

A hanging drop containing Stylonychia mytilus, Stentor sp., Lionotus fasciola, Lionotus sp., Chilodonella sp., and Cinetochilum margaritaceum were placed in the chamber. Stentor reacted by detaching itself from the substrate and becoming free swimming. It soon became toxic and died. Except for Lionotus sp. and Cinetochilum no ciliate survived twenty hours.

A hanging drop containing Enchelys sp., Chilodonella sp., Halteria grandinella, Stylonychia mytilus, and Lagynophrya sp. was placed in the chamber. Within an hour Halteria and Stylonychia were dead and Lagynophrya alone survived for six hours.

A hanging drop with Paramecium aurelia, and Lionotus sp. was placed in the chamber. The single Paramecium died within the first twenty four hours.

A hanging drop containing Paramecium aurelia, Lionotus sp., and Cyclidium glaucoma was placed in the chamber. The ciliates survived forty-eight hours.

A hanging drop containing three Paramecium aurelia, one Euplotes aediculatus, about fifteen Cinetochilum and three rotifers was placed in the chamber. The Euplotes was slightly toxic after six hours. The Paramecium and Cinetochilum survived seventy six hours.

A hanging drop containing two Stentor roeseli, one Paramecium bursaria, nine Cinetochilum, about twenty Halteria grandinella, nine Cyclidium glaucoma, four Chilodonella sp. and three Lionotus fasciola was placed in the chamber. After one and a quarter hours one Stentor was free swimming and after seventeen hours was dead. The second became free swimming after twenty six hours. The drop was returned to the air and the Stentor resettled. The other ciliates survived except for Halteria which was toxic within an hour and did not survive twenty four hours.

A hanging drop containing two Euplotes patella, Chilodonella, Cyclidium, and Cinetochilum was put in the chamber. After forty eight hours the two Euplotes were still alive.

Summary

The reactions of the fresh water ciliates tested show that the ciliates fall into two groups:

1. Those that blistered within a few hours, viz. Stylonychia mytilus, Halteria grandinella, Vorticella campanula and V. convallaria.

2. Those that generally survive for many hours, e.g.

Coleps hirtus, Cyclidium glaucoma and Cinetochilum margaritaceum.

The two Vorticella spp. although they blistered very quickly first reacted by detaching themselves from their stalks. Due to the rapidity with which they blistered however normal telotroch formation was interrupted by death.

The reaction of Stentor, another sessile ciliate, is in many ways similar. Stentor roeseli, the species studied, lives within a gelatinous tube attached at its base to the substrate. The ciliate reacts to lack of oxygen by detaching itself from its base and from its gelatinous tube and swimming freely in the drop. Locomotion is achieved by the same ciliary action which normally causes current of particles to stream towards the peristome. While swimming Stentor rotates slightly on its long axis. The period of survival of the free swimming form is limited perhaps partly by the inhibition of feeding, as the individual in the present experiments which became detached twenty four hours later did not blister.

ii Reactions of Soil Ciliates

Colpoda

The three species tested were C. steinii, C. cucullus, and C. inflata. They were cultured in yeast autolysate free of other protozoa.

Colpoda steinii

The ciliates become progressively more sluggish with slower ciliary movement when kept in the nitrogen chamber. When food is present they feed and increase in size. When there is no food they encyst. Temporary or unstable cysts are occasionally formed. Division is absolutely inhibited and so is excystment of resting cysts.

Inhibition of Division in Colpoda steinii

Hanging drops were prepared of ciliates washed and suspended in fresh yeast extract.

<u>A.</u>				
Time(Hours)	0	21	25	
Control	1	1 active,	1 div.cyst	5 active
Nitrogen	1	1		1
<u>B.</u>				
Time(Hours)	0	5.30	29.30	98
Nitrogen	3	3 sluggish	3 very sluggish & large	3
Control 1.	1	1	5 active	
Control 2.	2	2	c.10 active	
<u>C.</u>				
Time(Hours)	0	30	53.30	77.30
Control	2	3	5,2 div.	c.40 active, 2 resting cysts
Nitrogen	1	1 cyst	1 active	1 active

These experiments show conclusively that division is inhibited in the absence of oxygen, a fact established for ciliates by Thomas (1942) and Watson (1944), and known to be true for a wide variety of organisms and tissues (Medawar, 1947). The ciliates continue to feed and consequently may increase in size, e.g. Experiment B. In Experiment C the bubbling of nitrogen slowed down and stopped within the first thirty hours and the ciliate encysted. The bubbling was recommenced and the ciliate excysted and continued active in the drop. After eighty hours the bubbling again slowed down and stopped. It was not recommenced and the ciliate consequently began dividing. Similarly in Experiment B, the three ciliates which had been kept in nitrogen for ninety eight hours were returned to air and within two and a half hours had formed division cysts. Division continued and after forty six hours in air there were about a hundred active ciliates and two division cysts.

Effect on Encystment

Encystment in Colpoda is dependent upon the depletion of food and the concentration of the ciliates. Temporary and unstable cysts may be formed under other conditions. The following experiments show that resting cyst formation is independent of the oxygen tension. Ciliates placed in the chamber flushed with nitrogen and ciliates placed in the chamber flushed with oxygen and ciliates in air showed no significant difference of encystment rate. The rate of resting cyst formation however depended upon the initial concentration of ciliates.

Typical experiments are:

A hanging drop containing about four hundred active ciliates was placed in the nitrogen chamber. After twenty two hours all but twenty had encysted.

Four ciliates in a hanging drop were placed in the nitrogen chamber. After seventy hours all were encysted.

About thirty to forty active ciliates and three digenic cysts were placed in the nitrogen chamber. After sixty six hours there were none active and forty four cysts.

Effect of Oxygen Tension of Encystment and Division

Hanging drop preparations were made and put into the gas chamber. Nitrogen or pure oxygen was then passed through. Controls were kept in air.

Encystment

Typical experiments are:

A. Time (Hours)		0	5 $\frac{1}{2}$	% Encystment	
Control	Active	94	21	70	
	Cysts		73		
Control	Active	125	15	88	
	Cysts		110		
B. Time (Hours)		0	6	% Encystment	
Control	Active	101	6	95	
	Cysts				
Control	Active	29	6	80	
	Cysts	2	26		
Oxygen	Active	50	8	87	
	Cysts	11	65		
C. Time (Hours)		0	3.45	6.25	23.30 % Encystment
Control	Active	14	13	12	8 43
	Cysts		1	2	6
Oxygen	Active	25	23	19	8 68
	Cysts				17
D. Time (Hours)		0	1.45	2.35	23.45 % Encystment
Control	Active	4			4 0
	Cysts	6			6
Nitrogen	Active	12	7	7	5 42
	Cysts		3	5	5
		2 encysting		2 dead	
E. Time (Hours)		0	4.15	52.15	72.15 77.15 % Encystment
Control	Active	36			8 80
	Cysts	24		54	56
Nitrogen	Active	40	33	14	4 3 93
	Cysts	21	28	47	57 58
F. Time (Hours)		0	3.45	22.45	% Encystment
Control	Active	101	1	0	100
	Cysts		100	101	
Nitrogen	Active	43	24	3	93
	Cysts	3	22	43	
G. Time (Hours)		0	24	Division Rate	% Encystment
Control	Active	15	25	2.4	30
	Cysts		11		
Oxygen	Active	20	25	2.55	49
	Cysts		26		

These experiments show that the rate of encystment is dependent upon the concentration of the ciliates and not the gas phase in which they are suspended. They also show little difference in the division rate between ciliates suspended in pure oxygen and those suspended in air. There appears to be a slight increase in division rate of the ciliates in pure oxygen which agrees with the findings of Pace and Ireland (1945) for Tetrahymena pyriformis.

Effect on Excystment

Brown (1939) showed that excystment was dependent upon the presence of oxygen. This fact was confirmed in the present work.

Typical experiments are:

Cysts were washed and suspended in hanging drops with a loop of bacteria. One was placed in the nitrogen chamber, others were used as controls. After three and a half hours there was complete excystment of the controls. In the nitrogen chamber there were only 4 active ciliates out of 150 to 200 cysts. After twenty three hours there were only 18 active ciliates.

In a second case two control cysts excysted within the first five hours. There were 3 active ciliates in the nitrogen chamber after twenty three hours and about 15 cysts. There were 2 active and about 15 cysts still after forty six hours.

Using the alcohol-phosphate excysting medium (Strickland and Haagen-Smit, 1948) instead of bacteria about 500 to 600 cysts were suspended in a hanging drop for twenty hours in the nitrogen chamber. At the end of this period there was one active ciliate. After four hours in air there were about 300 active ciliates, i.e. 50% excystment.

Following the interesting discovery that an impure nitrogen stream stimulated excystment in Vorticella and Oxytricha, cysts of C. steinii were subjected to the same conditions.

Following is the result:

A hanging drop with 13 active ciliates and about 120 cysts was placed in the chamber and impure nitrogen turned on. After three hours there were 14 to 15 active and 20 cysts activated. After five hours there were 50 active, 5 activated cysts and 77 unactivated cysts. After twenty three hours there were about 41 active ciliates and 89 cysts. The nitrogen was turned off after twenty four hours and oxygen turned on. Later there were 14 active and about 100 cysts.

This experiment seems to confirm the findings with Vorticella microstoma and Oxytricha pellionella. The cysts appear to be activated by reduced oxygen tensions.

Colpoda inflata

The reactions of this ciliate are similar to those of C. steinii.

In one experiment 10 active ciliates were suspended in a hanging drop in the nitrogen chamber and 12 in a control. There were no cysts. A day later there were still 10 active in the nitrogen chamber and no cysts, and 20 active, 6 cysts and one dividing cyst in the control. A day later the nitrogen chamber had 5 active and 5 cysts; the control had 20 active and 10 cysts. A day later there were still only 5 active in the nitrogen and 8 active and 30 cysts in the control. Six days after the commencement

of the experiment there were 9 active in the nitrogen chamber and no cysts and 5 to 6 active in the control and about 30 cysts. A week after the commencement of the experiment there were 7 active and 1 cyst in the nitrogen chamber and 7 active and 30 cysts in the control.

In a second experiment 3 ciliates were suspended in a hanging drop preparation together with phosphate buffer and live yeast. After twenty two hours one of the ciliates had encysted; the other two encysted within sixty six hours. The hanging drop was then placed in air and within twenty four hours the ciliates had excysted. They were then replaced in the nitrogen chamber where however they did not again encyst but after seventy hours died. By this time the ciliates were much larger than they had been originally.

Colpoda cucullus

Although the reaction of this species is the same as the two preceding species it does not divide so rapidly under the conditions of culture and consequently the contrast between the nitrogen and control is not so great.

In one experiment there were initially 13 C.cucullus and 1 C.inflata in the nitrogen chamber and about the same number of C.cucullus and 1 C.inflata in the control. After twenty four hours C.inflata had encysted in both chambers but there were no C.cucullus cysts. After forty eight hours there were 11 cysts of C.cucullus and 2 active in the nitrogen chamber and 13 to 14 active with no cysts in the control. After three days there were no active ciliates in the nitrogen chamber; there were 12 C.cucullus cysts and 1 C.inflata cyst.

In the control there was 1 active, and 15 encysted C.cucullus and 1 encysted C.inflata. At the end of the fourth day there were no active ciliates in either drop: each had 1 C.inflata cyst and there were 12 encysted C.cucullus in the nitrogen and 14 in the control

Trichopelma sphagnetorium
Colpoda inflata
Cyclidium glaucoma
Mayorella vespertilio

A hanging drop containing these four species of soil protozoa was placed in the nitrogen chamber and remained there for twenty four days. This was the longest experiment made. Initially there were 27 active Trichopelma, 21 C.inflata and 14 Cyclidium. The Cyclidium both in this experiment and in a similar experiment died within twenty four hours. Trichopelma survived the entire period but there were only 13 active on the twenty fourth day. On the third day most of the C.inflata encysted and on the twenty fourth day there were only 6 active and 8 cysts.

Mayorella vespertilio survived for twelve days there being initially four amoebae. The amoebae showed slight movement and did not encyst. A comparable experiment with Trichamoeba sp. which lasted for twenty four hours showed survival and protoplasmic streaming, with no encystment. Hogue (1921) reported encystment of amoebae under anoxic conditions, but there appears to be no other evidence. The present experiments appear to demonstrate that encystment is not the general rule.

Vorticella microstoma

The reactions of this ciliate to suddenly reduced oxygen tensions or complete oxygen lack are quite definite. The trophic ciliate forms a posterior ciliary wreath, detaches itself from its stalk and becomes a free swimming non-trophic telotroch. The telotroch will not settle unless a trace of oxygen is present. The cysts are activated by suddenly decreased oxygen tensions and many excyst.

Following are some typical experiments:

A hanging drop containing 5 trophic and one dividing ciliate was placed in the nitrogen chamber. Within half an hour the first telotroch had formed and a second was being formed. Within four hours all 7 ciliates had formed telotrochs. After twenty four hours, 3 telotrochs were still free-swimming, 4 were stationary and attempting to settle. After thirty hours all 7 were attempting to settle. After forty eight hours only 4 viable telotrochs remained. These were still attempting to settle but no stalk or peristome was formed and there was no pulsation of the contractile vacuole. The drop was taken from the chamber after fifty hours and within an hour and a half one of the ciliates had formed a peristome and was feeding.

A hanging drop of 10 trophic ciliates was placed in the nitrogen chamber. Nitrogen was bubbled through for forty minutes. The ciliates stopped feeding and 1 telotroch was formed. The nitrogen was stopped for two hours and all the ciliates became trophic. The nitrogen was recommenced and after an hour they had all formed telotrochs. The nitrogen was again stopped. The ciliates settled and recommenced feeding and after sixty six

hours there were about 300 trophic ciliates in the chamber. The nitrogen was recommenced and after two hours there were about 120 telotrochs and after six and a half hours about 150. After twenty four hours there were still about 100 telotrochs and no trophic ciliates. The nitrogen was stopped and within two and a half hours all the telotrochs had settled and were normal trophic forms.

The effect of lowered oxygen tensions upon starved vorticellids was also tested. The apparatus developed a slight leak and a trace of oxygen entered the chamber. The reaction of the trophic and encysted forms and of the encysted forms of Oxytricha pellionella also suspended in the drop are given below.

A.				
Time (Hours)	0	0.30	0.45	18.30
<u>Vorticella</u>	9 trophic	telotroch formation	1 trophic 8 telotrochs c.140 excysting	c.100 trophic 2 telotrochs c.30 cysts
<u>Oxytricha</u>	0 trophic			c.45 trophic c.11 excysting
B.				
Time (Hours)	0	0.30	1.0	5.30
<u>Vorticella</u>	6 trophic	1 telotroch	c.30telotrochs	c.50 telotrochs c.200 trophic
<u>Oxytricha</u>	4 trophic			c.20 trophic

These experiments show first, that the trophic vorticellids react to a change in oxygen tension by forming telotrochs but that they become adapted to lower oxygen tensions and later settle again; secondly, that the cysts of both Vorticella and Oxytricha are stimulated by suddenly lowered oxygen tensions.

The following experiments show the effect of the different factors. A hanging drop with about 2000 cysts and about 20 trophic vorticellids plus a loop of bacteria was placed in the chamber and the nitrogen turned on. There was still a slight leak in the apparatus so that a trace of oxygen was present. Within 70 minutes there were about 100 trophic vorticellids and about 50 cysts remaining. The rest were all telotrochs. After three hours the majority of the telotrochs had settled and there were only about 100 telotrochs. The nitrogen was turned off after three hours ten minutes and fifty minutes later, when there was still about 100 unsettled telotrochs, the chamber was flushed through with carbon dioxide for ten minutes. Two hours later there were about 300 telotrochs and three and a half hours later about 1000 telotrochs, the other vorticellids having succumbed to the carbon dioxide. Twenty hours later there were still about 1000 telotrochs and no trophic forms. The chamber was then flushed through with the impure nitrogen thus removing the carbon dioxide and bringing a trace of oxygen. Within an hour all but 10 of the telotrochs had settled. The experiment was ended.

In a similar experiment, a fresh drop of about 28 trophic vorticellids was placed in the chamber and impure nitrogen bubbled through. Within twenty minutes posterior ciliary wreaths were being formed and within forty five minutes there were about 18 telotrochs formed. After an hour there was one settled and the rest still telotrochs. After two and a quarter hours, 6 had settled and after twenty one hours there were about 55 settled and dividing ciliates and about 8 telotrochs. The nitrogen was

turned off and carbon dioxide turned on for quarter of an hour. Two hours later there were about 60 telotrochs and 3 forming. Oxygen was then run through and 66 were settled, 1 was settling and 1 was about to settle within an hour.

Discussion

These experiments demonstrate quite clearly that in the absence of oxygen Vorticella microstoma does not encyst, as suggested by Brand (1923), but invariably forms a telotroch. This telotroch does not settle again unless oxygen is present. As it cannot feed the survival of the telotroch is limited and two days in a nitrogen atmosphere appears to be the maximum survival time. This is much longer however than the survival of the microgamete which cannot metamorphose into a trophic vorticellid and dies within five hours unless it fuses with a macrogamete (Finley, 1936). Brand (1946) records that Vorticella nebulifera also forms a telotroch in response to oxygen lack, and settles only when oxygen is present. Vorticella is therefore similar to Stentor in this respect, viz. that it is a sessile ciliate which becomes free swimming in response to oxygen lack and settles again only in the presence of oxygen. The ecological significance of this mechanism is obvious and is stressed by Brand (1946). Conversely it is possible with this information to criticise the records of Kolkwitz and Marsson (1909) who report V. microstoma in the absence of oxygen. The presence of a trophic vorticellid is adequate proof that oxygen must be present even if in very small quantities. The same criticism holds for Stentor recorded by a number of workers from 'oxygen-free' habitats.

The effect of carbon dioxide is dealt with more fully in the next section but it is enough to notice here that V.microstoma also forms a telotroch in response to high carbon dioxide tensions.

The effect of lowered oxygen tensions on the cysts of both V.microstoma and O.pellionella is interesting and is discussed later. It is apparent that the lowered oxygen tension definitely stimulates excystment although the mechanism of the stimulus is obscure.

One point worth noting is the fact that in response to a sudden fall in oxygen tension the vorticellids formed telotrochs but with a trace of oxygen present they later resettled. This indicates that the effect of lowered oxygen tension is relative and not absolute save only in the case when oxygen is altogether excluded. The vorticellids which at first respond to a sudden drop in oxygen tension by becoming telotrochs become adapted to the lower oxygen tension and form trophic vorticellids. This is an important demonstrable instance of ciliate adaptation to a changing environment. It is obvious that the telotroch-forming mechanism is not set off by a particular oxygen tension but by a sudden change in oxygen tension or by the complete absence of oxygen. A comparable mechanism is the excystment of Colpoda which is similarly affected by different salinities (Chapter V). The present evidence strongly supports the view of Juday (1908) and Brand (1946) that the differences between experimental and ecological evidence may be explained in terms of gradual adaptation under natural conditions.

Metopus sp.

Because it had been found that division in facultative anaerobes is inhibited by anoxia, experiments were carried out with this typical sapropelic ciliate to test the effects of anoxia on division in obligate anaerobes.

Hanging drops containing active Metopus were placed in the nitrogen chamber and in a control. After a week there were 10 ciliates in the control and 15 in the nitrogen chamber. Three of these ciliates were washed and resuspended with a loop of bacteria. After a week in the nitrogen chamber these three ciliates had not divided although they appeared quite normal and slightly increased in size. No dividing ciliates in fact were observed in either the control or nitrogen chambers.

The failure of these experiments, or rather their inconclusiveness, may have been due to the provision of an unsuitable bacterial strain as food or it may be that Metopus is not a bacteria-feeder at all (Sandon, 1932). Lackey (1938b) recorded good growth of Metopus in the absence of oxygen which was the point intended to be demonstrated in these experiments.

Summary

Colpoda and Trichopelma are facultative anaerobes in that they survive without adverse effects in an atmosphere devoid of oxygen. They feed but cannot divide. Rarely, Colpoda encysts in the presence of food to form a temporary or perhaps unstable cyst, one that excysts without special stimulus. Normally resting cysts are formed on the exhaustion of food. The resting or resistant cysts do not excyst in the absence of oxygen but are stimulated to excyst by lowered oxygen tensions.

Vorticella microstoma forms a telotroch in the absence of oxygen or in response to lowered oxygen tensions or high carbon dioxide tensions. It will not settle unless oxygen is present but will become adapted to lowered oxygen tensions. In the complete absence of oxygen the telotroch survives about fifty hours. Cysts are stimulated to excyst by lowered oxygen tensions.

Metopus was not observed to divide in the presence or absence of oxygen although the experiments lasted a week.

B. THE EFFECTS OF HIGH CARBON DIOXIDE TENSIONS

Introduction

The most comprehensive account of the effect of pure carbon dioxide on ciliate protozoa is that given by Jacobs (1912) in a paper that has been generally overlooked. This author also published a number of papers on carbon dioxide narcosis, the most important of which is that of 1922. Watson (1944) studied the effect of pure carbon dioxide on two ciliates, Balantiophorus minutus and Paramecium caudatum using apparatus identical with the present. Root (1930) and Pace and Ireland (1945) studied the effect of carbon dioxide tension on Paramecium caudatum and Tetrahymena pyriformis respectively.

The response of the ciliates is at first negative. They migrate from the edges of the hanging drop into the centre where the concentration of carbon dioxide is least. Within a few minutes the drop is saturated and the ciliates then swim freely in the drop. As narcosis takes place the ciliates swim more and more slowly and the body becomes more rounded. Finally they cease to move although the cilia may still beat freely. At this stage recovery may be obtained by returning the ciliates to air. Finally there is no further movement and the ciliates blister. This description drawn from the reactions of Paramecium fits most ciliates. Those that are very sensitive succumb immediately while those that are very resistant will continue to swim slowly but not feed for many hours. In Vorticella the cilia cease to beat almost immediately.

The effect of the carbon dioxide is first stimulatory, marked by acceleration of swimming, and then depressive. The cytoplasm first liquefies and then coagulates. As coagulation of the cytoplasm is the prelude to death it occurs earlier in the sensitive species and later in the more resistant species. Oxygen consumption is first stimulated and then depressed (Root, 1930).

Chang and Loomis (1946) after discussing carbon dioxide narcosis conclude that it results from rapid penetration, increases in cellular activity and the formation of specific but weakly bonded protein compounds.

Jacobs (1912) noted three factors that affected his experimental results. First, the rate of bubbling of carbon dioxide: longer survival was associated with slow bubbling and shorter survival with rapid bubbling. Secondly, temperature: survival being about twice as long at 12°C as at 22°C. Thirdly, the size of the drop: death occurring rapidly in small drops and more slowly in medium and large drops. He found further that there was great variation in the different experiments and that different clones and different cultural conditions affected the results. Old cultures and laboratory clones showed greater survival than fresh cultures or wild strains. However for any particular drop if there was exceptional survival for one species there was exceptional survival also for other species so that the conditioning factor lay in the suspension fluid. In the present experiments ciliates from fresh cultures survived better than ciliates from old cultures.

The period of survival of the different ciliates varied between almost instantaneous death (Coleps birtus) to prolonged survival

(Colpidium colpoda). In this latter case the ciliates survived up to a week in very high carbon dioxide concentrations moving slowly but not feeding. The longest survival in the present experiments was 24 hours by Colpoda steinii in an oxygen-carbon dioxide mixture. In this case movement was slow and the ciliates did not feed or divide.

The ecological significance of the experiments is apparent. Jacobs' results (in Table 14) show that four ciliates survive more than half an hour on the average and four less than half an hour, one dying within a few minutes. The more resistant species are all recorded by Noland (1925a) from samples rich in carbon dioxide and poor in oxygen and from laboratory cultures. None of the other four species are so recorded. The four resistant species may be termed then typical polysaprobies and the others mesosaprobies, the first three b-mesosaprobies and the last an a-mesosaprobe.

The relative sensitivity of oligo - and mesosaprobic species to carbon dioxide is an important distinction closely correlated with a greater sensitivity to anoxia. Rossbach (1872) found that Euplotes, Stylonychia and Chilodonella were completely killed by carbon dioxide in three minutes. Prowazek (1903) found that Colpidium survived anoxia better than Paramecium caudatum.

Jennings and Moore (1901) found that certain protozoa were attracted by carbon dioxide (including Paramecium caudatum, Colpidium colpoda and Chilomonas paramecium) while others were indifferent (Euplotes and Euglena). This is of interest for Pace and Ireland (1945) showed that Chilomonas paramecium grew

best at a carbon dioxide tension of 100mm.Hg and both Paramecium caudatum and Colpidium colpoda are found only or chiefly in association with rich bacterial flora. Faure-Fremiet (1950) has observed that Colpidium colpoda and C. campylum are attracted chemotactically by the slightly acid conditions of a Beggiotia colony. It can be seen therefore that not only is there a gradation of sensitivity to carbon dioxide but that the polysaprobic species are actually attracted by acid conditions and it therefore explains why they are rapidly associated with bacterial degradation.

The fact that these ciliates respond negatively to very great concentrations of carbon dioxide indicates clearly an ability to select the optimum conditions in the same manner as Spirostomum ambiguum selects the optimum pH and Loxodes rostrum the optimum oxygen tension. The optimum is both absolute and relative; absolute in that a certain concentration is invariably toxic, relative in that the ciliates can be slowly acclimatized to increased concentrations (Jennings, 1915).

It is also interesting to note that in the Beggiotia association Coleps hirtus appears as a detritus feeder only after the destruction of the colony.

Summing up it can be said that not only are the polysaprobic tolerant of high carbon dioxide tensions but that they select such conditions whereas mesosaprobic and oligosaprobic species are sensitive to such conditions.

Table 14

Survival of Ciliates in Pure Carbon Dioxide

<u>Species</u>	<u>Survival time</u>
<u>a. After Jacobs (1912)</u>	
<u>Colpidium colpoda</u>	6 hours to several days
<u>Blepharisma lateritia</u>	3 to 6 hours
<u>Paramecium caudatum</u>	20 minutes to 3½ hours (avg. 3 hrs)
<u>Vorticella nebulifera</u>	45 minutes
<u>Paramecium aurelia</u>	10 minutes to 2 hours (avg. <30mins)
<u>Euplotes patella</u>	< 30 minutes
<u>Paramecium bursaria</u>	5 to 60 minutes (avg. 10 to 20 mins)
<u>Coleps hirtus</u>	< 10
<u>b. After Watson (1944)</u>	
<u>Balantiophorus minutus</u>	25 to 30 minutes
<u>Paramecium caudatum</u>	72 minutes

While the present experiments generally confirm Jacobs' results especially with regard to Coleps hirtus there is one important difference, viz. Colpidium colpoda which was found in the present experiments to be relatively sensitive whereas it was the most resistant species recorded by Jacobs. Hetherington (1933) however points out that Colpidium colpoda is comparatively rare and that until the publication of a paper by Bresslau (1922) C. campylum was frequently called C. colpoda. Hetherington found that, unlike C. campylum, C. colpoda did not tolerate extremely acid conditions and was not found in laboratory infusions at the most intense period of bacterial degradation. It was more sensitive than the smaller species to bacteria. I am inclined to think therefore that Jacobs' Colpidium was C. campylum and not C. colpoda and this would agree better both with Hetherington's work and the present experiments.

Methods

Similar to the preceding experiments except that carbon dioxide was used instead of nitrogen or a mixture of carbon dioxide and oxygen. The latter was arranged by bubbling oxygen and carbon dioxide from the cylinders at approximately equal rates into a jar from which a lead passed to the chamber. With pure carbon dioxide the anaesthetic or narcotic effect of the carbon dioxide is combined with the concomitant effect of absence of oxygen. The mixed gas removes this second factor and therefore tests only the effect of high carbon dioxide tensions.

Results

The effects parallel those with nitrogen. Soil species proved exceedingly resistant and fresh water species generally not so resistant.

1. Reaction of Fresh Water Ciliates

The maximum survival time of fresh water ciliates both in pure carbon dioxide and in a 1:1 mixture of carbon dioxide and oxygen is given in Table 15. The ciliates fall into three groups. First, sensitive species, including Stylonychia, Stentor, Vorticella campanula, Lembadion bullinum, Coleps and Euplotes aediculatus. These succumbed very rapidly to the carbon dioxide even when mixed with oxygen. Stylonychia in particular reacting very quickly as with anoxia. Stentor likewise reacted by detaching itself from its base. Secondly, moderately sensitive species, viz. Glaucoma, Colpidium, Cinetochilum, Cyclidium, Paramecium bursaria and Podophrya. These ciliates did not survive more than quarter of an hour in the pure carbon dioxide atmosphere. Thirdly, the resistant species which survived over a quarter of an hour in pure carbon dioxide. These include Lacrymaria, Lionotus, Paramecium caudatum, Euplotes patella, Aspidisca costata and V. striata.

Typical experiments are given below:

A hanging drop containing two trophic Vorticella nebulifera was placed in the chamber and carbon dioxide bubbled through for fourteen minutes. The drop was then placed in air and within an hour both ciliates had recovered.

Five Paramecium aurelia were placed in the carbon dioxide chamber for twenty minutes and 4 recovered when returned to air. In a second test, 2 ciliates left for forty minutes blistered. There was no recovery in air. With 3 Paramecium caudatum left for forty minutes and 4 left for seventeen minutes there was no subsequent recovery in air.

Survival of Ciliates at High Carbon Dioxide Tensions

Fresh Water Species	Survival Time (Mins.)		Comment
	CO ₂	O ₂ /CO ₂	
<u>Coleps hirtus</u>		<10	
<u>Lacrymaria olor</u>	>15		
<u>Lionotus fasciola</u>	>30		
<u>Paramecium bursaria</u>	<15	<25	
<u>Paramecium caudatum</u>	>30		
<u>Lembadion bullinum</u>	<15	<10	
<u>Glaucoma scintillans</u>	<15	<15	
<u>Colpidium colpoda</u>	<15		
<u>Cinetochilum margaritaceum</u>	<15		
<u>Cyclidium glaucoma</u>	<15	<15	
<u>Halteria grandinella</u>		<25	
<u>Stentor sp.</u>	<15		Detached from base after 10 minutes
<u>Stylonychia mytilus</u>	<15	<10	
<u>Euplotes patella</u>	>15		
<u>Euplotes aediculatus</u>	<15		
<u>Aspidisca costata</u>	>30		
<u>Vorticella campanula</u>	<15		
<u>Vorticella convallaria</u>	>15		
<u>Vorticella striata</u>	>15		
<u>Vorticella nebulifera</u>	>14		
<u>Podophrya fixa</u>	<15		

Soil Species	Survival Time (CO ₂)		Comment
<u>Trichopelma sphagnetorium</u>	3 hours		
<u>Colpoda cucullus</u>	<15 mins.		
<u>Colpoda inflata</u>	2 hours 30 minutes		
<u>Colpoda steinii</u>	2 hours 50 minutes		Old culture
	6 hours		Fresh culture
	24 hours		No feeding or div.
<u>Vorticella microstoma</u>	6 hours		Telotroch form- ation.

Hanging drops of mixed ciliate species were subjected to pure carbon dioxide: 2 Paramecium caudatum, 1 Oxytricha pellionella 3 Lagynophrya sp. for fourteen minutes - no recovery; 4 Paramecium caudatum, 4 Oxytricha pellionella, 3 Lagynophrya sp., 1 Lionotus sp. for twenty five minutes - Oxytricha toxic within five minutes, Lionotus still active, and 2 Paramecium, after being motionless, recovered in air; 2 Paramecium caudatum, 3 Oxytricha pellionella, 1 Lionotus sp., 4 Lagynophrya sp., 3 Cyclidium glaucoma for five minutes (Oxytricha and Cyclidium toxic), bubbling stopped for three minutes then slow bubbling for an hour - at first Paramecium and Lionotus slightly and then markedly toxic, no recovery of Paramecium, Lionotus recovered; 6 Paramecium caudatum, 7 Oxytricha pellionella, 11 Lagynophrya, after seventeen minutes Paramecium still active, Oxytricha toxic, Lagynophrya still active, after thirty two minutes Paramecium still active, after forty minutes still active but toxic, stopped after an hour - 4 Paramecium recovered and 2 dead.

In experiments with Stentor this ciliate after ten minutes became detached and later toxic and shortly afterwards died.

Survival in mixed oxygen and carbon dioxide was longer although Stylonychia mytilus was still extremely sensitive as were Lembadion bullinum and Coleps hirtus. Halteria grandinella on the other hand survived up to twenty five minutes under these conditions.

ii. Reaction of Soil Ciliates

The maximum survival time of soil species in pure carbon dioxide is given in Table 15.

Colpoda

The three species C.steinii, C.inflata and C.cucullus were tested as well as their cysts. C.cucullus differs from the other two species in its sensitivity to carbon dioxide. C.steinii and C.inflata are both extremely resistant. Total anaesthesia occurred in pure carbon dioxide but recovery of both species was obtained after exposures of two and a half hours. C.steinii from a well fed culture was viable after six hours in pure carbon dioxide. Perhaps the most marked contrast was in the carbon dioxide-oxygen mixture. The same atmosphere which resulted in the death of other ciliates in half an hour or less only partially anaesthetised C.steinii. Although not very active ciliary movement continued for twenty four hours. At the end of this period the ciliates, there were three in the test, were showing some signs of toxemia being inactive but return to air resulted in the recovery of all the ciliates which began to feed again and to divide. It is important to note that the high carbon dioxide tensions did not cause encystment, although they did appear to stimulate excystment.

Colpoda steinii

The survival of the ciliates depended largely upon the nutritive state, well fed ciliates surviving better than starved ones. Following are typical experiments.

A hanging drop of ciliates was placed in the chamber and pure carbon dioxide bubbled through for an hour by which time the

ciliates were showing the initial stages of dedifferentiation. The carbon dioxide was turned off and the ciliates recovered and became active. The carbon dioxide was again turned on for six hours by which time dedifferentiation had reached an advanced stage. Very little movement was observed and no recovery was obtained.

Other results are:

Material	Exposure Time	Final Condition	Recovery
Old culture	1 hr.	Dedifferentiation	Majority
	6 hrs.	Cytolysis	None
	1 hr.	Dedifferentiation	50%
	1 hr.25 min.	"	50%
	40 min.	"	50%
	2 hrs.50mins.	"	50%
	2 hrs.25mins.	Cytolysis	None
Fresh culture	6 hrs.40mins.	Some still active	25%

Cysts were kept in the chamber for twenty four hours and thirty minutes and then taken from the chamber and fresh yeast extract added. Excystment took place within four hours.

Starved ciliates were placed in the chamber and a mixture of carbon dioxide and oxygen bubbled through. There was no encystment after several hours.

Three ciliates with a loop of bacteria were placed in the chamber and the carbon dioxide-oxygen mixture bubbled through. The ciliates were still active after twenty four hours though they were moving very slowly and they had not divided. They recovered fully when returned to air.

C.inflata

The resistance of this ciliate to carbon dioxide was comparable to that of C.steinii. Only tests with pure carbon dioxide were made and these are summarised below.

Material	Exposure Time	Final Condition	Recovery
Fresh Culture	2hrs.15min.	Cytolysis	None
" "	1hr. 10min.	Dedifferentiation	50%
Old culture	50min.	Cytolysis	1 recovered (2%)
" "	1hr. 35min.	"	None
Fresh culture	2hr.	"	None
" "	2hr. 30min.	Some still active	100%
" "	1hr. 30min.	" " "	50%
" "	3hrs.	" " "	25%
Cysts (10)	22hrs.25min.		1 excystment after 48 hours.
Cysts (100)	18hrs.45min.		25% excystment after 3 days.

C.cucullus

Because this species does not grow so well on yeast extract it was cultured on lettuce infusion, which unlike the former medium was unbuffered. Tests were made both with unbuffered and buffered medium. In the latter case C.steinii was used as a control. In both cases C.cucullus was far less resistant to carbon dioxide than the other two species and indeed resembled more closely typical fresh water species.

Following are the results:

Material	Exposure Time	Final Condition	Recovery
Fresh culture	10 min.	Cytolysis	None
" "	13 min.	"	"
Ditto 10 active & cysts	7½ min.	"	1 excystment after 2 hrs.
Ditto 50 active	15 min.	"	None of active ciliates
Ditto 7 active & 9 cysts	11 min.	"	"
Ditto 10 active & 1 cyst	11 min.	"	"

In these experiments there appeared to be some evidence that the carbon dioxide stimulated excystment.

Buffered lettuce infusion with C.cucullus and C.steinii

Ciliates		Time Exposed	Recovery
<u>C.cucullus</u>	10 active & cysts	11 minutes	3 excysted 2 still viable
<u>C.steinii</u>	3 active		
<u>C.cucullus</u>	6 active	12 minutes	- Still viable
<u>C.steinii</u>	20 to 30 active		
<u>C.cucullus</u>	3 active	18 minutes	- Still viable
<u>C.steinii</u>	20 active		
<u>C.cucullus</u>	5 active	25 minutes	- Still viable
<u>C.steinii</u>	20 active		

In all these experiments there was cytolysis of all the trophic C.cucullus after nine minutes, C.steinii alone showed recovery. The only exception were the cysts of C.cucullus in the first experiment which recovered and were apparently stimulated to excyst. The mechanism of the stimulus is obscure and will be discussed later.

Trichopelma sphagnetorium

Hanging drops of this ciliate and Colpoda inflata were placed in the carbon dioxide chamber and pure carbon dioxide passed through for one and three hours respectively in two separate tests. About 30 Trichopelma recovered from the short test and 6 from the longer test.

About 100 Trichopelma and C.inflata were subjected to pure carbon dioxide for one hour twenty five minutes and the drop left in the chamber a further forty five minutes after the gas had been turned off. One Trichopelma and two C.inflata recovered.

Vorticella microstoma

Some results with carbon dioxide and this ciliate have been recorded in the previous section where it was shown that flushing out the oxygen either with nitrogen or carbon dioxide caused telotroch formation. Moreover the telotroch proved very resistant to the high concentrations of carbon dioxide surviving twenty hours in a pure carbon dioxide atmosphere, or one with very high concentrations of this gas.

Typical experiments with pure carbon dioxide are recorded in an earlier section. Experiments with oxygen-carbon dioxide mixture are given here:

A hanging drop containing 6 Oxytricha pellionella and 26 trophic vorticellids and many cysts was subjected to pure oxygen for an hour. There was no change in either the encysted or trophic forms with the exception of a single vorticellid at the margin of the drop which formed a telotroch as the edge of the drop dried. The oxygen-carbon dioxide mixture was then bubbled through for twenty minutes. There was no excystment or telotroch formation but the ciliates became very toxic and the flow of carbon dioxide and oxygen was therefore stopped. Forty minutes later there were 6 telotrochs, the other ciliates were still very toxic, and after an hour and twenty five minutes there were no telotrochs, all the ciliates being either settled and feeding or toxic and dead. Even in the pure oxygen stream some of the ciliates appeared very toxic and inactive and failed to recover from the initial treatment with carbon dioxide. Pure carbon dioxide was then bubbled through the chamber for some minutes.

After twenty minutes there were 11 telotrochs; after 30 minutes 20 telotrochs and no trophic ciliates. A trace of oxygen was let in but the ciliates were left in a predominately carbon dioxide atmosphere for seventeen hours when there were still 35 to 40 telotrochs. The experiment was then stopped.

Further experiments subjecting trophic vorticellids to mixed oxygen-carbon dioxide atmosphere for half an hour generally showed no telotroch formation but narcosis and death of the trophic ciliates. However in one experiment under such conditions about 12 trophic vorticellids were placed in the chamber and after three hours in the carbon dioxide oxygen atmosphere they had formed telotrochs. The resistance of the trophic ciliates is much less than that of the telotrochs which survive many hours in high carbon dioxide tensions.

Generally then Vorticella microstoma responds to high carbon dioxide tensions by forming a telotroch but if it fails to form a telotroch the trophic ciliate succumbs fairly quickly, within half an hour. The telotroch however may survive twenty hours or more in an almost pure carbon dioxide atmosphere. With high carbon dioxide tensions it is difficult to separate the effect of the high carbon dioxide tension from the effect of lowered oxygen tension but it appears as if both stimulate telotroch formation. Neither stimulate encystment.

It is interesting to note that in one instance a trophic ciliate formed a telotroch in response to dessication. Again telotroch formation and not encystment is the rule (Brand, 1923).

Summary

Most fresh water species tested survive less than quarter of an hour in pure carbon dioxide. Stentor reacts by becoming free swimming. Coleps hirtus, Lembadion bullinum and Stylonychia mytilus are particularly sensitive even to carbon dioxide-oxygen mixture. Paramecium caudatum is the most resistant of these species, recovery being obtained after forty minutes exposure to pure carbon dioxide.

Colpoda steinii and C.inflata are extremely resistant. Recovery is obtained after two, three and even six hours exposure to pure carbon dioxide. C.steinii is relatively little affected by carbon dioxide-oxygen mixture although it does not divide in such an atmosphere. The cysts of these species are also resistant surviving exposures to pure carbon dioxide of twenty four hours.

Colpoda cucullus on the other hand is very sensitive succumbing in nine minutes to pure carbon dioxide. The cysts which survive longer appear to be stimulated as they occasionally excyst.

Trichopelma sphagnetorium is as resistant as Colpoda steinii and C.inflata, recovery being obtained after exposures of up to three hours of pure carbon dioxide.

Vorticella microstoma generally reacts to increased carbon dioxide tensions by forming a telotroch. When formed the telotroch is much more resistant than the trophic form to high carbon dioxide tensions surviving seventeen hours or more in an atmosphere of almost pure carbon dioxide whereas trophic forms if they do not form telotrochs rapidly succumb.

C. THE EFFECT OF AMMONIA

Reaction of Colpoda steinii

In an endeavour to find a volatile substance likely to increase in concentration by concentrating the ciliates, first carbon dioxide and then ammonia were tested for their effect on encystment.

Method

A dilute solution of ammonia was placed in a petri dish and hanging drops of the ciliates were suspended in glass rings in the petri dish so that the volatile ammonia would diffuse into the culture fluid.

Results

C.steinii was the only ciliate tested. In high concentrations of ammonia the ciliate died. In lower concentrations it first accelerated and then became adjusted. There was no encystment.

D. GENERAL DISCUSSION

The results obtained in the present experiments are generally similar to previous findings. The only species which survived indefinitely in Lindeman's experiments and which was recovered in sub-cultures was Coleps hirtus and the present experiments confirm that this species shows no marked adverse effects in the absence of oxygen. However it is highly susceptible to carbon dioxide as the present experiments and those of Jacobs (1942) demonstrate. Other species which proved capable of anoxic survival are Cyclidium glaucoma, Cinetochilum margaritaceum, Colpoda cucullus and Colpoda steinii all of which occur in soil and all of which are recorded by Noland (1925a) in the complete absence of oxygen. Other species such as Stylonychia mytilus, Vorticella campanula and Vorticella convallaria die very quickly when oxygen is removed.

The reaction of the sessile species, Stentor and Vorticella, is particularly instructive. In response both to raised carbon dioxide and lowered oxygen tensions these ciliates become detached and free swimming. However they settle quickly when normal aerobic conditions are restored. Brand (1946) recorded that V.nebulifera formed a telotroch in the absence of oxygen and settled again when oxygen was restored. This has been amply confirmed in the present work and moreover it has been shown that a marked change in oxygen tension as well as the complete absence of oxygen will cause telotroch formation in V.microstoma although this ciliate can become adapted to lower tensions and will settle again as long

as a trace of oxygen is present. It will not settle however if oxygen is absent and this proves that the report of Nikitinsky and Mudrezowa-Wyss of the anaerobic survival of V.microstoma is misleading for oxygen could not have been entirely absent if the ciliate was trophic. The same criticism applies to the record of Kolkwitz and Marsson (1909) who also recorded V.microstoma in the complete absence of oxygen. The reaction of these sessile ciliates to both carbon dioxide and anoxia helps to explain the distribution of these species in nature. Sprugel (1951) found that S.coeruleus occurred only within a limited range of pH (6.8 to 7.7), carbon dioxide concentration (3 to 24 p.p.m.), temperature (2° to 26° C.), and oxygen tension. This distribution is accounted for not only by the fact that the ciliates thrive only within a certain range of environmental conditions but also by their reaction to adverse conditions which enables them, by trial and error, to select a favourable habitat from one less favourable (Jennings, 1915, p.66). This selection together with the gradual acclimatization of species accounts very largely for their distribution in natural waters. Loxodes rostrum while not appearing or appearing exceptionally in water wholly devoid of oxygen does not appear in water with a saturation much greater than 60%. Rylov (1923) found this species in small numbers in oxygen free water but generally in water between 9.15% and 45% saturation and Noland (1925a) found it in a range of 14% to 64.3% with an average of 42.7%. Rylov found however that this species is sensitive to hydrogen sulphide which would again affect its distribution in natural waters. Generally however its distribution

is similar to that of Stentor coeruleus. Other species, such as Microthorax sulcatus, are less tolerant of high oxygen tensions. The range for this species recorded by Noland (1925a) is 0% to 16.9%, average 8.5%. Metopus sigmoides has a slightly wider range, 5.3% to 31.7%, average 15.2%. These figures explain why Lauterborn found Metopus and Microthorax only in the mud and Loxodes only at the surface, together with Paramecium caudatum and Spirostomum ambiguum. Noland's figures also confirm that Halteria grandinella and Stylonychia mytilus are more susceptible to low oxygen tensions than Cinetochilum margaritaceum and Cyclidium glaucoma. The figures given by Noland are:

Species	Range	Average
<u>Halteria grandinella</u>	16.9% to 173.6%	79.7%
<u>Stylonychia mytilus</u>	22.7% to 169.2%	70.6%
<u>Cinetochilum margaritaceum</u>	0.0% to 124.9%	66.9%
<u>Cyclidium glaucoma</u>	0.0% to 169.2%	66.0%

There is then an excellent correlation between the ecological and experimental findings. With some species, particularly the larger species such as Paramecium and Spirostomum, the differences may be attributed chiefly to the failure of the ciliate to become adapted quickly to the experimental conditions whereas in nature such adaptation is invariably gradual. Further these species are never permanently devoid of oxygen for any length of time.

One interesting point is the correlation between the experiments described in Chapter 3 and those described in the present chapter. Here again there is good correlation particularly with the results from the carbon dioxide tests. In the enrichment

cultures the three species which survived the most intense bacterial infusion were Trichopelma sphagnetorium, Colpoda inflata, and Colpoda steinii. Only the last species grew well in this culture and it proved by far the most resistant to carbon dioxide. Colpoda cucullus which failed to grow at all in this medium proved highly sensitive to carbon dioxide. Similarly in the experiment testing the effect of bacteria on a mixed fauna, Lembadion bullinum and Euplotes aediculatus both became very toxic and died. Both these species proved very sensitive to carbon dioxide whereas Vorticella striata is relatively resistant. It is not unreasonable then to conclude that an important factor affecting the protozoan fauna is the carbon dioxide concentration, associated as it generally is with increased acidity. It is this factor, rather than lack of oxygen, which appears to be of greatest significance as a selective ecological factor.

The experiments with Paramecium are of interest because of the immense amount of work that has been done on this ciliate. Unfortunately many of the experiments testing the effect of anoxia were vitiated either by the presence of algae in the hanging drop or by a small leak developing in the apparatus. It may be taken however that those experiments in which the ciliate survived for about three hours compare with the experiments of Kitching and Watson. Paramecium caudatum proved one of the species most resistant to carbon dioxide which is similar to Watson's findings. This confirms that for polysaprobic species resistance to carbon dioxide is the sine qua non. The anoxia

experiments with Paramecium give some evidence that gradual adaptation and adequate food supply are two factors which favour anaerobic survival particularly in the larger ciliates.

The experiments with Colpoda give the clearest demonstration of anaerobic survival. Adolph (1929) however found that Colpoda was killed when oxygen was suddenly taken away and further that when partly grown individuals were subjected to low oxygen tensions they finished growing and formed their division cysts several hours sooner than the controls under the oxygen tension of room air. The sizes were furthermore much smaller than would be accounted for by the shortened period of growth indicating also a lessened rate of assimilation. The smaller individuals each gave only two progeny in place of the usual four. In some experiments some of the individuals under low oxygen tension formed permanent cysts whereas no such cysts were found in the controls. Adolph cites Rhumbler (1888) who also stated that decrease of oxygen tension causes the formation of protective cysts in Colpoda. Adolph did not find any variation in oxygen consumption of Colpoda with oxygen tensions of 157 to 750 mm.Hg. but in an experiment with a tension of about 5mm.Hg. the oxygen consumption fell to 31% of its normal value.

Johnson and Evans (1941) found that Woodruffia metabolica dies within three hours of the removal of oxygen. With decreasing oxygen tensions there is first a retardation of encystment (stable cyst formation), then an inhibition of division, then a tendency to form unstable cysts (at an oxygen tension of .1%).

They also state that encystment of Woodruffia in the presence of food sometimes takes place in rich cultures which they ascribe to reduced oxygen tensions.

In the present experiments with Colpoda unstable cysts were formed on two occasions. Once a single ciliate (C.steinii) encysted when there was adequate food in the drop and then excysted without dividing, and secondly three ciliates (C.inflata) formed unstable cysts which excysted when the drop was placed in air and did not encyst again when they were returned to the nitrogen chamber. In this case also there was ample food and the ciliates continued to feed even in the nitrogen atmosphere. At no time however were resting cysts formed as described by Adolph for Colpoda and Johnson and Evans for Woodruffia. Such cysts are formed only on the exhaustion of food and their rate of formation is dependent upon the concentration of the ciliates and is independent of the oxygen tension. The formation of unstable cysts is very unusual but it may be due to a sudden, as contrasted with a gradual, change of oxygen tension. The inhibition of division and encystment is similar to the results with Woodruffia, Balantiothrus and Tetrahymena. Brown (1939) demonstrated that encystment was independent of oxygen tension above 15mm.Hg. and was completely inhibited below.

The Causes of Death in Anaerobiosis

The inhibition of respiration due to oxygen lack is not the sole nor perhaps the most important factor involved in anaerobiosis. Respiration can be inhibited by narcotics, such as cyanide, and the effect is reversible. Moreover removal of the cyanide block restores the ciliate to normal activity. It is therefore obvious that inhibition of respiration is not in itself fatal to ciliates.

Habituation, especially apparent in nature, results in greater tolerance of anaerobic conditions than sudden changes. This is in general accord with tolerance to increased or decreased salinity or temperature. It is obvious therefore that there is seldom an absolute barrier to anaerobiosis provided the ciliate is habituated, as in Lindeman's experiments, to anaerobic conditions. The fact that experimentally ciliates are more resistant in culture fluid than in buffer strongly suggests that secondary factors are of more consequence than oxygen lack.

The effect of some of these factors has been discussed in Chapter III and they will be briefly mentioned again.

Oxidation-Reduction Potentials

Falling redox potentials are almost inseparable from lowered oxygen tensions and redox potential has therefore been used as a measure of oxygen tension (Quispel, 1947a; Baker and Baumberger, 1941). Anaerobic habitats are invariably marked by strongly reducing potentials, e.g. the anaerobic digester (Chapter III). This potential is due not only to the low oxygen tension but also to redox poisoning systems such as ferrous-ferric or sulphide. It is because of these systems that habitats with

the same oxygen tension, such as lake bottoms, differ often in redox potential due to the presence or absence of such poisoning systems (Allgeier et al., 1941). It has been found that lakes similar in respect to oxygen tension but differing in redox potential have a different Chironomid fauna (Hutchinson et al., 1939). In other words the fauna is dependent less upon the oxygen tension than upon the oxidation reduction potential. This is also true of the anaerobic bacteria which can be cultured in the presence of oxygen provided the redox potential is strongly reducing (Eh - 125 mv. or more), a potential which can be obtained by using ascorbic acid (Kligler and Guggenheim, 1938; Sisler and Zobell, 1950). If the potential rises above a certain point it is presumed that the substrate is oxidised by the free oxygen and not by the bacterial enzymes. In the absence of oxygen growth can be obtained at higher potentials for then the substrate cannot react with oxygen and the metabolism of the cell is not inhibited. Conversely, with aerobic bacteria, e.g. Azotobacter (Quispel, 1947b) absence of oxygen inhibits growth and metabolism but is not lethal whereas a strongly reducing potential is lethal to the cell. The biochemical significance of the redox potential has been discussed by many authors and the redox potential of tissues and cells including protozoa measured (Needham, 1930; Thomas, 1942; Brand, 1946). These measurements give an indication of two things: first the metabolism of the organism and secondly its redox tolerance. A different rH was found for parasitic species such as Opalina and Nyctotherus cordiformis than for free-living Amoeba proteus which is to be expected from their different ecology.

It may well be then that the susceptibility of some species such as Stylonychia mytilus to oxygen lack is in reality a susceptibility to greatly reduced potential and the tolerance of the facultative anaerobes essentially a tolerance of greatly reduced potential and indicative of a metabolism which functions satisfactorily in such strongly reducing conditions.

Metabolic Wastes

The accumulation of metabolic wastes has been advanced as a possible explanation of death but considering that death in the case of sensitive species takes place within an hour or so in many cases this is not very plausible. It is true however that incompletely oxidized metabolites, such as lactic acid, do accumulate anaerobically and therefore a tolerance to such acids is essential for the prolonged survival of ciliates anaerobically. Hetherington (1933) has shown that different species vary in resistance to metabolic wastes and certainly it is the most tolerant species which best survive anaerobic conditions but, because of this tolerance, the anaerobic death of the resistant species can hardly be ascribed to the effect of these metabolites.

Food

Associated with anaerobic conditions and anaerobic habitats is a flora quite distinct from that found under aerobic conditions. Cyanophyceae and reducing bacteria are common, Chlorellae and aerobic bacteria are absent. Many ciliates are dependent upon bacteria or related organisms as a food source and they are selective in their feeding thriving only on certain floras. The absence of suitable food organisms in anaerobic habitats may

be one of the factors excluding many ciliates and conversely the sapropelic fauna may be dependent upon the bacterial or fungal flora associated with mud bottoms. In this respect it is interesting to note that there was no growth of Metopus when suspended in a thick suspension of aerobic bacteria. This may indicate that Metopus is not a bacteria feeder but a coenozoan.

in nature are increasing acidity, carbon dioxide tensions, and occasionally hydrogen sulphide, hydrogen and ammonia. Rylov (1923) showed that Loxodes rostrum was sensitive to hydrogen sulphide and the experiments of Nikitinsky and Mudrezowa-Wyss confirm the effects of these factors. They are not however generally significant under experimental conditions and it is anaerobic death under experimental conditions which is most difficult to explain.

Ecological Importance

It is apparent that anaerobic conditions obtain in the soil particularly in waterlogged soils and in those soils rich in organic matter and with a high clay content. These soils often have a large ciliate population and it is evident therefore that the ciliates are able to withstand such conditions either as cysts or free swimming forms. Soils with good granular structure, such as many of the Intrazonal and Pacific Island soils or granular litters, such as beech litters, are not liable to anaerobic conditions provided there is adequate drainage. Not only do these soils generally have the greatest ciliate fauna but they also have species not found in other soil types. This we attribute partly to the better structure and water retention of these granular soils and partly to the absence of intense bacterial fermentation causing anaerobic conditions to develop.

One of the most marked differences between the Zonal soils and most fresh water streams and ponds is this development of anaerobic conditions often accompanying bacterial fermentation and marked by increased acidity. These conditions, which develop

also in an ordinary hay infusion, are toxic to many fresh water ciliates and the ciliates which develop under such conditions are different from the common fresh water fauna (Chapter III, Noland, 1925a). The species which do develop are the species which are also found just above the mud level near the bottom of lakes or streams where anaerobic conditions are common particularly during winter (Lindeman, 1942). This fauna is largely bacteriophagous with a few carnivorous species, such as Dileptus, feeding on the bacteriophagous species (Chapter III and Noland, 1925a). In contradistinction the ordinary fresh water fauna is either carnivorous e.g. Stentor or herbivorous i.e. feeding on diatoms and flagellates (Picken, 1930; Gray, 1951, 1952). Noland's interpretation is that the fauna in each case is determined by its food requirements, a thesis also supported by Picken and Gray, and for this reason the diatom-feeders are associated with the diatom flora and the bacteriophagous species with bacterial infusion. However most species have a comparatively wide food range (Sandon, 1932) and the exclusion of most fresh water species from bacterial infusions is due not only to the absence of suitable food but also to the adverse conditions which develop. That this is so is amply borne out by the present experiments. Soil species generally show a marked tolerance to anaerobic conditions whereas typical fresh water species, such as the vorticellids and Stylonychia, are very sensitive. Many fresh water species however, and in particular Coleps hirtus, are not sensitive to anoxia a conclusion which confirms Lindeman's evidence. Moreover one common soil species, notably Halteria

grandinella, was remarkably sensitive to anoxia. The effect of high carbon dioxide tensions is perhaps more important even than lack of oxygen. It is interesting to find therefore that Coleps hirtus is very sensitive to high carbon dioxide tensions whereas Halteria grandinella is far less sensitive. It will be remembered that the pH range of Halteria extends further toward the acid side than that of Coleps which agrees with their carbon dioxide sensitivity.

Halteria is generally considered a bacteria-feeder whereas Coleps is generally associated with euglenoids or detritus (Noland, 1925a,b). It is interesting to find that Noland recorded the lowest oxygen saturation for Halteria as 16.9% and for Coleps 31.7% and the highest carbon dioxide concentration and average carbon dioxide concentration as 14.7 cc./litre (3.7cc./1.) and 10.7cc./litre (1.5cc./1.) respectively. That the carbon dioxide concentration is a better indication of the ecology of a species than the oxygen tension can be seen from other of Noland's figures, e.g. the average carbon dioxide concentration for Vorticella campanula was 1.3cc./1. (-3.1 to 6.6) and for V. nebulifera it was 4.3 cc./1. (0.8 to 10.1). The present experimental work confirms this difference between the two species.

The discussion has been concentrated on Coleps and Halteria because these two species are very common in fresh water and yet while one is common in soil the other has never been recorded from New Zealand soils and very rarely from other soils. Similarly Stylonychia a common fresh water ciliate is absent from New Zealand soils so far studied and has rarely been recorded in other soils. The absence of Stylonychia may be attributed partly to

its size and partly to its great sensitivity to anaerobic conditions. Coleps appears to be excluded partly because of its sensitivity to carbon dioxide, partly by the acidity of soil and perhaps partly because of the absence of suitable food. Halteria although sensitive to anoxia survives high carbon dioxide tensions, acid conditions and is essentially a bacteria feeder.

Although the effects of anaerobic conditions are sufficient to explain the absence of many fresh water species each species must be considered individually as can be seen from the example of Coleps birtus. It is improbable that anaerobic conditions are important factors in granular soils and as, in many of these soils, acid conditions are not present the exclusion of the fresh water fauna must be explained on other grounds, primarily perhaps in the mechanism of encystment and excystment. All that can be concluded is that the common soil fauna are ecologically similar to the infusion and benthic (though not mud) fauna and that a tolerance of one or other aspect of anaerobic conditions is common to all these species. The presence of anaerobic conditions in the heavier soils and particularly the waterlogged soils will also account for the occasional record of anaerobic species such as Metopus es and Trinnyema compressa and the incidence of several species in waterlogged soils which were not found in non waterlogged soils (Fantham et al. 1922-1931.)

It has been stressed that the formation of cysts by Colpoda greatly favours its survival in the soil. In respect to anaerobiosis the formation of cysts not only ensures that the ciliates do not die out under anoxic conditions but also that

under conditions of high carbon dioxide tension the ciliate can survive prolonged periods in the encysted condition. Certainly this is true for both C.inflata and C.steinii, for the cysts remained viable after exposures to pure carbon dioxide of twenty four hours.

Physiological Significance

Excluding the case of those ciliates which blistered or died, the effects of oxygen lack are: first, the complete inhibition of division; secondly, the inhibition of excystment; and thirdly, the general slowing down or retardation of ciliary movement. These effects were most clearly demonstrated with Colpoda but they are also true of other facultative anaerobes. The clear distinction between division and excystment on the one hand and feeding and movement on the other is common to other organisms and can be demonstrated by factors other than anoxia, such as increasing carbon dioxide tensions. The problem of the 'activity' and 'resting' systems, as they are called, will be discussed more fully in a later section together with evidence of other environmental effects.

Anaerobiosis has been closely studied in Tetrahymena pyriformis (Lwoff, 1932; Lwoff, 1934; Thomas, 1942). Baker and Baumberger (1941) showed that the oxygen consumption of this ciliate is inhibited at very low tensions (2.52mm.Hg). Pace and Ireland (1945) showed that oxygen consumption increases with increasing oxygen tension and this appears to be the general rule for ciliates (Adolph, 1920; Specht, 1934) despite Jahn's conclusion to the contrary (Jahn, 1941). The oxygen consumption

of Tetrahymena is also cyanide sensitive (Baker and Baumberger, 1941; Lwoff, 1934). This is also true for Colpoda (Taylor and van Wagtendonk, 1942) and for Paramecium (Clark, 1945; Humphrey and Humphrey, 1948). The effect of narcotics is not only to depress respiration but also to inhibit division (Jahn, 1941) and this is particularly true of narcotics affecting the carbohydrate cycle.

Under anaerobic conditions the carbohydrate metabolism of T.pyriformis is characterised by the production of lactic, acetic and succinic acids and the assimilation of carbon dioxide (Thomas, 1942), and anabolism is largely though not wholly inhibited. Under aerobic conditions these organic acids are completely oxidised and do not accumulate (Seaman, 1949 a, b, 1950, 1951, a, b, 1952). This is confirmatory evidence that Thomas's experiments were carried out under strictly anoxytrophic conditions. Thomas found that after anaerobiosis the rate of oxygen consumption of Tetrahymena increased and Specht (1934) found the same was true for Spirostomum. This rise was attributed by both authors to repayment of the oxygen debt contracted during anaerobiosis. The conclusion is that the ciliates derive their energy anaerobically from glycolysis in much the same manner as muscle tissue and that this energy while sufficient to maintain the 'resting' system is not sufficient to permit the 'activity' system to function. Although a similar study has not yet been made of the biochemistry of Colpoda it is not unreasonable to suppose that in all essentials it is similar to that of Tetrahymena, and the evidence that is available supports this assumption.

If the biochemistry of Tetrahymena pyriformis can be taken as typical of the facultative anaerobes it is important to distinguish between these ciliates and the obligate aerobes and anaerobes. It is immediately obvious that the biochemistry of Metopus must be profoundly different to that of Tetrahymena for both the 'activity' and 'resting' systems function in the complete absence of oxygen (Lackey, 1938b). Either the activity system must operate at a lower energy level or else metabolism, dependent upon respiration in Tetrahymena, is achieved anaerobically by Metopus. The obligate aerobes on the other hand, such as Woodruffia metabolica neither divide nor feed in the absence of oxygen. In other words even the resting system is curtailed in the absence of oxygen. It is worth noting however that whereas Woodruffia is a carnivore, feeding on Paramecium, the facultative anaerobes, e.g. Balantiophorus, Colpoda, and Tetrahymena are all bacteriophagous. Indeed it is invariably these bacteriophagous species, such as Paramecium, which constitute the polysaprophes and which are to a greater or lesser extent facultative anaerobes.

The period of anaerobic survival depends largely upon the nutrition of the ciliate, well fed ciliates surviving longer than starved ones. It is obvious then that the ability to continue feeding is essential for prolonged anaerobic survival unless the organism is able to accumulate large reserves. At high oxygen tensions Tetrahymena accumulates fat globules which are not formed at low oxygen tensions (Pace and Ireland, 1945). When starved Tetrahymena becomes increasingly smaller and then remains almost wholly inactive in the culture fluid. Cysts are rarely

if ever formed (Furgason, 1940; Watson, 1946b). When fresh culture fluid is added the ciliates again become active, feeding and dividing rapidly. This is also true of the other small facultative anaerobes such as Colpoda, although Colpoda survives periods of starvation as a cyst. Most fresh water species die out under similar circumstances if they do not encyst.

This ability to make use of a rich organic energy source and to survive long periods of starvation combined with a high division rate is characteristic not only of such facultative anaerobes but also of such parasitic ciliates as Balantidium and Nyctotherus. There is one conspicuous difference: parasitic species generally encyst under optimum growth conditions and not when starved (Balamuth, 1951, Everitt, 1950). Otherwise there is much in common between parasites and the ciliates found in soil, faeces, polluted streams and other places rich in decaying organic matter. For this reason it is not remarkable that two of the most typical members of this fauna, viz. Colpoda steinii and Tetrahymena pyriformis are both accidental parasites. It is also interesting that Balantidium should have been twice recorded from soil samples (once in New Zealand and once in South Africa). This shows that excystment of Balantidium is easily obtained and that it is able to survive the conditions of the soil culture. Both Balantidium and Nyctotherus are facultative anaerobes and not, as some gut parasites, obligate anaerobes.

The other ecological group to which the soil fauna is related is the sapropelic ciliates and in particular Metopus and Trimyxa. Again there is the rich bacterial fermentation but the conditions

are more strictly anaerobic. In this case as in the case of the more specialized parasites the ciliates are obligate anaerobes although Metopus es is tolerant of oxygen and Noland recorded a range of oxygen tensions of 5.3% to 31.7% (avg. 15.2%) saturation in his paper.

We may conclude then that part of the soil fauna has specialized nutritional habits thriving best in rich organic infusions but also surviving periods of relative starvation indefinitely. That they can feed and grow but not divide under anaerobic conditions and that only the most specialized physiologically are able to live parasitically.

V

AN EXPERIMENTAL STUDY OF THE EFFECT OF PH, SALINITY
AND TEMPERATURE ON THE LIFE HISTORY OF COLPODA ,
WITH A DISCUSSION OF CYST PHYSIOLOGY AND THE
'ACTIVITY' AND 'RESTING' SYSTEMS

- A. A DISCUSSION OF CYST PHYSIOLOGY
- B. THE EFFECT OF ENVIRONMENTAL FACTORS ON THE LIFE HISTORY OF COLPODA.
 - i. Hydrogen Ion Concentration
 - ii. Osmotic Pressure
 - iii. Temperature
- C. A DISCUSSION OF THE 'ACTIVITY' AND 'RESTING' SYSTEMS.

A DISCUSSION OF CYST PHYSIOLOGY

Cyst physiology covers the life of an organism as a cyst, the process of encystment and the process of excystment. Encystment involves the secretion of the cyst membrane and generally structural dedifferentiation. Excystment involves differentiation and escape from the cyst membrane either by fracturing or dissolving the membrane. During the encysted period there may be considerable cytoplasmic and nuclear reorganization. Division or conjugation may take place (Taylor, 1935).

Although encystment is a widespread phenomenon and is particularly evident amongst protozoa the following account will be confined almost wholly to ciliates and particularly to holotrichous ciliates, especially the Colpodidae, which have formed the principal subject of the present investigations.

Types of Cyst

In all cases encystment is marked by rounding of the ciliate and secretion of a membrane consisting, according to Goodey (1913) of a carbohydrate. The thickness of the cyst membrane may vary not only from species to species but within the species depending upon the type of cyst formed. In the Colpodidae the cyst membrane is typically triple composing an endo-, meso- and ectocyst. However in the permanent cysts this is much thicker than in the unstable cysts, particularly in *Woodruffia* (Johnson and Evans, 1940). In excystment of Colpodidae increasing osmotic tension splits the ecto- and mesocyst and the ciliate

escapes still within the endocyst which is subsequently dissolved. This is also the case in Didinium (Beers, 1945b). Cysts vary greatly in shape and appearance depending largely upon the conditions under which they are kept. Paramecium cysts ultimately become small and wrinkled, very like a grain of sand (Michelson, 1928).

In the Colpodidae, as in some other ciliates, there are two basic types of cyst, viz. stable and unstable. The stable cyst is formed in the absence of food. If the ciliates are crowded a true resting or resistant cyst will be formed which requires a definite stimulus before excystment takes place. If the ciliates are not crowded a temporary cyst is formed which may excyst and encyst again several times in a food free medium (Taylor and Strickland, 1939). Ultimately if conditions remain unfavourable the ciliate will die. In the temporary cyst there does not appear to be either dedifferentiation or nuclear reorganization. In the resting cyst there is nuclear reorganization which takes place within nine hours of encystment and which is considered by Taylor and Garnjobst (1939) to represent a suppressed mitosis (Burt, Kidder and Claff, 1941).

The unstable cyst is formed in the presence of food but in response to adverse conditions. In the Colpodidae division almost invariably takes place within a cyst. In this case the well fed ciliate secretes its cyst membrane, divides once, twice or three times and then generally excysts spontaneously. In response to adverse conditions both division and excystment may

be inhibited and this is shown to be the case with Colpoda in the absence of oxygen and under conditions of high and low temperatures and of increasing salinity. Johnson and Evans (1939, 1940, 1941a) have shown that these factors together with crowding and feeding the ciliates on starved Paramecium all favour the formation of unstable cysts in Woodruffia. In Woodruffia unstable cysts are also formed in which the ciliate digests its food, but does not divide. Johnson and Evans (1941b) have shown that complete dedifferentiation and differentiation of cilia takes place in encystment and excystment of both stable and unstable Woodruffia cysts but the process takes longer in stable cysts. This they attribute to the water content of the two types of cyst, stable cysts, unlike unstable cyst, losing most of their free water upon encystment.

Not only do the two cyst types differ in structure and function, they differ also in their resistance to environmental factors. This may attribute partly to the thicker cyst membrane and partly to the lower water content of the resting or resistant cyst. Woodruffia unstable cysts will not survive drying or freezing for long periods. Woodruffia stable cysts, like the resistant cysts of Colpoda, can withstand freezing and drying. If wet they are killed at about 50 to 60°C but if dry they are not killed until 100°C or more. Colpoda cysts can also withstand high vacua and various acids toxic to the active ciliate (Bodine, 1923; Taylor and Strickland, 1936).

The cysts of Colpoda continue to lose water after encystment

and decrease slightly in size (Allison, 1924b). Respiration is depressed but there is a marked increase in the respiratory rate with the onset of excystment and it has been shown that excystment can be blocked by oxygen lack (Thimann and Commoner, 1940; Brown, 1939). Interestingly enough absolute alcohol, immediately lethal to all animals, cannot penetrate the cyst membrane. Dilute alcohol however is lethal (Bodine, 1923). The presence of water appears to be necessary for the penetration of the cyst membrane by many chemicals and may explain why the dried cyst is more resistant than the wet.

Besides Colpoda and Woodruffia the other genus of Colpodidae in which cystment has been studied is Tillina. This genus very closely resembles Colpoda although little study seems to have been made of encystment. It appears however that both crowding and starvation are essential, as in Colpoda, and the cysts formed appear to be identical with those of Colpoda (Beers, 1943; 1945a; 1946a; Bridgeman, 1948).

Paramecium although systematically close to Colpoda does not commonly encyst. Indeed for many years it was thought that it did not encyst at all and there is so far no record of excystment in this genus. Kudo (1947) cites the following records of encystment: Prowazek for P.bursaria; Lindner for P.putrinum; Curtis and Guthrie for P.caudatum?; Cleveland for Paramecium sp.; and Michelson (1928) for P.caudatum. Cleveland obtained encystment by injecting the ciliate into the rectum of frogs. Michelson observed encystment under conditions of slow dessication. Despite the fact that no ciliate has been studied more intensively than

Paramecium these few records are the sole evidence of the cyst physiology of the ciliate. Yet Paramecium is a common infusion ciliate and Pruthi (1922) states that the ciliate can always be obtained from an infusion of dried hay.

In Spathidium encystment appears to be dependent upon food depletion. The effect of crowding has not been tested. Excystment is spontaneous. Division may take place, very rarely, within the cyst. There is nuclear reorganization (Moore, 1922; 1924).

In Didinium encystment is primarily dependent upon crowding, although starvation alone is sometimes effective. Excystment is dependent upon the presence of bacteria or bacterial products. Nuclear reorganization but not division takes place within the cyst, (Mast, 1917; Beers, 1927a,b, 1933, 1935, 1945b, 1946b, 1947).

Dileptus anser encysts in the absence of food or when too active fermentation develops. Only ciliates above a certain size are able to form cysts. Hayes (1938) did not record nuclear reorganization but stated that in the encysted ciliate the nuclei were angular masses staining well with the Feulgen reaction in contrast to the nuclei of the active forms. Excystment takes place through an especially thin part of the cyst membrane.

Prorodon griseus forms both temporary and permanent cysts with thin and thick cyst membranes respectively. The temporary cysts are formed often in response to adverse conditions such as extreme temperature, food lack or dessication. The ciliate may excyst in less than an hour. Division and conjugation may take place within a temporary cyst. The permanent cysts show complete cytoplasmic and nuclear reorganization and are inactive until this

process has taken place, that is for at least four days (Tannreuther, 1926). In excystment the cyst membrane is partly dissolved. The Prorodon species described earlier, appears similar in many respects to P. griseus.

Less is known of the cyst physiology of hymenostomatous ciliates. Hoare (1927) has described encystment in Cohnilembus pusillus and Watson (1944, 1946) in Glaucoma pyriformis and Balantiophorus minutus. In this last case Watson showed that encystment took place only within a limited pH range (5 to 5.5) and temperature (12 to 14°C).

Stolte (1922) found that crowding was an important factor in encystment of Blepharisma undulans. Noland (1927) recorded encystment of the larger exconjugant in Metopus es. The smaller exconjugant died. Beers (1948) found two types of cyst in Bursaria truncatella. A winter cyst formed in autumn which could not be stimulated to excyst till eleven to thirteen months later when maturity was marked by increased transparency and cytoplasmic change. There was no nuclear reorganization. Excystment was followed by differentiation which took an hour. Encystment could be obtained by starving the ciliates. The cysts formed however were temporary or unstable cysts which would excyst within a week or less.

Numerous studies have been made on hypotrichous ciliates. The more important papers are recorded by Penn (1935). Generally starvation, crowding, or dessication cause encystment although Darby (1929) showed that increased acidity or alkalinity, together with crowding, cause encystment in Stylonychia pustulata.

Excystment is caused by dilution, change in pH, or food. Nuclear reorganization is common. Division and conjugation within a cyst however have not so far been recorded for Hypotricha.

The cyst physiology of Verticella microstoma has been studied by Brand (1923). He concluded that absence of food, drying of culture media and lack of oxygen caused encystment and that the presence of bacteria, oxygen and a neutral medium were necessary for excystment. The second and third encystment factors mentioned by Brandt are here found to cause telotroch, and not cyst, formation. Apart from exceptional circumstances the excystment factors are correct. Brand also described nuclear changes during encystment.

Discussion

The brief summary of the principal evidence on cyst physiology clearly shows that the process is complex, is not under the control of any single factor and that no single explanation will account for all the phenomena.

Bridgeman (1948) working with Tillina proposed that basically encystment was due to an insufficient amount of food or an insufficient amount of oxygen or both and that it was accompanied by water loss. Excystment she considered occurred when food and oxygen levels are sufficiently high and there is an entrance of water into the cyst. She is in this case dealing with the resting or resistance cyst and for the family Colpodidae she is partially correct. However she is in error when she considers the two processes, viz. encystment and excystment, as complementary; both under the influence primarily of food and oxygen. Absence of

food or nutritional deficiency is an absolute condition of resting cyst formation in the Colpodidae but it is not the only condition. Crowding, for Colpoda, is equally important nor in this case is encystment affected by the oxygen tension. Indeed encystment, in marked distinction to excystment, is independent of oxygen tension. Moreover excystment both in Tillina and Colpoda can be achieved with distilled water. Excystment is then independent, in these cases, of food. Unlike encystment however it is dependent upon respiration, and is accompanied by a marked rise in the respiratory rate. The two processes then must be considered as quite distinct and the factors affecting them quite distinct also. This distinction is shown in the following table:

	Encystment	Excystment
Food Deficiency	Dependent	Independent
Food	Inhibits	Independent
Respiration	Independent	Dependent
Osmosis	Independent	Dependent

Encystment in Colpoda presents a number of unsolved problems. True resting cysts are formed only when the ciliates are concentrated. The proportion of resting cysts and the rate of encystment are dependent upon the size of the drop and the concentration of the ciliates respectively. Because of this Strickland (1940) inferred that the factor causing encystment was independent of the factor causing resting cyst formation. In other words Strickland is postulating two factors: one causing encystment and the second causing resting cyst formation. The fact that the latter was dependent upon the size of the drop led Taylor and Strickland (1939) to believe that it was a volatile or

unstable substance readily oxidised. The present researches attempted to find such a factor in the normal metabolites of ciliates, viz. carbon dioxide and ammonia as suggested by Taylor and Strickland, but neither of these factors were effective. The problem then remains unsolved. However it seems to me unnecessary to postulate the two factors suggested by Taylor and Strickland. In place of their hypothesis I suggest that there is one factor highly unstable which if it reaches sufficient concentration or is active for a sufficiently long period causes resting cyst formation, but that if it is less concentrated or acts for a shorter time causes only encystment, i.e. temporary cyst formation. It must be remembered that there is no initial distinction between the ciliates forming temporary and true resting cysts. The only distinction then is in the stimulus which in one case initiates complete nuclear reorganization and in the other case only the secretion of the cyst membrane. The nature of the putative factor appears to be highly specific and it seems unlikely therefore that it is one of the common metabolites such as carbon dioxide or ammonia. It may be such a factor as paramecin (von Wagtendonk et al. 1947; 1948;) a highly unstable antibiotic protein, secreted by Paramecium aurelia, specifically responsible for the lysis of sensitive clones and which is dependent upon genetic and cytoplasmic inheritance. Or it may be a byproduct formed in the secretion of the cyst membrane which diffuses in the medium. Or it may be a byproduct normally secreted by the ciliate and to which it is sensitive only when starved. This last hypothesis draws some support from the evidence of Stuart, Kidder and Griffin (1939)

that the ciliates are sensitive to medium containing recently autolysed Colpoda. Whatever be the factor it will only be defined by the use of sterile culture methods which are not generally employed for Colpoda since growth is slower than on a bacterial diet and the medium is not easily prepared.

Another problem is the efficacy of potassium ions and alcohol as excysting factors. The protective function of the potassium ion in the alcohol-potassium excystment medium has also not been satisfactorily explained (Thimann & Haagen-Smit, 1937; Haagen-Smit & Thimann, 1938; Strickland & Haagen-Smit, 1947, 1948). This is partly due to the fact that those who worked on these factors were unaware that excystment of Colpoda can be achieved simply with distilled water. Such substances as hay extract, organic acids and so on have not therefore the role of essential metabolites. In this case Bridgeman is also misleading for alcohol can hardly be considered as food. She is on safer ground when she earlier stressed the fundamental matter of osmotic differential. It has been shown (Bodine, 1923) that alcohol in low concentrations rapidly penetrates the cyst membrane. It may be presumed that metabolites such as the organic acids readily diffuse through the cyst membrane as was shown by Bodine (1923) and Haagen-Smit and Strickland (1938). The particular efficacy of the potassium ions found by these last authors may therefore be attributed to their ready diffusion through the membrane and consequently their stimulus to further osmosis. Once osmosis is established, for example with distilled water, the excystment process is largely though not wholly

automatic, provided oxygen is present (Brown, 1939). No further stimulus is needed although excystment is more rapid with a definite excystment medium than with distilled water. The stimulus must however be maintained until the ciliate is activated and differentiation has begun (McIntyre, 1949, Brown and Taylor, 1938). Differentiation of the ciliates without excystment does sometimes take place which shows that the mechanism is not always perfectly automatic. Nevertheless basically excystment and differentiation in Colpoda and Tillina are best understood as a mechanism set off by a trigger reaction and this trigger is osmosis. The extraordinary excystment both of Colpoda and Vorticella in the present work when the oxygen tension was reduced may have been due to an osmotic stimulus caused by the cyst and the medium being under different pressure or it may have caused the diffusion.

It is necessary now to consider Vorticella microstoma although less work was done on this ciliate and the only previous work is that of Brand's (1923) with which some of the present findings disagree. First it is important to distinguish the stable and unstable cyst. The latter ^{are} formed in heavy bacterial infusions due it is suggested to the low redox potential or the specific inhibition of reduced metabolites. Certainly they are not formed simply by the exclusion of oxygen. Nor are the ordinary resting cysts. These are formed however upon the depletion of the food supply with or without a conjugation or sexual cycle intervening. Excystment in this case is not obtained by washing or with yeast extract or hay infusion. It

is obtained with bacteria and in this respect Brand is correct and Vorticella microstoma therefore resembles ciliates such as Didinium nasutum which excyst only in the presence of bacteria or bacterial metabolites. Oxygen is also necessary as in the case of Colpoda.

The mechanics of excystment have been frequently described (Goodey, 1913; Beers, 1945b). In Vorticella it is worth noting that the ciliate escapes through a definite cyst pore and does not split the cyst membrane as in Colpoda. Brand does not make this point clear. Another point is that excystment and differentiation are distinct processes and the interrelation of the two may not always be the same. Thus sometimes if excystment is delayed the telotroch will be fully differentiated in the cyst membrane whereas normally differentiation takes place during and after excystment.

B. THE EFFECT OF ENVIRONMENTAL FACTORS ON
THE LIFE HISTORY OF COLPODA

Introduction

The experiments recorded in the previous chapter dealt with the effects of two environmental factors, viz. oxygen tension (and anoxia) and carbon dioxide tension. Colpoda steinii, one of the most common soil ciliates, proved to be a typical facultative anaerobe and extremely tolerant of high carbon dioxide tensions. The effect of anoxia was differential, division and excystment were inhibited but not feeding or encystment.

In the present chapter the effect of three other environmental factors on Colpoda, viz. pH, salinity, and temperature, are examined with particular reference to the tolerance of the ciliate and the effect on division, encystment and excystment. During the past half century a considerable body of evidence has grown up on the effect of these factors on Colpoda. Experiments were performed therefore to confirm or refute the prevailing opinion and to clarify conflicting opinions.

Culture of Colpoda

Colpoda is very easily cultured in 'pure' culture, i.e. free of other protozoa. It is more difficult to maintain in sterile (bacteria-free) culture. For the present work pure cultures free of other protozoa were generally used particularly of C.steinii and C.inflata.

The medium commonly employed was yeast autolysate. Yeast was cultured on glucose-peptone-agar and a loop of yeast added to a 5ml.balanced salt solution. This solution was .4% NaCl and .2% NaH₂PO₄. The yeast suspension was prepared in cotton wool stoppered glass vials and autoclaved at 15lbs. for 15 to 30 minutes. The autolysate was then decanted into Syracuse watch glasses which were inoculated with Colpoda. To establish pure cultures the ciliates were first washed in sterile distilled water or balanced salt solution.

Growth under such conditions is initially slow. After several days the Colpoda increase in numbers and then as the food is depleted encyst. Excystment can be obtained in a variety of ways most simply by adding fresh yeast autolysate.

More rapid growth is obtained in bacterial suspensions. The ultimate result is however the same. The bacteria are consumed and the ciliates encyst. Encystment is the invariable rule in a normal completion culture.

Colpoda cucullus, which does grow in the yeast autolysate, grows better in lettuce or hay infusion. Ultimately it too will encyst.

1. Hydrogen Ion Concentration

The significance of pH has been fully discussed in Chapter III. Colpoda has been found to tolerate a wide range of pH, and it seems unlikely that pH would ever be a factor limiting its distribution in nature except in the most extreme cases.

Colpoda steinii

Unbuffered yeast autolysate sub-cultures were prepared and the pH adjusted with HCl and KOH. The sub-cultures were inoculated and both the growth of the ciliate and the change of pH recorded. When the ciliates had encysted the pH of the cultures was again changed using KOH and HCl and the cultures examined for any signs of excystment.

Results

<u>Initial pH</u>	<u>After 7 days</u>	<u>Changed to</u>	<u>After 2 days</u>
3.5	4.5	9.4	7.1
5.25	5.6	10.7	7.4
6.5	6.1	7.7	6.5
9.2	6.1	10.0	6.7
10.2	6.5	3.0	2.0
10	8.0	4.8	5.0

After seven days the initial pH of the sub-cultures had changed and the sub-cultures were all within the acid range except for a culture buffered with phosphate. The ciliates had grown in all the sub-cultures but growth had been slower in the very acid (pH 3.5) culture wherein after two days there were only about 10 ciliates large and slow moving but also dividing. After seven days all the cultures had formed many cysts and these were tested for viability with yeast extract. The cultures were then tested with KOH and HCl to see if a marked change in pH

would cause excystment. However this was not the case the only result was the death of most of the remaining active forms a few surviving in four cultures two days later.

These results indicate that C. steinii grows and divides in a wide range of pH and that encystment is independent of pH. They also show that excystment is not brought about simply by change in pH, although excystment will take place within a wide range of pH if a favourable stimulus such as yeast extract is added.

2. The first experiment indicated that growth of Colpoda steinii took place within a wide range. The initial inhibition of growth in extremely acid culture may have been due either to the effect of pH on the bacterial flora required as food by Colpoda or the direct effect on the ciliate itself.

It is unfortunately as difficult to test the single factor of pH as to test Eh. The common practice is to use a range of buffered solutions but these buffers are not biologically inert and the buffers themselves affect the organism differentially. So two buffers at the same pH may differ in their effect on the organisms. This was shown by Thomas (1942) who found that the metabolism of Tetrahymena pyriformis grown in carbonate and phosphate buffers of the same pH differed. Bridgeman (1948) found that with Tillina magna acetate buffer gave different excystment results to phosphate though the pH was the same (Bodine, 1923).

For this reason the following tests in which buffers were employed must not be considered conclusive evidence of the effect

FIGURE 1

Fig.1. Division Rate of Colpoda steinii as a function of pH.

of pH particularly as the concentration of buffer required to stabilize the pH was little short of toxic concentrations.

The buffers used were tartrate (pH 3.57) and borate (pH 9.2) together with two standard pH buffers issued by the Macbeth Co. of pH 4.0 and 7.0.

The ciliates were suspended with a loop of bacteria in the buffer and their rate of division followed.

Results

Diagrammatically the results are given in Figure 1. In the drop of pH 9.2 the ciliate lived for twenty four hours, moved slowly but did not divide. It later died. The ciliates at pH 3.57 showed inhibition of division over the first twenty four hours and then divided rapidly. There was no apparent difference between the division rate at pH 4.0 and pH 7.0.

These results indicate that with an adequate food supply the division rate of the ciliate is independent of pH between 4.0 and 7.0 and probably independent of pH at 8.0. The initial inhibition of division at pH 3.57 is very similar to the results of the first experiment and indicate probably a pH shift in this period with maximum division rate at less acid conditions. The failure of the ciliate to divide at pH 9.2 may well have been due to the effect of the buffer rather than the pH as the first experiment shows that the ciliates certainly survive at higher pH.

3. To test the excystment of the ciliate with different concentrations of KOH and HCl, cysts were taken from a completion culture on strands of cotton wool and placed in small dishes and the alkali, acid or distilled water then added.

Results

	Initial pH	Final pH	Excystment
KOH	8.5	7.8	95%
HCl	4.7	5.5	75%
Dist. water	7.0	6.8	67%

Excystment took place in all three tests without any special excystment factor.

4. To cysts formed in buffer solution of pH 3.57 loops of bacteria were added. Excystment was obtained although the percentage was not high.

Discussion

The experiments on Colpoda have been to a large extent redundant for they merely confirm the conclusions reached by earlier investigators. Particular mention however must be made of Koffman's results (1924). Using C.steinii and C.cucullus he followed the pH of hay infusion cultures and correlated rising pH with encystment. The pH of his cultures rose steadily in seventy two hours from an initial 6.05 to 8.80. His cultures were started with cysts and after seventy two hours there were only cysts left in the cultures. The ciliates had excysted, multiplied and encysted. Koffman attributed this to the change in pH and concluded therefore that encystment in C.steinii took place between 7.75 and 8.30 and in C.cucullus at 8.30. Morea (Kudo, 1947) however recorded growth of C.cucullus between 5.5 and 9.5 and Nasir (Cutler and Crump, 1935) between 3.5 and 9.75. Koffman further recorded that the ciliates decreased in size and there can be no doubt that encystment in this case was simply due to the exhaustion of food. The present experiments show quite

clearly that it is this factor and not pH which is significant.

Bodine (1923) tested the effect of different acids organic and inorganic on cysts of Colpoda cucullus. He found the relative toxicity to be: salicylic > butyric > oxalic, phosphoric, hydrochloric, sulphuric > acetic. Bodine also remarked that different acids of the same pH had different toxicity. Acetic acid was found to be much more toxic than phosphoric or hydrochloric of the same pH. This may be significant for litters in which the high acidity of beech litter for example may be due to less toxic acids (Collett, 1919, 1921).

The present experiments also confirm that over a wide range of pH the division rate is affected solely by the food supply, other factors such as temperature being stable. The slight inhibition of division below pH 4.0 may have been due to the buffer solution or may have been due to an acidity factor. Considering that growth has been obtained above pH 9.0 by both Morea and Nasir it seems probable that failure of the ciliate to grow in buffer at 9.2 was probably not simply a pH effect.

11. Osmotic Pressure

Most free-living ciliates occur either in fresh or marine water. Some however occur in both and many will tolerate brackish conditions. Extreme salinities are reached in the salt lakes and Pack (1919) recorded two ciliates in Great Salt Lake, Utah, which has a salinity of 23%. Gurwitsch (1934) examined the fauna of four canals and lakes. One of these was definitely brackish (C1', 1.14 to 1.37%). Whereas the fauna of the fresh water lakes and canal was very changeable, the fauna of the brackish canal

was constant, most species being seen regularly during the period when they were studied. Certain species, Nassula halophila, Trachelocerca fusca, Condyllostoma patens and Euplotes charon were found only in this canal. Diffugia found in the other fresh water lakes and canal was never present in the brackish water canal. This illustrates the total exclusion of strictly fresh water groups and the incidence of brackish water species. It is interesting to find that brackish conditions judged by the protozoan fauna are more stable than fresh water conditions.

To some extent ciliates are able to adapt to changing salinities. Pack (1919) adapted his ciliates from an initial density of 1.22 to a final density of 1.010. Hoare (1927) worked with three euryhaline species Cohnilembus pusillus, Uronema nigricans and Cyclidium glaucoma. Using marine and fresh water strains of Uronema he adapted each strain to the opposite salinity. He transferred the fresh water Cohnilembus to marine salinity and the fresh water Cyclidium to 50% marine salinity. The salt concentration of fresh water used by Hoare was .0037% and of sea water 4% (NaCl 3.17%). Anigstein (1949) acclimatized a marine ciliate (Oxytrichidae) to 30% marine salinity. Yocom (1934) found that Euplotes patella could be adapted to 65% sea water, but Paramecium caudatum and Spirostomum ambiguum to only 20%.

Finley (1930) recorded the tolerance of a number of fresh water ciliates of direct and indirect transfer to higher salinities. Uronema nigricans withstood direct transfer to sea water. (Total salinity 3.51%, sodium 2.73% and magnesium .38%).

Of soil species mentioned by Finley Cinetochilum margaritaceum tolerated 15% and 52% (direct and indirect transfer); Colpoda cucullus 5% and 10%; C. steinii 25% and 40%; Cyclidium glaucoma 15% and 103%; Halteria grandinella 5% and 10%; Oxytricha pellionella 5% and 40%; and Uronema nigricans 100% and 103%.

Earlier work is reviewed by Hoare (1927). There is considerable discrepancy between the different reports, e.g. between Hoare's experience with Cyclidium glaucoma and Finley's. There are two points of importance: first, Finley used a short term survival for his tests, viz. three days; and secondly, Hoare records difficulty in transferring his acclimatized Uronema back into fresh water. This difficulty Hoare attributed to the difficulty of food supply, the marine bacteria not readily adapting themselves to fresh water.

The chief factor however is the increment of salinity to which the ciliate will acclimatize at one time. This varies with the species (Finley) and with the saline concentration (Chatton and Tellier, 1927 a, b). These French authors worked with five ciliates Glaucoma scintillans, Glaucoma (Syn.Tetrahymena) pyriformis, Paramecium caudatum, Colpidium colpoda and a ciliate Colpidium steini which I imagine might be Colpoda steinii as there is no ciliate of the former name and it is referred to throughout as C.steini. However they also refer to the two Colpidium species. They found the initial salinity tolerated by Glaucoma (Syn. Tetrahymena) pyriformis to be 1% and the final salinity to be 1.8%. The other ciliates acclimatized first to .65% and finally to 1.3% NaCl. Loefer (1939) employing sterile cultures of Colpidium campylum and Glaucoma (Syn.Tetrahymena) pyriformis found that the former ciliate could be acclimatized to 95% van't Hoff solution (total salinity 3.97%) and the latter to a van't Hoff solution of

70% or 2.78% total salinity. It is obvious then that adequate food is essential if the ciliate e.g. Glaucoma (Syn. Tetrahymena) pyriformis is to be acclimatized to higher salinities and the failure of Finley (1930) to acclimatize Colpoda steinii to a salinity higher than 1.4% and of Chatton and Tellier (1927a) to acclimatize G. (Syn. Tetrahymena) pyriformis to a salinity higher than 1.8% may be attributed to inadequate nutrition.

Effect on Cyst Physiology

Greig-Smith (1912) reports that Colpoda encysts when washed in .2% saline. Garnjobst (1928, 1937) found that dessication which is accompanied by marked increase in salinity caused encystment of Euplotes taylori and Stylonethes sterkii, two marine species, and that on dilution they excysted. Schewiakoff (1928) records the same reaction for Euplotes harpa.

Finley (1930) has some interesting observations on cyst physiology.

'Stylonychia occasionally encysts when transferred into the maximum concentration which it can survive. Soon after transfer the animals begin the 'encysting reaction' continuing until a cyst is formed. They probably excyst after making adjustments to suit the new environment, for after five to eight days comparatively few cysts are found, and the living organisms are rather numerous in the culture.

'The maximum indicated for direct transfer for Lionotus fasciola (25%), Cyclidium glaucoma (15%), and Paramecium aurelia 20% is believed not to be a true direct transfer maximum for in no case were animals recovered from cysts placed in those concentrations of sea water.

'Experiments indicated that those protozoa which encysted readily could be recovered from their cysts by placing them in a saline medium; but the concentration of that medium was in no case greater than the maximum concentration tolerated in direct transfer. This conclusion is supported by attempts to recover Colpoda, Stylonychia, Euplotes, Halteria and Chilodon. In no case were they recovered from cysts placed in salt water unless the concentration was equal to or below the maximum direct transfer. Colpoda aspera and Bodo uncinatus were recovered in 100% artificial sea water inoculated with unsterilized dry hay'.

Soil Conditions

As far as soil organisms are concerned the effective salinity and osmotic pressure of the soil is that of the soil solution. This will vary greatly depending both upon the type of soil and the conditions of leaching and also will vary with the moisture content of the soil.

Most soils have an osmotic tension at the permanent wilting point of less than 2 atmospheres (Kramer, 1949). However salt concentration may increase due to irrigation, the application of fertilizer or the deposition of cyclic salt. Cyclic salt is of particular interest in New Zealand for no part of the country is more than 70 miles from the sea and under conditions of low to moderate rainfall deposition of cyclic salt may average over 300 lbs. NaCl per acre per annum in coastal areas. This has been shown to be of considerable pedological importance particularly for soils with 10 to 20% clay and a high proportion of sand and

its effect can be judged in the changed soil profile (Gibbs, 1948).

Microscopically the presence of efflorescences shows that increasing evaporation leads to greatly increased salt concentration and Kubiens (1938) has shown that this affects the microflora, and in particular cites degeneration of the sporangiophores of Rhizopus nodosus and the retreat of the fungus into spaces deeper in the soil.

Soils having osmotic tensions of 4 atmospheres or more seriously reduce plant growth (Kramer, 1949) and osmotic pressures of 12 to 15 atmospheres (NaCl) inhibit germination, while there is high mortality of seedlings with pressures of 7 to 9 atmospheres (NaCl).

Methods

Basically there are two methods of testing the osmotic tolerance of an organism: either by transferring the organism to a solution of known salinity, or by gradually increasing the salinity in which the organism is living. Both methods were employed in these researches on Colpoda.

Method 1. Transfer of the ciliate to culture media of increasing salinity. The salinity of yeast extract prepared with double glass distilled water was adjusted with sodium chloride to the following concentrations: .4% (the standard preparation); 1%; 2%; 2.5%; 3%; and 4% NaCl. The ciliates were inoculated into media of progressively increased salinity and their growth and reactions studied.

Method 2. Increasing the salinity of the culture medium by the addition of NaCl. In this case small volumes (c.1cc.) of culture medium containing the ciliates were placed in small dishes and a concentrated solution of NaCl (4%) added progressively. Again the reactions and growth of the ciliates were studied.

Results

Method 1. The ciliates were cultured successfully in saline concentrations up to 3%. Attempts to culture the ciliates in 4% NaCl were unsuccessful. In the 3% medium the ciliates appeared sluggish and though a few ciliates divided growth was restricted and slow with the ciliates ultimately forming unstable cysts. Growth was better at $2\frac{1}{2}\%$ and 2% although there was a lag period before the ciliates began to divide. Growth was normal at 1%. The ciliates grow quite normally in double glass distilled water with yeast autolysate. The range of salinity tolerated then by C.steinii is 0% to 3% with growth increasingly inhibited above 2.4%.

Dilution of the $2\frac{1}{2}\%$ and 3% cultures with double glass distilled water resulted in excystment and rapid growth.

Method 2. As a control in these experiments the tests were prepared in duplicate: one dish receiving .4% NaCl and the other 4% NaCl. The ciliates in the .4% medium grew normally and formed resistant cysts. The ciliates in the 2 to $2\frac{1}{2}\%$ medium occasionally divided after a lag period of 48 hours but generally formed unstable cysts. If the salinity was further increased division was entirely inhibited and the ciliates all formed unstable cysts. Dilution of these cultures resulted in excystment and division.

DIVISION
RATE
48 HOURS

FIGURE 2

Fig.2. Division rate of *Colpoda steinii* as a function of salinity.

Effect of Salinity on Division Rate

The experiments show that division is entirely inhibited by salinities above 3% (NaCl) and that lower salinities considerably retard division. Hanging drop preparations were made of ciliates in yeast autolysate of different salinities, viz. 0.4% and 2% NaCl. Following are the results.

Time (Hours)	0	48	D.R. (48 hrs.)
0.4%	3	15 (3 quadragenetic cysts)	3
2%	3	5 (3 monogenic cysts)	1.6

This shows that increased salinities depress the division rate and agrees with the observations on mass cultures.

The effect of salinity on the division rate is shown diagrammatically in Figure 2. The relation is shown as linear but it may possibly be curvilinear.

Effect on Encystment

In salinities in which normal growth takes place, i.e. in salinities up to 3% the ciliates form normal completion cultures ending in encystment. At higher salinities division is inhibited and consequently the food is not exhausted. If the ciliates survive, a period of sluggish activity and feeding intervenes and then an unstable cyst is formed or rarely division might take place. If the culture is diluted excystment or division takes place and normal growth ensues.

In no case whether with starved or fed ciliates in food free or yeast autolysate medium is there immediate encystment following transfer to higher salinities. With ciliates in yeast autolysate

unstable cysts are formed within twenty four hours but not within six hours. In some cases the ciliates were still trophic after over sixty hours although division is completely inhibited.

Effect on Excystment

Hypertonic solutions inhibit excystment. Resistant cysts were obtained either from completion cultures or by centrifuging ciliates from an old culture in which the food has been exhausted. The residue containing the concentrated ciliates was pipetted into watch glasses and all the ciliates rapidly encysted. The cysts were then placed in watch glasses and washed with double glass distilled water; .4% NaCl; 1% NaCl; and 2% NaCl. Results of typical experiments are given below:-

Effect of Salinity on Excystment

Colpoda steinii

A. Cysts taken from 0.4% NaCl completion culture.

Salinity	Time (Hours)	0	4	% Excystment	2	3.30
0.4%	Active	-	450	90		
	Cysts	c.500	28			
1%	Active	-	-	0 + aq. dist. 80		320(20)
				(260 activated)		
	Cysts	c.400	c.400		30	10

B. Cysts taken from 2.4% NaCl completion culture

Salinity	Time (Hours)	0	5	24	% Excystment
0%	Active	-	175	200	100
	Cysts	c.200	-	-	
1%	Active	-	19	22	71
	Cysts	30		8	
2%	Active	-	6	5	10
	Cysts	50			
3%	Active	-	-	-	0
	Cysts	50			
4%	Active	-	-	-	0
	Cysts	400			

Effect of Salinity on Excystment - Colpoda steinii (Continued)

C. Cysts from centrifuged 0.4% NaCl completion culture.

Salinity	Time (Hours)	0	5	% Excystment
0%	Active	-	90	90
	Cysts	100	6	
0.4%	Active	3	200	100
	Cysts	200	-	

D. Cysts from 3% NaCl completion culture.

Salinity	Time (Hours)	0	22.30	% Excystment
0%	Active	-	6	100
	Cysts	5	-	
1%	Active	1	28	100
	Cysts	30	-	

E. Cysts from 2.4% NaCl completion culture

Salinity	Time (Hours)	0	6	% Excystment
0%	Active	6	200	98
	Cysts	200	5	
0.4%	Active	4	200	100
	Cysts	200	-	
1%	Active	-	280	98
	Cysts	300	10	
2%	Active	1	5	15
	Cysts	30	27	
3%	Active	-	-	0
	Cysts	25	25	

F. Cysts from 2% NaCl completion culture

Salinity	Time (Hours)	0	3.40	22	% Excystment
1%	Active	-	20	32	60 and 90
	Cysts	35	15	3	
2%	Active	3	7	32	
	Cysts	120	120	90	3 and 25

Effect of Salinity on Excystment (Continued)
Colpoda Steinii

G. Cysts from 0.4% NaCl completion culture

Salinity	Time (Hours)	0	5	% Excystment	28	% Excystment
0%	Active	-	30	50		
	Cysts	60	30			
0%	Active	-	15	50		
	Cysts	30	15			
1%	Active	-	-	0 + aq. dist.	25	96
	Cysts	25	25		1	
1%	Active	-	-	0 + aq. dist.	30	90
	Cysts	35	35		5	

Colpoda inflata

Cysts from 0.4% NaCl completion culture.

Salinity	Time (Hours)	0	19	42	% Excystment
1%	Active	-	5	25	0.5%
	Cysts	c.1000	c.1000	c.1000	
0.4%	Active	-	13	65	50
	Cysts	26	14	6	
0.4%	Active	-	42	300	80
	Cysts	60	12	-	
0%	Active	-	23	160	95
	Cysts	24	1	1	

In the first experiment when the cysts failed to excyst in 1% NaCl distilled water was added and as shown excystment was then obtained. Generally cysts taken from the completion culture of normal salinity (.4%NaCl) excysted in distilled water and .4%NaCl but rarely in higher salinities. Cysts from completion cultures of 2.4% NaCl excysted in 1%NaCl and occasionally in higher salinities. In all cases 3% NaCl and 4% NaCl gave negative results.

Tests with Colpoda inflata showed that cysts taken from completion cultures of .4% NaCl showed decreasing excystment with solutions of increasing salinity.

Instead of washing the cysts with different saline solutions, cysts from the 3% NaCl completion culture were placed in watch glasses and bacteria and yeast added. No excystment was obtained. The addition of distilled water resulted in excystment and division. This showed that salinity is a crucial factor in excystment.

These experiments also show ^{that} increasing the salinity of the excystment milieu not only inhibits excystment but also slows the excystment process. So in experiment F with C. steinii cysts, of the 32 cysts which excysted in both the 1% and 2% saline solutions 20 or 62% excysted within four hours in the 1% solution and only 7 or 22% in the 2% solution.

Discussion

Colpoda steinii is plainly tolerant of high osmotic pressure. Growth takes place at tensions of 12 atmospheres which must be reached very rarely under ordinary soil conditions. Increasing osmotic pressure however affects the activity of the ciliates and particularly division so that at high osmotic pressure the ciliate encysts to form an unstable cyst and division and excystment only take place when the salinity is decreased.

Excystment of the resting cyst is dependent within limits upon the relative tensions of the milieu interieur and the milieu exterieur. The experiments indicate that the osmotic tension of the ciliate increases as the osmotic pressure or salt concentration of the culture fluid increases and that for this reason there can be gradual adaptation of the ciliate to

increased osmotic tensions up to a certain critical value. Pack (1919) recorded an increase in size both of the active and encysted forms of his ciliates as the salinity was decreased. In the present work there was an increase in size with increasing salinity due to the increasing inhibition of division and ultimately the formation of large unstable cysts. The resting cysts however were smaller in higher salinities than lower.

The failure to achieve excystment of resting cysts at high salinities shows that it is osmotic diffusion which is of primary importance, a conclusion also reached by Bridgeman (1948). This is confirmed by the fact that isotonic or hypertonic solutions are ineffective but that if the ciliate is pre-cultured at a higher salinity response may be obtained with these cysts not obtained by the same solution with cysts formed at lower salinity.

Penn (1935) working with Pleurotricha lanceolata found a slight increase in the rate of encystment (10% to 20%) with increasing salinity. Garnjobst (1928) found that evaporation caused encystment in Euplotes taylori and Stylonethes sterkii. Neither evaporation nor increased salt concentration cause resting cyst formation in Colpoda unless evaporation leads to the concentration of starved ciliates in which case this is the effective factor. The initial effect of increased salinity is to slow the metabolism of the ciliate but it does not evoke encystment.

Finley (1930) failed to acclimatize C.steinii to salinity greater than 1.4% (NaCl 1.09%). Yakimoff, Wassilewski, and Zweitkoff (1925) found that C.steinii lived only fifteen days in

0.07% NaCl. Thimann & Barker (1934) allege that C.steinii is sensitive to saline. These differences may be ascribed to two causes: failure to adapt the ciliate slowly to increased salinities (Hoare 1927) and failure to provide adequate food. Doflein (1916) apparently transferred C.cucullus to almost pure sea water. It is probable from Finley's data that the species actually used was C.aspera which is euryhaline. Woker and Pecker (1914) tested the effect of blood serum and salt solutions on cysts of C.cucullus.

Effect of Salinity on Excystment of Colpoda

It is generally agreed that the first excystment reaction is osmotic and Brown (1939) demonstrated that this reaction took place irrespective of the inhibition of the second major reaction which is dependent upon oxygen. The present experiments have explored more fully the effect of increasing salinity and osmotic tension on excystment in Colpoda. It is clear that there is a critical salinity above which excystment will not take place. But this critical salinity is not absolute but relative and apparently the essential point is that the osmotic tension of the milieu exterieur must be less than the osmotic tension of the milieu interieur. The cysts formed in cultures of high salinity excyst in solutions of comparatively high salinity whereas cysts grown in cultures of low salinity do not. This may be interpreted as demonstrating the acclimatization of the ciliates to increased salinities by increased osmotic tension of the milieu interieur.

This explains why Chatton and Tellier (1927a) found that there was a proximal and distal limit to which the ciliates could

be acclimatized for it is obvious that if there is a critical osmotic difference between the milieu interieur and milieu exterieur which can be tolerated by the ciliate then this difference will be reached at the proximal limit, i.e. in the first transfer when the osmotic difference is between the ciliate acclimatized to initially low salinity and subjected to an increased salinity. If the initial concentration is progressively raised, then the distal limit will be progressively increased as was found by Chatton and Tellier (1927b) until the distal limit, i.e. the highest salinity tolerated by the ciliate, is reached. If the ciliate is transferred beyond its proximal limit it will die because it cannot overcome the osmotic difference between the two milieux. In other words the acclimatization to increased salinity is interpreted as involving a change in the internal osmotic tension and vice versa. It should be correlated then with decrease in size and increase in density and this appears to be the case for Pack's ciliates. It is also the case with Glaucoma (Syn.Tetrahymena) pyriformis which Loefer (1939) found to average 50u in the 1% van't Hoff's solution but only 42u in the 35% solution. Because of the inhibition of division in Colpoda the ciliates in the higher salinities were generally larger than the ciliates in lower salinities although resting cysts in the higher salinities were smaller. At the highest salinity tolerated by Glaucoma (Syn.Tetrahymena) pyriformis Loefer found that the contractile vacuoles did not function. This may indicate that there was no osmotic difference between the two milieux.

Besides absolute inhibition of excystment at certain concentrations increasing salinity increases the excystment time. This strengthens the view that the action of the excystment media (yeast extract etc.) which have been equalled by alcohol and the phosphate ions is due to their rapid penetration and consequently their acceleration of the osmotic reaction.

Finley's results may be readily interpreted on this basis. He found that the ciliates excysted in direct transfer but not in the ultimate saline concentrations. This agrees with the present work. Cysts formed at low saline concentrations will excyst only within the proximal limit. However had the cysts been formed at higher salinities Finley might have found excystment at higher concentrations.

iii. Temperature

Introduction

Noland (1925a) concluded from his study of the distribution of fresh water ciliates that temperature was a significant factor for some species. Most species recorded had a wide temperature range. Certain species however were found more frequently at higher and others at lower temperatures. Noland carried out an experiment to test this factor. Identical field samples were cultured in the dark at two different temperatures (4.0° to 5.5° and 20° to $22^{\circ}\text{C}.$). Coleps hirtus and Halteria grandinella occurred only in the warm cultures. Interpreting these results as indicative of the influence of temperature Noland conceded that temperature might act through affecting bacterial numbers and the oxygen supply. However it seems very probable that as the ciliates were not observed initially in the cultures they were probably present in an encysted condition and excystment is not likely to take place at such low temperatures. The development of bacteria at the higher temperatures would both stimulate excystment and growth of these ciliates.

Wang (1928) also attached considerable importance to temperature correlating temperatures with the presence and maxima for certain species. However Wang's evidence may also be treated as the effect of food, oxygen supply or the ecological balance of the different groups.

Of soil species mentioned by these authors Oxytricha pellionella is mentioned as a cold water species but it can

be cultured at room temperatures. Cinetochilum margaritaceum and Cyclidium glaucoma are considered by Noland to be eurythermic forms, as are Paramecium caudatum and Chilodonella uncinatus.

Soil Conditions

Soil temperatures in New Zealand are generally higher than air temperatures (Simmons, 1951) and are more stable (Cutler and Crump, 1938). In the months November to April soil temperatures in New Zealand are about 50 to 60°F. Soil temperatures decrease with depth. Records of soil temperatures are not very common. They include those of Russell and Appleyard (1915) and Boynton and Compton (1944). Their readings also indicate greater stability and slightly higher temperatures in soil than in air. In New Zealand the difference between soil and air temperatures is about 5 to 6°F.

Extreme measurements have been recorded however in non-temperate climates. Very high temperatures have been recorded in the Sheraqi period in Egypt and Kraus (1911) found a patch of bare soil in central Germany 28°C. higher than the maximum air temperature. Although no measurements appear to have been made it is probable that microscopically there is considerable local variation.

The soils sampled in New Zealand came from sub-antarctic and tropical islands from sub-alpine and lowland regions, from the semi-arid Mackenzie plains and humid North Auckland. Every range of temperature was therefore represented and although indirectly temperature affects both soil processes and biological

activity there was no evidence of species restricted to a particular temperature range. The distributional evidence does not support the views of Noland and Wang.

Material and Methods

Colpoda steinii was employed and maintained either on yeast extract or fed with loops of bacteria. The effect on excystment had been fully tested by Bodine (1923), Thimann and Barker (1934) and Goodey (1913). Present tests were made on the division rate, the effect of low temperatures and the effect of high temperatures. Hanging drop preparations were generally used for quantitative work and sub-cultures in Syracuse watch glasses for qualitative work.

Effect of Temperature on Division Rate

Hanging drops were prepared and maintained at room temperature (c.18°C.), at 14°C. and at 28°C. They were also placed in an oven at 37°C.

Typical experiments are given below:-

Colpoda steinii

Hanging drops with yeast autolysate

A. Hours	0	26.30	48	D.R.	26	24
13°C.	2	4	16	3 (48hrs.)	37°C. 20 cysts	
18°C.	1	2	16	4 (48hrs.)		
28°C.	1	8 (2 div. cysts)	3.3 (26hrs.)	37°C. 11 cysts	18°C. 23	1 div. cyst
						5 rest."

B. Hours	0	24	27	D.R.
28°C.	3	c.44		3.8 (24 hrs.)
28°C.	3	c.38 (3 div. cysts)		3.7 (24 hrs.)
28°C.	1	2 (15 quadragenic cysts)	c.62	5.9 (27 hrs.)

C. Hours	0	24	72	D.R. (72 hrs.)
12°C.	8	7 (1 div. cyst)	c. 80 (5. cysts)	3.5
18°C.	7	12	c.150 (35 cysts)	4.4
28°C.	4	13 (3 quadr. cysts)	c.200 (40 cysts)	5.9

Hanging drops with bacteria

Hours	0	19.30	D.R. (19.30hrs.)
18°C.	1	4 (10 div. cysts)	4
28°C.	1	c.220 (22 div. systs)	8

It is obvious from the first experiment that the division rate increases with increase in temperature and this is clearly demonstrated also in the third experiment. These experiments employed yeast autolysate and it was thought that the effect of the

Fig.3. Division Rate of Colpoda steinii as a function of temperature.

temperature may have been indirect in affecting the bacterial population rather than directly affecting the ciliate division rate. The last experiments, in which the ciliates were suspended with loops of bacteria, shows however that this is not so although food obviously has a limiting effect. For this reason the absolute division rate is not a useful guide as it varies at the same temperature with different cultural conditions, as is shown by the second experiments and the difference between the division rate of the ciliates in yeast autolysate and with bacteria. When other conditions are identical however the division rate varies directly with temperature and this is shown graphically in Figure 3. This curve is very similar to the excystment curve given by Thimann and Barker (1934).

An interesting fact concerning these experiments is that the highest division rate was recorded with a hanging drop initially inoculated with a single ciliate (B.). Robertson (1921, 1924, 1927) founded his theory of allelocatalysis largely on his study of the growth of Colpoda. His theory is generally rejected (Richards, 1941) and it is interesting that the present experiments with Colpoda do not confirm Robertson's views.

Effect of High Temperature

Hanging drops were placed in the oven at 37°C. The ciliates in these drops continue to swim normally and to feed for several hours. After a time they encyst prior to division but at this temperature division (and excystment) is entirely inhibited and consequently unstable cysts are formed. If the hanging drop is returned to room temperature division and excystment take place. These effects are shown for the first experiment in which all the hanging drops were placed in the high temperature oven. After 26 hours there were only cysts and no active forms. When the drops were returned to air temperature the ciliates excysted and began to feed and divide again.

The effect on excystment of resistant cysts is similar (Goodey, 1913 and Bodine, 1923).

Effect of Low Temperatures

Cysts of Colpoda may be frozen and yet remain viable (Taylor and Strickland, 1936). This was confirmed. It was also found that active ciliates survived twenty four hours at 20°F. provided the culture did not freeze. At this temperature the ciliates become inactive and settle at the bottom of the culture dish. There is no division or encystment.

Ciliates could be kept for comparatively long periods at temperatures just above freezing point. The ciliates at this temperature are extremely sluggish but do feed and form unstable cysts prior to division. Division is however entirely inhibited and the ciliates remain encysted unless returned to room temperatures when excystment, or division and excystment, take place. Typical experiments are given as follows:-

Colpoda steinii

Hanging drops with yeast autolysate

Temperature	1° C.			18°C.		
Time (Hours)	0	67 $\frac{1}{2}$	140	0	26	31
Active Cysts	10	7	3	3	c.150 & division cysts	
	10	13	17	17		
Active Cysts	10	8	3	3	c.25	
		1	3	3		
Active Cysts	1	1	1	1	1 quadragenic cyst	4

These experiments show results similar to those obtained with high temperatures, viz. the inhibition of division and excystment and the formation of unstable cysts. In the third test the cyst still showed cyclosis after one hundred and forty hours at 1°C. but after four hours at room temperature the cyclosis had ceased and twenty four hours later division had taken place, later to be followed by excystment. The normal life cycle was resumed.

Discussion

Cysts of Colpoda survive indefinitely whether in the wet or dry state (Taylor and Strickland, 1936) but they react differently to high temperatures. Wet cysts are much more sensitive to high temperatures than dry cysts. The lethal temperature for wet cysts is 44°C for a one hour exposure and for dry cysts is 106°C. (Doflein, 1928; Taylor and Strickland, 1936). Similar figures are given by Bodine (1923). Taylor and Strickland also subjected the cysts to pressures of 1×10^{-5}

atmospheres for three days and found that viability of the cysts was not affected by slow evacuation. Using liquid air they also found that the cysts could survive a temperature of about -180°C .

Active ciliates are killed at about 40°C ., i.e. about the same temperature as the wet cysts. The ciliates can be gradually acclimatized to higher temperatures (Bodine, 1923). As already remarked the active ciliates cannot be frozen.

Encystment, unlike excystment, is not affected by temperature changes but at low temperatures activity is so reduced that I did not observe resting cysts to be formed, although of course unstable cysts are.

Excystment takes place within a temperature range of about 8° to 32°C . (Thimann and Barker, 1934; Bodine, 1923; Goodey, 1913). The rate of excystment or rather the speed varies directly with the temperature with an optimum about 25° to 28°C .

The present experiments have shown that division shows the same response to temperature as excystment and that it is inhibited above and below a certain temperature range. Because of this unstable cysts are formed under such conditions and the normal life cycle is restored only with a return to a favourable temperature.

Compared with other ciliates the effect of temperature on the division rate of Colpoda is very similar, e.g. Tetrahymena (Phelps, 1946) and Paramecium (Woodruff and Baitsell, 1911). Although the division rate appears to vary directly with temperature Watson (1944) found that the population growth of Balantiophorus was at a maximum at 13° to 16°C . This is difficult to understand but he was using a complex culture medium and the

effect of temperature may not have been direct.

The failure of Colpoda to divide at 37°C. indicates that growth will be inhibited at body temperatures and for this reason Colpoda could not successfully parasitize warm blooded animals, despite its tolerance of a wide range of environmental conditions. Moreover it seems unlikely that cysts would remain viable after passing through the alimentary canal of man, contrary to Watson's (1946c) assumption (Hoare, 1927). Certainly the ciliates would not excyst at such temperatures. It must be concluded therefore that Colpoda is not physiologically adapted to a parasitic existence in warm blooded animals and for this reason the earlier records of Schulz (1899), Yakimoff and Kolpakoff (1921) and Demianov (1943) must be discounted.

Conclusions

The experiments in this chapter have been concerned with the growth of Colpoda under varying conditions of hydrogen ion concentration, salinity, and temperature, and the effect of these factors upon the life history of the ciliate. The growth of the ciliate is relatively unaffected throughout a wide range of environmental conditions. The division rate however is affected both by increasing salinity and increasing temperature and there are extreme limits of temperature, pH and salinity beyond which division does not take place. Encystment does not appear to be affected directly by any of these factors but excystment, like division, is similarly inhibited by high and low temperatures and high salinities. It appears from the experiments that excystment will not take place unless the

osmotic tension of the milieu exterieur is less than that of the milieu interieur and this confirms the general belief that the first critical reaction in excystment is osmotic diffusion.

The effect both of high and low temperatures is reversible. The inhibited ciliates returned to a favourable temperature range will divide or excyst. In the latter case then a second critical reaction other than the osmotic diffusion is inhibited. This second reaction is known to involve respiration (Brown, 1939; Thimann and Commoner, 1940). Patently encystment is not dependent upon either of these reactions but division is, for in every case excystment and division are inhibited by the same factors.

These two reactions, division and excystment, are separate from feeding, movement and encystment which are not affected by oxygen lack or by the other environmental factors. This separation of the two reactions may also be achieved by various drugs or narcotics (Burt, 1945). The separation of two physiological systems by these means is also demonstrated by a variety of other organisms, such as the grasshopper egg and the sea urchin egg. In the case of sea urchin eggs it has been shown that lack of oxygen (Harvey, 1927), high carbon dioxide tensions (Haywood and Root, 1930) and cyanide (Clement, 1940) all inhibit division in the same way as they inhibit division of ciliates. Because of this separation two physiological systems are now recognized, the 'activity' system concerned with division, and the 'resting' system (Fisher and Henry, 1944).

C. A DISCUSSION OF THE 'ACTIVITY' AND 'RESTING'
SYSTEMS

If we now consider in retrospect the effect of the various factors on Colpoda, and on other ciliates, we are struck by two things: first, that the primary effect of all these factors is differential, suppressing one physiological process and not another; and secondly, that the response of Colpoda is similar to the response of other types of plant and animal.

The first phenomenon is known as dissociation (Needham, 1942) and the two systems dissociated by these factors are the 'activity' and 'resting' systems (Fisher and Stern, 1942; McElroy, 1947).

Recent study has confirmed that the activity system is generally associated with the aerobic carbohydrate metabolism (Bullough, 1948; Bullough and Johnson, 1951; Medawar, 1947; O'Connor, 1950a,b) and in particular oxidative phosphorylation (Keltch et al., 1950; Clowes et al., 1950; Brachet, 1951a). Inhibitors therefore which are most effective in suppressing the 'activity' system are those which interfere with the carbohydrate cycle and it is these inhibitors, and these inhibitors alone, which prevent cells entering the prophase of division (Hughes, 1950; Green and Ghadially, 1951). In the event of the cytochrome-cytochrome oxidase system being the only oxygen transport system, cyanide, by inhibiting this mechanism will also interrupt oxidative phosphorylation (Syrett, 1951). If an alternative oxidative system is provided however, such as ferricyanide, phosphorylation will take place anaerobically (Hersey and Ajl, 1951, a, b.).

Naturally oxidative phosphorylation will be inhibited in the absence of oxygen in the case of an aerobic organism and further the reduced carbohydrates of the Krebs cycle will accumulate as with Tetrahymena (Thomas, 1942). The effect of oxygen lack then can be attributed simply to the suppression of the phosphorylation which normally takes place under aerobic conditions. Anaerobes must either have an alternative system of phosphorylation or else division must be independent of the high energy phosphates. This latter hypothesis seems the more probable for although division in most animal and plant cells depends upon oxygen and the Krebs cycle there are two notable exceptions, namely embryonic tissue and neoplasms both of which continue to grow for a short period in the absence of oxygen or are relatively insensitive to carbohydrate inhibitors (Green & Savigear, 1951.). Indeed it seems certain that glucolysis rather than glycolysis is the chief carbohydrate mechanism in embryonic tissue and that only later does the phosphorylation mechanism become established (Needham, 1942). It also seems that neoplasms resemble embryonic tissue more closely than normal tissue. This difference in metabolic pathways is shown by sea urchin eggs in which there is a marked change between the unfertilized and fertilized eggs (Fisher et al., 1944). The former is insensitive to azide while the latter is sensitive. There is also an interesting distinction between endogenous respiration and exogenous respiration. Both for Chlorella (Syrett, 1951) and Paramecium endogenous respiration is cyanide insensitive whereas exogenous respiration is about 60% cyanide sensitive (Clark, 1945; Humphrey and Humphrey, 1948). Assuming that exogenous respiration inhibits

endogenous respiration, the cyanide sensitive mechanism can be associated with carbohydrate assimilation.

While the effect of anoxia may be attributed directly to the inhibition of phosphorylation by interfering with the oxidation of the carbohydrate substrate the effect of the other factors is patently different. It has already been stressed that Chang and Loomis (1945), after an exhaustive review of carbon dioxide toxæmia, attributed its inhibitory effect to the formation of a hydrogen bond linkage perhaps with the one-alpho-amino nitrogen of a protein. The coagulation of the cytoplasm indicates that molecular structure has been affected. In other words the effect of carbon dioxide is more fundamental than anoxia in that the enzyme itself is reversibly affected. Kavanau (1950) in discussing the effect of pH and temperature on enzyme kinetics also suggests that the inactivation of the enzyme system is caused by the formation of intramolecular hydrogen bridges. Kavanau considers that there is a thermodynamic equilibrium between catalytically active and catalytically inactive forms of the enzyme. The reactive configuration of the enzyme is possessed in only a relatively narrow temperature band, being lost at both high and low temperatures. Increasing the temperature from the point of low temperature inactivation results in the equilibrium between inactive and active configuration of the enzyme changing from the inactive to the active form. This continues until the optimum temperature is reached after which the equilibrium changes from the active to the inactive form. The rate of biological processes can then be explained in terms of an absolute reaction rate with a changing

equilibrium of active and inactive enzyme. This theory accounts satisfactorily for the curves illustrating biological activity such as division or excystment in Colpoda. For these processes an optimum temperature is about 25°C. and above and below this optimum the rate falls off at much the same rate in a linear manner to the point of low and high temperature inhibition. One can imagine therefore that at 25°C. the enzyme connected with the activity system or with excystment is largely if not wholly in its active form, whereas with increasing and decreasing temperatures it becomes increasingly inactive until at about 8°C. and 37°C. it is wholly or very largely inactive. While this interpretation may be accepted it is important to stress the differential effect of these factors for it is only the enzymes of the activity system that are affected by temperatures outside the range 8° to 37°C. Other enzyme systems function quite normally, such as those connected with encystment and ciliary movement.

It is also necessary to consider the effect of increasing osmotic tensions. It has been emphasised that an organism will tolerate only a limited osmotic difference between its own cytoplasm and the surrounding medium. In most fresh water animals adaptation to increasing salinity cannot be taken past the point where the medium is hypertonic to the organism. It is probable that euryhaline organisms are exceptions to this rule and this view is strengthened for protozoa by the fact that the contractile vacuole often ceases to function in higher salinities. However this may only indicate isotonic conditions. It is apparent that Colpoda steinii is stenohaline and that increasing salinity

seriously affects its physiology. It is difficult to attribute the inhibition of division in this case either to metabolic interference or inactivation of the protein, although the latter possibility is not unreasonable as we have shown that with increasing external salinity the internal salinity also increases. However a simpler explanation may be that as the salinity approaches the distal limit the increasing inhibition of division is due to the decreasing osmotic difference of organism and medium and the increasing difficulty of the organism to absorb water against this gradient.

It is now necessary to consider the action of the excystment promoting substances on the metabolism of Colpoda. First it is necessary to remember that in Colpoda as in Tillina excystment can be stimulated simply by a hypotonic solution such as distilled water. Whatever further role the excystment promoting substances may have as metabolites is secondary. After a series of extensive researches Strickland and Haagen-Smit (1948) reported that .3M ethyl alcohol was as efficient by itself as any of the recorded complex substances such as hay infusion or yeast extract. However this concentration of alcohol proved fatal to the ciliates as they emerged from the cyst membrane. The lethal effect of alcohol was removed by a concentration of 0.0001 potassium phosphate, itself a stimulatory substance. In their researches Thimann, Strickland and Haagen-Smit found that potassium was particularly active and that increasing activity was correlated with decreasing atomic weight and the presence of the hydroxy group (Thimann and Haagen-Smit, 1937). It is

apparent that the activity of the potassium ion and alcohol is dependent upon their mobility and is not due to any metabolic significance and this is confirmed by Garnjobst (1947) who found that the basic growth medium did not stimulate excystment. The activity of the K ion in cells is well known (Ponder, 1950; Raker et al. 1950). It is known to stimulate respiration and in particular there is an uptake of K following fertilization of sea urchin eggs accompanying increased oxygen uptake and increased acidity (Oddo and Esposito, 1951). It is considered that the role of K in these exchanges is the replacement of H ions. If this is so the effect of the potassium ion in accelerating excystment in Colpoda, a phenomenon we know to be comparable to division, may be interpreted in terms of Kavanau's theory. During the encysted period respiration is depressed but it rises rapidly in response to the excystment stimulus and this respiratory increase is cyanide sensitive (van Wagtendonk and Taylor, 1942; Thimann and Commoner, 1940). It is not unreasonable to presume that the increased metabolic activity is due to increasing activity of the enzymes connected with the activity system. That the increased respiration is due to increased enzyme activity and not to a new substrate is shown by the fact that distilled water may stimulate excystment, although it is patently not a metabolic substrate. If then there is a changeover between the encysted ciliate before and after stimulation comparable to the difference between the unfertilized and fertilized egg and this changeover is due, as in the case of the sea urchin egg, to the introduction of an

alternative respiratory mechanism then its activity will be governed by the principles outlined by Kavanau. Namely, with increasing temperature up to an optimum there will be increased acceleration as the equilibrium increasingly favours the active form of the enzyme. Past the optimum acceleration will decrease. This is the case (Thimann and Barker, 1934). Accepting this theory then we attribute the activity of the K ion to the displacement of the hydrogen bond which characterises the inactive form (Seifriz, 1942). With increased concentrations of the K ion up to an optimum there will be increased acceleration of excystment. This is also the case (Haagen-Smit and Thimann, 1938; Strickland and Haagen-Smit, 1947). However this initial activation of the enzyme system must be separated from the ultimate respiration of this system for the two are separable, the second is absolutely dependent upon oxygen and the first is not. The effect of the alcohol is less certain (Strickland and Haagen-Smit, 1948). It may be that, like the substituted phenols, in dilute concentrations it stimulates respiration but the mechanism of such stimulation is less certain. It may be that the hydroxyl group is active in accelerating the formation of active enzyme, and that its role is complementary to that of the K ions. As this role is performed by a large number of substances, although with greatest efficiency by alcohol, it is difficult to define precisely what this action is (Thimann and Haagen-Smit, 1937; Haagen-Smit and Thimann, 1938). These metabolic changes are accompanied by a marked uptake of water and as we have seen such uptake is possible only if there is a

sufficient gradient of osmotic tension. This water uptake which is definitely dependent upon excystment metabolism is comparable to the water uptake during division which is also dependent upon a favourable osmotic gradient. The fact that considerably smaller salinities are required to inhibit excystment than those required to inhibit division strengthens the view already proposed that this water uptake is not dependent upon absolute but upon relative tensions within certain limits.

There remains one phenomenon still to be explained, namely the difference of high temperature on dry and wet cysts. The former are irreversibly affected at 106°C and the latter at 44°C . Taylor and Strickland (1936) assume that the temperature of coagulation of a protein in colloidal solution varies with the amount of free water but they do not discuss why this should be so. There is evidence (Chase, 1950) that reversible and irreversible denaturation of protein takes place at different temperature levels but in this case irreversible denaturation takes place at two different temperatures depending on whether the cyst is wet or dry. It may be, as with erythrocytes, that with increasing temperature and increasing rate of diffusion there is increased loss of cations, such as K, which is naturally prevented in the dry cyst. This loss of cations, if indeed it takes place, may cause irreversible denaturation before the critical temperature is reached. Considering the importance and mobility of the K ions such an explanation is not improbable. It is interesting to note that the lethal limit of the wet cysts is much the same as the lethal limit of the active ciliates (Bodine, 1923). Encystment per se does not therefore raise the resistance of the ciliate to ^{high} temperature.

It has been shown that soil ciliates are not ecologically or physiologically a homogenous group. On an ecological basis three major groups are distinguished: a litter group, a soil group and a saprobic group. The first differs from the other two in consisting of larger species which are morphologically more specialized and which are confined to soils and litters of granular structure. These ciliates cannot be cultured in enriched media although they tolerate a wide pH range. It is concluded that they are associated with a different microflora and that they require a well aerated habitat with good waterholding capacity but which is not subject to waterlogging. Such conditions are offered only by granular conditions for sands to not have good water retention and loams are liable to anaerobic conditions and waterlogging. As they are found in strongly acid beech litters and neutral or slightly alkaline soils pH cannot be a limiting factor and will not account for their absence from fresh water. Indeed they are more closely related to the fresh water fauna than any other soil group and possibly they differ only in their acid tolerance and a more effective cyst physiology.

The second and third groups consist of species which are found throughout the whole range of soil types and are conspicuously smaller in size than fresh water species. They are tolerant of a wide range of pH while the third group is distinguished by a tolerance of enriched culture conditions. It is essentially this group which is also found in polluted streams, sewage and coprophilic habitats and which occasionally

is parasitic. It also includes the most common of soil ciliates. Their small size is correlated with the restricted moisture of the soil capillaries and inter- and intragranular spaces. The exclusion of typical fresh water species is attributed partly to this size restriction and partly to the more acid conditions of soil.

It is the characteristics of the third group which have been most closely studied and in particular the genera Colpoda and Vorticella. It is emphasised that these species are generally bacteriophagous and that not only do they tolerate rich bacterial infusions but they prefer and indeed thrive only under such conditions. Some species are capable of facultative anaerobiosis although they do not divide in the complete absence of oxygen. They are conspicuously resistant to carbon dioxide anaesthesia and toxæmia and in this respect, more than any other, differ from typical fresh water species which avoid, where the polysaprophes seek, conditions of bacterial decomposition and high carbon dioxide tensions. They are tolerant of a wide range of temperatures and of high salinity. In this they are again conspicuously different from fresh water species and this is correlated with the fluctuating osmotic soil conditions. These conditions of temperature, salinity, and bacterial activity accompanied by low oxygen and high carbon dioxide tensions are the discriminating features of the soil ecology and the species adapted to such conditions are therefore able to tolerate the parasitic environment, at least in cold blooded animals. Many fresh water species on the other hand

are extremely sensitive to carbon dioxide or anoxia and are thereby excluded from such habitats.

But the most important feature of the bionomics of those species which are most widespread in soil is their cyst physiology which plays an extremely important part in their life history. In Colpoda the most common of all soil ciliates the cyst mechanism is most highly developed. In fresh water where there is a continuous and varied habitat species may avoid adverse conditions by migration whether vertical or horizontal. In this way Paramecium may migrate to the surface or spend the period of winter senescence at the bottom of a lake. Loxodes selects a favourable oxygen tension and Spirostomum a favourable pH. In these cases therefore a cyst physiology is not very important and indeed is generally absent. In a few cases such as Stylonychia pustulata the ciliates encyst in response to adverse conditions, viz. pH and excyst under favourable conditions. Generally, however, as there is no fear of dessication or great change in salinity, changes in temperature, pH, oxygen and carbon dioxide tension can be circumvented by the avoiding reaction. (Jennings, 1904, 1915). With sessile species such as Stentor or Vorticella the ciliate may become free-swimming under adverse conditions and settle again only when it has swum clear of these conditions. Ciliates are also attracted by chemotaxis when favourable conditions arise and collect together to feed and divide (Fauré-Fremiet, 1950). Soil offers no such continuous and varied habitat and is generally subject to alternating conditions

of dessication and wetting. Such conditions are not unfavourable for protozoan activity provided the intervening periods of dessication can be overcome. The cyst physiology provides a survival mechanism. However encystment is seldom a response to such adverse conditions as dessication and survival depends on the fact that encystment is a normal feature of the ciliate life history and for this reason there is always a number of encysted ciliates in the soil as well as trophic forms (Cook, 1932). What is important is the regularity with which the ciliates encyst, the resistance of the cysts to every kind of treatment, and the readiness with which they excyst. Excystment is not always a response to favourable conditions and the encysted protozoan may be activated by stimuli which cause the death of the ciliate as it escapes from the cyst membrane. There is then no absolute connection between the life history of the ciliate and its environmental conditions although the latter greatly influences the former. Rather it is that the bionomics of these species enable them to take advantage of soil conditions whereas other species are prevented by one cause or another.

The soil ciliates are closely connected with the bacterial flora and bacterial growth is invariably followed by great ciliate activity (Kubiena, 1938). The effect of this activity is the rapid consumption of the bacteria, unless a constant supply of fresh substrate is forthcoming, as e.g. in polluted streams. With the bacteria consumed the ciliates encyst. Not all bacteria are equally suitable as food and some are even toxic to the ciliates. Consequently they exert a selective effect on the

bacterial flora and conversely the bacterial flora determines the growth and activity of the ciliate fauna. Their role in the soil economy is therefore indirect rather than direct and is both an indication of bacterial activity and a check on such activity.

The general problem of ciliate bionomics, to which the present studies offer a contribution, may now be re-examined. There is on the one hand the problem of ciliate ecology and on the other the behaviour, the life history and the physiology of the ciliates and there is finally the relationship between these two. As has been recently stressed by Wigglesworth (1951) a proper understanding of the ecology of an insect is impossible without a knowledge of its physiology, and further "the study of diverse physiological mechanisms serves to codify our knowledge, to put it on a rational basis and to point the way to further advances". What is true of insects is equally true of ciliate protozoa. The difficulties which arise from an uncritical interpretation of ecological data have been stressed by Lackey (1938b). However there appear to be only two alternatives: either to pass such generalizations as are made by Noland (1925a), Wang (1930), Picken (1938) and other ecologists (and which have been criticised by Lackey), or to confine oneself to a single species, as in the case of Watson's study of Balantiophorus minutus. Neither method will enable any conclusions to be made on the bionomics of soil ciliates or indeed on the bionomics of ciliates generally. To overcome this difficulty and to place their bionomics on a rational basis the following scheme is proposed.

The ecology of any organism or of any fauna may be considered a function of three attributes of the organism or fauna - behaviour, life history, and physiology. Applying this equation to any particular habitat such as soil the limitations of the habitat upon these three attributes can be defined. Only an organism or fauna which possesses these defined attributes will be found in such a habitat. For soil, e.g., the behaviour of the ciliate fauna will be limited by the small spaces in which water is retained and by the predominately bacterial flora.

① Consequently the soil ciliates must be small forms feeding on bacteria. Again, because of the fluctuating soil conditions

② their life history must have alternating resistant and trophic stages. Finally because of the fluctuations in the chemical and physical soil conditions and because of the activity of the

③ bacterial flora soil ciliates must be tolerant of a wide range of environmental conditions. Alternatively with another habitat, such as litter or granular soils, the first and the third qualifications are less significant but the second is important. For sewage or polluted water the first and second qualifications are not significant while the third is particularly significant.

Finally for fresh water the essential qualification is the ability of the ciliates to select a favourable environment of temperature, pH, oxygen tension or carbon dioxide tension, to be able to avoid unfavourable conditions generally by migration and occasionally by encystment (Jennings, 1915). This concerns primarily the first two qualifications and the third is less significant.

Table 16 gives a diagrammatic representation of these differences

and thereby defines the differences between various ecological niches. It overcomes the difficulty of defining the ecology of a species individually which is difficult for widespread species and tends to blur significant distinctions, while it also permits the essential characteristics of a particular fauna to be defined and contrasted with the characteristics of other faunas.

Table 16

Diagram showing the characteristic behaviour, life history, and physiology of the ciliate fauna of different habitats and the relation between them.

Ecology	f	Behaviour	Life History	Physiology
Soil		Small, free-swimming, without elaborate feeding mechanism	Alternate resistant and trophic stages	Wide range of tolerance to environmental conditions.
Litter and Granular Soils		-	"	-
Sewage and Polluted Water		-	-	"
Fresh Water		Able to migrate from adverse to favourable conditions.	-	-

Nevertheless it remains true that, as Jennings (1915, p.67) remarked, 'acclimatization to non-optimal conditions is an ever present factor in the behaviour of organisms'. This statement is as applicable to soil ciliates as to any other group of organisms.

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P L A T E S

Generally the figures are drawn from life using a camera lucida. High power ($1/6$ inch), water immersion ($1/7$ inch, $1/10$ inch) and oil immersion ($1/12$ inch) objectives were used. Nuclear detail was obtained from fixed and stained material, methyl green, Schaudinn's fixative and Erlich's haematoxylin being generally employed.

The dimensions given indicate the length of the ciliate. The position of the ciliary meridians is indicated by lines except at the margins of the ciliate where the cilia are shown individually. An exception is the drawings of Dileptus in which there are many more ciliary meridians than indicated. In the Trichopelmidae cilia are sparse and are indicated individually. The lines in these drawings indicate ridges and grooves on the body surface. In Phacodinium the position of the individual cilia, with the exception of the marginal and peristomal cilia, is indicated by dots and the same convention is used generally for the Hypotricha, the exact position and relative size of the cirrhi being thereby indicated.

PLATE 1

1.	<u>Spathidium sp.1</u>	(70mu)	x 1150	
2.	<u>Spathidium sp.2</u>	(140mu)	x 610	
3.	<u>Spathidium sp.3</u>	(190mu)	x 550	
4.	<u>Spathidium sp.4</u>	(100mu)	x 610	
5.	<u>Didinium (Monodinium) balbiani?</u>	(25mu)		x 610
6.	<u>Urotricha agilis</u>	(20mu)	x 610	
7.	<u>Pithothroax ovata</u>	(25mu)	x 610	
8.	<u>Enchelys farcimen?</u>	(45mu)	x 1150	
9.	<u>Prorodon sp.</u>	(85mu)	x 610	
10, 11.	<u>Lacrymaria sp.</u>	(70mu extended; 45mu contracted)		x 610
12.	<u>Chaenea limicola?</u>	(140mu)	x 610

PLATE I

PLATE 2

1,
2,
5.	<u>Lionotus</u> sp.	(70mu)	x 610, x 1150
3.	<u>Loxophyllum</u> <u>utriculariae</u>	(90mu)	x 610
4.	<u>Bryophyllum</u> <u>spathidioides</u>	(140mu)	x 610
6.	<u>Dileptus</u> <u>gigas</u>	(250mu)	x 610
7.	<u>Dileptus</u> sp.	(130mu)	x 610
8.	<u>Dileptus</u> <u>americanus</u>	(240mu)	x 610
9.	<u>Trichopelma</u> <u>sphagnetorium</u>	(25mu)	x 1150
10.	<u>Paramecium</u> sp.	(55mu)	x 610
11,
12.	<u>Drepanomonas</u> <u>revoluta</u>	(22mu)	x 1150
13.	<u>Drepanomonas</u> <u>sphagni</u>	(32mu)	x 610

PLATE 2

PLATE 3

1.	<u>Chilodonella uncinatus?</u>	(55mu)	x 610
2,			
3.	<u>Chilodonella uncinatus?</u>	(70mu)	x 610
4,			
5.	<u>Chilodonella uncinatus?</u>	(60mu)	x 610
6,			
7,			
8.	<u>Chilodonella gouraudi?</u>	(40mu)	x 610
9.	<u>Chilodonella cucullus?</u>	(100mu)	x 610
10,			
11.	<u>Chilodonella gouraudi?</u>	(45mu)	x 610
12,			
13,			
14.	<u>Plagiopyla sp.?</u>	(60mu)	x 610
15,			
16.	<u>Plagiopylidae</u>	(60mu)	x 610

PLATE 3

PLATE 4

1.	<u>Cyclidium pellucidum?</u>	(35mu)	x 610
2.	<u>Cohnilembus fusiformis</u>	(75mu)	x 610
3.	<u>Cyrtolophosis mucicola</u>	(30mu)	x 1150
4.	<u>Homalogastra setosa</u>	(25mu)	x 1150
5.	<u>Colpidium sp.?</u>	(30mu)	x 1150
6.	<u>Cinetochilum margaritaceum</u>	(23mu)	x 1150
7.	<u>Saprophilus agitatedus?</u>	(35mu)	x 610
8.	<u>Saprophilus muscorum?</u>	(35mu)	x 610
9.	<u>Saprophilus sp.</u>	(35mu)	x 610
10.	<u>Pseudoglaucoma muscorum?</u>	(12mu)	x 1150

PLATE 4

PLATE 5.

1.	<u>Blepharisma</u> <u>sp.</u>	(100mu)	x 1150
2.	<u>Blepharisma</u> <u>steini</u>	(120mu)	x 610
3.	<u>Bryometopus</u> <u>pseudochilodon</u>	(65mu)	x 1150
4.	<u>Balantidium</u> <u>sp.</u>	(45mu)	x 610
5,
6.	<u>Phacodinium</u> <u>metschnikoffi</u>	(110mu)	x 610

PLATE 5

PLATE 6

1.	<u>Oxytricha fallax</u>	(150mu)	x 610
2.	<u>Oxytricha pellionella</u>	(110mu)	x 610
3.	<u>Oxytricha sp.</u>	(90mu)	x 610
4.	<u>Oxytricha setigera?</u>	(45mu)	x 610
5,			
6.	<u>Psilotricha sp.</u>	(60mu)	x 610
7,			
8.	<u>Gonostomum affine</u>	(95mu)	x 610
9,			
10,			
11,			
12.	<u>Euplotes sp.</u>	(60mu)	x 610
13.	<u>Aspidisca lynceus?</u>	(32mu)	x 610
15,			
16.	<u>Aspidisca costata</u>	(40mu)	x 610

PLATE 6

PLATE 7

1.	<u>Uroleptus mobilis?</u>	(120mu)	x 610
2.	<u>Uroleptus musculus</u>	(110mu)	x 610
3.	<u>Uroleptus piscis?</u>	(160mu)	x 610
4.	degenerate form of 6.	(160mu)	x 610
5.	<u>Hemicycliostyla sp.</u>	(210mu)	x 610
6.	<u>Holosticha sp.</u>	(200mu)	x 610
7.	<u>Onychodromus grandis?</u>	(180mu)	x 610

PLATE 7

PLATE 8

1.	<u>Hemicycliostyla</u> sp.	(220mu)	x 610
2.	<u>Onychodromus grandis</u> ?	(160mu)	x 610
3.	<u>Trachelophyllum apiculatum</u> ?	(160mu)	x 610
4,
5.	<u>Psilotricha</u> sp.	(70mu)	x 610
6.	<u>Thecacineta</u> sp.	(50mu)	x 610

PLATE 8

PLATE 9

1.	Suctorian	(30mu)	x 610
2,			
3.	<u>Sphaerophrya magna</u>	(20mu)	x 610
4,			
5.	Suctorian	(35mu)	x 610

PLATE 9

PLATE 10

1. Colpoda saprophila x 2200
2. Colpoda saprophila: ciliary meridians
3. Colpoda cucullus x 1150
4. Colpoda inflata x 1150

3

4

PLATE 10

PLATE 11

Colpoda steinii

(30mu)

x 2000

MI
MA

miconucleus
macronucleus

PLATE II

PLATE 12

- 1 - 5 Vorticella striata
- 1,
2,
3. Normal 'utriculus' form x 1800,x 520
4. Well fed form x 520
5. Fed on bacteria x 520
- 6 - 12 Vorticella microstoma
6. typical soil form x 520
7. an abnormal two-stalked form x 520
8. a typical 'microstoma' form x 520
- 9,
10. a starved form and cyst x 520
11. after feeding on bacteria, the 'hians' form x 520
12. another large 'hians' form x 520

P	peristome
CV	contractile vacuole
SC	scopula
ST	striations
FV	food vacuole
V	vestibule
NU	macronucleus

PLATE 12

PLATE 13

Excystment of large Vorticella microstoma

x 630

- 1 - 7. excystment and formation of the telotroch
- 8. cyst membrane
- 9. telotroch

CV contractile vacuole
V vestibule
VM vestibular membrane
CP cyst pore
CW ciliary wreath

100 μ

PLATE 13

PLATE 14

Prorodon sp.

x 660

1. temporary cyst
2. permanent cyst
3. ciliary meridians
4. Prorodon feeding on Vorticella microstoma (free hand)

CM (figs. 1, 2)	cyst membrane
CM (fig. 3)	ciliary meridians
CV	contractile vacuole
NU	nucleus
PR	pharyngeal rods

PLATE 14